Zirconia Publication Abstracts - Sorted by Subject and Chronologically

(Rev. 07/26/11)

Reviews

16. P.W. Carr, J.A. Blackwell, T.P. Weber, W.A. Schafer, and M.P. Rigney, "Zirconium Oxide Based Supports for Biochromatographic Applications," in *Chromatography in Biotechnology*, C. Horvath and L.S. Ettre, (Eds.), ACS Symposium Series No. 529, 146-64 (1993).

    Abstract: Porous microparticulate zirconium oxide is an excellent alternative to silica-based and functionalized organic resin-based supports for the separation of biomolecules. The complex surface chemistry of this chemically and physically stable support can be exploited in a number of ways to yield biocompatible surfaces with unique selectivities. Treatment of the particles with phosphoric acid converts the amphoteric metal oxide surface to an efficient cation exchanger. When using the bare particles, the Lewis acid-base interactions between the surface zirconium (IV) ions and Lewis base solutes can be controlled by the use of competing eluent Lewis bases to give a novel ligand exchange support.

17. J. Nawrocki, M.P. Rigney, A. McCormick, and P.W. Carr, Review, "Chemistry of Zirconia and Its Use in Chromatography," *J. Chromatogr*. A **657**, 229-82 (1993).

    Abstract: The purpose of this review is to shed some light on the complex properties of zirconia's surface chemistry in order to better understand its behavior under chromatographic conditions. We emphasize the great differences between the much better known chemistry of a silica surface and the chemistry of zirconia's surface. The review describes both the physical and chemical properties of zirconium dioxide from a chromatographic point of view. The chemistry of monoclinic zirconia surface is developed from its underlying crystalline structure. The paper describes the dependence of the specific surface area, pore volume, porosity and mechanical strength on thermal treatment. Methods of synthesis of chromatographically useful zirconia are outlined. The view also covers the adsorption properties of zirconia a both gas-solid and liquid-solid interfaces. Adsorption of water, carbon dioxide, carbon monoxide and ammonia are described and the controversies concerning the surface concentration of adsorption sites are presented. The complex chemistry of a zirconia surface is pointed out and the importance of ligand exchange reactions is emphasized. In contrast to a silica surface, ligand exchange plays an important role in liquid chromatographic applications of zirconia. Strong, hard Lewis acid sites, present on a zirconia surface, can interact with hard Lewis bases and these interactions, sometimes troublesome, can be successfully exploited even for protein separations. Zirconia's surface can be modified in many ways: dynamically, by addition of competing Lewis bases to the mobile phase, or permanently, by covering its surface with polymers or by depositing carbon. The review also shows that the main difficulty in achieving a wider variety of applications is probably our lack of knowledge and poor understanding of zirconia's surface chemistry.

23. J Nawrocki, C.J. Dunlap, P.W. Carr, and J.A. Blackwell, Review, "New Materials for Biotechnology: Chromatographic Stationary Phases Based on Zirconia," *Biotechnol. Prog.* **10**, 561-73 (1994).

    Abstract: This review explores the usefulness of zirconia-based materials in separations in biotechnology. The physical and chemical properties of zirconia are discussed briefly to familiarize the reader with the advantages of zirconia. The use of native zirconia is then examined, with a study of the Lewis acid/base chemistry that defines chromatography with zirconia. Modification of the zirconia surface with small molecules is then discussed. Finally, polymer-coated zirconia materials are examined. Examples of separations using these various-materials are shown. The advantages and disadvantages of each of these materials are presented.

51. P.T. Jackson and P.W. Carr, “Improving Reversed-Phase Liquid Chromatography,” *CHEMTECH* **28**, 29-37 (1998).

    Abstract: With the wide array of problems associated with the current suite of RPLC stationary phases, new materials, which are immune to chemical susceptibilities of silica, bonded phases, and polymers, are needed. Zirconia, a chemically and thermally stable alternative to silica developed at the University of Minnesota, is now manufactured by ZirChrom Separations.

65. C. McNeff, L. Zigan, K. Johnson, P.W. Carr, A. Wang, A.M. Weber-Main, “Analytical Advantages of Highly Stable Stationary Phases for Reversed-Phase LC,” *LCGC*, **18(5)**, 514-529 (2000).

    Abstract: During the past five years, many manufacturers of high performance liquid chromatography (HPLC) columns have focused on improving stationary-phase stability and reproducibility. Improved column stability – both chemical and thermal – offers new advantages, such as decreased analysis time and new methods of selectivity optimization. More stable HPLC packing materials have been achieved through advances in silane chemistry; however, the greatest improvements in stability have resulted from the use of alternative nonsilica supports such as synthetic organic polymers, alumina, and zirconia. In this article, the authors describe their use of various test solutes to compare the efficiency, selectivity, and hydrophobic retention mechanisms of five commercially available HPLC columns based on silica, alumina, zirconia, and polystyrene cross-linked with divinlybenzene (PS-DVB).

71. Christopher J. Dunlap, Peter W. Carr, Clayton V. McNeff, Dwight Stoll, “Zirconia Stationary Phases for Extreme Separations,” *Anal. Chem.*, 598A-607A (2001).

    Abstract: Composite materials surpass standard silicon and carbon for stability and robustness in complicated reversed-phase column applications.

77. R.A. Henry, “Highly Selective Zirconia-based Phases for HPLC Applications,” *American Laboratory*, **34**(**22**), 18-25 (2002).

    Abstract: Zirconia-based phases (Z-phases) for HPLC have been available for almost a decade, and are finding a wide range of applicability because they are very complementary to silica-based phases (S-phases) and often replace total-polymer phases (P-phases), particularly in reversed-phase (RP) applications.

87. J. Nawrocki, C. Dunlap, A. McCormick, P. W. Carr, “Part I. Chromatography Using Ultra-Stable Metal Oxide-Based Stationary Phases for HPLC,” *Journal of Chromatography A*, **1028**, 1-30 (2004).

    Abstract: The first part of the review contrasts the main drawbacks of silica-based packings such as their relative thermal and chemical instability with excellent stability of metal oxides. The paper concerns mainly ZrO2, TiO2 and Al2O3. Methods of preparation of spherical particles for HPLC are described. Surface chemistry of the oxides is, however, very different from that of silica. Ability of the oxides to ion- and ligand exchange is discussed from a chromatographic point of view.

88. J. Nawrocki, C. Dunlap, J. Li, J. Zhao, C.V. McNeff, A. McCormick, P.W. Carr, “Part II. Chromatography Using Ultra-Stable Metal Oxide-Based Stationary Phases for HPLC,” *Journal of Chromatography A*, **1028**, 31-62 (2004).

    Abstract: In this part of the review authors discuss methods used for modification of metal oxide surfaces. On the basis of literature data it is shown, that silanization of the surfaces do not form stable supports for chromatography. On the other hand, the success of polymer modified surfaces such as polybutadiene (PBD) and polystyrene (PS) is emphasized. Permanent modification of metal oxide surfaces with Lewis bases is also widely discussed. Chromatographic properties of polymer-modified surfaces of zirconia are discussed in details. The perspectives of carbon-coated metal oxide surfaces in HPLC and high temperature separations are described.

89c. H.A. Claessens and M.A. van Straten, “Review on the Chemical and Thermal Stability of Stationary Phases for Reversed-phase Liquid Chromatography,” *Journal of Chromatography A*, **1060(1-2)**, 23-41 (2004).

    Abstract: At present, in high-performance liquid chromatography (HPLC) for the majority of analyses, reversed-phase liquid chromatography (RPLC) is the separation mode of choice. Faster method development procedures using aggressive eluents under elevated temperature conditions, the need for improved selectivities, efficiencies and resolution, the reduction of solvent consumption and also the decrease of analysis times require reversed-phase (RP) columns of high chemical and thermal stability. Until now, the majority of columns for RPLC separations are manufactured from silica substrates. Silica has many favorable properties making this material nearly ideal as a support for RP columns. However, its solubility, that increases considerably in eluents of pH above ±7, is a drawback preventing its widespread use over the entire pH range. In addition, also the thermal stability of silica is limited. Recently, however, substantial progress has been made in the synthesis of RPLC silica-based stationary phases showing satisfactory thermal and chemical stability under many different experimental conditions. Also, new substrates mainly based on other inorganic substrates like, e.g. alumina and zirconia have been developed now as a starting material for the preparation of RPLC stationary phases of improved chemical and thermal stability. In addition, for the same reasons, many efforts have also been made to synthesize polymer and also polymer-coated phases. These latter phases, more particularly those based on zirconia, but also polymer phases show a high degree of chemical and thermal stability compared to silica counterparts. In this paper, an overview will be given of the state-of-the-art of the thermal and chemical stability of the different available stationary phases for RPLC.

105.C.V. McNeff, B. Yan, D.R. Stoll, R.A. Henry, “Review: Practice and Theory of High Temperature Liquid Chromatography,” *Journal of Separation Science,* **30**, 1672-1685 (2007).

Abstract: High temperature liquid chromatography (HTLC) exists in a temperature region beyond ambient (ca. 40 C) and below super critical temperatures. The promises of HTLC, such as increased analysis speed, enhanced separation productivity, “green” LC with pure water mobile phases coupled to universal FID detection, and fast analysis of complex samples by combination with fast 2-D techniques, have become an option for routine practice. The focus of this paper is to review the key developments that have made the application of HTLC a practical technique and draw attention to new developments in 2-D techniques that incorporate HTLC that offer an opportunity to vastly increase the usefulness of HPLC for the analysis of complex samples.

108. N. Wu, A. Clausen, “Fundamental and Practical Aspects of Ultrahigh Pressure Liquid Chromatography for Fast Separations,” *J. Sep Sci*., **30**, 1167-1182 (2007).

Abstract: The ongoing development of HPLC has been focused on increasing the speed and efficiency of separations over the past decade. The advances in separation speed have been primarily related to the development of column technology and instrumentation. Relatively short columns packed with sub-2 lm particles provide highspeed separations while maintaining or increasing resolution. Ultrahigh pressure pump systems have been developed to overcome the high-pressure drop generated by such sub-2 lm packings. In this review, fundamental and practical aspects of ultrahigh pressure or ultrahigh performance liquid chromatography (U-HPLC) are discussed. Applications of fast U-HPLC separations are also presented.

115. G. Vanhoenacker, P. Sandra, “High Temperature and Temperature Programmed HPLC: Possibilities and Limitations,” *Anal Bioanal Chem*, 390, 245 – 258 (2008).

Abstract: Temperature plays an important role in high-performance liquid chromatography (HPLC) since both the kinetics and thermodynamics of the chromatographic process are a function of temperature. To date, the possibilities of using temperature to improve LC separations have not been fully investigated. Most researchers mainly focus on column chemistry and dimensions, and on mobile phase composition and pH to optimize their separation. Temperature is rarely examined over a broad range and most separations are performed and/or optimized in the temperature region 20–50 °C. Moreover, currently a large number of liquid chromatographs are still operated at room temperature instead of using a column thermostat to control column temperature and thus retention time. We have recently reviewed the use of elevated temperature and temperature programming in HPLC [1].

117. B. Wenclawiak, S. Giegold, T. Teutenberg, “Mini-Review: High-Temperature Liquid Chromatography,” *Analytical Letters*, 41, 1097-1105 (2008).

Abstract: The influence of temperature on separation is discussed. Elevated temperature can significantly reduce the retention times in liquid chromatography and thus the throughput and running costs of a LC instrument. A decrease of viscosity at increasing temperatures allows the use of columns packed with very small particles and of higher flow rates as well. Modern reversed phase columns are stable up to (and >)

90_C. Metal oxide columns (Zirconium oxide, Titanium dioxide, Aluminium oxide) and polymer packed columns are temperature stable up to 200_C. The consumption of organic solvents can significantly be reduced and thus also the disposal costs.

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1. M.P. Rigney, T.P. Weber, P.W. Carr, "Preparation and Evaluation of a Polymer-Coated Zirconia Reversed-Phase Chromatographic Support, "*J. Chromatogr*. **484**, 273-91 (1989).

   Abstract: The preparation of a reversed-phase high-performance liquid chromatographic support by deposition and cross-linking of polybutadiene on the surface of microporous zirconia is described. It is demonstrated that acidic solutes undergo very strong interactions with the zirconia surface even in the presence of a thick polymer layer. These interactions can be minimized by the use of phosphate in the mobile phase, in which case the small solute retention characteristics of the polymer-coated zirconia are comparable to alkylsilane-derivatized silica-based reversed-phase supports. Most importantly, the polymer-coated zirconia was stable in alkaline solution. There was no evidence for degradation of the support even when it was exposed to a mobile phase of 1M sodium hydroxide at 100^{o}C. In contrast, polybutadiene-coated alumina was not stable under these conditions.

27. L. Sun and P.W. Carr, "Mixed-Mode Retention of Peptides on Phosphate-Modified Polybutadiene-Coated Zirconia," *Anal. Chem*. **67**, 2517-23 (1995).

   Abstract: Zirconia HPLC packing materials were found to be potentially advantageous for large scale protein separations due to their excellent pH stability and mechanical stability. However, Lewis acid sites on zirconia’s surface cause irreversible adsorption of proteins due to their interactions with hard Lewis bases such as the carboxyl groups in proteins. Although the Lewis acid sites can be effectively blocked by adsorbing phosphate ions onto zirconia’s surface, proteins and peptides cannot be eluted using a typical reversed-phase mobile phase. In this work, we found that the separation of peptides on a phosphate-modified polybutadiene-coated zirconia (PBD-ZrO$_2$) can be brought about by using a mobile phase containing both an organic modifier and a high concentration of sodium perchlorate. The salt is needed to cancel the coulombic interactions between negatively charge stationary phase and the positively charged proteins. To understand the retention mechanism of proteins and peptides on phosphate-modified PBD-ZrO$_2$, this work was aimed at the study of the surface characteristics of the phosphate-modified ZrO$_2$. We found that the phosphate-modified ZrO$_2$ phase has both reversed-phase and cation-exchange characteristics under the acidic mobile-phase conditions used for proteins and peptide separations. The PBD coating provides hydrophobic moieties, and the phosphate ions adsorbed on zirconia’s surface provide cation-exchange sites. Reversed-phase separation of a peptide standard mixture and cation-exchange separation of a cationic peptide standard mixture on the same phosphate-modified PBD-ZrO$_2$ column shows excellent column resolution in both modes. Although mixed-mode stationary phases provide unique selectivity, the secondary equilibrium on phosphate-modified PBD-ZrO$_2$ can cause peak broadening. Applications of the phosphate-modified PBD-ZrO$_2$ to peptide separations are demonstrated here.

30. L. Sun and P.W. Carr, "Chromatography of Proteins Using Polybutadiene-Coated Zirconia," *Anal. Chem*. **67,** 3717-21 (1995).

   Abstract: Polybutadiene-coated zirconia (PBD-ZrO$_2$), when used as a stationary phase in conjunction with a mobile phase containing phosphates, constitutes a reversed-phase/cation-exchange mixed-mode chromatographic system. The separation of proteins on this phase can be achieved only through the use of mobile phases containing the correct combination of phosphoric acid, displacing salt and organic cosolvent. We found that *excessive* coulombic interactions between proteins and the stationary phase impair the system performance for the separation of proteins. The effects of mobile phase conditions on the separation on proteins using phosphate-adsorbed PBD-ZrO$_2$ are studied in this work. Factors such as the presence of a multivalent cation, mobile phase pH, phosphate concentration, and slat concentration can be manipulated to reduce the new negative charge on the surface and thereby improve the performance of the system toward the separations of proteins.

35. J.W. Li and P.W. Carr, “The Retention Characteristics of Polybutadiene-Coated Zirconia and Comparison to Conventional Bonded Phases,” *Anal. Chem.*, **68(17)**, 2857-2868 (1996).

Abstract: This paper presents a detailed study of retention on a reversed-phase material made by coating polybutadiene (PBD) on porous zirconia. PBD-coated zirconia particles with six different carbon loads (0.25-5.6% carbon by weight) were prepared by evaporatively depositing and cross-linking PBD on microparticulate porous zirconia. Retention data of a homologous series of alkylbenzenes were obtained on the six PBD phases as a function of mobile phase composition in methanol-water and acetonitrile-water mixtures from 20 to 50% (v/v). The results obtained for the phase were compared to those for conventional octadecylsilane (ODS) bonded phases, and the effect of the amount of PBD on retention was studied in detail. We find that, per amount of bonded phase, the PBD phase is less retentive than is the ODS phase, but it has comparable hydrophobic selectivity. Furthermore, the PBD phase has about the same sensitivity toward changes in mobile phase composition as does the ODS phase, and its solute shape selectivity is similar to that of a monomeric ODS phase. Finally, we conclude that retention arises primarily from a partition-like process.

38. J. Li and P.W. Carr, ”Characterization of Polybutadiene-Coated Zirconia and Comparison to Conventional Bonded Phase by Use of Linear Solvation Energy Relationships,” *Analytica Chimica Acta,* **334**, 239-250 (1996).

Abstract: This paper describes the use of linear solvation energy relationships (LSERs) to thermodynamically characterize retention on a reversed-phase type material based on polybutadiene (PBD)-coated zirconia. Retention data were obtained for a large set of judiciously selected test solutes on four PBD phases (carbon loads varying from 1.5 to 5.6 % by weight) in various methanol-water mobile phases. Based on the LSERs, the free energy of retention was dissected into contributions from cavity formation/dispersive interactions, dipolar interactions, and hydrogen bond (HB) donor-acceptor interactions. The PBD-zirconia phases were compared to conventional silica-based bonded reversed phases. As is the case with conventional bonded phases, the solute’s size and HB acceptor basicity are the predominant retention determining factors, and on the whole, PBD-zirconia phases closely resemble conventional chemically bonded reversed phase materials. Interestingly, the solute’s basicity has a larger effect on its retention on the PBD-zirconia phase than on conventional bonded phases, so relative to their behavior on conventional phases, strong hydrogen bases and highly dipolar analytes, when compared to nonpolar solutes, are less strongly retained on PBD-zirconia than on conventional phases. PBD-zirconia and conventional phases are so similar that there should be little difficulty in transferring separation methods between phases.

40. D.H. Reeder, J.W. Li, P.W. Carr, M.C. Flickinger and A.V. McCormick, “Models for Polybutadiene Pore Wall Coatings in Porous Zirconia,” *J. Chromatogr.*, **760(1)**, 71-79 (1997).

Abstract: We present three models of the changes in measured pore size distribution for cylindrical pores when a polymer is deposited in the pores by evaporation from a volatile solvent. The predicted results serve as an aid in interpreting experimental nitrogen adsorption data for polybutadiene (PBD) coating on porous zirconia. At low loadings, PBD appears to deposit in thin layers on the surface with no preference for filling either large or small pores. At higher PBD loadings, the polymer deposits preferentially in smaller pores. This is in qualitative agreement with PBD coatings on porous silica.

41. J.W. Li and P.W. Carr, “Effect of Temperature on the Thermodynamic Properties, Kinetic Performance, and Stability of Polybutadiene-Coated Zirconia,” *Anal. Chem.*, **69(5)**, 837-843 (1997).

Abstract: This article describes the results of a study of the effect of temperature on the performance of a reversed-phase material prepared by coating polybutadiene (PBD) on porous zirconia. We examined the effect of temperature on retention, efficiency, and stability of this phase. The thermodynamic properties were evaluated via the separation of alkylbenzenes and a set a tricyclic antidepressant drug at different temperatures, while the intrinsic kinetic performance of the PBD phase at elevated temperatures was examined by using alkylbenzenes as probe solutes. Moreover, the thermal stability was determined by measuring the drift in *k’* while continuously pumping a mobile phase at 100°C. We found that enthalpy changes were between -2 and -4 kcal/mol and that changes in selectivity varied with the type of solute. High temperatures improved the column efficiency by 30%, mainly by accelerating the *solute diffusion rate* in the stationary phase. Finally, the PBD-coated zirconia phase was very stable at a temperature of 100°C for at least 7000 column volumes.

42. J.W. Li and P.W. Carr, “A Study of the Efficiency of Polybutadiene-Coated Zirconia as a Reversed-Phase Chromatographic Support,” *Anal. Chem.*, **69(11)**, 2193-2201 (1997).

Abstract: The work describes the dynamic characteristics of columns packed with polybutadiene (PBD)-coated zirconia. Reduced plate height (*h*) vs. reduced velocity (*v*) data for alkylbenzenes were acquired for three phases coated with different amount of PBD. Additional data for benzene (*k'* = 0) on uncoated and four coated phases were also collected. Their h vs. *v* plots have a minimum between 4 and 5, indicated good but not excellent column efficiency. By fitting the Know equation to these data, the A (packing quality), B (axial diffusion), and c (mass transfer resistance) coefficients were determined. The A coefficients were about 3 in all cases, and the B coefficients had values between 2 and 3. The C coefficients varied form 0.05 to 0., depending on the polymer load. The data suggest that the quality of packing and the efficiency of mass transfer resistance in the "film" of the polymeric stationary phase, not the stagnant mobile phase, are key to further improvements in column performance. The amount of polymer loaded significantly affects the efficiency through the mass transfer resistance in the stationary phase.

43. J.W. Li and P.W. Carr, "Evaluation of Temperature Effects on Selectivity in RPLC Separations Using Polybutadiene-Coated Zirconia," *Anal. Chem.*, **69(11)**, 2202-2206 (1997).

    Abstract: The effect of temperature on selectivity on RPLC method development has been evaluated on polybutadiene-coated zirconia. We find that the influence of temperature on selectivity depends strongly on solute type. For solutes of similar structure such as polyaromatic hydrocarbons, temperature has almost no effect on selectivity; however, for solutes with very different functional groups such as chlorophenols, temperature changes did significantly affect selectivity. *We feel that simple mixtures with one dominant retention mechanism e.g., solvophobic retention – will not be helped appreciably by adjusting temperature. However, in complex mixtures with polar and ionizable solutes, optimization by varying the temperature may well be fruitful.*

44. J.W. Li, Y. Hu, and P.W. Carr, "Fast Separations at Elevated Temperatures on Polybutadiene-Coated Zirconia Reversed-Phase Material," *Anal. Chem.* **69,** 3884-8 (1997).

    Abstract: Ion-exchange chromatography is a major method used for large-scale protein separations. New zirconia-based polymeric cation-exchange HPLC stationary phases have been developed for protein synthesis. In one method, polyethyleneimine (PEI) was adsorbed onto porous zirconia particles and cross-linked with 1,4-butanediol diglycidyl ether (BUDGE). Succinic anhydride was then reacted with the remaining primary and secondary amine groups on PEI to afford anionic functionalities. The second method utilizes poly(acrylic acid) anhydride as both the cross-linker and the stationary phase. The resulting stationary phases act to separate proteins by a weak cation-exchange mechanism with a slight contribution to retention from hydrophobic interactions. In the presence of 20 mM phosphate buffer, Lewis acid/base interactions between the zirconia support and the proteins, which can significantly broaden the peaks, are sufficiently suppressed. The effects of ionic strength, mobile phase pH, and salt type are discussed. Proteins mass recovery and loading capacity for protein separations on these phases have no limitation on the type of salt used as the eluent, and they exhibit unique selectivities. Therefore, they offer interesting alternatives for protein separations. To our knowledge, this work represents the first successful examples of protein separations using porous zirconia-based polymeric phases under normal chromatographic conditions, which will definitely help make zirconia-based supports more useful for bioseparations.

48. J.W. Li, D.H. Reeder, A.V. McCormick, and P.W. Carr, "Factors Influencing Polybutadiene Deposition within Porous Chromatographic Zirconia," *J. Chromatogr. A* **791,** 45-52 (1998).

    Abstract: We have studied the effect of the conditions for the deposition of polymers in the preparation of polybutadiene-coated porous zirconia particles for reversed-phase chromatography. Chromatographic performance improves when the particle surface is pre-coated with elemental carbon by a chemical vapor deposition (CVD) process. Conversely, performance is significantly degraded when the solvent is removed very slowly during the deposition process. No improvement results when the particles are coated using small sequential load of polymer. We hypothesize that the polymer deposition process is controlled by the rate at which the solvent meniscus recedes during solvent evaporation, by the affinity of the polymer for the zirconia surface, and by polymer-solvent and polymer-polymer interactions.

50. J. Zhao and P.W. Carr, "Comparison of the Retention Characteristics of Aromatic and Aliphatic Reversed Phases for HPLC Using Linear Solvation Energy Relationships," *Anal. Chem.* **70,** 3619-28 (1998).

Abstract: The similarities and differences in retention characteristics of aromatic and aliphatic phases have been elucidated by the use of linear solvation energy relationships (LSERs). Three aromatic phases and three aliphatic phases were investigated in a series of mobile phases. The results of LSERs on a polymer-based aromatic phase, poly(styrene-divinylbenzene) resin (PRP-1) are very different from those on either silica- or zirconia-based aromatic and aliphatic phases. Retention on all aromatic and aliphatic phases except PRP-1 is markedly influenced by the solute hydrogen bond donor acidity. We believe that PRP-1 exhibits a more adsorption-like retention mechanism than do the other phases. With the inorganic oxide-based phases, the aromatic phases are less retentive that the aliphatic phases but show a large dependence on molecular polarizability. The enhanced polarizability of aromatic phases is the likely cause of some differences in their chromatographic selectivity relative to the aliphatic phases.

56a. N. Wu, Q. Tang, Y. Shen, M.L. Lee, "Polybutadiene-Coated Zirconia Packing Materials in Solvating Gas Chromatography Using Carbon Dioxide as Mobile Phase," *Chromatographia*, **49(7-8)**, 431-435 (1999).

Abstract: In this paper, practical considerations of column efficiency, separation speed, thermal stability, and column polarity of capillary columns packed with polybutadiene-coated zirconia were investigated under solvating gas chromatography (SGC) conditions using carbon dioxide as mobile phase. When compared with results obtained from conventional porous octadecyl bonded silica (ODS) particles, PBD-zirconia particles produced greater change in mobile phase linear velocity with pressure than conventional ODS particles under the same conditions. The maximum plate number per second (N_t) obtained with a 30 cm PBD-zirconia column was approximately 1.5 times higher than that obtained with an ODS column at 100 °C. Therefore, the PBD-zirconia phase is more suitable for fast separations than conventional ODS particles in SGC. Maximum plate numbers per meter of 76,900 and 63,300 were obtained using a 57 cm x 250 μm i.d. fused silica capillary column packed with 3μm PBD-zirconia at 50 °C and 100 °C, respectively. The PBD-zirconia phase was stable at temperatures up to 320 °C under SGC conditions using carbon dioxide as mobile phase. Polarizable aromatic compounds and low molecular weight ketones and aldehydes were eluted with symmetrical peaks from a 10 cm column packed with 3 μm PBD-zirconia. Zirconia phases with greater inertness are required for the analysis of more polar compounds by SGC.

56b. Q. Tang, Y. Shen, N. Wu, M.L. Lee, "In Situ Crosslinked Polybutadiene-Encapsulated Zirconia as a Monolithic Column for Fast Solvating Gas Chromatography," *J. Microcolumn Separations*, **11(6)**, 415-420 (1999).

Abstract: A new monolithic column type was developed by crosslinking polymer-encapsulated packing materials in polymer-coated fused silica open tubular columns in situ using a free radical reaction. A 25 cm x 250 μm i.d. PSB-Octyl fused silica capillary column coated with a 0.25-μm film of polyoctylmethylsiloxane was packed with 3% polybutadiene-encapsulated zirconia packing materials (2.5 μm, 250 A, 30 m^2/g). The packing material was immobilized using a dynamic crosslinking reaction at 260°C with azo-*tert*-butane as a free radical initiator. A scanning electron micrograph of the crosslinked packing in the column showed that the particles were interconnected to each other and attached to the capillary wall, forming a monolith. This monolithic column was found to be stable in carbon dioxide mobile phase at 200 atm column inlet pressure and 260°C. The column efficiency, solute retention, and permeability of the monolithic column were similar to those measured for columns freshly packed, but not crosslinked, with the same material under the same conditions. The potential use of these monolithic columns for fast solvating gas chromatography was demonstrated by the rapid separation of a mixture of hydrocarbons.

58. Y. Mao and P.W. Carr, “Adjusting Selectivity in Liquid Chromatography by Use of the Thermally Tuned Tandem Column Concept,” *Anal. Chem.* **72**, 110-118 (2000).

Abstract: In this study, we propose the novel “thermally tuned tandem column (T^3C)” concept for the optimization of selectivity in LC by continuous adjustment of the stationary phase. Two columns with distinctly different chromatographic selectivities (e.g., polybutadiene- and carbon-coated zirconia) are serially coupled and *independently* temperature-controlled. Selectivity is “tuned” by adjusting the individual temperatures of the two columns. The effect of changing column temperature is quite analogous to changing the relative column lengths, thereby altering the relative and absolute contribution each column makes to the overall retention time in T^3C. The distinct selectivity differences between polybutadiene- and carbon-coated zirconia as well as the extraordinary thermal stability of zirconia-based phases (thermally stable to 200º C) allow us to tune the overall chromatographic selectivity over a very substantial range. We have developed a simplified useful model, which characterizes retention and selectivity for the T^3C system as a function of the two column temperatures. The model is in good agreement with the experimental

results. We also describe a simple computer-assisted optimization strategy based on the window diagram method, which facilitates the optimization of the T^3C system with only four or five initial runs.

60. B. Yan, J. Zhao, J.S. Brown, J. Blackwell and P.W. Carr, "High-Temperature Ultrafast Liquid Chromatography," *Anal. Chem.* **72**, 1253-1262 (2000).

    Abstract: A novel liquid chromatographic system which enables high temperature ultrafast liquid chromatography (HTU-FLC) has been designed through the careful consideration of heat transfer, band broadening, and pressure drop. Studies of the effect of linear velocity on the HETP show that column efficiency at high velocity, especially of well-retained solutes, dramatically improves at higher temperatures. At 150°C, at a flow rate of 15 mL/min with a 5 cm by 4.6mm (i.d.) column packed with 3 micron polystyrene-coated zirconia porous particles, long chain alkylphenones were completely resolved, and the analysis time could be decreased by a factor of 50 compared to that at room temperature (25°C) at a conventional flow rate (4 mL/min). In addition, using pure water as the mobile phase, five phenols were separated in less than 30 s.

61. Y. Mao and P.W. Carr, "Application of the Thermally Tuned Tandem Column Concept to the Separation of Several Families of Environmental Toxicants," *Anal. Chem*., **72**, 2788-2796 (2000).

    Abstract: Separations of several families of environmental toxicants were optimized by means of the thermally tuned tandem column (T^3C) concept. We use a tandem combination of an octadecylsilane (ODS) and a carbon-coated zirconia ($C\text{-}ZrO_2$) column; and tune the selectivity by independently adjusting the isothermal temperatures of the two columns. This results in the change in the contribution that each column makes to the overall retention and selectivity. The separation was optimized by locating the optimum pair of column temperatures which give the best separation of the critical solute pair. For both triazine herbicides and carbamate pesticides samples, dramatically different selectivities and different critical pairs were observed for the two types of phases. Although neither individual phase gave adequate separation, the T^3C approach provided baseline separations using only four preliminary trial separations. We also showed that, for the triazine samples, the T^3C approach gave a better separation than did conventional mobile phase optimization with an ODS column. The combination of superior selectivity of T^3C and high flow rate allows the baseline separation of complex mixtures in just a few minutes.

65. C. McNeff, L. Zigan, K. Johnson, P.W. Carr, A. Wang, A.M. Weber-Main, "Analytical Advantages of Highly Stable Stationary Phases for Reversed-Phase LC," *LCGC*, **18(5)**, 514-529 (2000).

    Abstract: During the past five years, many manufacturers of high performance liquid chromatography (HPLC) columns have focused on improving stationary-phase stability and reproducibility. Improved column stability – both chemical and thermal – offers new advantages, such as decreased analysis time and new methods of selectivity optimization. More stable HPLC packing materials have been achieved through advances in silane chemistry; however, the greatest improvements in stability have resulted from the use of alternative nonsilica supports such as synthetic organic polymers, alumina, and zirconia. In this article, the authors describe their use of various test solutes to compare the efficiency, selectivity, and hydrophobic retention mechanisms of five commercially available HPLC columns based on silica, alumina, zirconia, and polystyrene cross-linked with divinlybenzene (PS-DVB).

67. Y. Mao and P.W. Carr, "Separation of Barbiturates and Phenylthiohydantoin Amino Acids Using the Thermally Tuned Tandem Column Concept," *Anal. Chem*., **73**, 1821-1830 (2001).

    Abstract: There are many more choices of column type than of eluent type for method development in reversed-phase liquid chromatography. It is common to switch between different column types or between the same type from different suppliers to achieve the desired separations. The key difficulty in modulation band spacing by adjusting the column type is that it is a discontinuous, "hit or miss" proposition. The thermally tuned tandem column (T^3C) concept effectively solves this problem by connecting two columns in series and independently controlling the two column temperatures. The columns are chosen to have distinctively different chromatographic selectivities (band spacing), so that the unresolved peaks on one column are separated by the other. The optimized separation in the T^3C is achieved by simultaneously tuning the two column temperatures. In this study, we used the T^3C combination of a carbon and a conventional bonded phase for separation of barbiturates and phenylthiohydantoin amino acids (PTH-amino acids). Good peak shapes and comparable retention times were observed on the two phases at room temperature. The selectivities on the two phases were quite different. Baseline separations were easily achieved with the T^3C set although neither column could individually resolve all the peaks. We further compared the separation of barbiturates optimized by the T^3C approach with that optimized by adjusting the mobile phase. We found that T^3C gave a better separation. We believe that the T^3C combination of a carbon phase and a bonded conventional reversed-phase material provides a powerful and general method to optimize the separation of various mixtures.

68. Brain C. Trammell, Marc A. Hillmyer, Peter W. Carr, "A Study of the Lewis Acid-Base Interactions of Vinylphosphonic Acid-Modified Polybutadiene-Coated Zirconia," *Anal. Chem.*, **73**, 14, 3323-3331 (2001).

Abstract: Polybutadiene-coated zirconia (PBD-ZrO_2) is very useful for reversed-phase separation under a wide variety of conditions. Its excellent chemical (pH = 1-13) and thermal (up to 150ºC) stability distinguish it from silica-based reversed phases. Just as with silica-based phases, zirconia's surface chemistry significantly influences the chromatography of certain classes of analytes. Zirconia's *hard* Lewis acid sites can be chromatographically problematic. Analytes such as carboxylic acids strongly interact with these sites on PBD-ZrO_2 and do not elute. Addition of phosphate or other strong, hard Lewis bases to the eluent brings about elution, but the resulting peak is often tailed and broad. Typically, cationic solutes are more retained in the presence of phosphate or fluoride due to adsorption of the Lewis base additives and the concomitant development of a negative charge on the surface. This Coulombic interaction can be used to optimize selectivity, but the reversed-phase-cation-exchange retention can produce broad peaks with excessive retention. As an alternative to adding Lewis bases to the eluent, we studied the effect of permanently modifying PBD-ZrO_2 by covalently attaching vinylphosphonic acid (VPA) to PBD which was predeposited in the pores of zirconia. We have investigated the chromatography of acids, bases, and small peptides on VPA-modified PBD-ZrO_2 (VPA-PBD-ZrO_2) and compared it to PBD-ZrO_2. VPA-PBD-ZrO_2 is a reversed-cation-exchange phase with properties quite different fromPBD-ZrO_2. The chemical stability of both phases led us to explore how low-pH (1.5-3), ultralow ph (0), and high-pH (12) eluents effect the retention properties of these mixed-mode phases. Ultralow-pH eluents effectively separate small peptides on both phases. This approach gives lower retention, without sacrificing resolution, and much higher efficiency for small peptides than previously reported.

71. Christopher J. Dunlap, Peter W. Carr, Clayton V. McNeff, Dwight Stoll, "Zirconia Stationary Phases for Extreme Separations," *Anal. Chem.*, 598A-607A (2001).

Abstract: Composite materials surpass standard silicon and carbon for stability and robustness in complicated reversed-phase column applications.

71a. Yun Mao and Peter W. Carr, "Separation of Selected Basic Pharmaceuticals by Reversed-Phase and Ion-Exchange Chromatography Using Thermally Tuned Tandem Columns," *Anal. Chemistry*; **73**(18); 4478-4485 (2001).

Abstract: The separation of basic pharmaceuticals is usually performed on C8 or C18 bonded silica supports. Silanolphilic interactions between basic analytes and surface silanol groups often lead to tailed peaks, poor efficiency, and irreproducible retention times. To solve these problems, many new types of silica-, zirconia-, and polymer based columns, which provide unique selectivities, improved stability at high pH, or both, have been developed for the analysis of basic compounds. The essence of method development for the chromatographic analysis of basic compounds is to choose a system in which the band spacing can be varied dramatically, quickly, and conveniently while minimizing the tailing due to silanolphilic interactions. The thermally tuned tandem column (T3C) approach has been shown to provide an effective way to adjust stationary-phase selectivity for nonionic compounds. In this study, a tandem combination of an octadecylsilane (ODS) and a polybutadiene-coated zirconia (PBD-ZrO2) phase was used to separate nine antihistamines. Selectivity is tuned by independently adjusting the isothermal temperatures of the two columns. We found dramatic differences in the retention factors, elution sequences, and band spacing for the above set of basic drugs on the two types of columns. The T3C model has been used successfully to locate the optimal temperatures based on only four exploratory runs. The nine antihistamines were baseline separated on the tandem column combination even though they could not be separated on the individual phases. The effect of the buffer concentration on retention of the basic antihistamines was also studied. We conclude that the cation-exchange interactions predominate on the PBD-ZrO2 phase, while reversed phase interactions are more important on the ODS phase. Interestingly, an increase in column temperature causes a significant increase in the retention on the ODS column and a decrease of retention on the PBD-ZrO2 column. This can be explained by the change in the analyte's degree of ionization with temperature. The T3C combination of silica- and zirconia-based RPLC columns is demonstrated to be a powerful approach for the separation of the mixture of basic analytes.

71b. Steven M. Fields, Christine Q. Ye, Dee Dee Zhang, B. Russell Branch, X. Jason Zhang, Ngozi Okafo, "Superheated Water as Eluent in High-temperature High-performance Liquid Chromatographic Separations of Steroids on a Polymer-coated Zirconia Column," *Journal of Chromatography A*, **913**, 197 - 204 (2001).

Abstract: High-temperature liquid chromatography (HTLC), with superheated water mobile phase, has been shown to be a feasible replacement for medium-polarity acetonitrile-water mixtures as an eluent in reversed-phase HPLC. Instrumental parameters of flow-rate, injection volume and mobile phase preheating were shown to have significant effects on the quality of the chromatographic peaks. The selectivity and retention patterns of testosterone and several related compounds were investigated on a porous zirconia, polybutadiene-coated column at temperatures up to 200°C and compared with that of porous silica, octadecylsilane-coated column and the zirconia column under traditional reversed-phase conditions of an acetonitrile-water mobile phase at 40°C. The selectivity differences observed for testosterone and related compounds show that the separation mechanisms are complementary and unique selectivity is obtained with the zirconia column under HTLC conditions.

71c. N. Wu, Q. Tang, J.A. Lippert, M.L. Lee, "Packed Capillary Column Solvating Gas Chromatography Using Neat Water Mobile Phase and Flame Ionization Detection," *J. Microcolumn Separations*, **13(2)**, 41-47 (2001).

Abstract: Neat water at temperatures above its normal boiling point was used as a mobile phase for packed capillary column chromatography. The "universal" flame ionization detector (FID) was used for detection. No restrictor or back pressure was imposed at the column outlet. The effects of temperature on column efficiency and retention were investigated using particle-bonded continuous bed columns packed and crosslinked with 3 μm polybutadiene-encapsulated zirconia particles. It was found that when the column inlet pressure was held constant and the temperature was increased to values that were higher than 100°C, the pressure drop decreased rapidly, allowing the use of long columns or small particles. Solute retention factors decreased with increasing temperature; however, column inlet pressure had little effect on retention factor. Mass transfer resistance also decreased with an increase in temperature. Mixtures of alcohols and phenols could be separated within several minutes after the interface between the column and the FID was optimized.

72. Jonathan D. Thompson and Peter W. Carr, "A Study of the Critical Criteria for Analyte Stability in High-Temperature Liquid Chromatography," *Anal. Chem.*, **74**, 1017-1023 (2002).

Abstract: There are three major impediments to the use of high-temperature ultrafast liquid chromatography. First, the stationary phase must be thermally stable. Over the past decade, a series of thermally stable, highly efficient stationary phases have been developed that can withstand temperatures exceeding 200ºC. Second, the temperature mismatch between the incoming eluent and the column must be minimized (<5ºC), because such a mismatch is a very serious cause of peak broadening, especially in ultrafast separations. The thermal mismatch problem can be significantly ameliorated at high column linear velocities by using narrow-bore columns (2.1-mm i.d.). Third, analytes that are exposed to high temperatures must be thermally stable *on the time scale of the chromatographic run*. We report here a study of the ability of several pharmaceuticals to withstand super ambient temperatures on the time scale of fast separations. We propose criteria by which a particular analyte may be rejected as a candidate for high-temperature analysis, and we demonstrate that complex molecules are amenable to quantitation, even at temperatures in excess of 100ºC in an aqueous media. We also show that as the time an analyte spends on a hot column decreases, the extent of on-column reactions decreases for those analytes that do react. Although the seminal work of Antia and Horvath addresses these issues from a theoretical perspective, we hope to further alleviate fear of high temperatures in liquid chromatography through the empirical approach used here.

74. Y. Hu, X. Yang and P.W. Carr, "Mixed-mode Reversed-phase and Ion-exchange Separations of Cationic Analytes on Polybutadiene-coated Zirconia," *Journal of Chromatography A*, **968**, 17-29 (2002).

Abstract: The retention and selectivity of the chromatographic separation of basic (cationic) analytes on a polybutadiene-coated zirconia (PBD-ZrO2) stationary phase have been studied in greater detail than in previous studies. These separations are strongly influenced by the chemistry of the accessible surface of zirconia. In the presence of buffers which contain hard Lewis bases (e.g., phosphate, fluoride, carboxylic acids) zirconia's surface becomes negatively charged due to adsorption of the buffer anion at the hard Lewis acid sites. Consequently, under most conditions (e.g., neutral pH), cationic analytes undergo both hydrophobic and cation-exchange interactions. This mixed-mode retention process generally leads to greater retention factors for cations relative to those on silica-based reversed phases despite the lower

surface areas of the zirconia phase, but, more importantly, adsorption of hard Lewis bases can be used to control the chromatographic selectivity for cationic analytes on these zirconia-based stationary phases. In contrast to our prior work, here we show that when mixed-mode retention takes place, both retention and selectivity are easily adjusted by changing the type of hard Lewis base buffer anion, the type of buffer counter-ion (e.g., sodium, potassium, ammonium), the pH, and the ionic strength of the eluent as well as the type and amount of organic modifier.

76. Y. Hu and P.W. Carr, "The Special Effect of Fluoride on the Chromatography of Acidic Analytes on Polybutadiene-Coated Zirconia," *Chromatographia*, **56(7-8)**, 439-444 (2002).

Abstract: The special effect of fluoride as a Lewis base additive in suppressing the ligand-exchange interactions for acidic analytes on polybutadiene-coated zirconia (PBD-ZrO2) has been investigated. We found that fluoride is more effective than phosphate in improving the separation efficiency for strong acids. The improvement is attributed to that fluoride has a smaller size and a more flexible coordination chemistry towards zirconium centers than phosphate; consequently, fluoride can more effectively improve the kinetics of the ligand-exchange processes for strongly acidic analytes. We demonstrated that using a small amount of fluoride in combination with a larger quantity of phosphate is a practical way to improve the separation efficiency and resolution for acidic analytes. Some example separations are presented.

77. R.A. Henry, "Highly Selective Zirconia-based Phases for HPLC Applications," *American Laboratory*, **34**(**22**), 18-25 (2002).

Abstract: Zirconia-based phases (Z-phases) for HPLC have been available for almost a decade, and are finding a wide range of applicability because they are very complementary to silica-based phases (S-phases) and often replace total-polymer phases (P-phases), particularly in reversed-phase (RP) applications.

77a. T.S. Kephart, P.K. Dasgupta, "Superheated Water Eluent Capillary Liquid Chromatography," *Talanta*, **56**, 977-987 (2002).

Abstract: A capillary scale reverse phase liquid chromatography (LC) system using a super hot water eluent is described. The system, constructed in-house from readily available components, has been shown to operate at temperatures as high as 370 °C and pressures in excess of 10,000 psi. The capability of the system is demonstrated with the separation of a mixture of polar and non-polar benzene derivatives on polybutadiene and elemental carbon modified zirconia packings with or without temperature gradients. Six benzene derivatives can be separated in ~ 2 min.

78. Y. Xiang, B. Yan, B. Yue, C.V. McNeff, P.W. Carr, M.L. Lee, "Elevated-temperature Ultrahigh-pressure Liquid Chromatography Using Very Small Polybutadiene-coated Nonporous Zirconia Particles," *Journal of Chromatography A*, **983**, 83-89 (2003).

Abstract: Capillary columns packed with small diameter particles typically lead to low permeability and long separation times in high-performance liquid chromatography. Ultrahigh pressures (>10000 p.s.i.; 1 p.s.i. =6894.76 Pa) can be used to overcome the limitations that small particles impose. Ultrahigh-pressure liquid chromatography (UHPLC) has demonstrated great potential for high-speed and high-efficiency separations. Decreasing the viscosity of the mobile phase by elevating the temperature could additionally reduce the pressure drop and facilitate the use of longer columns or smaller particles to achieve even higher total plate numbers. For this reason, we investigated the use of elevated temperatures in UHPLC. Water-resistant, flexible heater tape covered with insulation was used to provide the desired heat to the column. Polybutadiene-coated 1 um nonporous zirconia particles were used because of their chemical stability at elevated temperature. A column efficiency as high as 420,000 plates m-1 was obtained. The effects of temperature and pressure on the separation of parabens were investigated. Separation of five herbicides was completed in 60 s using 26,000 p.s.i. and 90 °C.

78a. E. Van Gyseghem, S. Van Hemelryck, M. Daszykowski, F. Questier, D.L. Massart, Y. Vander Heyden, "Determining Orthogonal Chromatographic Systems Prior to the Development of Methods to Characterise Impurities in Drug Substances," *Journal of Chromatography A*, **988(1)**, 77-93 (2003).

Abstract: To define starting conditions for the development of methods to separate impurities from the active substance and from each other in drugs with an unknown impurity profile, the parallel application of generic orthogonal chromatographic systems could be useful. The possibilities to define orthogonal chromatographic systems were examined by calculation of the correlation coefficients between retention factors k for a set of 68 drugs on 11 systems, by visual evaluation of the selectivity differences, by using principal component analysis, by drawing color maps and evaluating dendrograms. A zirconia-based stationary phase coated with a polybutadiene (PBD) polymer and three silica-based phases (base-deactivated, polar-embedded and monolithic) were used. Besides the stationary phase, the influence of pH and of organic modifier, on the selectivity of a system were evaluated. The dendrograms of hierarchical clusters were found good aids to assess orthogonality of chromatographic systems. The PBD-zirconia phase/methanol/pH 2.5 system is found most orthogonal towards several silica-based systems, e.g. a base-deactivated C16-amide silica/methanol/pH 2.5 system. The orthogonality was validated using cross-validation, and two other validation sets, i.e. a set of non-ionizable solutes and a mixture of a drug and its impurities.

80. Y. Mao and P.W. Carr, "The Thermally Tuned Tandem Column Approach to Optimizing Selectivity in HPLC," *LCGC North America*, **21**(**2**), 150-167 (2003).

    Abstract: The authors describe the thermally tuned tandem column concept for the optimization of selectivity in liquid chromatography by effectively continuously adjusting the stationary phase. They serially connected two columns with distinctly different chromatographic selectivities (band spacings) and used independent temperature control for the columns. They tuned the overall selectivity of the tandem column set by individually adjusting the two column temperatures. The authors compare thermally tuned tandem column separations with some single-column optimization strategies, including the variation of temperature, eluent type, eluent composition, and simultaneously varying the temperature and eluent composition.

81. X. Yang, J. Dai, P.W. Carr, "Analysis and Critical Comparison of the Reversed-phase and Ion-exchange contributions to Retention on Polybutadiene Coated Zirconia and Octadecyl Silane Bonded Silica Phases," *Journal of Chromatography A*, **996**, 13-31 (2003).

    Abstract: The two major modes of retention of basic compounds in reversed-phase liquid chromatography on both octadecyl silane bonded silica-based (ODS) and polybutadiene coated zirconia (PBD-ZrO2) materials are hydrophobic and ion-exchange (Coulombic) interactions. Although the influence of reversed-phase and Coulombic interactions on the chromatography of organic cations is qualitatively well recognized, the quantitative relationship between hydrophobic and ion-exchange interactions remains unclear. In this work, the retention mechanisms on both of the above types of phases were probed by studying the retention of a homologous series of *p*-alkylbenzylamines as a function of the ammonium concentration in the eluent. The various columns tested were studied in terms of plots of retention factor vs. the inverse of the displacing cation concentration. The analysis of such plots as well as plots of log *k'* vs. number of methylene groups in the solutes and plots of log *k'* vs. log[NH4+] clearly shows that at least two types of sites - a pure reversed-phase site and a "hydrophobically-assisted ion-exchange site" similar to the type of site described by Neue [J. Chromatogr. A 925 (2001) 49] are needed to explain the observations. In addition, we have found a quantitative measure of the relative amount of reversed-phase and ion-exchange interaction on a given solute has on a given stationary phase which allows unambiguous classification of columns. It is now clear that ion-exchange contributions to retention on PBD-ZrO2, sometimes exceeding 90%, are even more important than previously thought and relative to hydrophobic interaction much more significant on PBD-ZrO2 than on ODS type-B silicas.

82. Y. Xiang, B. Yan, B. Yue, C.V. McNeff, P.W. Carr, M.L. Lee, "Synthesis of Micron Diameter Polybutadiene-encapsulated Non-porous Zirconia Particles for Ultrahigh Pressure Liquid Chromatography," *Journal of Chromatography A*, **1002**, 71-78 (2003).

    Abstract: In this study, 1-µm diameter polybutadiene-encapsulated non-porous zirconia particles were synthesized, slurry packed into 50-µm I.D. fused-silica capillary columns, and evaluated using ultrahigh pressure liquid chromatography. The dependencies of column efficiency and solute retention factor on pressure were investigated. Efficiencies as high as 280 000 plates per meter were obtained for the separation of anti-inflammatory drugs at a pressure of 1351 MPa. Comparing the reversed-phase behavior of the polybutadiene-encapsulated non-porous zirconia with octadecylsilane bonded non-porous silica, greater selectivity was found using the zirconia-based material for the applications reported in this study. The encapsulated non-porous zirconia particles demonstrated excellent thermal stability in the separation of polycyclic aromatic hydrocarbons at a temperature of 100 °C and a pressure of 1351 MPa.

82a. J. Dai, X. Yang, P.W. Carr, "Comparison of the Chromatography of Octadecyl Silane Bonded Silica and Polybutadiene-Coated Zirconia Phases Based on a Diverse Set of Cationic Drugs," *Journal of Chromatography A*, **1005(1-2)**, 63-82 (2003).

Abstract: In this study, we compare the separation of basic drugs on several octadecyl silane bonded silica (ODS) phases and a polybutadiene-coated zirconia (PBD–ZrO2) phase. The retention characteristics were investigated in detail using a variety of cationic drugs as probe solutes. The ODS phases were selected to cover a relatively wide range in silanol activity and were studied with ammonium phosphate eluents at pH 3.0 and 6.0. Compared to any of the ODS phases, the PBD–ZrO2 phase showed very significant differences in selectivities towards these drugs. Due to the presence of both reversed-phase and ion-exchange interactions between the stationary phase and the basic analyte on ODS and PBD–ZrO2, mixed-mode retention takes place to some extent on both types of phases. However, very large differences in the relative contributions from ion-exchange and reversed-phase interactions on the two types of phases led to quite different selectivities. When phosphate is present in the eluent and adsorbs on the surface, the PBD–ZrO2 phase takes on a high negative charge over a wide pH range due to phosphate adsorption on its surface. On ODS phases, ion-exchange interactions result from the interactions between protonated basic compounds and ionized residual silanol groups. Since the pH of the eluent influences the charge state of the silanol groups, the ion-exchange interactions vary in strength depending on pH. At pH 6.0, the ion-exchange interactions are strong. However, at pH 3.0 the ion-exchange interactions on ODS are significantly smaller because the silanol groups are less dissociated at the lower pH. Thus, not only are the selectivities of the ODS and PBD–ZrO2 phases different but quite different trends in retention are observed on these two types of phases as the pH of the eluent is varied. More importantly, by using the large set of "real" basic analytes we show the extreme complexity of the chromatographic processes on the reversed stationary phases. Both the test condition and solute property influence the column performance. Therefore, use of only one or two probe solutes is not sufficient for column ranking.

83. R.P. Hunter, D.E. Koch, A. Mutlow, R. Isaza, "Extraction and Quantitation of Carfentanil and Naltrexone in Goat Plasma with Liquid Chromatography-Mass Spectrometry," *Journal of Chromatography B*, **793**, 351-355 (2003).

Abstract: This method is the first analytical method for the detection and quantitation of carfentanil and naltrexone at clinically relevant concentrations using liquid chromatography-mass spectrometry. Samples were alkalinized with 100 µl of 1 M NaOH and extracted 2× with 2 mL of toluene. The extractions were combined and dried under N2 at 40ºC in a H2O bath. Chromatography was performed using a ZirChrom-PBD column and a mobile phase of 30:70 acetonitrile/10 mM ammonium acetate and 0.1 mM citrate (pH= 4.4) at a flow rate of 0.3 ml/min. The lower limit of quantitation was 8.5 pg/ml for carfentanil and 0.21 ng/ml for naltrexone.

84. T. Andersen, Quynh-Nhu T. Nguyen, R. Trones, T. Greibrokk, "Mesoporous Polybutadiene-Modified Zirconia for High-Temperature Packed Capillary Liquid Chromatography: Column Preparation and Temperature Programming Stability," *Journal of Chromatography A*, **1018**, 7-18 (2003).

Abstract: In the present study, three different methods for packing of 3 µm PBD-ZrO2 particles in 0.5 mm i.d. glass-lined stainless steel columns have been examined. The two first methods were based on a traditional downstream high-pressure technique using tetrachloromethane (Method I) or aqueous Triton X-100 (Method II) as slurry solvents, while Method III was an upstream high-pressure flocculating method with stirring, using isopropanol both as the slurry and packing solvent. Method I was found to be superior in terms of efficiency, producing 0.5 mm i.d. x 10 cm columns with almost 90,000 plates m-1 for toluene (R.S.D.= 8.7%, n = 3), using a slurry concentration of 600 mg ml-1, ACN-water (50:50 (v/v)) as the packing solvent and a packing pressure of 650 bars. For Method I, the slurry concentration, column i.d., column length and initial packing pressure were found to have a significant effect on column efficiency. Finally, the long-term temperature stability of the prepared columns was investigated. In isothermal mode, using ACN-20 mM phosphate buffer, pH 7 (50:50 (v/v)) as the mobile phase, the columns were found to be stable for at least 3,000 void volumes at 100°C. At this temperature, the solute efficiencies changed about 5-18% and the retention factors changed about 6-8%. In temperature programming mode (not exceeding 100°C), on the other hand, a rapid decrease in both column efficiency and retention factors was observed. However, when the columns were packed as initially described, ramped up and down from 50 to 100°C for 48 h and refilled, fairly stable columns with acceptable efficiencies were obtained. Although not fully regaining their initial efficiency after refilling, the solute efficiencies changed about 19-28% (32-37%) and the retention factors changed about 4-5% (13-17%) after running 3,000 (25,000) void volumes or 500 (3,900) temperature programs.

85. D.E. Koch, R. Isaza, J.W. Carpenter, R.P. Hunter, “Simultaneous Extraction and Quantitation of Fentanyl and Norfentanyl from Primate Plasma with LC/MS Detection,” *Journal of Pharmaceutical and Biomedical Analysis*, **34**, 577-584 (2004).

> Abstract: The quantitation of both fentanyl and its desalkyl metabolite, norfentanyl, in plasma using LC/MS has not been previously described. The detection and quantitation of fentanyl and norfentanyl was achieved using LC/MS detection. The liquid-liquid extraction used toluene as the organic phase. Chromatography was carried out using a ZirChrom-PBD (50 mm × 2.1 mm, 3 µm) column with a mobile phase of acetonitrile-ammonium acetate (10 mM), citrate (0.1 mM, pH 4.4) (45:55, v/v) with a flow rate of 0.3 ml/min. Mass spectroscopy detection was performed using ESI in the positive mode. The LOQ for fentanyl was 25 pg/ml and norfentanyl was 50 pg/ml. For the concentrations of 75, 250, 750 pg/ml, respectively, fentanyl had inter-day precisions of 6.6, 7.2, and 6.6% with accuracies of 4.0, 5.1, and 5.1% and intra-day precisions of 1.6, 1.9, and 1.9% with accuracies of 11.6, 9.4, and 8.4%, and norfentanyl had inter-day precisions of 7.4, 0.3, and 0.7% with accuracies of 9.1, 8.8, and 12.3% and intra-day precisions of 5.3, 1.4, and 0.1% with accuracies of 10.9, 8.9, and 12.8%. The recoveries of fentanyl were 85, 92, and 75% and of norfentanyl were 40, 49, and 46% at the 75, 250, 750 pg/ml concentrations, respectively.

86. E. Schleyer, S. Pursche, C.H. Köhne, U. Schuler, U. Renner, H. Gschaidmeier, J. Freiberg-Richter, T. Leopold, A. Jenke, M. Bonin, T. Bergemann, P. le Coutre, M. Gruner, M. Bornhäuser, O.G. Ottmann, G. Ehninger, “Liquid Chromatographic Method for Detection and Quantitation of STI-571 and its Main Metabolite N-Desmethyl-STI in Plasma, Urine, Cerebrospinal Fluid, Culture Medium and Cell Preparations,” *Journal of Chromatography B*, **799**, 23-36 (2004).

> Abstract: An isocratic online-enrichment HPLC-assay was developed allowing for the simple and fast separation and quantitation of STI-571 and its main metabolite N-desmethyl-STI (N-DesM-STI) in plasma, urine, cerebrospinal fluid (CSF), culture media and cell preparations in various concentrations using UV-detection at 260 nm. The analytical procedure consists of an online concentration of STI-571 and N-DesM-STI in the HPLC system followed by the elution on a ZirChrom-PBD analytical column. Time of analysis is 40 min including the enrichment time of 5 min. The detection limit is 10 ng/ml in plasma, CSF, culture medium (RPMI) and 25 ng/ml in urine for both STI-571 and N-DesM-STI. The intra-day precision, as expressed by the coefficient of variation (CV), in plasma samples ranges between 1.74 - 8.60% for STI-571 and 1.45 - 8.87% for N-DesM-STI. The corresponding values for urine measurements are 2.17 - 7.54% (STI-571) and 1.31 - 9.51% (N-DesM-STI). The inter-day precision analyzed over a 7-month time period was 8.31% (STI-571) or 6.88% (N-DesM-STI) and 16.45% (STI-571) or 14.83% (N-DesM-STI) for a concentration of 1000 ng/ml in plasma and 750 ng/ml in urine, respectively. Moreover, we demonstrate that with an alternative, but more time and labor consuming sample preparation and the implementation of electrochemical detection, a detection limit < 10ng/ml can be achieved. The method described was used to perform pharmacokinetic measurements of STI-571 and N-desmethyl-STI in patient samples and for kinetic measurements of intracellular STI-571 and N-DesM-STI following in vitro incubation.

86a. E. Van Gyseghem, I. Crosiers, S. Gourvenec, D.L. Massart, Y. Vander Heyden, “Determining Orthogonal and Similar Chromatographic Systems from the Injection of Mixtures in Liquid Chromatography-Diode Array Detection and the Interpretation of Correlation Coefficients Color Maps,” *Journal of Chromatography A*, **1026**, 117-128 (2004).

> Abstract: Generic orthogonal chromatographic systems might be helpful tools as potential starting points in the development of methods to separate impurities and the active substance in drugs with unknown impurity profiles. The orthogonality of 38 chromatographic systems was evaluated from weighted-average-linkage dendrograms and color maps, both based on the correlation coefficients between the retention factors on the different systems. On each chromatographic system, 68 drug substances were injected as mixtures of three or four components to increase the throughput. The (overlapping) peaks were identified and resolved with a peak purity algorithm, orthogonal projection approach (OPA). The visualization techniques applied allowed a simple evaluation of orthogonal and (groups of) similar systems.

87. J. Nawrocki, C. Dunlap, A. McCormick, P. W. Carr, “Part I. Chromatography Using Ultra-Stable Metal Oxide-Based Stationary Phases for HPLC,” *Journal of Chromatography A*, **1028**, 1-30 (2004).

> Abstract: The first part of the review contrasts the main drawbacks of silica-based packings such as their relative thermal and chemical instability with excellent stability of metal oxides. The paper concerns mainly ZrO2, TiO2 and Al2O3. Methods of preparation of spherical particles for HPLC are described.

Surface chemistry of the oxides is, however, very different from that of silica. Ability of the oxides to ion- and ligand exchange is discussed from a chromatographic point of view.

88. J. Nawrocki, C. Dunlap, J. Li, J. Zhao, C.V. McNeff, A. McCormick, P.W. Carr, "Part II. Chromatography Using Ultra-Stable Metal Oxide-Based Stationary Phases for HPLC," *Journal of Chromatography A*, **1028**, 31-62 (2004).

    Abstract: In this part of the review authors discuss methods used for modification of metal oxide surfaces. On the basis of literature data it is shown, that silanization of the surfaces do not form stable supports for chromatography. On the other hand, the success of polymer modified surfaces such as polybutadiene (PBD) and polystyrene (PS) is emphasized. Permanent modification of metal oxide surfaces with Lewis bases is also widely discussed. Chromatographic properties of polymer-modified surfaces of zirconia are discussed in details. The perspectives of carbon-coated metal oxide surfaces in HPLC and high temperature separations are described.

89b. M.M Sanagi, H.H. See, W.A. Ibrahim, A.A. Naim, "High Temperature Liquid Chromatography of Triazole Fungicides on Polybutadiene-coated Zirconia Stationary Phase," *Journal of Chromatography A*, **1059**, 95-101 (2004).

    Abstract: High temperature liquid chromatography using water-rich and superheated water eluent is evaluated as a new approach for the separation of selected triazole fungicides, hexaconazole, tebuconazole, propiconazole, and difenoconazole. Using a polybutadiene-coated zirconia column at temperatures of 100-150 degrees C, clear separations were achieved when 100% purified water was utilized as organic-free eluent. Excellent limits of detection down to pg level were obtained for the separation of the triazole fungicides under optimum conditions. Van't Hoff plots for the separations were linear suggesting that no changes occurred in the retention mechanism over the temperature range studied.

93. M.M. Sanagi, H.H. See, W.A.W. Ibrahim, A.A. Naim, "High Temperature Liquid Chromatography of Tocol-Derivatives on Polybutadiene-Coated Zirconia Stationary Phases," *Chromatographia*, **61(11/12)**, 567-571 (2005).

    Abstract: High temperature Reversed-phase high performance liquid chromatography is evaluated as a new approach for the separation of vitamin E isomers (alpha-, gamma-, delta-tocopherol, and alpha-, gamma- ,delta-tocotrienol) and alpa-tocopherol acetate. The separations of each analyte were examined by varying the eluent composition and column temperature. Using a polybutadiene-coated zirconia column at temperatures of 80 degrees C to 140 degrees C, complete separations were achieved within 10-20 min using organic modifier (acetonitrile) in the range of 40-50%. Irregularity of the Van't Hoff analysis was noted over the temperature studied. The plot tends to deviate from linearity at higher operating temperatures (140 degrees C and 150 degrees C) with linear deviation errors of 12.7% and 41.6%, respectively, for the mobile phase examined.

94. J. Murphy, G. Deshpande, "The Use of Zirconium-Based HPLC Columns for Heterocyclic Amines Analysis," *American Laboratory*, **37(15)**, 26-27 (2005).

    Abstract: The analysis of heterocyclic amines present in over-the-counter (OTC) and prescription products presents unique problems for HPLC analysis. Many of the degradant products present in the various dosage forms are difficult to separate due to the compounds' highly similar characteristics. The first method detailed below currently achieves acceptable separation of these byproducts, but does so at the price of short column life since the chromatographic conditions push beyond the recommended limitations of the silica based column. Another trade-off was a long run time of 30 min per injection. The use of a zirconium-based column was investigated to see if an improvement in resolution and run time could be achieved. The ZirChrom® column (ZirChrom, Anoka, MN) is a radical departure from the traditional silica-based chemistry that the bulk of current HPLC analysis rests upon.

95d. G. Vanhoenacker and P. Sandra, "High Temperature Liquid Chromatography and Liquid Chromatography–mass Spectroscopy Analysis of Octylphenol Ethoxylates on Different Stationary Phases," *Journal of Chromatography A*, **1082(2)**, 193-202 (2005).

    Abstract: Temperature was investigated as active parameter in the liquid chromatography (LC) analysis of octylphenol ethoxylates. Significant differences in selectivity were observed when the oligomers were analyzed by reversed phase LC (RPLC) on silica-, zirconia- and polystyrene/divinylbenzene based stationary phases at low (ambient), medium and elevated temperature with acetonitrile/water as mobile phase. As ascertained by LC–mass spectroscopy (MS), in most cases the elution order of the oligomers was completely reversed comparing ambient and high temperature separations. On a graphitized carbon type column, the selectivity remained unchanged, regardless the analysis temperature. Also in normal phase LC, the elution order remained unaffected by temperature variations both for acetonitrile/water and methanol/water mixtures as mobile phase. Surprisingly, when reversed phase LC on a octadecylsilicagel column at different temperatures was repeated with methanol instead of acetonitrile as mobile phase ingredient, the reversal of elution order did not take place. Results are evaluated in terms of thermodynamic parameters.

95e. K. Soukupová, E. Krafková, Jana Suchánková and E. Tesařová, "Comparison of Zirconia- and Silica-based Reversed Stationary Phases for Separation of Enkephalins," *Journal of Chromatography A*, **1087(1-2)**, 104-111 (2005).

Abstract: In this study, the separation of biologically active peptides on two zirconia-based phases, polybutadiene (PBD)-ZrO2 and polystyrene (PS)-ZrO2, and a silica-based phase C18 was compared. Basic differences in interactions on both types of phases led to quite different selectivity. The retention characteristics were investigated in detail using a variety of organic modifiers, buffers, and temperatures. These parameters affected retention, separation efficiency, resolution and symmetry of peaks. Separation systems consisting of Discovery PBD-Zr column and mobile phase composed of a mixture of acetonitrile and phosphate buffer, pH 2.0 (45:55, v/v) at 70 °C and Discovery PS-Zr with acetonitrile and phosphate buffer, pH 3.5 in the same (v/v) ratio at 40 °C were suitable for a good resolution of enkephalin related peptides. Mobile phase composed of acetonitrile and phosphate buffer, pH 5.0 (22:78, v/v) was appropriate for separation of enkephalins on Supelcosil C18 stationary phase.

97a. T. Teutenberg, H.-J. Goetze, J. Tuerk, J. Ploeger, T.K. Kiffmeyer, K.G. Schmidt, W. gr. Kohorst, T. Rohe, H.-D. Jansen and H. Weber, "Development and Application of a Specially Designed Heating System for Temperature-programmed High-performance Liquid Chromatography Using Subcritical Water as the Mobile Phase," *Journal of Chromatography A*, **1114(1)**, 89-96 (2006).

Abstract: A specially designed heating system for temperature-programmed HPLC was developed based on experimental measurements of eluent temperature inside a stainless steel capillary using a very thin thermocouple. The heating system can be operated at temperatures up to 225 °C and consists of a preheating, a column heating and a cooling unit. Fast cycle times after a temperature gradient can be realized by an internal silicone oil bath which cools down the preheating and column heating unit. Long-term thermal stability of a polybutadiene-coated zirconium dioxide column has been evaluated using a tubular oven in which the column was placed. The packing material was stable after 50 h of operation at 185 °C. A mixture containing four steroids was separated at ambient conditions using a mobile phase of 25% acetonitrile:75% deionized water and a mobile phase of pure deionized water at 185 °C using the specially designed heating system and the PBD column. Analysis time could be drastically reduced from 17 min at ambient conditions and a flow rate of 1 mL/min to only 1.2 min at 185 °C and a flow rate of 5 mL/min. At these extreme conditions, no thermal mismatch was observed and peaks were not distorted, thus underlining the performance of the developed heating system. Temperature programming was performed by separating cytostatic and antibiotic drugs with a temperature gradient using only water as the mobile phase. In contrast to an isocratic elution of this mixture at room temperature, overall analysis time could be reduced two-fold from 20 to 10 min.

100. C. Ovens, D. Sievwright and A.J. Silcock, "Unusual Retention Behaviour of 4-substituted Piperidines on Polybutadiene and Polystyrene Coated Zirconia by Comparison to Reverse Phase Silica," *Journal of Chromatography A*, **1137(1)**, 56-62 (2006).

Abstract: The retention properties of a range of N-methylated and N-des-methyl 4-substituted piperidines on polybutadiene (PBD) and polystyrene (PS) coated zirconia have been studied and compared to those of Xterra RP18 and Genesis C18 silica. The effect of buffer type and pH are investigated with regard to the elution order on all three stationary phases. The change in the elution order is linked to the degree of substitution of the piperidine nitrogen and appears to be independent of the rest of the structure, indicating that this moiety is most heavily involved in the separation mechanism on the zirconia phases.

101. V. Žižkovský, R. Kučera and J. Klimeš, "Potential Employment of Non-silica-based Stationary Phases in Pharmaceutical Analysis," *Journal of Pharmaceutical and Biomedical Analysis*, **44(5)**, 1048-1055 (2007).

Abstract: The absolute majority of the HPLC applications use silica-based columns for the separation of active substance and its impurities. However, stationary phases based on metal oxides appear as an interesting alternative. The aim of our study was to investigate the potential utilization of metal oxide-based stationary phases in analytical evaluation of ondansetron and its five pharmacopoeial impurities. In our study commercially available ZrO2-based columns (e.g. Zr-PBD, Zr-PS, Zr-C18) and TiO2-based column were used. The effect of an organic modifier (type and ratio), a buffer (type, pH and concentration) and the influence of temperature was investigated. The separation of ondansetron and its five pharmacopoeial impurities was successfully accomplished on a Zirchrom®-PBD column using a mobile phase consisting of acetonitrile-ammonium phosphate (25 mM, pH 7.0) (18:82, v/v). Detection was performed at 216 nm and the analysis was completed within 7.5 min. The paper proves metal oxide-based stationary phases as an alternative to classical silica-based stationary phases in pharmaceutical analysis.

103. S. Pursche, O.G. Ottmann, G. Ehninger and E. Schleyer, "High-performance Liquid Chromatography Method with Ultraviolet Detection for the Quantification of the BCR-ABL Inhibitor Nilotinib (AMN107) in Plasma, Urine, Culture Medium and Cell Preparations," *Journal of Chromatography B*, **852(1-2)**, 208-216 (2007).

Abstract: An isocratic and sensitive HPLC assay was developed allowing the determination of the new anticancer drug nilotinib (AMN107) in human plasma, urine, culture medium and cell samples. After protein precipitation with perchloric acid, AMN107 underwent an online enrichment using a Zirchrom-PBD recolumn, was separated on a Macherey-Nagel C18-HD column and finally quantified by UV-detection at 258 nm. The total run time is 25 min. The assay demonstrates linearity within a concentration range of 0.005–5.0 μg/ml in plasma (r2 = 0.9998) and 0.1–10.0 μg/ml in urine (r2 = 0.9913). The intra-day precision expressed as coefficients of variation ranged depending on the spiked concentration between 1.27–9.23% in plasma and 1.77–3.29% in urine, respectively. The coefficients of variation of inter-day precision was lower than 10%. Limit of detection was 0.002 μg/ml in plasma and 0.01 μg/ml in urine. The described method is stable, simple, economic and is routinely used for in vivo and in vitro pharmacokinetic studies of AMN107.

104. V. Žižkovský, R. Kučera, J. Klimeš and J. Dohnal, "Titania-based Stationary Phase in Separation of Ondansetron and Its Related Compounds," *Journal of Chromatography A*, **online 15 December 2007**.

Abstract: Improvements in stationary phase stability have been and remain a great task for research of new stationary phases. Metal oxide-based stationary phases appear as one of perspective alternatives to classical silica based stationary phases regarding to their similar effectiveness, different selectivity, different retention mechanism and mainly better chemical and thermal stability. In this study, the retention behaviour of ondansetron and its five pharmacopoeial impurities on TiO2-based reversed phase was investigated. The influence of buffer type, pH and concentration on retention was studied. Different types and amount of organic solvent in mobile phase were tested. The effect of temperature and flow rate on separation was investigated. The separation conditions were optimized and developed method validated. The retention parameters – retention time (tR), retention factor (k′), theoretical plate number (N), resolution between peaks due to nearby peaks (Rs) and symmetry factor (As) have been compared to parameters achieved on polybutadiene-coated zirconia column. The thermodynamic parameters of retention of analysed compounds – enthalpy, entropy and Gibbs free energy – were calculated and compared to those achieved on polybutadiene-coated zirconia column. This work proves similarity of retention behaviour of ondansetron and its five related compounds on zirconia-based and titania-based stationary phases and potential utilisation of polyethylene covered TiO2-based reversed stationary phase as an alternative to polybutadiene-coated ZrO2 stationary phase in pharmaceutical analysis of ondansetron.

105. C.V. McNeff, B. Yan, D.R. Stoll, R.A. Henry, "Review: Practice and Theory of High Temperature Liquid Chromatography," *Journal of Separation Science,* **30**, 1672-1685 (2007).

Abstract: High temperature liquid chromatography (HTLC) exists in a temperature region beyond ambient (ca. 40 C) and below super critical temperatures. The promises of HTLC, such as increased analysis speed, enhanced separation productivity, "green" LC with pure water mobile phases coupled to universal FID detection, and fast analysis of complex samples by combination with fast 2-D techniques, have become an option for routine practice. The focus of this paper is to review the key developments that have made the application of HTLC a practical technique and draw attention to new developments in 2-D techniques that incorporate HTLC that offer an opportunity to vastly increase the usefulness of HPLC for the analysis of complex samples.

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3. T.P. Weber, P.W. Carr, and E.F. Funkenbusch, "Evaluation of a Zirconia-Based Carbon-Polymer Composite Reversed-Phase Chromatographic Support," *J. Chromatogr*. **519**, 31-52 (1990).

   Abstract: Three zirconia-based composite reverse-phase high-performance liquid chromatography supports with exceptional alkaline stability are described. The first support was prepared by chemical vapor deposition of carbon onto a porous zirconia substrate, the second by hydrogen treatment of the carbon coated zirconia particle at elevated temperature, and the third by deposition and cross-linking of a hydrophobic polymer layer over the carbon-coated zirconia particle. The alkaline stability, and chromatographic selectivity, efficiency and loading capacity of these packing materials were examined and compared. Hydrogen treatment of the carbon particles served to remove or modify high-energy adsorption sites on the carbon surface. The polymer coating on the carbon particles increased the chromatographic efficiency and loading capacity over that of the bare carbon supports. These materials appear to be indefinitely stable at 80^{o}C in a mobile phase of methanol-water (50:50) buffered at pH 12.

4. T.P. Weber, P.W. Carr, "Comparison of Isomer Separation on Carbon-Clad Microporous Zirconia and on Conventional Reversed-Phase High-Performance Liquid Chromatography Supports," *Anal. Chem*. **62**, 2620-5 (1990).

   Abstract: A carbon-clad zirconia-based support is used for the separation of a variety of structural isomers. The separation of these isomers on the carbon-clad zirconia surface is compared to that on a conventional reversed-phase column of higher efficiency. The mobile phases were individually optimized for each isomer separation on both columns. The relative retention of the solutes of interest on carbon and ODS columns are also compared. In general the carbon support has much greater selectivity for the separation of both nonpolar and polar isomers and is considerably more retentive than the conventional reverse-phase support. The retention of the carbon-clad zirconia support is especially high for solutes with polar functional groups attached to an aromatic ring (i.e. nitrotoluene, alkyl aryl ketones, nitroxylenes). Despite the lower chromatographic efficiency of the carbon support, it proved to have better resolving power and provided faster separations than did the conventional column.

28. T.P. Weber, P.T. Jackson, and P.W. Carr, "Chromatographic Evaluation of Porous Carbon-Clad Zirconia Microparticles," *Anal. Chem*. **67,** 3042-50 (1995).

    Abstract: The chemical vapor deposition of hydrocarbons on porous zirconia (ZrO_2) microparticles creates a reversed-phase support that is significantly different from conventional bonded reversed-phase supports. This carbon-overload zirconia support (C/ZrO_2) is stable to conditions of extreme pH (0-12) and temperature (80^{o} C). It differs greatly from conventional supports in its chromatographic selectivity; the separation of positional isomers is facile, and they are well resolved compared to the separation on ODS silica. The unique chromatographic nature of this material was studied in detail to better understand and find applications for this novel stationary phase. The results of loading capacity and chromatographic efficiency studies are also discussed.

39. P.T. Jackson, M.R. Schure, T.P. Weber, and P.W. Carr, "Intermolecular Interactions Involved in Solute Retention on Carbon Media in Reversed-Phase High Performance Liquid Chromatography," *Anal. Chem*., **69**, 416-425 (1997).

    Abstract: Carbon adsorbents for RPLC separations are greatly underutilized due to the poor chromatographic properties of the earliest commercially available materials and our limited understanding of solute interactions with the solid surface. Previously, we reported on the properties of a carbon surface prepared by vapor deposition on porous zirconia microspheres. The resulting material is a new type of carbon sorbent with considerably improved chromatographic properties. Here we present a fundamental study of the intermolecular interaction influencing solute retention on these novel carbon phases under RPLC conditions. Retention on seven unique carbon phases has been correlated with solute descriptors of dispersion, dipolarity/polarizability, and hydrogen bond basicity through the use of linear solvation energy relationships (LSERs). In stark contrast, conventional bonded phases do not show the large contribution from dipolarity/polarizability that is observed on these types of carbon. The presence of this interaction indicates a distinct difference between carbon and conventional bonded RPLC phases. Other results suggest that solvent sorption plays a significant sole in controlling solute retention on carbon. In addition, we investigated the temperature dependence of retention on carbon and found typical RPLC-like behavior.

46. P.T. Jackson, T.-Y. Kim, and P.W. Carr, “Diastereomeric Selectivity of Carbon-Coated Zirconia Reversed-Phase Liquid Chromatographic Media,” *Anal. Chem.* **69,** 5011-17 (1997).

> Abstract: The determination of enantiomeric excess, that is, the relative amount of any pair of optical antipodes, constitutes an integral part of the work of analytical and synthetic chemists involved in natural products research or pharmaceutical development. Mosher’s reagent [α-methoxy-α-(trifluoromethyl)phenylacetyl chloride] has evolved into a major tool for the determination of absolute configuration by NMR. We report here on the separation of diastereomers formed by derivatizing enantiomers with Mosher’s reagent. We have shown that reversed-phase liquid-solid adsorption chromatography on carbon surfaces frequently gives considerably superior resolution of diastereomeric pairs than does RPLC on conventional bonded phases. The improved resolution results from the very high sensitivity of solid carbon surfaces to the geometric organization of the solute rather than from differences in column efficiency. We compare the separation of pharmaceutically and biologically important stereoisomeric mixtures, including (±)-warfarin and (±)-amino acid esters, on both conventional bonded phases and carbon surfaces prepared by chemical vapor deposition of organic compounds on porous zirconia microparticles.

51. P.T. Jackson and P.W. Carr, “Improving Reversed-Phase Liquid Chromatography,” *CHEMTECH* **28**, 29-37 (1998).

> Abstract: With the wide array of problems associated with the current suite of RPLC stationary phases, new materials, which are immune to chemical susceptibilities of silica, bonded phases, and polymers, are needed. Zirconia, a chemically and thermally stable alternative to silica developed at the University of Minnesota, is now manufactured by ZirChrom Separations.

57a. T. Scott Kephart and Purnendu K. Dasgupta, "Hot Eluent Capillary Liquid Chromatography Using Zirconia and Titania Based Stationary Phases," *Analytica Chimica Acta* **414**, 71-78 (2000).

> Abstract: High speed capillary liquid chromatographic separations using a simple home made system constructed from readily available inexpensive components have been studied. Using thermally stable zirconia and titania based packing, the separation of eight alkylbenzene derivatives in less than 2 min is shown. The system is capable of operating at temperatures as high as 200°C and pressures up to 10,000 psi and can, thus, take advantage of the reduction in eluent viscosity and enhanced mass transport at elevated temperatures to operate at very high linear velocities without sacrificing separation efficiency.

58. Y. Mao and P.W. Carr, “Adjusting Selectivity in Liquid Chromatography by Use of the Thermally Tuned Tandem Column Concept,” *Anal. Chem.* **72**, 110-118 (2000).

> Abstract: In this study, we propose the novel “thermally tuned tandem column (T^3C)” concept for the optimization of selectivity in LC by continuous adjustment of the stationary phase. Two columns with distinctly different chromatographic selectivities (e.g., polybutadiene- and carbon-coated zirconia) are serially coupled and *independently* temperature-controlled. Selectivity is “tuned” by adjusting the individual temperatures of the two columns. The effect of changing column temperature is quite analogous to changing the relative column lengths, thereby altering the relative and absolute contribution each column makes to the overall retention time in T^3C. The distinct selectivity differences between polybutadiene- and carbon-coated zirconia as well as the extraordinary thermal stability of zirconia-based phases (thermally stable to 200º C) allow us to tune the overall chromatographic selectivity over a very substantial range. We have developed a simplified useful model, which characterizes retention and selectivity for the T^3C system as a function of the two column temperatures. The model is in good agreement with the experimental results. We also describe a simple computer-assisted optimization strategy based on the window diagram method, which facilitates the optimization of the T^3C system with only four or five initial runs.

61. Y. Mao and P.W. Carr, “Application of the Thermally Tuned Tandem Column Concept to the Separation of Several Families of Environmental Toxicants,” *Anal. Chem.*, **72**, 2788-2796 (2000).

> Abstract: Separations of several families of environmental toxicants were optimized by means of the thermally tuned tandem column (T^3C) concept. We use a tandem combination of an octadecylsilane (ODS) and a carbon-coated zirconia (C-ZrO_2) column; and tune the selectivity by independently adjusting the isothermal temperatures of the two columns. This results in the change in the contribution that each column makes to the overall retention and selectivity. The separation was optimized by locating the

optimum pair of column temperatures which give the best separation of the critical solute pair. For both triazine herbicides and carbamate pesticides samples, dramatically different selectivities and different critical pairs were observed for the two types of phases. Although neither individual phase gave adequate separation, the T^3C approach provided baseline separations using only four preliminary trial separations. We also showed that, for the triazine samples, the T^3C approach gave a better separation than did conventional mobile phase optimization with an ODS column. The combination of superior selectivity of T^3C and high flow rate allows the baseline separation of complex mixtures in just a few minutes.

65. C. McNeff, L. Zigan, K. Johnson, P.W. Carr, A. Wang, A.M. Weber-Main, "Analytical Advantages of Highly Stable Stationary Phases for Reversed-Phase LC," *LCGC*, **18(5)**, 514-529 (2000).

Abstract: During the past five years, many manufacturers of high performance liquid chromatography (HPLC) columns have focused on improving stationary-phase stability and reproducibility. Improved column stability – both chemical and thermal – offers new advantages, such as decreased analysis time and new methods of selectivity optimization. More stable HPLC packing materials have been achieved through advances in silane chemistry; however, the greatest improvements in stability have resulted from the use of alternative nonsilica supports such as synthetic organic polymers, alumina, and zirconia. In this article, the authors describe their use of various test solutes to compare the efficiency, selectivity, and hydrophobic retention mechanisms of five commercially available HPLC columns based on silica, alumina, zirconia, and polystyrene cross-linked with divinlybenzene (PS-DVB).

67. Y. Mao and P.W. Carr, "Separation of Barbiturates and Phenylthiohydantoin Amino Acids Using the Thermally Tuned Tandem Column Concept," *Anal. Chem.*, **73**, 1821-1830 (2001).

Abstract: There are many more choices of column type than of eluent type for method development in reversed-phase liquid chromatography. It is common to switch between different column types or between the same type from different suppliers to achieve the desired separations. The key difficulty in modulation band spacing by adjusting the column type is that it is a discontinuous, "hit or miss" proposition. The thermally tuned tandem column (T^3C) concept effectively solves this problem by connecting two columns in series and independently controlling the two column temperatures. The columns are chosen to have distinctively different chromatographic selectivities (band spacing), so that the unresolved peaks on one column are separated by the other. The optimized separation in the T^3C is achieved by simultaneously tuning the two column temperatures. In this study, we used the T^3C combination of a carbon and a conventional bonded phase for separation of barbiturates and phenylthiohydantoin amino acids (PTH-amino acids). Good peak shapes and comparable retention times were observed on the two phases at room temperature. The selectivities on the two phases were quite different. Baseline separations were easily achieved with the T^3C set although neither column could individually resolve all the peaks. We further compared the separation of barbiturates optimized by the T^3C approach with that optimized by adjusting the mobile phase. We found that T^3C gave a better separation. We believe that the T^3C combination of a carbon phase and a bonded conventional reversed-phase material provides a powerful and general method to optimize the separation of various mixtures.

71. Christopher J. Dunlap, Peter W. Carr, Clayton V. McNeff, Dwight Stoll, "Zirconia Stationary Phases for Extreme Separations," *Anal. Chem.*, 598A-607A (2001).

Abstract: Composite materials surpass standard silicon and carbon for stability and robustness in complicated reversed-phase column applications.

73b. P.T. Jackson and P.W. Carr, "Study of Polar and Nonpolar Substituted Benzenes and Aromatic Isomers on Carbon-coated Zirconia and Alkyl Bonded Phases," *Journal of Chromatography A*, **958(1-2)**, 121-129 (2002).

Abstract: Retention factors of polar and non-polar mono- and di-substituted benzene derivatives were measured on carbon coated zirconia (C/ZrO2) and an alkyl modified silica using water–acetonitrile mobile phases. Published data on porous graphitic carbon phases (PGC) were used to facilitate comparisons between the two types of carbon media. This work showed that retention on both C/ZrO2 and PGC is much more sensitive to the solute polarizability, dipolarity, and shape than on aliphatic phases. For simple disubstituted benzenes there was no general clear cut advantage in terms of chromatographic selectivity to using a carbon-based phase over a bonded phase silica; however, the selectivities towards such isomers are quite different on the two types of media. In contradistinction to their effect on alkyl bonded phase retention, addition of a dipolar substituent and weak hydrogen bond acceptor to a benzene ring almost always increases the solute's retention on C/ZrO2 and PGC.

75. M. Gray, G.R. Dennis, P. Wormell, R.A. Shalliker and P. Slonecker, "Two Dimensional Reversed-phase-reversed-phase Separations: Isomeric Separations Incorporating C18 and Carbon Clad Zirconia Stationary Phases," *Journal of Chromatography A*, **975**, 285-297 (2002).

Abstract: Informational theory and a geometric approach to factor analysis were employed to evaluate the degree of orthogonality of a two-dimensional reversed-phase-reversed-phase chromatographic system. The system incorporated a C18 column as one dimension and a carbon clad zirconia column as the second dimension. In order to study the resolving power of this system, the separation of a sample matrix containing an artificial mix of 32 isomers (structural and diastereoisomers) was evaluated. Using this system, between 25 and 28 of the 32 isomers could be separated, depending on the mobile phase combinations- with resolution that could not possibly be achieved in a single one dimensional separation. The results from this study indicate that in order to fully evaluate the resolving power of a 2D system multiple methods of analysis are most appropriate. This becomes increasingly important when the sample contains components that are very closely related and the retention of solutes is clustered in one quadrant of the 2D space. Ultimately, the usefulness of the 2D separation is determined by the goals of analyst.

77. R.A. Henry, "Highly Selective Zirconia-based Phases for HPLC Applications," *American Laboratory*, **34**(**22**), 18-25 (2002).

Abstract: Zirconia-based phases (Z-phases) for HPLC have been available for almost a decade, and are finding a wide range of applicability because they are very complementary to silica-based phases (S-phases) and often replace total-polymer phases (P-phases), particularly in reversed-phase (RP) applications.

77a. T.S. Kephart, P.K. Dasgupta, "Superheated Water Eluent Capillary Liquid Chromatography," *Talanta*, **56**, 977-987 (2002).

Abstract: A capillary scale reverse phase liquid chromatography (LC) system using a super hot water eluent is described. The system, constructed in-house from readily available components, has been shown to operate at temperatures as high as 370 °C and pressures in excess of 10,000 psi. The capability of the system is demonstrated with the separation of a mixture of polar and non-polar benzene derivatives on polybutadiene and elemental carbon modified zirconia packings with or without temperature gradients. Six benzene derivatives can be separated in ~ 2 min.

80. Y. Mao and P.W. Carr, "The Thermally Tuned Tandem Column Approach to Optimizing Selectivity in HPLC," *LCGC North America*, **21**(**2**), 150-167 (2003).

Abstract: The authors describe the thermally tuned tandem column concept for the optimization of selectivity in liquid chromatography by effectively continuously adjusting the stationary phase. They serially connected two columns with distinctly different chromatographic selectivities (band spacings) and used independent temperature control for the columns. They tuned the overall selectivity of the tandem column set by individually adjusting the two column temperatures. The authors compare thermally tuned tandem column separations with some single-column optimization strategies, including the variation of temperature, eluent type, eluent composition, and simultaneously varying the temperature and eluent composition.

82b. M.J. Gray, G.R. Dennis, P.J. Slonecker, R.A. Shalliker, "Evaluation of the Two-Dimensional Reversed-Phase-Reversed-Phase Separations of Low-Molecular Mass Polystyrenes," *Journal of Chromatography A*, **1015(1-2)**, 89-98 (2003).

Abstract: The resolving power of four reversed-phase–reversed-phase two-dimensional (2D) chromatographic systems was evaluated using information theory (IT) and a geometric approach to factor analysis. The first separation dimension employed a C18 column, while the second separation dimension employed a carbon clad zirconia column. Mobile phases in each dimension were either methanol or acetonitrile. The sample matrix that was employed in this study contained a mixture of 58 components, comprised of stereoisomers and structural isomers of a systematic variation in molecular weight. Each of the components were oligostyrenes, with between two and five configurational repeating units having either n-butyl, sec-butyl or tert-butyl end groups.

In the two-dimensional systems employed in this study, between 46 and 49 of the 58 components could be separated, depending on the mobile phase combinations—with apparent resolution that could not possibly be achieved in a single one-dimensional separation. The results from this study indicate that in order to fully evaluate the resolving power of a 2D system multiple methods of analysis that evaluate the separation potential are most appropriate. This becomes increasingly important when the sample contains components that are very closely related and the retention of solutes displays a high degree of solute crowding.

88. J. Nawrocki, C. Dunlap, J. Li, J. Zhao, C.V. McNeff, A. McCormick, P.W. Carr, "Part II. Chromatography Using Ultra-Stable Metal Oxide-Based Stationary Phases for HPLC," *Journal of Chromatography A*, **1028**, 31-62 (2004).

    Abstract: In this part of the review authors discuss methods used for modification of metal oxide surfaces. On the basis of literature data it is shown, that silanization of the surfaces do not form stable supports for chromatography. On the other hand, the success of polymer modified surfaces such as polybutadiene (PBD) and polystyrene (PS) is emphasized. Permanent modification of metal oxide surfaces with Lewis bases is also widely discussed. Chromatographic properties of polymer-modified surfaces of zirconia are discussed in details. The perspectives of carbon-coated metal oxide surfaces in HPLC and high temperature separations are described.

89. M. Gray, G.R. Dennis, P.J. Sloneckerb, R.A. Shalliker, "Separation of Oligostyrene Isomers in a Complex Mixture Using Two-Dimensional Heart-Cutting Reversed-Phased Chromatography," *Journal of Chromatography A*, **1028**, 247-257 (2004).

    Abstract: The development of a two-dimensional liquid chromatographic system requires a process of assessment that can yield an optimum performing system with minimal experimental evaluation. Information Theory and a geometric approach to Factor Analysis are two techniques that when used in combination, provide important information on the expected two-dimensional performance. In the present study, we compare the predicted separation performance of two-dimensional systems that have been subjected to analysis by Information Theory and Factor Analysis to that of actual chromatographic separation performance. Our test separation comprised a mixture of 32 oligostyrene structural isomers and stereoisomers. The optimal combination as determined by Information Theory and Factor Analysis consisted of a C18 column with a methanol mobile phase in the first dimension and a carbon clad zirconia column with an acetonitrile mobile phase in the second dimension. This system was also shown to be the most successful practical system when a heart-cutting approach was employed. The practical results were in total agreement with the results from Information Theory and Factor Analysis. The number of isomers resolved using this system was 27. A second system, namely one comprising of a C18 column and methanol mobile phase in the first dimension and a carbon clad zirconia column with a methanol mobile phase in the second dimension was also predicted to be a system with high separation potential. However, practical assessment of this system did not realize the theoretical predictions, largely due to the long separation times required in the second dimension. Furthermore, all combinations that employed a C18 column with an acetonitrile mobile phase in the first dimension failed to realize the theoretical separation potential due to high solute crowding, low orthogonality and a disordered arrangement of bands along the first separation axis. This was also predicted by the theoretical assessment.

89a. J.H. Park, J.W. Lee, S.H. Kwon, J.S. Cha, P.W. Carr, C.V. McNeff, "Separation of Racemic 2,4-Dinitrophenyl Amino Acids on 9-O-(phenyloxycarbonyl)quinine-bonded Carbon-Clad Zirconia in Reversed-Phase Liquid Chromatography," *Journal of Chromatography A*, **1050**, 151-157 (2004).

    Abstract: Zirconia is known to be one of the best materials for the chromatographic support due to its excellent chemical, thermal, and mechanical stability. In this work, we report preparation and use of 9-O-(phenyloxycarbonyl)quinine-bonded carbon-clad zirconia (QNCZ) as a chiral stationary phase (CSP) for separation of N-(2,4-dinitrophenyl) (DNP)-amino acids (AAs) enantiomers in reversed-phase liquid chromatography. Retention and enantioselectivity of the QNCZ CSP were compared with those of quinine 3-triethoxysilylpropylcarbamate-coated zirconia (QNZ) and quinine 3-triethoxysilylpropylcarbamate-bonded silica (QNS). The QNCZ CSP showed in general the better enantioselectivity for most of the amino acids studied.

95a. J.C. Reepmeyer, J.F. Brower, H. Ye, "Separation and Detection of the Isomeric Equine Conjugated Estrogens, Equilin Sulfate and delta8,9-dehydroestrone Sulfate, by Liquid Chromatography-Electrospray-Mass Spectrometry using Carbon-coated Zirconia and Porous Graphitic Carbon Stationary Phases," *Journal of Chromatography A*, **1083**, 42-51 (2005).

<u>Abstract:</u> Equilin-3-sulfate and delta8,9-dehydroestrone-3-sulfate are two isomers found in equine conjugated estrogens that differ in structure only by the position of a double bond in the steroid B-ring. These geometric isomers were not resolved on a C18 column during the analysis of conjugated estrogen drug products by LC-MS using acetonitrile-ammonium acetate buffer as the mobile phase. While no separations of these two isomers were observed on C18 or other alkyl-bonded silica based phases using a variety of mobile phase conditions, partial separations were achieved on phenyl bonded silica phases with a resolution of 1.5 on a diphenyl phase, and baseline separations were readily achieved on two carbonaceous phases with resolutions routinely exceeding three on graphitic carbon-coated zirconia (Zr-CARB) and resolutions as high as 19 on porous graphitic carbon (Hypercarb). An examination of a selected few conjugated estrogens in the complex drug substance by LC-MS on Hypercarb is presented.

98. D.R. Stoll, J.D. Cohen, P.W. Carr, "Fast, Comprehensive Online Two-dimensional High Performance Liquid Chromatography through the use of High Temperature Ultra-fast Gradient Elution Reversed-phase Liquid Chromatography," *Journal of Chromatography A*, **1122**, 123-137 (2006).

<u>Abstract:</u> A new approach to high speed, comprehensive online dual gradient elution 2DLC (LCxLC) based on the use of ultra-fast, high temperature gradient elution reversed phase chromatography is described. Entirely conventional gradient elution instrumentation and columns are assembled in a system which develops a total peak capacity of about 900 in 25min; this is equivalent to roughly one peak/2s. Each second dimension gradient is done in a cycle time of 21s and the peak retention times measured for a set of twenty six indole-3-acetic acid (IAA) derivatives are reproducible to 0.2s. Each peak eluting from the first dimension column is sampled at least twice across its width, as the corresponding peaks on the second dimension column appear in two or three consecutive second dimension chromatograms, clearly indicating that there is little loss in the resolution gained in the first dimension separation. Application to the separation of the low molecular weight components of wild-type and mutant maize seedlings indicates the presence of about 100 peaks on a timescale of 25min. Compelling illustrations of the analytical potential of fast, high temperature 2DLC are evident in the clear presence of nine distinct peaks in a single second dimension chromatogram from a single quite narrow first dimension peak, and the great power of 2DLC to solve the "analytic dynamic range" problem inherent in the measurement of small peaks that are neighbors to a gigantic peak.

98b. F. Cacciola, P. Jandera, E. Blahová, L. Mondello, "Development of Different Comprehensive Two Dimensional Systems for the Separation of Phenolic Antioxidants," *Journal of Separation Science*, **29(16)**, 2500-2513 (2006).

<u>Abstract:</u> Three different comprehensive 2-D HPLC systems for the separation of phenolic antioxidants have been developed on the basis of different selectivities of a PEG-silica column in the first dimension and a packed or monolithic C18 or a ZR-CARBON column, respectively, in the second dimension. Two-dimensional comprehensive liquid chromatography using a serially connected short PEG-silica column and a conventional C18-silica or a ZR-CARBON column in the second dimension was tested to improve the resolution of the earlier eluting compounds in the first dimension. Various types of interface were used to connect the columns in the first and in the second dimension: i) two injection sampling loops of 100 L in conventional arrangement; ii) a 10-port 2-position valve equipped with two trapping X-Terra columns instead of loops; and iii) two analytical D2 columns in parallel. The mobile phase in the first dimension has a lower elution strength than in the second dimension, allowing band compression of the solutes transferred from the first to the second dimension. This effect was enhanced using trapping columns instead of sampling loops as the interface between the two dimensions, thus allowing a decrease in the time of analysis. These systems were used for the analysis of beer samples. The relative location of the components in the 2-D retention plane varied in relation to their chemical structure in each instrumental set-up and allowed positive peak identification.

101. V. Žižkovský, R. Kučera and J. Klimeš, “Potential Employment of Non-silica-based Stationary Phases in Pharmaceutical Analysis,” *Journal of Pharmaceutical and Biomedical Analysis*, **44(5)**, 1048-1055 (2007).

<u>Abstract:</u> The absolute majority of the HPLC applications use silica-based columns for the separation of active substance and its impurities. However, stationary phases based on metal oxides appear as an interesting alternative. The aim of our study was to investigate the potential utilization of metal oxide-based stationary phases in analytical evaluation of ondansetron and its five pharmacopoeial impurities. In our study commercially available ZrO2-based columns (e.g. Zr-PBD, Zr-PS, Zr-C18) and TiO2-based column were used. The effect of an organic modifier (type and ratio), a buffer (type, pH and concentration) and the influence of temperature was investigated. The separation of ondansetron and its five pharmacopoeial

impurities was successfully accomplished on a Zirchrom®-PBD column using a mobile phase consisting of acetonitrile-ammonium phosphate (25 mM, pH 7.0) (18:82, v/v). Detection was performed at 216 nm and the analysis was completed within 7.5 min. The paper proves metal oxide-based stationary phases as an alternative to classical silica-based stationary phases in pharmaceutical analysis.

102. S.D. Chambers and C.A. Lucy, "Surfactant Coated Graphitic Carbon Based Stationary Phases for Anion-exchange Chromatography," *Journal of Chromatography A*, **1176(1-2)**, 178-184 (2007).

Abstract: Separations of common inorganic anions were carried out on three different surfactant coated media using carbonate/bicarbonate eluents with suppressed conductivity detection. Graphitic carbon columns (porous graphitic carbon and carbon-clad zirconia) packed with 3 μm particles have been converted into anion-exchange stationary phases by equilibration with the cationic surfactants: didodecyldimethylammonium bromide (DDAB); cetyltrimethylammonium bromide (CTAB); and cetylpyridinium chloride (CPC). Additionally, an ethylene-bridged silica column was studied with CPC coatings. Porous graphitic carbon (PGC) columns coated with DDAB exhibited pressure increases and loss of resolution at higher capacities. CPC coatings on PGC exhibited better repeatability and efficiencies of 5.0 × 104 plates/m. However, CPC coatings exhibited a 15% loss in retention factor with <1.2 × 103 column volumes on PGC. Conversely, the ethylene-bridged silica column showed complete failure in less than 8 h of use. As with PGC, carbon-clad zirconia coated with CPC showed an initial loss of capacity, but thereafter was stable for more than 1.7 × 103 column volumes (tr RSD < 2%).

105. C.V. McNeff, B. Yan, D.R. Stoll, R.A. Henry, "Review: Practice and Theory of High Temperature Liquid Chromatography," *Journal of Separation Science*, **30**, 1672-1685 (2007).

Abstract: High temperature liquid chromatography (HTLC) exists in a temperature region beyond ambient (ca. 40 C) and below super critical temperatures. The promises of HTLC, such as increased analysis speed, enhanced separation productivity, "green" LC with pure water mobile phases coupled to universal FID detection, and fast analysis of complex samples by combination with fast 2-D techniques, have become an option for routine practice. The focus of this paper is to review the key developments that have made the application of HTLC a practical technique and draw attention to new developments in 2-D techniques that incorporate HTLC that offer an opportunity to vastly increase the usefulness of HPLC for the analysis of complex samples.

107. F. Cacciola, P. Jandera, L. Mondello, "Temperature Effects on Separation on Zirconia Columns: Applications to One- and Two-Dimensional LC Separations of Phenolic Antioxidants," *J. Sep. Sci.*, **30**, 462-474 (2007).

Abstract: The effects of temperature and mobile phase on LC chromatographic separation of phenolic antioxidants on zirconia-based columns were investigated. Unlike silicabased materials, zirconia columns show excellent thermal stability over a wide range of temperatures and enable high-temperature separations. Enthalpic and entropic contributions to the retention of phenolic compounds on ZR-Carbon and ZR-Carbon C18 columns were determined from retention versus temperature plots in order to elucidate the retention mechanism of sample compounds over the temperature range up to 1408C. High-temperature liquid chromatography on ZR-Carbon columns was used for comprehensive LC6LC two-dimensional separation systems based on the different selectivity of a Zorbax SB micro-column used in the first dimension and a ZR-Carbon column used in the second dimension. Two-dimensional LC6LC systems employing a setup with two alternately operated parallel ZRCarbon columns in the second dimension were used for the analysis of phenolic antioxidants in beer and wine samples.

110. C. Milroy, G. Dennis, R.A. Shalliker, "Ultra-High Resolution Separation of Diastereomers on Carbon Adsorption Stationary Phases," *Journal of Liquid Chromatography & Related Technologies*, **30**, 991-999 (2007).

Abstract: Reversed-phase adsorption liquid chromatography was used to successfully resolve diastereomers in a complex mixture of low molecular weight polystyrene oligomers. The separation was achieved by incorporating a two-dimensional liquid chromatography system where the polystyrene oligomers were transported from the first dimensional column, a C18, to an adsorbent phase in the second dimension, a carbon clad zirconia (CCZ) column. Using this system up to 46 diastereomers, each consisting of eight configurational repeat units, were able to be resolved in ultra high separations.

112. S. Giegold, M. Holzhauser, T. Kiffmeyer, J. Tuerk, T. Teutenberg, M. Rosenhagen, D. Hennies, T. Hoppe-Tichy, B. Wenclawiak, “Influence of the Stationary Phase on the Stability of Thalidomide and Comparison of Different Methods for the Quantification of Thalidomide in Tablets using High-Temperature Liquid Chromatography,” *Journal of Pharmaceutical and Biomedical Analysis*, **46**, 625-630 (2008).

> Abstract: In this paper, three different HPLC methods for the quantification of thalidomide in tablets were developed and compared. The comparison of a conventional method at 30 ◦C with two high-temperature methods at 180 ◦C showed equal results. Using high-temperature HPLC (HT-HPLC), faster analysis times could be achieved.We have also focused on analyte stability and could show that the stationary phase has a pronounced effect on the on-column degradation of thalidomide at high temperatures. Virtually no degradation occurs if a polystyrene divinylbenzene column is used, whereas thalidomide is completely degraded at 180 ◦C when a carbon clad zirconium dioxide column is used.

114. M. Beldean-Galea, P. Jandera, S. Hodisan, “Retention and Separation Selectivity of Natural Phenolic Antioxidants on Zirconia Based Stationary Phases,” *Journal of Liquid Chromatography & Related Technologies*, 31, 807-818 (2008).

> Abstract: Many columns for reversed phase HPLC, based on silica gel, frequently used for separation of natural antioxidants, have a limited thermal and pH stability. In contrast, zirconium dioxide based stationary phases have an extremely different surface chemistry and are chemically stable over a wide rage of pH (1 to 14). Chromatographic properties associated with attractive and repulsive ionic interactions were investigated by measuring the retention data of isomeric naphthalene disulphonic acids on three zirconia based columns with carbon, carbon C18, and polystyrene bonded stationary phases. A comparative study on the retention behavior of natural antioxidants on zirconia based stationary phases is reported. The results suggest that zirconia based columns could be successfully used for separation of natural phenolic antioxidants.

115. G. Vanhoenacker, P. Sandra, “High Temperature and Temperature Programmed HPLC: Possibilities and Limitations,” *Anal Bioanal Chem*, 390, 245 – 258 (2008).

> Abstract: Temperature plays an important role in high-performance liquid chromatography (HPLC) since both the kinetics and thermodynamics of the chromatographic process are a function of temperature. To date, the possibilities of using temperature to improve LC separations have not been fully investigated. Most researchers mainly focus on column chemistry and dimensions, and on mobile phase composition and pH to optimize their separation. Temperature is rarely examined over a broad range and most separations are performed and/or optimized in the temperature region 20–50 °C. Moreover, currently a large number of liquid chromatographs are still operated at room temperature instead of using a column thermostat to control column temperature and thus retention time. We have recently reviewed the use of elevated temperature and temperature programming in HPLC [1].

120. R. A. Shalliker, “Two-dimensional HPLC Analysis of Oligostyrenes: Comprehensive and Online Heart-cutting Techniques,” *Journal of Separation Science*, **32**, 2903-2911 (2009).

> Abstract: 2-D HPLC incorporating two reversed phase (RP) environments was employed for the isolation of oligomers and their diastereomers of low molecular weight oligostyrenes. The operation of a comprehensive method of analysis was compared to a heart-cutting approach. The comprehensive approach employed a high resolution diastereomer separation in the first dimension and a low peak capacity C18, high speed separation according to molecular weight. Because of solvent incompatibility between the dimensions in the comprehensive method, successful separation of the diastereomers of the oligomers was not possible. The heart-cutting approach used a C18 monolith in the first dimension, which was selective only for molecular weight. Entire molecular weight fractions were then transported to the second dimension in an online heart-cutting process for the separation of diastereomers. The heart-cutting process was more successful in that 228 components of the 511 within the sample were recognized. This series of separations was undertaken in less than 6 h.

125. M. F. Wahab, C. A. Pohl, C. Lucy, “Ion Chromatography on Carbon Clad Zirconia Modified by Diazonium Chemistry and Functionalized Latex Particles,” *Royal Society of Chemistry*, DOI: 10.1039/c1an15284j (2011).

> Abstract: This work explores the potential of 3 mm carbon coated zirconia particles as a stationary phase for ion chromatography for the separation of organic acids and inorganic ions. A 4-phenylsulfonic acid functionality is introduced onto the carbon surface by reducing 4-phenylsulfonic acid diazonium chloride

with borohydride in the presence of carbon clad zirconia particles. The elemental sulfur analysis gave 132 meq–SO3H/g carbon clad zirconia and 2% S atomic concentration by XPS analysis. The –SO3_ groups serve as electrostatic anchors for latex nanoparticles bearing quaternary triethylamine functional groups. The agglomeration step in 5 _ 0.4 cm i.d. columns converts the packed particles into an anion exchanger. The breakthrough curves with nitrate indicate a capacity of 3 meq/column. Separation of common organic acids and inorganic ions using carbonate eluent and suppressed conductivity detection yield plate heights (H) of 0.023–0.05 mm.

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50. J. Zhao and P.W. Carr, "Comparison of the Retention Characteristics of Aromatic and Aliphatic Reversed Phases for HPLC Using Linear Solvation Energy Relationships," *Anal. Chem.* **70,** 3619-28 (1998).

    Abstract: The similarities and differences in retention characteristics of aromatic and aliphatic phases have been elucidated by the use of linear solvation energy relationships (LSERs). Three aromatic phases and three aliphatic phases were investigated in a series of mobile phases. The results of LSERs on a polymer-based aromatic phase, poly(styrene-divinylbenzene) resin (PRP-1) are very different from those on either silica- or zirconia-based aromatic and aliphatic phases. Retention on all aromatic and aliphatic phases except PRP-1 is markedly influenced by the solute hydrogen bond donor acidity. We believe that PRP-1 exhibits a more adsorption-like retention mechanism than do the other phases. With the inorganic oxide-based phases, the aromatic phases are less retentive that the aliphatic phases but show a large dependence on molecular polarizability. The enhanced polarizability of aromatic phases is the likely cause of some differences in their chromatographic selectivity relative to the aliphatic phases.

53. J. Zhao and P.W. Carr, "An Approach to the Concept of Resolution Optimization through Changes in the Effective Chromatographic Selectivity," *Anal. Chem.* **71**, 2623-2632 (1999).

    Abstract: It is very common chromatographic practice to optimize resolution by making changes in selectivity by systematically varying key retention controlling factors. In many instances, a change in conditions merely results in monotonic, systematic variation in the relative retention of all pairs of peaks. Useful or "effective" changes in selectivity generally result when we see peak crossovers, changes in elution order or differential changes in band position of three or more peaks upon changing some operating condition. In this work, we demonstrate that changes in what we now call the *effective selectivity* can *only* take place when retention depends on a minimum of two solute molecular properties and further the dependencies must differ for the two sets of conditions. To verify our concept, real chromatographic data are examined from the viewpoint of linear solvation energy relationships (LSERs) and linear solvent strength theory. Five different RPLC stationary phases in different eluents are compared to elucidate the similarities and differences in their effective selectivities. Of major importance is our finding that the effective selectivity can only be understood when it is viewed in terms of the *ratios* of system-dependent interaction coefficients, such as the LSER coefficients, and not merely the *absolute* values of the coefficients. We confirm, both theoretically and experimentally, that a change in mobile-phase volume fraction and in column temperature is not as powerful a mechanism for tuning the effective selectivity, as is a change in stationary-phase type.

55. J. Zhao and P.W. Carr, "Synthesis and Evaluation of an Aromatic Polymer-Coated Zirconia for Reversed-Phase Liquid Chromatography," *Anal. Chem.* **71**, 5217-5224 (1999).

    Abstract: We synthesized a novel aromatic polymer-coated zirconia-based RPLC stationary phase by chemical adsorption of a copolymer of chloromethylstyrene and diethoxymethylvinylsilane onto zirconia (CMS/VMS-ZrO_2). Characterization of the pore structure of the support by nitrogen porosimetry and inverse size-exclusion chromatography indicates that CMS/VMS-ZrO_2 maintains the well-defined pore structure of the base material. Flow studies show that CMS/VMS-ZrO_2 has good mass transfer characteristics. The reversed-phase retention characteristics of the new support are comparable to those of conventional silica-bonded phases. We have also evaluated the mechanical, thermal, and pH stability of CMS/VMS-ZrO_2. The results show that CMS/VMS-ZrO_2 is stable over a very wide range of pH (pH = 1-13) and at temperatures as high as 160º C. Chromatographic separations of some low molecular weight aromatic analytes on CMS/VMS-ZrO_2 and octadecyl-bonded silica phases indicate that there are some subtle but significant differences in the chromatographic selectivity of these two types of phases.

59. J. Zhao and P.W. Carr, “A Comparative Study of the Chromatographic Selectivity of Polystyrene-Coated Zirconia and Related Reversed-Phase Materials,” *Anal. Chem.* **72**, 302-309 (2000).

    Abstract: The chromatographic selectivities of polystyrene-coated zirconia (PS-ZrO_2) have been investigated in detail by means of homologue series retention studies and studies of a variety of nonpolar aromatic positional isomers. This material has been compared to polybutadiene-coated zirconia, octadecyl- and phenyl-bonded silica, and polymer-based reversed phases. In addition, the separations of a wide variety of compound classes on PS-ZrO_2 were compared with those on other aromatic and aliphatic RPLC phases. The PS-ZrO_2 material exhibits substantial differences in selectivity toward nonpolar and polar analytes as compared to other phases, especially with respect to polybutadiene-coated zirconia (PBD-ZrO_2). On PS-ZrO_2, polynuclear aromatic hydrocarbons (PAHs) are retained much more than are alkylbenzenes, whereas on PBS-ZrO_2 the opposite is observed. In addition, PS-ZrO_2 has much greater selectivity than does PBD-ZrO_2 for the separation of structural isomers which differ in the position of phenyl groups. In general, even though the carbon content of PS-ZrO_2 is much lower than that of conventional reversed-phase materials, such as octadecyl-bonded silica (C18-SiO_2), polar analytes exhibit sufficient retention and differential selectivity on PS-ZrO_2 that resolution comparable to that on C18-SiO_2 can be obtained but with substantially improved analysis times.

60. B. Yan, J. Zhao, J.S. Brown, J. Blackwell and P.W. Carr, “High-Temperature Ultrafast Liquid Chromatography,” *Anal. Chem.* **72**, 1253-1262 (2000).

    Abstract: A novel liquid chromatographic system which enables high temperature ultrafast liquid chromatography (HTU-FLC) has been designed through the careful consideration of heat transfer, band broadening, and pressure drop. Studies of the effect of linear velocity on the HETP show that column efficiency at high velocity, especially of well-retained solutes, dramatically improves at higher temperatures. At 150°C, at a flow rate of 15 mL/min with a 5 cm by 4.6mm (i.d.) column packed with 3 micron polystyrene-coated zirconia porous particles, long chain alkylphenones were completely resolved, and the analysis time could be decreased by a factor of 50 compared to that at room temperature (25°C) at a conventional flow rate (4 mL/min). In addition, using pure water as the mobile phase, five phenols were separated in less than 30 s.

77. R.A. Henry, “Highly Selective Zirconia-based Phases for HPLC Applications,” *American Laboratory*, **34**(**22**), 18-25 (2002).

    Abstract: Zirconia-based phases (Z-phases) for HPLC have been available for almost a decade, and are finding a wide range of applicability because they are very complementary to silica-based phases (S-phases) and often replace total-polymer phases (P-phases), particularly in reversed-phase (RP) applications.

79a. P. He, Yu Yang, “Studies on the Long-Term Thermal Stability of Stationary Phases in Subcritical Water Chromatography”, *Journal of Chromatography A*, **989**, 55-63 (2003).

    Abstract: The long-term thermal stability of five commercially available reversed-phase columns has been evaluated under subcritical water conditions (100 and 150°C). The five columns included Zorbax RX-C8, Nucleosil C18 AB, Hypersil BDS C18, PRP-1 (poly(styrene-divinylbenzene)), and ZirChrom-PS (polystyrene) columns. Retention factors, plate numbers, and peak areas were monitored over a prolonged period of time. Comparing the three silica-based columns, the Zorbax RX-C8 column was the most stable followed by the Nucleosil C18 AB column. The Hypersil BDS C18 column was the least stable under subcritical water conditions. The ZirChrom-PS column was stable at 100 °C for at least 7600 column volumes. Of all five columns, the polymeric PRP-1 column was the most stable under subcritical water conditions.

84a. L.J. Lamm, Yu Yang, “Off-Line Coupling of Subcritical Water Extraction with Subcritical Water Chromatography via a Sorbent Trap and Thermal Desorption”, *Analytical Chemistry*, **75**, 2237-2242 (2003).

    Abstract: In this study, the off-line coupling of subcritical water extraction (SBWE) with subcritical water chromatography (SBWC) was achieved using a sorbent trap and thermal desorption. The sorbent trap was employed to collect the extracted analytes during subcritical water extraction. After the extraction, the trap was connected to the sub-critical water chromatography system, and thermal desorption of the trapped analytes was performed before the SBWC run. The thermally desorbed analytes were then introduced into the subcritical water separation column and detected by a UV detector. Anilines and phenols were extracted from sand and analyzed using this off-line coupling technique. Subcritical water extraction of flavones from orange peel followed by subcritical water chromatographic separation was also investigated. The effects of water volume and extraction temperature on flavone recovery were determined. Because a sorbent trap was used to collect the extracted analytes, the sensitivity of this technique was greatly enhanced as compared to that of subcritical water extraction with solvent trapping. Since no organic solvent-water extractions were necessary prior to analysis, this technique eliminated any use of organic solvents in both extraction and chromatography processes.

86a. E. Van Gyseghem, I. Crosiers, S. Gourvenec, D.L. Massart, Y. Vander Heyden, "Determining Orthogonal and Similar Chromatographic Systems from the Injection of Mixtures in Liquid Chromatography-Diode Array Detection and the Interpretation of Correlation Coefficients Color Maps," *Journal of Chromatography A*, **1026**, 117-128 (2004).

Abstract: Generic orthogonal chromatographic systems might be helpful tools as potential starting points in the development of methods to separate impurities and the active substance in drugs with unknown impurity profiles. The orthogonality of 38 chromatographic systems was evaluated from weighted-average-linkage dendrograms and color maps, both based on the correlation coefficients between the retention factors on the different systems. On each chromatographic system, 68 drug substances were injected as mixtures of three or four components to increase the throughput. The (overlapping) peaks were identified and resolved with a peak purity algorithm, orthogonal projection approach (OPA). The visualization techniques applied allowed a simple evaluation of orthogonal and (groups of) similar systems.

88. J. Nawrocki, C. Dunlap, J. Li, J. Zhao, C.V. McNeff, A. McCormick, P.W. Carr, "Part II. Chromatography Using Ultra-Stable Metal Oxide-Based Stationary Phases for HPLC," *Journal of Chromatography A*, **1028**, 31-62 (2004).

Abstract: In this part of the review authors discuss methods used for modification of metal oxide surfaces. On the basis of literature data it is shown, that silanization of the surfaces do not form stable supports for chromatography. On the other hand, the success of polymer modified surfaces such as polybutadiene (PBD) and polystyrene (PS) is emphasized. Permanent modification of metal oxide surfaces with Lewis bases is also widely discussed. Chromatographic properties of polymer-modified surfaces of zirconia are discussed in details. The perspectives of carbon-coated metal oxide surfaces in HPLC and high temperature separations are described.

95c. R. Kučera, J. Sochor, J. Klimeš and J. Dohnal, "Use of the Zirconia-based Stationary Phase for Separation of Ibuprofen and Its Impurities," *Journal of Pharmaceutical and Biomedical Analysis*, **38(4)**, 609-618 (2005).

Abstract: A new reversed-phase liquid chromatographic method using zirconia-based stationary phase was developed for determination of ibuprofen, its related compounds and its main degradation products. The chromatographic separation was successfully achieved on the Discovery®Zr-PS column (150 mm × 4.6 mm i.d., 5 μm), using a mobile phase methanol–phosphate buffer (pH 4.5; 0.05 M)–tetrahydrofurane (21:74:5, v/v/v) and the flow rate 0.5 ml min^{-1}. The UV detection was performed in dual wavelength mode (219 and 258 nm) to detect all compounds of interest. The column temperature was set on 60 °C to shorten the analysis time and improve the peak symmetry. The method is simple, rapid and cuts down the amount of hazardous waste produced in the analysis. The assay is completed within 22 minutes.

95e. K. Soukupová, E. Krafková, Jana Suchánková and E. Tesařová, "Comparison of Zirconia- and Silica-based Reversed Stationary Phases for Separation of Enkephalins," *Journal of Chromatography A*, **1087(1-2)**, 104-111 (2005).

Abstract: In this study, the separation of biologically active peptides on two zirconia-based phases, polybutadiene (PBD)-ZrO2 and polystyrene (PS)-ZrO2, and a silica-based phase C18 was compared. Basic differences in interactions on both types of phases led to quite different selectivity. The retention characteristics were investigated in detail using a variety of organic modifiers, buffers, and temperatures. These parameters affected retention, separation efficiency, resolution and symmetry of peaks. Separation systems consisting of Discovery PBD-Zr column and mobile phase composed of a mixture of acetonitrile and phosphate buffer, pH 2.0 (45:55, v/v) at 70 °C and Discovery PS-Zr with acetonitrile and phosphate buffer, pH 3.5 in the same (v/v) ratio at 40 °C were suitable for a good resolution of enkephalin related peptides. Mobile phase composed of acetonitrile and phosphate buffer, pH 5.0 (22:78, v/v) was appropriate for separation of enkephalins on Supelcosil C18 stationary phase.

100.C. Ovens, D. Sievwright and A.J. Silcock, "Unusual Retention Behaviour of 4-substituted Piperidines on Polybutadiene and Polystyrene Coated Zirconia by Comparison to Reverse Phase Silica," *Journal of Chromatography A*, **1137(1)**, 56-62 (2006).

> Abstract: The retention properties of a range of N-methylated and N-des-methyl 4-substituted piperidines on polybutadiene (PBD) and polystyrene (PS) coated zirconia have been studied and compared to those of Xterra RP18 and Genesis C18 silica. The effect of buffer type and pH are investigated with regard to the elution order on all three stationary phases. The change in the elution order is linked to the degree of substitution of the piperidine nitrogen and appears to be independent of the rest of the structure, indicating that this moiety is most heavily involved in the separation mechanism on the zirconia phases.

101.V. Žižkovský, R. Kučera and J. Klimeš, "Potential Employment of Non-silica-based Stationary Phases in Pharmaceutical Analysis," *Journal of Pharmaceutical and Biomedical Analysis*, **44(5)**, 1048-1055 (2007).

> Abstract: The absolute majority of the HPLC applications use silica-based columns for the separation of active substance and its impurities. However, stationary phases based on metal oxides appear as an interesting alternative. The aim of our study was to investigate the potential utilization of metal oxide-based stationary phases in analytical evaluation of ondansetron and its five pharmacopoeial impurities. In our study commercially available ZrO2-based columns (e.g. Zr-PBD, Zr-PS, Zr-C18) and TiO2-based column were used. The effect of an organic modifier (type and ratio), a buffer (type, pH and concentration) and the influence of temperature was investigated. The separation of ondansetron and its five pharmacopoeial impurities was successfully accomplished on a Zirchrom®-PBD column using a mobile phase consisting of acetonitrile-ammonium phosphate (25 mM, pH 7.0) (18:82, v/v). Detection was performed at 216 nm and the analysis was completed within 7.5 min. The paper proves metal oxide-based stationary phases as an alternative to classical silica-based stationary phases in pharmaceutical analysis.

105.C.V. McNeff, B. Yan, D.R. Stoll, R.A. Henry, "Review: Practice and Theory of High Temperature Liquid Chromatography," *Journal of Separation Science*, **30**, 1672-1685 (2007).

> Abstract: High temperature liquid chromatography (HTLC) exists in a temperature region beyond ambient (ca. 40 C) and below super critical temperatures. The promises of HTLC, such as increased analysis speed, enhanced separation productivity, "green" LC with pure water mobile phases coupled to universal FID detection, and fast analysis of complex samples by combination with fast 2-D techniques, have become an option for routine practice. The focus of this paper is to review the key developments that have made the application of HTLC a practical technique and draw attention to new developments in 2-D techniques that incorporate HTLC that offer an opportunity to vastly increase the usefulness of HPLC for the analysis of complex samples.

111. R. Bagnati, G. Bianchi, E. Marangon, E. Zuccato, R. Fanelli, E. Davoli, "Direct Analysis of Isopropylthioxanthone (ITX) in Milk by High-Performance Liquid Chromatography/Tandem Mass Spectrometry," *Rapid Communications in Mass Spectrometry*, **21**, 1998-2002 (2007).

> Abstract: A fast screening method is presented for detecting isopropylthioxanthone (ITX) contamination in milk. The method is based on direct high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) analysis of milk samples. Sample preparation is limited to the addition of a deuterated ITX solution in acetonitrile that serves both as internal standard and to precipitate proteins. The method is highly accurate and sensitive. Isomeric specific analyses of 2-ITX and 4-ITX are possible at 6mg/L levels with about 5% precision and accuracy. This approach has been used to check contamination in samples like milk, soy milk, baby milk, in their packaging material. Out of 37 milk samples analyzed, 16 were positive with concentrations ranging from 173–439mg/L for 2-ITX and from <6 (lower than limit of quantification) to 25mg/L for 4-ITX.

114. M. Beldean-Galea, P. Jandera, S. Hodisan, "Retention and Separation Selectivity of Natural Phenolic Antioxidants on Zirconia Based Stationary Phases," *Journal of Liquid Chromatography & Related Technologies*, 31, 807-818 (2008).

> Abstract: Many columns for reversed phase HPLC, based on silica gel, frequently used for separation of natural antioxidants, have a limited thermal and pH stability. In contrast, zirconium dioxide based stationary phases have an extremely different surface chemistry and are chemically stable over a wide rage of pH (1 to 14). Chromatographic properties associated with attractive and repulsive ionic interactions were investigated by measuring the retention data of isomeric naphthalene disulphonic acids on three zirconia

based columns with carbon, carbon C18, and polystyrene bonded stationary phases. A comparative study on the retention behavior of natural antioxidants on zirconia based stationary phases is reported. The results suggest that zirconia based columns could be successfully used for separation of natural phenolic antioxidants.

118. P. Kalafut, R. Kucera, J. Klimes, J. Sochor, "An Innovative Approach to the Analysis of 3-[4-(2-methylpropyl)phenyl]propanoic Acid as an Impurity of Ibuprofen on a Carbon-Coated Zirconia Stationary Phase," *Journal of Pharmaceutical and Biomedical Analysis*, 49, 1150-1156 (2009).

Abstract: 3-[4-(2-Methylpropyl)phenyl]propanoic acid has been introduced as impurity F to the European Pharmacopoeia in its Supplement 4.2. In contrast to other impurities, which are evaluated by HPLC, the content of impurity F is determined by gas chromatography after previous derivatization. Thus a novel reversed-phase HPLC method was developed to simplify the evaluation of pharmacopoeial impurity F of ibuprofen. Favourable properties of zirconia stationary phases were employed for this purpose. The HPLC separation was achieved on a Zr-CARB column (150mm×4.6mm i.d., 5_m) using the mobile phase acetonitrile–phosphate buffer (pH 3.5, 25mM) (38:62, v/v), temperature 80 ◦C and the flow rate 1.2mlmin−1. The fluorescence detection was employed to enhance the sensitivity of the method. Optimal detection parameters were chosen on the basis of fluorescence spectra of the analytes. The excitation and emissionwavelengths were 220nm and 285 nm, respectively. The analysiswas completed within 25 min. The subsequent validation of the method confirmed the applicability of method for the analytical assay of impurity F.

121. L. Janeckova, K. Kalikova, Z. Bosakova, E. Tesarova, "Study of Interaction Mechanisms on Zirconia-based Polystyrene HPLC Column," *Journal of Separation Science*, **33**, 3043-3051 (2010).

Abstract: Separation systems with a zirconia-based polystyrene HPLC column were characterized by different approaches, which allowed the recognition of interactions participating in the separation environments. Zirconia-based HPLC columns as an alternative to silica-based ones offer unique interaction mechanism based on Lewis acid–base theory. Besides hydrophobic interactions with the modified surface of the zirconia carrier it includes ionexchange and ligand-exchange interactions that are helpful in the separation of many bioactive compounds. Three distinct approaches were applied for description of the complex separation mechanism. General chromatographic tests by Walters, Engelhardt and Galushko were applied to evaluate the fundamental properties of the systems – hydrophobicity and polarity. The complex model of linear free energy relationship described the interactions from the qualitative and quantitative points of view more in detail. Application of a set of basic compounds revealed the contribution of ion-exchange interactions participating in the separation systems.

Ion-Exchange

11. J.A. Blackwell and P.W. Carr, "Ligand Exchange Chromatography of Free Amino Acids on Phosphated Zirconium Oxide Supports," *J. Liq. Chromatogr*. **15**, 727-51 (1992).

Abstract: Phosphated zirconium oxide particles loaded with copper ions are a mechanically superior alternative to resin based supports for ligand exchange separations of amino acids. They show good stability under acidic and alkaline conditions and afford different selectivity than resin based exchangers. Acidic amino acids are more strongly retained on this phase than on silica or resin based supports due to interactions with the underlying zirconia surface. These interactions occur despite rigorous phosphation of the zirconia particle. The operational aspects of amino acid separations on this material are examined and compared to conventional resin based models.

12. J.A. Blackwell and P.W. Carr, "Role of Lewis Acid-Base Processes in Ligand-Exchange Chromatography of Benzoic Acid Derivatives on Zirconium Oxide," *Anal. Chem*. **64**, 853-62 (1992).

Abstract: Porous microparticulate zirconium oxide shows very different selectivities and pH dependencies for the separation of benzoic acid derivatives than do conventional bonded-phase anion-exchange supports. This results from a very significant ligand-exchange contribution to the retention of hard Lewis bases on the surface of transition-metal oxide supports. We have found that the capacity factors of a wide variety of derivatives of benzoic acid are closely correlated with their Bronsted acidities. The eluent pH is also a critical factor in determining the magnitude of the capacity factor, but it does not have much influence on chromatographic selectivity. The differential selectivity of this phase in comparison to conventional

polymeric and bonded-phase anion exchangers can be attributed to complexation and steric effects which profoundly alter the elution patterns of certain solutes.

22. C. McNeff, Q.H. Zhao, and P.W. Carr, "High-Performance Anion-Exchange of Small Anions with Polyethyleneimine Coated Porous Zirconia," *J. Chromatogr.* A **684**, 201-11 (1994).

    Abstract: The preparation and chromatographic characterization of an ion-exchange high-performance liquid chromatographic support by deposition and crosslinking of polyethyleneimine (PEI) on the surface of porous zirconia is described. Adsorption and evaporation methods were used for coating PEI onto the zirconia particles. These two stationary phases were compared by elemental analysis, ion-exchange capacity and by chromatography. High efficiency and good selectivity were observed for inorganic and organic anions. The addition of a strong, hard Lewis base to the mobile phase dramatically improved the peak shape and efficiency of *para* benzoic acid derivatives. PEI-coated zirconia showed a distinct elution sequence for organic anions when compared to *bare* zirconia or silica-based phases. The polyamine coated zirconia was stable over a pH range from 2.75 to 9. Flow studies, using nitrite as a probe solute, showed that both coating procedures produced packed columns with good mass-transfer properties.

26. C. McNeff and P.W. Carr, "High-Performance Anion-Exchange Chromatography of Oligonucleotides and Oligodeoxynucleotides on Quaternized Polyethyleneimine-Coated Zirconia," *Anal. Chem.* **67**, 2350-3 (1995).

    Abstract: The use of porous, quaternized polyethyleneimine-coated zirconia particles for the separation of nucleosides, nucleotides, oligonucleotides, and oligodeoxynucleotides is described. Separations of these biomolecules on quaternized PEI-zirconia are presented. Quaternized PEI-zirconia and a quaternary amine functionalized silica are compared chromatographically. Quaternized PEI-zirconia was able to separate oligonucleotides and oligodeoxynucleotides differing in length by a single nucleotide unit. It could also separate RNAs of the same length but differing by only one deoxynucleotide. In contrast, the silica-based phase was unstable at elevated temperature (75° C), and all retention toward anions was lost quite rapidly.

31. C. McNeff and P.W. Carr, "Synthesis and Use of Quaternized Polyethyleneimine-Coated Zirconia for High-Performance Anion-Exchange Chromatography," *Anal. Chem.* **67,** 3886-92 (1995).

    Abstract: The synthesis of an alkali-stable strong anion-exchange stationary phase by deposition of polynethylenimine (PEI), followed by cross-linking and quaternization, onto porous zirconia particle is described. Physical characterization of quaternized PEI-zirconia and PEI-zirconia shows that 50% and 24% of the amine groups are cross-linked, respectively. A plot of log *k'* versus log (competing ion concentration) is linear for three homopeptides, suggesting that ion exchange is the primary mechanism of retention on quaternized PEI-zirconia. Column efficiency for two 2,4-dinitrophenyl amino acids increased by 80% upon increasing the temperature from 50 °C to 100 °C. The hydrophobicity of quaternized PEI-zirconia was studied using a homologous series of *p*-alkoxybenzoic acids. For quaternized PEI-zirconia and PEI-zirconia, we found that the free energy of transfer of a methylene unit from the mobile phase to the stationary phase was –2.0 and –0.90 kJ/mol, respectively. The free energy of transfer of a methylene unit on quaternized PEI-zirconia is similar to that of a typical ODS phase (-2.4 kJ/mol). A van't Hoff plot for the above two 2,4-dinitrophenyl amino acids showed that the enthalpies of transfer are exothermic and fairly large (~ -14 kJ/mol). Isocratic separations on quaternized PEI-zirconia of inorganic and organic anions are presented. Quaternized PEI-zirconia is more efficient than the silica-based phase in the separation of benzoic acid derivatives but slightly less efficient than PEI-zirconia. The major virtue of quaternized PEI-zirconia is that it is chemically stable in the pH range of 1-13 and is also stable at temperatures up to 100 °C.

47. A.M. Clausen and P.W. Carr, “Chromatographic Characterization of Phosphonate Analog EDTA-Modified Zirconia Support for Biochromatographic Applications,” *Anal. Chem.* **70,** 378-85 (1998).

    Abstract: Zirconium dioxide (zirconia) has a great affinity for inorganic and organic phosphate. Previous work from is laboratory demonstrated the utility of phosphate-modified microparticulate zirconia as a support for protein separations. We have extended this investigation to include the study of ethylenediamine-N,N'-tetramethylphosphonic acid (EDTPA), a phosphonate analog of EDTA, as a surface modifier for zirconia. Our work explores the use of EDTPA-modified zirconia (PEZ) for its potential use as a high-performance inorganic cation-exchange support for the separation of proteins. The phosphate groups in EDTPA very effectively block the sites responsible for strong interactions of hard Lewis bases with zirconia's surface. Modification of zirconia with EDTPA provides a “biocompatible” stationary phase, resulting in high mass recoveries of proteins. We compare PEZ with inorganic phosphate-modified zirconia to show increased efficiency, as well as unique selectivities for chromatography of proteins on the chelator-modified surface. Finally, the selectivity, efficiency, and separation mechanism are reported. The studies show the PEZ is a useful high-performance ion-exchange support for the separation of cationic proteins and for modulating the sites responsible for the high affinity of zirconia toward certain classes of anions.

49. Y. Hu and P.W. Carr, "Synthesis and Characterization of New Zirconia-Based Polymeric Cation-Exchange Stationary Phases for High-Performance Liquid Chromatography of Proteins," *Anal. Chem.* **70,** 1934-42 (1998).

Abstract: Ion-exchange chromatography is a major method used for large-scale protein separations. New zirconia-based polymeric cation-exchange HPLC stationary phases have been developed for protein separations. Two routes were employed for the synthesis. In one method, polyethyleneimine (PEI) was adsorbed onto porous zirconia particles and cross-linked with 1,4-butanediol diglycidyl ether (BUDGE). Succinic anhydride was then reacted with the remaining primary and secondary amine groups on PEI to afford anionic functionalities. The second method utilizes poly9acrilic acid) anhydride as both the cross-linker and the stationary phase. The resulting stationary phases act to separate proteins by a weak cation-exchange mechanism with a slight contribution to retention from hydrophobic interactions. In the presence of 20 mM phosphate buffer, Lewis acid/base interactions between the zirconia support and the proteins, which can significantly broaden the peaks, are sufficiently suppressed. The effects of ionic strength, mobile phase pH and slat type are discussed. Protein mass recovery and loading capacity for protein separations on these phases have been evaluated. These weak cation-exchange stationary phases exhibit good stability under normal separation conditions for months and are stable in alkaline solution up to pH 10. In contrast to zirconia supports modified with small anionic species, these new phases have no limitation on the type of salt used as the eluent, and they exhibit unique selectivities. Therefore, they offer interesting alternatives for protein separations. To our knowledge, this work represents the first successful example of protein separations using porous zirconia-based polymeric phases under normal chromatographic conditions, which will definitely help make zirconia-based supports more useful for bioseparations.

56. C.V. McNeff, Q. Zhao, E. Almolof, M. Flickinger, P.W. Carr, "Synthesis and Use of Quaternized PEI-Zirconia for the Efficient Removal of Endotoxins from Proteins," *Anal. Biochem.,* 274, 181-187 (1999).

Abstract: The synthesis and use of a zirconia-based, alkali-stable strong anion-exchange stationary phase are described for the removal of pyrogenic lipopolysaccharides (LPS) from insulin. The strong anion-exchange material is produced by deposition of polyethyleneimine (PEI) onto porous zirconia particles, followed by cross-linking with a novel reagent, 1,2-bis-(2-iodoethoxy) ethane, and quaternization with iodomethane. Physical characterization of the chromatographic support shows that it has an ion-exchange capacity of 0.6 mmol/g, and 82% of the amine sites on the surface are in quaternized form. Isocratic elution of small benzoic acid derivatives shows good column efficiency. The two primary virtues of this material are its chemical stability under alkali conditions (up to pH 13) and its lower hydrophobicity compared to previously described alkali-stable PEI-coated zirconia supports cross-linked with 1,10-diiododecane. Using this new zirconia-based phase, a purification protocol is developed for the efficient removal of Escherichia coli 0111:B4 LPS from bovine insulin samples. An endotoxin clearance rate of up to 1.3 × 108 was attained. Endotoxin levels were reduced to less than 5 endotoxin units/ml even at initial contamination levels as high as 5.0×10^6 endotoxin units/ml. Furthermore, endotoxin adsorbed to the porous zirconia column may be easily remove (depyrogenated) using alkali for repeated purification cycles.

56c. A. Mullick and M.C. Flickinger, "Expanded Bed Adsorption of Human Serum Albumin from Very Dense Saccharomyces cerevesiae Suspensions on Fluoride-Modified Zirconia," *Biotechnology and Bioengineering,* Vol. 65, 282-290 (1999).

Abstract: The adsorption of proteins from high cell density yeast suspensions on mixed-mode fluoride-modified zirconia (FmZr) particles (38 to 75 um, surface area of 29 m^2/g and density of 2.8 g/cm^3) was investigated using human serum albumin (HSA) added to Saccharomyces cerevesiae as the model expression host. Because of the high density of the porous zirconia particles, HSA (4 mg/mL) can be adsorbed from a 100 g dry cell weight (DCW)/L yeast suspension in a threefold-expanded bed of FmZr. The expanded bed adsorption of any protein from a suspension containing >50 g DCW/L cells has not been previously reported. The FmZr bed expansion characteristics were well represented by the Richardson-Zaki correlation with a particle terminal velocity of 3.1 mm/s and a bed expansion index of 5.4. Expanded bed hydro-dynamics were investigated as a function of bed expansion using residence time distribution studies with sodium nitrite as the tracer. The adsorption of HSA on FmZr exhibited features of multicomponent adsorption due to the presence of dimers. The protein binding capacity at 5% breakthrough decreased from 22 mg HSA/mL settled bed void volume for 20 g DCW/L yeast to 15 mg HSA/mL settled bed void volume for 40 g DCW/L yeast and remained unchanged for the higher yeast concentrations (60 to 100 g DCW/L). However, the batch (or equilibrium) binding capacity decreased monotonically as a function of yeast concentration (20 to 100 g DCW/L) and the binding capacity at 100 g DCW/L yeast was fivefold lower compared with that at 20 g DCW/L yeast. The lower batch binding capacity at high cell concentrations resulted from the adsorption of cells at the surface of the particles restricting access of HSA to the intraparticle surface area. Batch (or equilibrium) and column HSA adsorption results indicated that the adsorption of HSA on FmZr occurred at a time scale that may be much faster than that of yeast cells. The zirconia particles were cleaned of adsorbed HSA and yeast with a total of 1500 to 2000 column volumes (over many cycles) of 0.25 M NaOH, without any significant effect on the chromatographic performance.

63. J. Zhao, P.W. Carr, "Quaternized Trimethylaminated Polystyrene-Coated Zirconia as a Strong Anion Exchange Material for HPLC," *Anal. Chem.* **72**, 4413-4419 (2000).

    <u>Abstract</u>: The synthesis and characterization of a new, base-stable, strong anion exchange phase by amination of polystyrene-coated zirconia (PS-ZrO_2) are described. Even though the ion exchange capacity of the quarternized trimethylaminated PS-ZrO_2 (QTMA-PS-ZrO_2) is only 0.07 mequiv/g, it is able to separate various inorganic anions, benzoic acid derivatives, and nucleotides in their deprotonated states. The effects of ionic strength, eluent pH, and counterion type are discussed. In the presence of both phosphate and fluoride ions in the eluent, band broadening caused by Lewis acid/base interactions between zirconia and analytes is greatly suppressed. The mixed retention modes (ion exchange, hydrophobic interaction, and Lewis acid/base interactions) on QTMA-PS-ZrO_2 offer a different selectivity toward various anionic analytes than do other zirconia and nonzirconia-based ion exchangers.

74. Y. Hu, X. Yang and P.W. Carr, "Mixed-mode Reversed-phase and Ion-exchange Separations of Cationic Analytes on Polybutadiene-coated Zirconia," *Journal of Chromatography A*, **968**, 17-29 (2002).

    <u>Abstract:</u> The retention and selectivity of the chromatographic separation of basic (cationic) analytes on a polybutadiene-coated zirconia (PBD-ZrO2) stationary phase have been studied in greater detail than in previous studies. These separations are strongly influenced by the chemistry of the accessible surface of zirconia. In the presence of buffers which contain hard Lewis bases (e.g., phosphate, fluoride, carboxylic acids) zirconia's surface becomes negatively charged due to adsorption of the buffer anion at the hard Lewis acid sites. Consequently, under most conditions (e.g., neutral pH), cationic analytes undergo both hydrophobic and cation-exchange interactions. This mixed-mode retention process generally leads to greater retention factors for cations relative to those on silica-based reversed phases despite the lower surface areas of the zirconia phase, but, more importantly, adsorption of hard Lewis bases can be used to control the chromatographic selectivity for cationic analytes on these zirconia-based stationary phases. In contrast to our prior work, here we show that when mixed-mode retention takes place, both retention and selectivity are easily adjusted by changing the type of hard Lewis base buffer anion, the type of buffer counter-ion (e.g., sodium, potassium, ammonium), the pH, and the ionic strength of the eluent as well as the type and amount of organic modifier.

81. X. Yang, J. Dai, P.W. Carr, "Analysis and Critical Comparison of the Reversed-phase and Ion-exchange contributions to Retention on Polybutadiene Coated Zirconia and Octadecyl Silane Bonded Silica Phases," *Journal of Chromatography A*, **996**, 13-31 (2003).

    <u>Abstract:</u> The two major modes of retention of basic compounds in reversed-phase liquid chromatography on both octadecyl silane bonded silica-based (ODS) and polybutadiene coated zirconia (PBD-ZrO2) materials are hydrophobic and ion-exchange (Coulombic) interactions. Although the influence of reversed-phase and Coulombic interactions on the chromatography of organic cations is qualitatively well recognized, the quantitative relationship between hydrophobic and ion-exchange interactions remains unclear. In this work, the retention mechanisms on both of the above types of phases were probed by studying the retention of a homologous series of *p*-alkylbenzylamines as a function of the ammonium concentration in the eluent. The various columns tested were studied in terms of plots of retention factor vs. the inverse of the displacing cation concentration. The analysis of such plots as well as plots of log *k'* vs. number of methylene groups in the solutes and plots of log *k'* vs. log[NH4+] clearly shows that at least two types of sites - a pure reversed-phase site and a "hydrophobically-assisted ion-exchange site" similar to the type of site described by Neue [J. Chromatogr. A 925 (2001) 49] are needed to explain the observations. In addition, we have found a quantitative measure of the relative amount of reversed-phase and ion-exchange interaction on a given solute has on a given stationary phase which allows unambiguous classification of

columns. It is now clear that ion-exchange contributions to retention on PBD-ZrO2, sometimes exceeding 90%, are even more important than previously thought and relative to hydrophobic interaction much more significant on PBD-ZrO2 than on ODS type-B silicas.

82a. J. Dai, X. Yang, P.W. Carr, "Comparison of the Chromatography of Octadecyl Silane Bonded Silica and Polybutadiene-Coated Zirconia Phases Based on a Diverse Set of Cationic Drugs," *Journal of Chromatography A*, **1005(1-2)**, 63-82 (2003).

Abstract: In this study, we compare the separation of basic drugs on several octadecyl silane bonded silica (ODS) phases and a polybutadiene-coated zirconia (PBD–ZrO2) phase. The retention characteristics were investigated in detail using a variety of cationic drugs as probe solutes. The ODS phases were selected to cover a relatively wide range in silanol activity and were studied with ammonium phosphate eluents at pH 3.0 and 6.0. Compared to any of the ODS phases, the PBD–ZrO2 phase showed very significant differences in selectivities towards these drugs. Due to the presence of both reversed-phase and ion-exchange interactions between the stationary phase and the basic analyte on ODS and PBD–ZrO2, mixed-mode retention takes place to some extent on both types of phases. However, very large differences in the relative contributions from ion-exchange and reversed-phase interactions on the two types of phases led to quite different selectivities. When phosphate is present in the eluent and adsorbs on the surface, the PBD–ZrO2 phase takes on a high negative charge over a wide pH range due to phosphate adsorption on its surface. On ODS phases, ion-exchange interactions result from the interactions between protonated basic compounds and ionized residual silanol groups. Since the pH of the eluent influences the charge state of the silanol groups, the ion-exchange interactions vary in strength depending on pH. At pH 6.0, the ion-exchange interactions are strong. However, at pH 3.0 the ion-exchange interactions on ODS are significantly smaller because the silanol groups are less dissociated at the lower pH. Thus, not only are the selectivities of the ODS and PBD–ZrO2 phases different but quite different trends in retention are observed on these two types of phases as the pH of the eluent is varied. More importantly, by using the large set of "real" basic analytes we show the extreme complexity of the chromatographic processes on the reversed stationary phases. Both the test condition and solute property influence the column performance. Therefore, use of only one or two probe solutes is not sufficient for column ranking.

Bioseparation and Proteins

6. J.A. Blackwell and P.W. Carr, "Fluoride-Modified Zirconium Oxide as a Biocompatible Stationary Phase for High-Performance Liquid Chromatography," *J. Chromatogr.* **549**, 59-75 (1991).

Abstract: Previously we found that porous microparticulate zirconium oxide strongly adsorbs fluoride from aqueous solutions. The adsorption is due in large part to a Lewis acid-base interaction between coordination sites on the zirconium oxide surface and fluoride. The resulting complex is very stable relative to many other species and therefore fluoride is hard to displace. The chromatographic properties of small molecules and proteins on zirconium oxide particles when fluoride is present in the mobile phase is investigated in this work. In the presence of fluoride, zirconium oxide is a very biocompatible adsorbent, the selectivities of which are analogous to those of calcium hydroxyapatite. The effect of fluoride is very reproducible even after the adsorbed fluoride is stripped by strong base and regenerated in fluoride buffer.

7. D.W. Karl, J.C. Magnusson, P.W. Carr, and M.C. Flickinger, "Preliminary Assessment of Removal of Pyrogenic Lipopolysaccharides with Colloidal Zirconia Adsorbents," *Enzyme Microb. Technol.* **13**, 708-15 (1991).

Abstract: Preliminary evaluation of bare or polymer-coated colloidal monoclinic zirconia of nominal particle size 100 nm indicated that it is an effective adsorbent for pyrogenic lipopolysaccharides (LPS) as measured by chemical and Limulus amebocyte lysate (LAL) assays. Zirconia at 50 $\mu g\ ml^{-1}$ adsorbed 99.95% of added E. coli O128 LPS. Residual LPS levels below 0.1 $ng\ ml^{-1}$ were easily attained. Colloidal zirconia was able to remove LPS from solution in the presence of bovine serum albumin BSA). Some LPS contaminating BSA lacked affinity for zirconia. Preadsorption of phosphate onto bare zirconia blocked LPS adsorption. However, phosphated-oligomeric glycidyl (epoxy) pentaerythritol-coated colloidal zirconia could be derivatized with imidazole-containing ligands to produce an LPS-binding surface. Preliminary results of adsorption of LPS by the coated particles indicated a reduced level of LPS binding compared to bare zirconia, probably because the particles aggregated during the derivatization process, reducing the effective surface available for LPS adsorption.

10. W.A. Schafer, P.W. Carr, "Chromatographic Characterization of a Phosphate Modified Zirconia Support for Bio-chromatographic Applications," *J. Chromatogr*. **587**, 149-60 (1991).

    <u>Abstract</u>: A phosphate-modified zirconia was investigated for its potential use as a high-performance inorganic cation-exchange support for the separation of proteins. This phosphate modification effectively blocks the sites responsible for the strong interactions of certain Lewis bases with the zirconia surface. It provides a more “bio-compatible” stationary phase, resulting in high recoveries for proteins and enzymes and retention of their enzymatic activity. The stability, loading capacity, selectivity, efficiency and separation mechanism on the phosphate-modified zirconia are reported. These studies have shown that phosphate-modified zirconia is a useful high-performance ion-exchange support for the separation of cationic proteins and for blocking the sites responsible for the high affinity of zirconia towards certain anions. This makes the phosphate modification interesting in its own right and as an intermediate stage for the development of other zirconia-based chromatographic supports.

14. J.A. Blackwell and P.W. Carr, "Ligand Exchange Chromatography of Free Amino Acids and Proteins on Porous Microparticulate Zirconium Oxide," *J. Liq. Chromatogr*. **15**(9), 1487-1506 (1992).

    <u>Abstract</u>: The Lewis acid sites present on the underlying zirconium oxide particles are responsible for the unusual elution sequence for amino acids on copper (II) loaded, phosphated zirconium oxide supports reported in an earlier study. To more thoroughly examine the effect of these strong Lewis acid sites, we have studied ligand exchange chromatography on copper (II) loaded zirconium oxide particles. It is shown here that carboxylate functional groups on amino acid solutes strongly interact with surface Lewis acid sites. Addition of competing hard Lewis bases to the eluent attenuates these specific interactions. The result is a chromatographic system with high selectivity which is also suitable for ligand exchange chromatography of proteins.

15. J.A. Blackwell and P.W. Carr, "Ion- and Ligand-Exchange Chromatography of Proteins Using Porous Zirconium Oxide Supports in Organic and Inorganic Lewis Base Eluents," *J. Chromatogr*. **596**, 27-41 (1992).

    <u>Abstract</u>: The applicability of an eluotropic scale pertaining to the desorption of low molecular weight Lewis base solutes from zirconium oxide is examined for its ability to rationalize the retention of proteins on this substrate. The strongest Lewis base eluents (phosphate and fluoride) are able to bring about elution of nearly all proteins provided that their initial mobile phase concentration almost saturates the eluent’s adsorption isotherm. In contrast, weaker Lewis bases such as borate, sulfate and bromide are able to elute only those proteins which are retained primarily by ionic interactions. In weak eluents, proteins that contain a large number of accessible Lewis base sites are not eluted from the support. The effect of ionic strength and a variety of Lewis base eluents were also examined.

16. P.W. Carr, J.A. Blackwell, T.P. Weber, W.A. Schafer, and M.P. Rigney, "Zirconium Oxide Based Supports for Biochromatographic Applications," in *Chromatography in Biotechnology*, C. Horvath and L.S. Ettre, (Eds.), ACS Symposium Series No. 529, 146-64 (1993).

    <u>Abstract</u>: Porous microparticulate zirconium oxide is an excellent alternative to silica-based and functionalized organic resin-based supports for the separation of biomolecules. The complex surface chemistry of this chemically and physically stable support can be exploited in a number of ways to yield biocompatible surfaces with unique selectivities. Treatment of the particles with phosphoric acid converts the amphoteric metal oxide surface to an efficient cation exchanger. When using the bare particles, the Lewis acid-base interactions between the surface zirconium (IV) ions and Lewis base solutes can be controlled by the use of competing eluent Lewis bases to give a novel ligand exchange support.

21. M.H. Glavanovich and P.W. Carr, "Easily Regenerable Affinity Chromatographic Zirconia-Based Support with Concanavalin A as a Model Ligand," *Anal. Chem*. **66**, 2584-9 (1994).

    <u>Abstract</u>: The goal of this work was to develop a generic approach for producing affinity chromatographic columns which can be regenerated. Concanavalin (Con A0 was immobilized adsorptively by an in situ method onto a zirconium dioxide (zirconia) chromatographic support and used to resolve chromophorically labeled monosaccharides. The Con A was then removed from the zirconia by flushing with base. The same column was regenerated by applying a fresh aliquot of Con A. This cycle was repeated several times to demonstrate consistency in the loading capacity and the stability of the underlying zirconia support. Finally we used glutaraldehyde to cross-link the Con A to increase the long-term stability of the column.

Hydrolyzing the protein with acid allowed it to be removed under alkaline conditions and the column regenerated simply by adding more Con A followed by glutaraldehyde cross-linking.

23. J Nawrocki, C.J. Dunlap, P.W. Carr, and J.A. Blackwell, Review, "New Materials for Biotechnology: Chromatographic Stationary Phases Based on Zirconia," *Biotechnol. Prog.* **10**, 561-73 (1994).

    Abstract: This review explores the usefulness of zirconia-based materials in separations in biotechnology. The physical and chemical properties of zirconia are discussed briefly to familiarize the reader with the advantages of zirconia. The use of native zirconia is then examined, with a study of the Lewis acid/base chemistry that defines chromatography with zirconia. Modification of the zirconia surface with small molecules is then discussed. Finally, polymer-coated zirconia materials are examined. Examples of separations using these various-materials are shown. The advantages and disadvantages of each of these materials are presented.

26. C. McNeff and P.W. Carr, "High-Performance Anion-Exchange Chromatography of Oligonucleotides and Oligodeoxynucleotides on Quaternized Polyethyleneimine-Coated Zirconia," *Anal. Chem.* **67**, 2350-3 (1995).

    Abstract: The use of porous, quaternized polyethyleneimine-coated zirconia particles for the separation of nucleosides, nucleotides, oligonucleotides, and oligodeoxynucleotides is described. Separations of these biomolecules on quaternized PEI-zirconia are presented. Quaternized PEI-zirconia and a quaternary amine functionalized silica are compared chromatographically. Quaternized PEI-zirconia was able to separate oligonucleotides and oligodeoxynucleotides differing in length by a single nucleotide unit. It could also separate RNAs of the same length but differing by only one deoxynucleotide. In contrast, the silica-based phase was unstable at elevated temperature (75° C), and all retention toward anions was lost quite rapidly.

27. L. Sun and P.W. Carr, "Mixed-Mode Retention of Peptides on Phosphate-Modified Polybutadiene-Coated Zirconia," *Anal. Chem.* **67**, 2517-23 (1995).

    Abstract: Zirconia HPLC packing materials were found to be potentially advantageous for large scale protein separations due to their excellent pH stability and mechanical stability. However, Lewis acid sites on zirconia's surface cause irreversible adsorption of proteins due to their interactions with hard Lewis bases such as the carboxyl groups in proteins. Although the Lewis acid sites can be effectively blocked by adsorbing phosphate ions onto zirconia's surface, proteins and peptides cannot be eluted using a typical reversed-phase mobile phase. In this work, we found that the separation of peptides on a phosphate-modified polybutadiene-coated zirconia (PBD-ZrO_2) can be brought about by using a mobile phase containing both an organic modifier and a high concentration of sodium perchlorate. The salt is needed to cancel the coulombic interactions between negatively charge stationary phase and the positively charged proteins. To understand the retention mechanism of proteins and peptides on phosphate-modified PBD-ZrO_2, this work was aimed at the study of the surface characteristics of the phosphate-modified ZrO_2. We found that the phosphate-modified ZrO_2 phase has both reversed-phase and cation-exchange characteristics under the acidic mobile-phase conditions used for proteins and peptide separations. The PBD coating provides hydrophobic moieties, and the phosphate ions adsorbed on zirconia's surface provide cation-exchange sites. Reversed-phase separation of a peptide standard mixture and cation-exchange separation of a cationic peptide standard mixture on the same phosphate-modified PBD-ZrO_2 column shows excellent column resolution in both modes. Although mixed-mode stationary phases provide unique selectivity, the secondary equilibrium on phosphate-modified PBD-ZrO_2 can cause peak broadening. Applications of the phosphate-modified PBD-ZrO_2 to peptide separations are demonstrated here.

30. L. Sun and P.W. Carr, "Chromatography of Proteins Using Polybutadiene-Coated Zirconia," *Anal. Chem.* **67,** 3717-21 (1995).

    Abstract: Polybutadiene-coated zirconia (PBD-ZrO_2), when used as a stationary phase in conjunction with a mobile phase containing phosphates, constitutes a reversed-phase/cation-exchange mixed-mode chromatographic system. The separation of proteins on this phase can be achieved only through the use of mobile phases containing the correct combination of phosphoric acid, displacing salt and organic cosolvent. We found that *excessive* coulombic interactions between proteins and the stationary phase impair the system performance for the separation of proteins. The effects of mobile phase conditions on the separation on proteins using phosphate-adsorbed PBD-ZrO_2 are studied in this work. Factors such as the presence of a multivalent cation, mobile phase pH, phosphate concentration, and slat concentration can be manipulated to reduce the new negative charge on the surface and thereby improve the performance of the system toward the separations of proteins.

33. C.J. Dunlap and P.W. Carr, “The Effect of Mobile Phase on Protein Retention and Recovery Using Carboxymethyl Dextran-Coated Zirconia Stationary Phases," *J. Liq. Chrom. & Relat. Technol.* **19,** 2059-76 (1996).

    Abstract: We have examined the effect of mobile phase conditions on the elution and recovery of proteins from a carboxymethyl dextran coated zirconia stationary phase. Mobile phases containing the Lewis bases phosphate and fluoride were examined as a function of concentration. We found that proteins only eluted from the stationary phase when some minimum concentration of Lewis base was present in the mobile phase. The effect of ionic strength was also examined. We found that a fairly high ionic strength is needed to elute the proteins with good recovery. The concentrations of the Lewis bases and the ionic strength needed to elute the proteins depended on the identity of the protein and the Lewis base. The pH of the mobile phase also plays a role; when the pH of the system is below the isoelectric point of a protein, it is difficult to elute that protein.

36. C.J. Dunlap and P.W. Carr, “Synthesis and Chromatographic Characterization of Dextran-Coated Zirconia High Performance Liquid Chromatographic Stationary Phases," *J. Chromatogr. A* **746,** 199-210 (1996).

    Abstract: Porous zirconia particles made by the oil emulsion (OE) method and the polymerization-induced colloid aggregation (PICA) method have been coated with a small, carboxymethylated (~5%) dextran polymer and crosslinked in place. The parameters of the coating process (dextran concentration, adsorption time and crosslinker concentration) have all been examined and an optimum value for each determined. The coated and uncoated materials were characterized by nitrogen sorptometry and size-exclusion chromatography (SEC) using solutes (polystyrenes and dextrans) of well-defined molecular masses. Nitrogen sorptometry results show that the PICA material has a much lower pore volume and smaller pore diameter than do the OE materials. Despite this, the elution volumes of the SEC probes change very little upon polymer coating the PICA material while the OE material shows a very large change upon coating.

47. A.M. Clausen and P.W. Carr, “Chromatographic Characterization of Phosphonate Analog EDTA-Modified Zirconia Support for Biochromatographic Applications,” *Anal. Chem.* **70,** 378-85 (1998).

    Abstract: Zirconium dioxide (zirconia) has a great affinity for inorganic and organic phosphate. Previous work from is laboratory demonstrated the utility of phosphate-modified microparticulate zirconia as a support for protein separations. We have extended this investigation to include the study of ethylenediamine-N,N’-tetramethylphosphonic acid (EDTPA), a phosphonate analog of EDTA, as a surface modifier for zirconia. Our work explores the use of EDTPA-modified zirconia (PEZ) for its potential use as a high-performance inorganic cation-exchange support for the separation of proteins. The phosphate groups in EDTPA very effectively block the sites responsible for strong interactions of hard Lewis bases with zirconia’s surface. Modification of zirconia with EDTPA provides a “biocompatible” stationary phase, resulting in high mass recoveries of proteins. We compare PEZ with inorganic phosphate-modified zirconia to show increased efficiency, as well as unique selectivities for chromatography of proteins on the chelator-modified surface. Finally, the selectivity, efficiency, and separation mechanism are reported. The studies show the PEZ is a useful high-performance ion-exchange support for the separation of cationic proteins and for modulating the sites responsible for the high affinity of zirconia toward certain classes of anions.

52. A.M. Clausen, A. Subramanian, and P.W. Carr, “Purification of Monoclonal Antibodies from Cell Culture Supernatants Using a Modified Zirconia Based Cation-Exchange Support,” *J. Chromatogr. A* **831,** 63-72 (1999).

    Abstract: A method suitable for the isolation of monoclonal antibodies (Mabs) is described. The protocol utilizes a zirconia based column modified with ethylenediamine-*N,N'*-tetra(methylenephosphonic) acid to create a novel cation-exchange chromatographic support. Initial experiments using a linear salt gradient demonstrate the ability of this support to efficiently separate Mab from transferrin and bovine serum albumin in a model matrix. Results of the purification of Mab from an actual cell culture supernatant over a range in protein concentrations are also shown. Analyses by enzyme-linked immunosorbent assay and gel electrophoresis demonstrate that Mabs can be recovered from a cell culture supernatant at high yield (92-98%) and high purity (>95%) in a single chromatographic step.

56. C.V. McNeff, Q. Zhao, E. Almolof, M. Flickinger, P.W. Carr, “Synthesis and Use of Quaternized PEI-Zirconia for the Efficient Removal of Endotoxins from Proteins,” *Anal. Biochem.*, 274, 181-187 (1999).

Abstract: The synthesis and use of a zirconia-based, alkali-stable strong anion-exchange stationary phase are described for the removal of pyrogenic lipopolysaccharides (LPS) from insulin. The strong anion-exchange material is produced by deposition of polyethyleneimine (PEI) onto porous zirconia particles, followed by cross-linking with a novel reagent, 1,2-bis-(2-iodoethoxy) ethane, and quaternization with iodomethane. Physical characterization of the chromatographic support shows that it has an ion-exchange capacity of 0.6 mmol/g, and 82% of the amine sites on the surface are in quaternized form. Isocratic elution of small benzoic acid derivatives shows good column efficiency. The two primary virtues of this material are its chemical stability under alkali conditions (up to pH 13) and its lower hydrophobicity compared to previously described alkali-stable PEI-coated zirconia supports cross-linked with 1,10-diiododecane. Using this new zirconia-based phase, a purification protocol is developed for the efficient removal of Escherichia coli 0111:B4 LPS from bovine insulin samples. An endotoxin clearance rate of up to 1.3 × 108 was attained. Endotoxin levels were reduced to less than 5 endotoxin units/ml even at initial contamination levels as high as 5.0×10^6 endotoxin units/ml. Furthermore, endotoxin adsorbed to the porous zirconia column may be easily remove (depyrogenated) using alkali for repeated purification cycles.

56c. A. Mullick and M.C. Flickinger, "Expanded Bed Adsorption of Human Serum Albumin from Very Dense Saccharomyces cerevesiae Suspensions on Fluoride-Modified Zirconia," *Biotechnology and Bioengineering*, Vol. 65, 282-290 (1999).

Abstract: The adsorption of proteins from high cell density yeast suspensions on mixed-mode fluoride-modified zirconia (FmZr) particles (38 to 75 um, surface area of 29 m^2/g and density of 2.8 g/cm^3) was investigated using human serum albumin (HSA) added to Saccharomyces cerevesiae as the model expression host. Because of the high density of the porous zirconia particles, HSA (4 mg/mL) can be adsorbed from a 100 g dry cell weight (DCW)/L yeast suspension in a threefold-expanded bed of FmZr. The expanded bed adsorption of any protein from a suspension containing >50 g DCW/L cells has not been previously reported. The FmZr bed expansion characteristics were well represented by the Richardson-Zaki correlation with a particle terminal velocity of 3.1 mm/s and a bed expansion index of 5.4. Expanded bed hydro-dynamics were investigated as a function of bed expansion using residence time distribution studies with sodium nitrite as the tracer. The adsorption of HSA on FmZr exhibited features of multicomponent adsorption due to the presence of dimers. The protein binding capacity at 5% breakthrough decreased from 22 mg HSA/mL settled bed void volume for 20 g DCW/L yeast to 15 mg HSA/mL settled bed void volume for 40 g DCW/L yeast and remained unchanged for the higher yeast concentrations (60 to 100 g DCW/L). However, the batch (or equilibrium) binding capacity decreased monotonically as a function of yeast concentration (20 to 100 g DCW/L) and the binding capacity at 100 g DCW/L yeast was fivefold lower compared with that at 20 g DCW/L yeast. The lower batch binding capacity at high cell concentrations resulted from the adsorption of cells at the surface of the particles restricting access of HSA to the intraparticle surface area. Batch (or equilibrium) and column HSA adsorption results indicated that the adsorption of HSA on FmZr occurred at a time scale that may be much faster than that of yeast cells. The zirconia particles were cleaned of adsorbed HSA and yeast with a total of 1500 to 2000 column volumes (over many cycles) of 0.25 M NaOH, without any significant effect on the chromatographic performance.

57. A. Subramanian, P.W. Carr, C.V. McNeff, "Use of Spray-Dried Zirconia Microspheres in the Separation of Immunoglobulins from Cell Culture Supernatant," *Journal of Chromatography A*, **890(1)**, 15-23 (2000).

Abstract: A method suitable for the isolation of monoclonal antibodies (Mabs) on novel zirconia microspheres (20-30 micron) is described. Zirconia microspheres were generated by spray drying colloidal zirconia. Spray-dried zirconia microspheres were further classified and characterized by X-ray diffraction, BET porosimetry and scanning electron microscopy. Spray-dried zirconia microspheres were modified with ethylenediamine-N,N'-tetra(methylenephosphonic) acid (EDTPA) to create a cation-exchange chromatographic support. The chromatographic behavior of a semi-preparative column packed with EDTPA-modified zirconia microspheres was evaluated and implications for scale-up are provided. EDTPA-modified zirconia microspheres were further used to purify Mabs from cell culture supernatant. Analysis by enzyme linked immunosorbent assay and gel electrophoresis demonstrate that Mabs can be: recovered from a cell culture supernatant at high yield (92-98%) and high purity (>95%) in a single chromatographic step.

73. Anuradha Subramanian and Sabyaschi Sarkar "Use of a Modified Zirconia Support in the Separation of Immunoproteins," *Journal of Chromatography A*, **944**, 179-187 (2002).

Abstract: Zirconia beads (25–38 µm in diameter) were modified with *N*,*N*,*N*',*N*'-ethylenediaminetetramethylenephosphonic acid to generate a zirconia based pseudoaffinity support, further referred to as r_PEZ. The influence of pH, salt concentration and temperature on the binding of human immunoglobulin G (hIgG) to r_PEZ was studied. Temperature had no significant impact on the maximum binding capacity (Q_{max}), and the equilibrium-binding constant (K_d), whereas pH and the salt concentration had a noticeable impact on both Q_{max} and K_d. The Q_{max} value of 55 mg hIgG/ml of bead was obtained at a pH of 5.5 and found to decrease with an increase of pH. The modified zirconia support allowed the separation of immunoglobulins (IgG, IgA and IgM) from untreated human serum. Elution was possible under mild conditions with a step salt gradient. Overall protein recoveries in the range of 109–125% were obtained with human serum. Human IgG, human IgA, and human IgM yields of 29.50±6.3, 3.22±0.7, and 6.84±0.7%, respectively, were obtained at a linear velocity of 4.32 cm/min. Purity of products, obtained from a single chromatographic step was estimated to be greater than 89.0±2.6%. The utility of r_PEZ in the selective removal of immunoglobulins, as in immunoadsorption was discussed.

79. S. Sarkar, P.W. Carr, C.V. McNeff, A. Subramanian, "Characterization and Optimization of a Chromatographic Process Based on Ethylenediamine-N,N ,N' ,N' -tetra(methylphosphonic) acid-modified Zirconia Particles," *Journal of Chromatography B*, **790**, 143-152 (2003).

    Abstract: The primary objective of work was to characterize, optimize and model a chromatographic process based on ethylenediamine-N,N,N',N'-tetra(methylphosphonic) acid (EDTPA)-modified zirconia particles. Zirconia particles were produced by spray-drying colloidal zirconia. Zirconia spheres produced were further classified, calcined and modified with EDTPA to yield a solid-phase support for use in bio-chromatography (r-PEZ). Specifically, the ability of r-PEZ to selectively bind and enrich IgG, IgA, and IgM from biological fluids was evaluated and demonstrated. To better understand the force of interaction between the IgG and the r-PEZ, the equilibrium disassociation constant (Kd) was determined by static binding isotherms, as a function of temperature and by frontal analysis at different linear velocities. The maximum static binding capacity (Qmax) was found to be in the range 55-65 mg IgG per ml of beads, and unaffected by temperature. The maximum dynamic binding capacity (Qx) was found to be in the range 20-12 mg IgG per ml of beads. The adsorption rate constant (ka) was determined by a split-peak approach to be between 982 and 3242 l mol-1 s-1 depending on the linear velocity. The standard enthalpy and entropy values were estimated for this interaction of IgG with this novel support.

79b. A. Subramanian and S. Sarkar, "Interaction of Immunoglobulin G with N,N,N′,N′-ethylenediaminetetramethylenephosphonic Acid-modified Zirconia," *Journal of Chromatography A*, **989(1)**, 131-138 (2003).

    Abstract: Zirconia beads (25–38 μm in diameter) were modified with N,N,N′,N′-ethylenediaminetetramethylenephosphonic acid to generate a pseudo-biospecific support, r_PEZ. To better understand the force of interaction between the IgG and the r_PEZ, the equilibrium dissociation constant (Kd) was determined by static binding isotherms, as a function of temperature and by frontal analysis at different linear velocities. Temperature had no significant impact on the maximum static binding capacity (Qmax) and the equilibrium-binding constant (Kd), whereas pH and the salt concentration had a noticeable impact on both Qmax and Kd values. Qmax was found to be in the range of 55–65 mg IgG per ml of beads and unaffected by temperature. The maximum dynamic binding capacity (Qx) was found to be in the range of 20–12 mg IgG per ml of beads. The adsorption rate constant (ka) was determined by a split-peak approach to be between 982 and 3242 l mol−1 s−1 depending on the linear velocity. Adsorption rate of IgG on r_PEZ was studied as a function of both feed concentration and linear velocity. The standard enthalpy and entropy values were estimated for the interaction of IgG with this novel support. The binding constants were also determined by modeling the batch protein-uptake data.

91. D.R. Stoll, P.W. Carr, "Fast, Comprehensive Two-Dimensional HPLC Separation of Tryptic Peptides Based on High-Temperature HPLC," *J. Am. Chem. Soc.*, **127**, 5034 (2005).

    Abstract: The dramatic improvement in the speed of reversed-phase gradient elution through the use of high-temperature HPLC and modest instrument modifications has led to a significant improvement in the performance of 2D-LC. Further optimization of this system will lead to improvements in the peak capacity productivity and make 2D high-temperature HPLC a practical approach for the analysis of complex biological mixtures.

92. A. Subramanian, "Chromatographic Purification of MAbs with Non-affinity Supports; Purifying MAbs with Novel Supports Works as well as Protein A," *BioPharm International*, June 2005, 64-70.

Abstract: An anionic column with modified chitosan bead matrix performs well in purifying cell culture. A pair of cationic-exchange columns shows promise in purifying S25 antibody.

95b. S. Sarkar and A. Subramanian, “Modeling of Immunoglobulin Uptake by N,N,N′,N′-ethylenediaminetetramethylenephosphonic Acid-modified Zirconia Particles under Static and Dynamic Conditions,” *Journal of Chromatography B*, **821(1)**, 81-87 (2005).

Abstract: A matrix developed from N,N,N′,N′-ethylenediaminetetramethylenephosphonic acid-modified zirconia beads (further referred to as r_PEZ); 25–38 µm in diameter and with a pore size of 22 ± 3 nm, was utilized for the separation of immunoglobulins (Igs). r_PEZ has been shown to bind to various Igs originating from a wide variety of species. To understand the mechanisms controlling the uptake of Igs by r_PEZ, static protein uptake experiments were carried out. The protein uptake profiles were further modeled with a kinetic rate constant model. Individual studies were undertaken for human immunoglobulin A, G and M (HIgA, HIgG and HIgM). The kinetic rate constant model indicated that HIgG binding to r_PEZ was more favorable than its disassociation. The equilibrium rate constants were found to decrease with increasing concentration. The effect of continuous loading in a packed bed system utilizing r_PEZ matrix was evaluated by carrying out frontal studies, using different feed concentrations and linear velocities. The breakthrough profiles obtained for the uptake of HIgG were modeled with the pore diffusion model. The model was found to best describe the breakthrough profiles obtained at a feed concentration of 2.0 mg of HIgG per milliliter. The NTU for the packed bed was found to be equal to 2.

95e. K. Soukupová, E. Krafková, Jana Suchánková and E. Tesařová, “Comparison of Zirconia- and Silica-based Reversed Stationary Phases for Separation of Enkephalins,” *Journal of Chromatography A*, **1087(1-2)**, 104-111 (2005).

Abstract: In this study, the separation of biologically active peptides on two zirconia-based phases, polybutadiene (PBD)-ZrO2 and polystyrene (PS)-ZrO2, and a silica-based phase C18 was compared. Basic differences in interactions on both types of phases led to quite different selectivity. The retention characteristics were investigated in detail using a variety of organic modifiers, buffers, and temperatures. These parameters affected retention, separation efficiency, resolution and symmetry of peaks. Separation systems consisting of Discovery PBD-Zr column and mobile phase composed of a mixture of acetonitrile and phosphate buffer, pH 2.0 (45:55, v/v) at 70 °C and Discovery PS-Zr with acetonitrile and phosphate buffer, pH 3.5 in the same (v/v) ratio at 40 °C were suitable for a good resolution of enkephalin related peptides. Mobile phase composed of acetonitrile and phosphate buffer, pH 5.0 (22:78, v/v) was appropriate for separation of enkephalins on Supelcosil C18 stationary phase.

95f. S. Sarkar, P.W. Carr and A. Subramanian, “Identification of the Mass Transfer Mechanisms Involved in the Transport of Human Immunoglobulin-G in N,N,N′,N′-ethylenediaminetetramethylenephosphonic Acid-modified Zirconia,” *Journal of Chromatography B*, **821(2)**, 124-131 (2005).

Abstract: Zirconia particles modified with N,N,N′,N′-ethylenediaminetetramethylenephosphonic acid (EDTPA), further referred to as r_PEZ, were studied as a support material for use in chromatography. Our previous studies have demonstrated the utility of r_PEZ in the separation of immunoglobulins from biological fluids. In the present study we sought to understand the underlying factors and identify the rate-limiting mechanisms that govern the transport of biomolecules in r_PEZ. Pulse injection techniques were used to elucidate the individual mass transfer parameters. Elution profiles obtained under retained and unretained conditions were approximated by the Gaussian equation and the corresponding HETP contributions were estimated. The dependence of the HETP values on incremental salt concentration in the mobile phase was determined. Resulting data in conjunction with the equations outlined in literature were used to estimate the theoretical number of transfer units for the chromatographic separation process. Our results indicate that surface diffusion probably plays a minor role; however pore diffusion was established to be the rate limiting mechanism for immunoglobulin G adsorption to r_PEZ. The HETP based methodology may be used to estimate the rate limiting mechanisms of mass transfer for any given chromatographic system under appropriate conditions.

97. H.K. Kweon and K. Håkansson, "Selective Zirconium Dioxide-Based Enrichment of Phosphorylated Peptides for Mass Spectrometric Analysis," *Analytical Chemistry*, **78(6)**, 1743 -1749 (2006).

Abstract: Due to the dynamic nature and low stoichiometry of protein phosphorylation, enrichment of phosphorylated peptides from proteolytic mixtures is often necessary prior to their characterization by mass spectrometry. Several phosphopeptide isolation strategies have been presented in the literature, including immobilized metal ion affinity chromatography. However, that technique suffers from poor selectivity and reproducibility. Recently, titanium dioxide-based columns have been successfully employed for phosphopeptide enrichment by several research groups. Here, we present, to our knowledge, the first demonstration of the utility of zirconium dioxide microtips for phosphopeptide isolation prior to mass spectrometric analysis. These microtips display similar overall performance as TiO2 microtips. However, more selective isolation of singly phosphorylated peptides was observed with ZrO2 compared to TiO2 whereas TiO2 preferentially enriched multiply phosphorylated peptides. Thus, these two chromatographic materials possess complementary properties. For - and -casein, Glu-C digestion provided no evident advantage compared to trypsin digestion when combined with TiO2 or ZrO2 phosphopeptide enrichment.

98c. A. Subramanian, B. Martinez, J. Holm, P.W. Carr, C.V. McNeff, "A Comparative Study of Monoclonal Antibodies (Mabs) Purified from Cell Culture Supernatant on EDTPA-Modified Zirconia Beads and Protein A-Hyper D Support," *Journal of Liquid Chromatography & Related Technologies*, **29(4)**, 471-484 (2006).

Abstract: Colloidal zirconia was spray dried to yield zirconia particles, which were further modified with N, N, N', N'- Ethylenediamine tetra methylenephosphonic acid (EDTPA) to yield a support for use in bioseparations. EDTPA modified zirconia particles will be further referred to as, r_PEZ. Cell culture supernatants rich in monoclonal antibody (Mab) subtypes IgG1, IgG2a, IgG2b, and IgG3 were chromatographed on a r_PEZ column, and on a protein A-hyper D column that was purchased commercially. All Mab subtypes bound to r_PEZ and process yields in the range of 88 to 99% were obtained. The purity of the Mab products were ascertained by gel electrophoretic analysis and were estimated to be greater than 95%. The purified Mab products obtained from r_PEZ and protein A columns were compared to the reference Mab standard in biological and enzymatic assays. The value of the dissociation constant (Kd) was found to be comparable and was in the range to that obtained with reference Mab standard (0.231±0.03 M). In addition, Mabs purified with r_PEZ had the same deglycosylation profile as the reference Mab standard. Thus, it appears that the r_PEZ purified Mab is similar in activity to Mab purified with a protein A support and in addition, the zirconia surface does not adversely impact the activity of the purified Mab.

103. S. Pursche, O.G. Ottmann, G. Ehninger and E. Schleyer, "High-performance Liquid Chromatography Method with Ultraviolet Detection for the Quantification of the BCR-ABL Inhibitor Nilotinib (AMN107) in Plasma, Urine, Culture Medium and Cell Preparations," *Journal of Chromatography B*, **852(1-2)**, 208-216 (2007).

Abstract: An isocratic and sensitive HPLC assay was developed allowing the determination of the new anticancer drug nilotinib (AMN107) in human plasma, urine, culture medium and cell samples. After protein precipitation with perchloric acid, AMN107 underwent an online enrichment using a Zirchrom-PBD recolumn, was separated on a Macherey-Nagel C18-HD column and finally quantified by UV-detection at 258 nm. The total run time is 25 min. The assay demonstrates linearity within a concentration range of 0.005–5.0 µg/ml in plasma (r2 = 0.9998) and 0.1–10.0 µg/ml in urine (r2 = 0.9913). The intra-day precision expressed as coefficients of variation ranged depending on the spiked concentration between 1.27–9.23% in plasma and 1.77–3.29% in urine, respectively. The coefficients of variation of inter-day precision was lower than 10%. Limit of detection was 0.002 µg/ml in plasma and 0.01 µg/ml in urine. The described method is stable, simple, economic and is routinely used for in vivo and in vitro pharmacokinetic studies of AMN107.

122. M. Mazanek, E. Roitinger, O. Hudecz, J. Hutchins, B. Hegemann, G. Mitulovic, T. Taus, C. Stingl, J. Peters, K. Mechtler, "A New Acid Mix Enhances Phosphopeptide Enrichment on Titanium- and Zirconium Dioxide for Mapping of Phosphorylation Sites on Protein Complexes," *Journal of Chromatography B*, **878**, 515-524 (2010).

Abstract: The selective enrichment of phosphorylated peptides prior to reversed-phase separation and mass spectrometric detection significantly improves the analytical results in terms of higher number of detected phosphorylation sites and spectra of higher quality. Metal oxide chromatography (MOC) has been recently described for selective phosphopeptide enrichment (Pinkse et al., 2004 [1]; Larsen et al., 2005 [2]; Kweon and Hakansson, 2006 [3]; Cantin et al., 2007 [4]; Collins et al., 2007 [5]). In the present work we have tested the effect of a modified loading solvent containing a novel acid mix and optimized wash conditions on the efficiency of TiO2based phosphopeptide enrichment in order to improve our previously published method (Mazanek et al., 2007 [6]). Applied to a test mixture of synthetic and BSAderived peptides, the new method showed improved selectivity for phosphopeptides, whilst retaining a high recovery rate. Application of the new enrichment method to digested purified protein complexes resulted in the identification of a significantly higher number of phosphopeptides as compared to the previous method. Additionally, we have compared the performance of TiO2 and ZrO2 columns for the isolation and identification of phosphopeptides from purified protein complexes and found that for our test set, both media performed comparably well. In summary, our improved method is highly effective for the enrichment of phosphopeptides from purified protein complexes prior to mass spectrometry, and is suitable for large-scale phosphoproteomic projects that aim to elucidate phosphorylation dependent cellular processes.

124. A. Gonzalvez, B. Preinerstorfer, “Selective Enrichment of Phosphatidylcholines from Food and Biological Matrices Using Metal Oxides as Solid-phase Extraction Materials Prior to Analysis by HPLC-ESI-MS/MS,” *Anals of Bioanalytical Chemistry*, **396**, 2965-2975 (2010).

Abstract: A zirconia (ZrO_2)-modified solid-phase extraction sorbent has been evaluated for selective extraction of phosphatidylcholines from biological samples, followed by analysis of the isolated solutes by reversed-phase liquid chromatography–electrospray ionization–tandem mass spectrometry. The clean-up process was optimized using seven standard phosphatidylcholines including two lyso derivatives. Different acidic conditions were tested for the bonding and washing steps; for elution, various aqueous or methanolic bases were studied. Experiments were conducted hydrodynamically using extraction cartridges, and statically in batch mode; the performance of the sorbent was significantly better when used in the flow-through mode. The developed clean-up procedure was used to selectively enrich phosphatidylcholines from whole milk, human plasma, and mouse plasma, to show the wide applicability of the method. For the preceding extraction of total lipids from the matrix, different solvent mixtures (methanol–chloroform, methanol–methyl tert-butyl ether, and ethanol–ethyl acetate) were compared. Accuracy and reproducibility of the proposed sample-preparation procedure were evaluated. Matrix effects possibly affecting mass spectrometric analysis were studied before and after the of phosphatidylcholines from biological samples, followed by analysis of the isolated solutes by reversed-phase liquid chromatography–electrospray ionization–tandem mass spectrometry. The clean-up process was optimized using seven standard phosphatidylcholines including two lyso derivatives. Different acidic conditions were tested for the bonding and washing steps; for elution, various aqueous or methanolic bases were studied. Experiments were conducted hydrodynamically using extraction cartridges, and statically in batch mode; the performance of the sorbent was significantly better when used in the flow-through mode. The developed clean-up procedure was used to selectively enrich phosphatidylcholines from whole milk, human plasma, and mouse plasma, to show the wide applicability of the method. For the preceding extraction of total lipids from the matrix, different solvent mixtures (methanol–chloroform, methanol–methyl tert-butyl ether, and ethanol–ethyl acetate) were compared. Accuracy and reproducibility of the proposed sample-preparation procedure were evaluated. Matrix effects possibly affecting mass spectrometric analysis were studied before and after the solid-phase extraction. They were found to be significant for several analytes, stressing the importance of a sample clean-up procedure. Under identical experimental conditions, recovery of bound phosphatidylcholines by zirconia was superior to that by other metal oxides, for example titania (TiO_2) and stannia (SnO_2).

Chiral

54. Cecelia B. Castells and P.W. Carr, “Cellulose *tris*(3,5-dimethylphenylcarbamate) Coated Zirconia as a Chiral Stationary Phase for HPLC,” *Anal. Chem*., 71, 3013-3021 (1999).

Abstract: In this work we explore the use of microparticulate porous zirconia coated with cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC) as a support for separation of chiral compounds by HPLC. The surface of zirconia, previously sintered but not rehydroxylated, provides a stable surface for depositing the chiral polymer. Zirconia’s surface prior to coating was investigated by diffuse reflectance FT-IR. The spectra indicated the presence of residual hydroxyl groups even after treatment at 750°C for 5h. The amount of chiral polymer deposited was systematically varied, and the pore structure of the resulting particles was assessed by nitrogen sorptometry. Dynamic studies of columns packed with these stationary phases were also conducted. We found that columns packed with about 3-4% (w/w) CDMPC coated on 2.5 micron zirconia particles provide an excellent compromise between loading need to impart good chiral recognition ability to the stationary phase and column’s chromatographic efficiency. Preliminary results show resolutions higher than 1 for 9 out of 16 racemic mixtures in packed 5-cm columns. The use of shorter columns combined with reduced particle size to provide sufficient resolution has the advantage of decreasing the analysis times and reducing eluent volumes. CDMPC-coated zirconia columns exhibit high stability under normal-phase conditions at relatively high linear velocities.

57b. C.B. Castells, P.W. Carr, "Fast Enantioseparations of Basic Analytes by High-Performance Liquid Chromatography Using Cellulose Tris(3,5-dimethylphenylcarbamate)-Coated Zirconia Stationary Phases," *Journal of Chromatography A*, **904(1)**, 17-33 (2000).

Abstract: In this work, we study the influence of the mobile phase and column temperature on the enantioresolution of basic compounds on microparticulate porous zirconia coated with cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC). The chiral analytes are amino compounds, including a number of -blockers. Analytes are eluted with hexane–alcohol mobile phases. We investigated the effect of alcohol (type and concentration), basic eluent additives, and column temperature on the parameters that control resolution (column efficiency, retention and selectivity). Conditions for achieving an adequate separation in the least time have been determined for numerous racemic mixtures. For most solutes, baseline resolution of the enantiomeric pair was achieved in less than 1 min; 12 of 13 pairs were separated in less than 2 min.

64. C.B. Castells, P.W. Carr, “A Study of Thermodynamics and Influence of Temperature of Chiral High-Performance Liquid Chromatographic Separations Using Cellulose *tris*(3,5-dimethylphenylcarbamate) Coated Zirconia Stationary Phases,” *Chromatographia*, **52(9-10)**, 535-542 (2000).

Abstract: In chiral HPLC, the separation is based on the differential interaction of a pair of enantiomeric molecules with a chiral selector. Temperature will affect such interactions. Most studies indicate that a decrease in temperature increase chromatographic selectivity. This is consistent with an enthalpy-controlled separation, but a more complete characterization of the physicochemical interactions is required to understand the driving forces for chiral recognition. In this work, we studied the separation of a number of enantiomers on cellulose *tris*(3,5-dimethylphenylcarbamate) supported on porous zirconia, over the temperature range of 0 to 55°C using *n*-hexanes/2-propanol mixtures as the eluent. The differences in the enthalpy (delta(deltaH°)) and entropy (delta(deltaS°)) of transfer of the enantiomers from the mobile to the chiral stationary phase were estimated from the van’t Hoff plots. These relationships allow the study of the origin of the differences in interaction energies. The most interesting findings is that while most solutes show a negative delta(deltaH°) difference, the two most easily resolved enantiomeric pairs were separated by an entropy dominated process. Studies of the relationship between the thermodynamics of transfer of these two entropically controlled separations and the eluent composition showed a substantial change in the interaction energies of these two solutes with the chiral polymer when the alcohol was reduced by 2% (*v/v*). Finally, we show that there is virtually no correlation between delta(deltaG°) and overall retention, between delta(deltaH°) and deltaH°, and little or no enthalpy-entropy compensation. These findings indicate the extreme difficulty in predicting or even correlating chiral selectivity with overall intermolecular interactions.

66. J.H. Park, J.K. Ryu, J.K. Park, C.V. McNeff, P.W. Carr, “Separation of Enantiomers on Bovine Serum Albumin Coated Zirconia in Reversed-Phase Liquid Chromatography,” *Chromatographia*, **53**, 7/8, 405-408 (2001).

Abstract: Zirconia is known to be one of the best materials for the chromatographic support due to its excellent chemical, thermal, and mechanical stability. In this work we report preparation and use of bovine serum albumin (BSA)-immobilized zirconia as a chiral stationary phase for separation of some enantiomers in reversed-phase liquid chromatography. The BSA-zirconia showed good enantioselectivity for some of the enantiomers studied and could be used for RPLC separations in mobile phases of alkaline pH.

70. Sung Yeh Park, Jung Kon Park, Jung Hag Park, Clayton V. McNeff, Peter W. Carr, “Separation of Racemic 2,4-dinitrophenyl amino acids on carboxymethyl-β-cyclodextrin Coated Zirconia in RPLC,” *Microc. J.*, **70**, 179-185 (2001).

Abstract: We report preparation and use of carboxymethyl-β-cyclodextrin-coated zirconia as a chiral stationary phase (CSP) for separation of enantiomers of 2,4-dinitrophenyl (DNP) amino acids in reversed-phase high performance liquid chromatography. The CSP showed good enantioselectivity for some of the amino acids studied. Effects of pH and amount of methanol in the mobile phases on retention and enantioselectivity for the analytes were examined.

84b. I.W. Kim, J.K. Ryu, S.D. Ahn, J.H. Park, K.P. Lee, J.J. Ryoo, M.H. Hyun, Y. Okamoto, C. Okamoto, P.W. Carr, "Comparison of Chiral Separation on Amylose and Cellulose tris(3,5-dimethylphenylcarbamate)-coated Zirconia in HPLC," *Bulletin of the Korean Chemical Society*, **24(2)**, 239-242 (2003).

Abstract: In this work we compared chromatographic performances of chiral separation for ADMPC and CDMPC coated on 3-mm zirconia particles by measuring retention of a set of racemic compounds on them. We used narrow-bore (1-mm ID) columns that lead to many advantages such as low consumption of both mobile and stationary phases etc.

84c. J.H. Park, Y.C. Whang, Y.J. Jung, Y. Okamoto, C. Yamamoto, P.W. Carr, C.V. McNeff, "Separation of Racemic Compounds on Amylose and Cellulose Dimethylphenylcarbamate-coated Zirconia in HPLC", *Journal of Separation Science*, **26(15-16)**, 1331-1336 (2003).

Abstract: Zirconia particles are a very robust material and have received considerable attention as a stationary phase support for HPLC. Chromatographic performances of ADMPC- and CDMPC-coated zirconia as chiral stationary phases (CSPs) were compared by measuring the chiral discrimination of a set of 14 racemic compounds on the two columns. On the whole CDMPC-zirconia showed better chiral selectivity for the test compounds than ADMPC-zirconia. The two CSPs show complementary chiral recognition capability for the types of racemates studied. Chiral selectivities of ADMPC- and CDMPC-coated zirconia were also compared with those of ADMPC- and CDMPC-coated silica. The zirconia CSPs exhibited comparable and sometimes better enantioselectivity than the corresponding silica CSPs.

89a. J.H. Park, J.W. Lee, S.H. Kwon, J.S. Cha, P.W. Carr, C.V. McNeff, "Separation of Racemic 2,4-Dinitrophenyl Amino Acids on 9-O-(phenyloxycarbonyl)quinine-bonded Carbon-Clad Zirconia in Reversed-Phase Liquid Chromatography," *Journal of Chromatography A*, **1050**, 151-157 (2004).

Abstract: Zirconia is known to be one of the best materials for the chromatographic support due to its excellent chemical, thermal, and mechanical stability. In this work, we report preparation and use of 9-O-(phenyloxycarbonyl)quinine-bonded carbon-clad zirconia (QNCZ) as a chiral stationary phase (CSP) for separation of N-(2,4-dinitrophenyl) (DNP)-amino acids (AAs) enantiomers in reversed-phase liquid chromatography. Retention and enantioselectivity of the QNCZ CSP were compared with those of quinine 3-triethoxysilylpropylcarbamate-coated zirconia (QNZ) and quinine 3-triethoxysilylpropylcarbamate-bonded silica (QNS). The QNCZ CSP showed in general the better enantioselectivity for most of the amino acids studied.

96. R.A. Henry, C.V. McNeff, B. Yan, T.R. Hoye, "A Novel Chemical Route to Stable, Regenerable Zirconia-Based Chiral Stationary Phases for HPLC," *American Laboratory (News Edition)*, **37(21)**, 22-24 (2005).

Abstract: A promising new route to preparing chiral stationary phases using a zirconia substrate has been developed. Chiral selectors that can be easily removed and replaced by simple, low-temperature reactions are targeted for commercialization.

98a. I.W Kim, S.H. Kwon, C.V. McNeff, P.W. Carr, M.D. Jang, J.H. Park, "9-O-(Phenylcarbamoyl)quinine-bonded Carbon-Clad Zirconia for Chiral Separation of Racemic 2,4-Dinitrophenyl Amino Acids in RPLC", *Bulletin of the Korean Chemical Society*, **27(4)**, 589-592 (2006).

Abstract: In this work we prepared 9-O-(phenylcarbamoyl)quinine-bonded carbon-clad zirconia (QNCCZ) in the hope of obtaining better synthetic and bonding yields than those for QNOCZ. It was also expected that different functionality in the linkage (carbamoyl vs. oxycarbonyl) would show different retention and selectivity behavior. QNCCZ was used as the chiral stationary phase (CSP) for separation of enantiomers of DNP-amino acids in RPLC. Retention and enantioselectivity for QNCCZ were compared to those for QNOCZ and quinine carbamate-bonded silica (QNS).

99. I.W. Kim, H.M. Choi, H.J. Yoon and J.H. Park, "β-Cyclodextrin-hexamethylene Diisocyanate Copolymer-coated Zirconia for Separation of Racemic 2,4-dinitrophenyl Amino Acids in Reversed-phase Liquid Chromatography," *Analytica Chimica Acta*, **569(1-2)**, 151-156 (2006).

Abstract: We report use of β-cyclodextrin (CD)-hexamethylene diisocyanate (HMDI) copolymer(CDPU)-coated zirconia (CDPUZ) as a chiral stationary phase for separation of enantiomers of a set of 2,4-dinitrophenyl (DNP) amino acids in reversed-phase liquid chromatography. The effects of polymer loading on zirconia and HMDI/CD molar ratio in the preparation of CDPU on retention and selectivity in the separation of DNP-amino acids in were examined to find optimum polymer loading and HMDI/CD ratio. It was observed that 8% loading of the CDPU prepared with the HMDI/CD molar ratio of 4 gave the best separation of the amino acids investigated. Retention and chiral selectivity of CDPUZ were also compared to those for previously reported carboxymethyl-β-CD-coated zirconia (CMCDZ). CDPUZ gave better chiral separation than CMCDZ for the DNP-amino acids.

123. M. Lee, J. Gwon, J. H. Park, “Enantioseparation of Neutral Compounds on a Quinine Carbamate-Immobilized Zirconia in Reversed-Phase Capillary Electrochromatography,” *Bulletin of Korean Chemical Society,* **31**, 82-86 (2010).

> Abstract: Quinine (QN) is a weak anion-exchange type chiral selector and QN-based silica stationary phases have been widely used for enantioseparation of *acidic* chiral analytes in HPLC and recently in CEC. In this work we report enantioseparation of non-acidic chiral analytes on a quinine carbamate-immobilized zirconia (QNZ) in reversed-phase (RP) CEC. Influences of pH, composition of the buffer, acetonitrile content and the applied voltage on enantioseparation were examined. Enantiomers of the analytes investigated are well separated in acetonitrile/phosphate buffer mobile phases. Separation data on QNZ were compared to those on QN-bonded silica (QNS). Retention was longer but better enantioselectivity and resolution were obtained on QNZ than QNS.

General Information

2. M.P. Rigney, E.F. Funkenbusch, and P.W. Carr, "Physical and Chemical Characterization of Microporous Zirconia," *J. Chromatogr*. **499**, 291-304 (1990).

> Abstract: Small (< 10 μm) microporous zirconia has been prepared and evaluated as an alkaline stable high-performance liquid chromatographic support. Zirconia is an amphoteric metal oxide which exhibits both anion- and cation-exchange properties depending on the solution pH and the nature of the buffer. The affinity of zirconia for polyoxy anions, particularly the effect of phosphate on the properties of the zirconia, is examined. Most significantly, the exceptional stability of zirconia, even under extreme conditions, is demonstrated and compared to the stability of alumina.

19. L. Sun, M. Annen, C. Francisco Lorrenzano-Porras, P.W. Carr and A. McCormick, "Synthesis of Porous Zirconia Spheres for HPLC by Polymerization-Induced Aggregation (PICA)," *J. Colloid Interface Sci*. **163**, 464-73 (1994).

> Abstract: Porous, spherical zirconia particles with a narrow particle size distribution, which are useful as chromatographic packing materials for high performance liquid chromatography (HPLC), were synthesized by polymerization-induced colloid aggregation (PICA) first described by Iler and McQueston (U.S. Patent 4,010,242, 1977) and the effects of a number of crucial processing variables were examined. In this method, an aqueous zirconia sol consisting of 700 Å (mean diameter) particles is mixed with urea and formaldehyde, which are polymerized by the acidic sol. Urea-formaldehyde polymer adsorbs onto the ZrO_2 colloids, entraining the colloids in the precipitation of the polymer gel and thus allowing the colloids to aggregate. Features of the aggregation process are elucidated from responses of the process to variations in temperature, reaction mixture composition, and solvent polarity. Our results suggest that the aggregation process resembles those reported for the bridging flocculation of colloids by adsorbed polymers. Porous zirconia particles obtained after polymer combustion and sintering of the aggregates are 3.5 μm in diameter with a surface area of 13 m^2/g, a porosity of 29% and pores ranging from <50 to 350 Å in diameter. The particles are strong enough to withstand the packing of a HPLC column.

20. C. Francisco Lorrenzano-Porras, P.W. Carr and A.V. McCormick, "Relationship between Pore Structure and Diffusion Tortuosity of ZrO_2 Colloidal Aggregates," *J. Colloid Interface Sci*. **164**, 1-8 (1994).

> Abstract: The performance of chromatographic materials is extremely sensitive to variations in pore architecture. In this study, porous zirconia particles (5-10 μm) manufactured with an oil emulsion process have been characterized with the aim of explaining its success in protein separations with liquid chromatography (HPLC). Its chromatographic performance is surprising since it is an aggregate of colloidal spheres; if the spheres are near close packed, small constrictions in the pore network should drastically reduce the effective diffusion coefficient and thus diminish the chromatographic resolution of proteins. Moreover, if small constrictions are avoided, such materials may serve as catalyst supports requiring reaction of large molecules (e.g., block copolymers). To characterize the pore structure we use electron microscopy, SEM and TEM; nitrogen adsorption; and mercury porosimetry. Since the latter techniques are compromised by the presence of pore constrictions, we also use the NMR spin lattice relaxation and NMR self diffusion experiment to determine the average hydraulic diameter and tortuosity of the pore space. It is shown that while many small constrictions are present, the spheres are quite loosely packed. There is still a high degree of connection between pore spaces through larger constrictions than

would be expected for close packed spheres. The particles are very porous and the effective tortuosity for diffusion is surprisingly low.

24. M.J. Annen, R. Kizhappali, P.W. Carr, and A. McCormick, "Development of Porous Zirconia Spheres by Polymerization-Induced Colloid Aggregation-Effect of Polymerization Rate," *J. Mater. Sci*. **29**, 6123-30 (1994).

    Abstract: Polymerization-induced colloid aggregation is used to synthesize spheres of narrow size distribution which are porous aggregates of ZrO_2 colloids. Variation of the reaction pH has been investigated to determine the optimum rate of polymerization of the urea-formaldehyde resin. At the optimum rate, a colloid packing structure is formed where a balance of high porosity and high strength of the aggregates is achieved. This optimum coincides with the maximum yield of the ~5 μm sintered (polymer-free) particles. Particles synthesized at pH values below the optimum are mechanically weak; some are hollow spheres. Variation of the pore structure, and thus colloid packing structure, is elucidated by nitrogen adsorption and apparent density measurements. Differences on either side of the optimum pH are related to the efficiency of polymer-bridge formation between colloids.

25. C. Francisco Lorrenzano-Porras, M.J. Annen, M.C. Flickinger, P.W. Carr and A.V. McCormick, "Pore Structure and Diffusion Tortuosity of Porous ZrO_2 Synthesized by Two Different Colloid-Aggregation Processes," *J. Colloid Interface Sci.* **170,** 299-307 (1995).

    Abstract: The pore structure of column-packing materials plays a significant role in high-performance liquid chromatography (HPLC) and will particularly important for the separation of synthesized by the controlled aggregation of 700-Å colloids. Two different aggregation methods were used: oil emulsion processing and polymerization-induced colloid aggregation (PICA). The pore structures of the resulting materials were characterized by electron microscopy, nitrogen adsorption/desorption, mercury (Hg) intrusion/extrusion, pulsed field gradient spin-echo NMR self diffusion measurements, and NMR spin-lattice relaxation measurements. The tortuosity and hydraulic diameter resulting from the two aggregation methods are compared. The PICA-generated sample is less porous but shows a more well-connected pore structure. These results show the need to restore high porosity while retaining the beneficial pore structure and the high degree of control of aggregate size that are characteristics of the PICA process.

29. C. Francisco Lorrenzano-Porras, D.H. Reeder, M.J. Annen, P.W. Carr and A.V. McCormick, "Unusual Sintering Behavior of Porous Chromatographic Zirconia Produced by Polymerization-Induced Colloid Aggregation," *Ind. Eng. Chem. Res.* **34,** 2719-27 (1995).

    Abstract: The effects of sintering temperature and duration on the pore structure of chromatographic zirconia particles produced by the controlled polymerization-induced aggregation of 1000 angstrom colloids are studied with an eye toward optimally strengthening the aggregates and eliminating small pores while preserving large pores. Nitrogen adsorption and mercury porosimetry are used to estimate the surface area, pore volume, and pore size distribution. Pulsed field gradient NMR measurements of solvent diffusion are used to estimate the diffusion tortuosity of the pore space. Initially of course, the pore volume and surface area decrease significantly, the decrease being more pronounced at higher temperatures. With prolonged sintering, the pore size, pore volume, and surface area change much more slowly, but the diffusion tortuosity seems to be minimized at a sintering temperature and time at which pores are allowed to redistribute so as to optimize large pores. The aggregates synthesized by this aggregation method apparently produce metastable large pores which are not easily collapsed.

32. C.J. Dunlap, P.W. Carr and A.V. McCormick, “A Chromatographic Comparison of the Pore Structures of Zirconia High Performance Liquid Chromatographic Materials Made by the Polymerization Induced Colloidal Aggregation and the Oil Emulsion Methods,” *Chromatographia*, **42(5-6)**, 273-282 (1996).

    Abstract: The pore structures of zirconium oxide particles prepared by two different methods (PICA and Oil Emulsion processes) are compared. Nitrogen sorptometry and size exclusion chromatography are used to characterize the two different types of particles. Significant and unexpected differences were found in the accessible pore volumes and the bed packing densities of the two materials. The PICA material was found to have a higher totally included and totally excluded volume than would be normally expected. The chromatographic method provides results which are more useful for the application of these materials to separation science.

34. J. Nawrocki, P.W. Carr, M.J. Annen and S. Froelicher, “A TGA Investigation of Hydrated Monoclinic Zirconia,” *Analytica Chimica Acta*, **327(3)**, 261-266 (1996).

Abstract: Thermogravimetric analysis (TGA) was used to monitor the surface hydroxyl and carbonate concentrations on porous zirconia after various vapor- and liquid-phase treatments. After equilibration with humid nitrogen, each of the surface zirconium atoms bears a single hydroxyl group and all surface oxygen atoms are present as bridged hydroxyl groups. Hydrochloric acid and sodium hydroxide treatments further increase the surface hydroxyl group concentration. In the presence of sufficient water, carbon dioxide chemisorption is inhibited. Once adsorbed, however, surface carbonates and bicarbonates are not removed by hydrochloric acid, sodium hydroxide or sodium fluoride washings.

36. C.J. Dunlap and P.W. Carr, “Synthesis and Chromatographic Characterization of Dextran-Coated Zirconia High Performance Liquid Chromatographic Stationary Phases," *J. Chromatogr. A* **746,** 199-210 (1996).

    Abstract: Porous zirconia particles made by the oil emulsion (OE) method and the polymerization-induced colloid aggregation (PICA) method have been coated with a small, carboxymethylated (~5%) dextran polymer and crosslinked in place. The parameters of the coating process (dextran concentration, adsorption time and crosslinker concentration) have all been examined and an optimum value for each determined. The coated and uncoated materials were characterized by nitrogen sorptometry and size-exclusion chromatography (SEC) using solutes (polystyrenes and dextrans) of well-defined molecular masses. Nitrogen sorptometry results show that the PICA material has a much lower pore volume and smaller pore diameter than do the OE materials. Despite this, the elution volumes of the SEC probes change very little upon polymer coating the PICA material while the OE material shows a very large change upon coating.

37. D.H. Reeder, A.M. Clausen, M.J. Annen, P.W. Carr, M.C. Flickinger, and A.V. McCormick, Note, "An Approach to Hierarchically-Structured Porous Zirconia Aggregates," *J. Colloid Interface Sci.* **184,** 328-30 (1996).

    Abstract: Presented here is a simple, inexpensive approach to aggregating colloids into hierarchically structured spherical particles. Successive aggregation step are used to assemble a particle that is self-similar on two size scales and is permeated by an ordered pore network with a bidisperse size distribution. The structure of the micro- and macropore networks as well as the mechanical integrity of the structure can be controlled by varying sintering conditions.

40. D.H. Reeder, J.W. Li, P.W. Carr, M.C. Flickinger and A.V. McCormick, “Models for Polybutadiene Pore Wall Coatings in Porous Zirconia,” *J. Chromatogr.*, **760(1)**, 71-79 (1997).

    Abstract: We present three models of the changes in measured pore size distribution for cylindrical pores when a polymer is deposited in the pores by evaporation from a volatile solvent. The predicted results serve as an aid in interpreting experimental nitrogen adsorption data for polybutadiene (PBD) coating on porous zirconia. At low loadings, PBD appears to deposit in thin layers on the surface with no preference for filling either large or small pores. At higher PBD loadings, the polymer deposits preferentially in smaller pores. This is in qualitative agreement with PBD coatings on porous silica.

45. M.J. Robichaud, A.R. Sathyagal, P.W. Carr, A.V. McCormick, and M.C. Flickinger, Technical Note, “An Improved Oil Emulsion Synthesis Method for Large, Porous Zirconia Particles for Packed- or Fluidized-Bed Protein Chromatography,” *Sep. Sci. Technol.* **32,** 2547-59 (1997).

    Abstract: A previously reported oil emulsion technique for the synthesis of large, porous zirconia particles has been modified to overcome problems of nonreproducability, formation of non-spherical or broken particles, and low yield of chromatographically useful particle. The improved technique described here uses a concentrated sol and an in-line mixer to successfully solve these problems. The results indicate that the stirring rate used to maintain the emulsion during drying has a strong effect on the final particle size.

48. J.W. Li, D.H. Reeder, A.V. McCormick, and P.W. Carr, “Factors Influencing Polybutadiene Deposition within Porous Chromatographic Zirconia,” *J. Chromatogr. A* **791,** 45-52 (1998).

    Abstract: We have studied the effect of the conditions for the deposition of polymers in the preparation of polybutadiene-coated porous zirconia particles for reversed-phase chromatography. Chromatographic performance improves when the particle surface is pre-coated with elemental carbon by a chemical vapor deposition (CVD) process. Conversely, performance is significantly degraded when the solvent is removed very slowly during the deposition process. No improvement results when the particles are coated using small sequential load of polymer. We hypothesize that the polymer deposition process is controlled by the rate at which the solvent meniscus recedes during solvent evaporation, by the affinity of the polymer for the zirconia surface, and by polymer-solvent and polymer-polymer interactions.

57. A. Subramanian, P.W. Carr, C.V. McNeff, "Use of Spray-Dried Zirconia Microspheres in the Separation of Immunoglobulins from Cell Culture Supernatant," *Journal of Chromatography A*, **890(1)**, 15-23 (2000).

    Abstract: A method suitable for the isolation of monoclonal antibodies (Mabs) on novel zirconia microspheres (20-30 micron) is described. Zirconia microspheres were generated by spray drying colloidal zirconia. Spray-dried zirconia microspheres were further classified and characterized by X-ray diffraction, BET porosimetry and scanning electron microscopy. Spray-dried zirconia microspheres were modified with ethylenediamine-N,N'-tetra(methylenephosphonic) acid (EDTPA) to create a cation-exchange chromatographic support. The chromatographic behavior of a semi-preparative column packed with EDTPA-modified zirconia microspheres was evaluated and implications for scale-up are provided. EDTPA-modified zirconia microspheres were further used to purify Mabs from cell culture supernatant. Analysis by enzyme linked immunosorbent assay and gel electrophoresis demonstrate that Mabs can be: recovered from a cell culture supernatant at high yield (92-98%) and high purity (>95%) in a single chromatographic step.

62. Akio Yuchi, Yasuharu Mizuno, Tomonori Yonemoto, "Ligand-Exchange Chromatography at Zirconium(IV) Immobilized on IDA-Type Chelating Polymer Gel," *Anal. Chem*., **72**, 15, 3642-3646 (2000).

    Abstract: Ligand-exchange chromatography of organo-acidic compounds at Zr(IV) immobilized on IDA-type chelating polymer gel using an aqueous mobile phase was studied, to obtain some insight into their retention on zirconia. Zirconium(IV) has no contribution to the retention of phenols, while appreciably enhancing that of benzoic acids and strongly or occasionally irreversible adsorbing the potentially chelating substances. The presence of two stationary-phase species adsorbing benzoates was confirmed, and the retention over a wide pH range of 3.5-9.5 was quantitatively formulated. The correlation of the retention factor with the Bronsted acidity of samples and its dependence on pH were theoretically explained, and the performance of this chromatographic system was compared with that of zirconia.

69. B. Yan, C.V. McNeff, F. Chen, P.W. Carr, A.V. McCormick, "Control of Synthesis Conditions to Improve Zirconia Microspheres for Ultrafast Chromatography," *J. Am*. Ceram*. Soc*., **84(8)**, 1721-1727 (2001).

    Abstract: Though it has been known for some time that monodisperse, micron-scale spherical zirconia particles can be synthesized by the hydrolysis of zirconium alkoxides, the particles so made are frequently aggregated. Moreover, it is not clear whether they can be made nonporous without suffering aggregation; this would limit their usefulness in applications like ultrafast chromatography, which require that the particles be spherical and unaggregated (which we then term "microspheres") and that they be nonporous. Here we report that when using an alkoxide hydrolysis synthesis process, critical washing steps can eliminate irreversible aggregation and so enable reliable production of microspheres. We clarify important but practical precautions that easily ensure the reproducibility of the synthesis method. Finally, we demonstrate that the microspheres can be made nonporous with a suitable heating schedule.

77b. B.A. Musial, A.J. Sommer, N.D. Danielson, "Microchannel Thin Layer Chromatography with in Situ Plate Scanning Micro-DRIFTS Detection Using Plain and Polybutadiene Modified Zirconia Stationary Phases," *Applied Spectroscopy,* **56(8)**, 1059-1066 (2002).

    Abstract: Thin layer chromatography (TLC) of various dyes is compared in microchannels packed with either bare zirconia (normal phase) or polybutadiene (PBD) modified zirconia (reversed phase). In situ micro-diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) of the analyte spots is possible due to the low absorption background of both plain and PBD zirconia. An instrument coupling DRIFTS with a motorized stage that could profile the microchannel TLC plate is developed. The retention order of anthracene and pyrene probes as a function of percent acetonitrile are generally as expected on PBD zirconia but opposite for plain zirconia. Impurities in technical grade methylene blue can be determined after TLC separation on plain zirconia. The separation and infrared identification of various dyes such as dichlorofluorescein and rhodamine B on PBD zirconia is improved using a mobile phase with an additive such as phosphate or dihexylamine that prevents band streaking.

87. J. Nawrocki, C. Dunlap, A. McCormick, P. W. Carr, "Part I. Chromatography Using Ultra-Stable Metal Oxide-Based Stationary Phases for HPLC," *Journal of Chromatography A*, **1028**, 1-30 (2004).

Abstract: The first part of the review contrasts the main drawbacks of silica-based packings such as their relative thermal and chemical instability with excellent stability of metal oxides. The paper concerns mainly ZrO2, TiO2 and Al2O3. Methods of preparation of spherical particles for HPLC are described. Surface chemistry of the oxides is, however, very different from that of silica. Ability of the oxides to ion- and ligand exchange is discussed from a chromatographic point of view.

90. B. Yan, C.V. McNeff, P.W. Carr, A.V. McCormick, "Synthesis and Characterization of Submicron-to-Micron Scale, Monodisperse, Spherical, and Nonporous Zirconia Particles," *J. Am. Ceram. Soc.*, **88(3)**, 707-713 (2005).

    Abstract: Monodisperse, spherical, and nonporous zirconia particles ranging from submicrons to microns for use as supports for capillary electrophoresis and chromatography were synthesized by the controlled hydrolysis of zirconium tetra-alkoxides in alcohol solutions in the presence of long-chain organic acids. Particle characteristics varied as a function of water concentration, the chain length of the organic acid, aging time, temperature, and extent of stirring. Changing these experimental parameters affected particle size, particle size distribution, and the degree of particle aggregation. Submicron particles were made by increasing the water concentration and decreasing aging time. Particle size increases as temperature during the aging period increases. Most importantly, large monodisperse particles (4 μm) could be made by extended stirring. In general, we found that particle size increases as the chain length of the organic acid increases. We also found that gentle rotation during the aging period prevented both particle settling and secondary nucleation. This allowed the production of large particles. BET nitrogen adsorption uptake measurements demonstrate that the particles could be sintered, without aggregating them, to remove virtually all internal porosity.

91a. M.J. Gray, G.R. Dennis, P.J. Slonecker, R.A. Shalliker, "Utilising Retention Correlation for the Separation of Oligostyrenes by Coupled-column Liquid Chromatography," *Journal of Chromatography A*, **1073**, 3-9 (2005).

    Abstract: The separation of n = 2-5 n-butyloligostyrenes has been illustrated by reversed-phase reversed-phase (RP-RP) coupled-column liquid chromatography. The coupled-column separation has been achieved by use of a C18 column with methanol as the mobile phase followed by a DiamondBond C18 column with acetonitrile (ACN) mobile phase. The DiamondBond C18 is a hybrid carbon clad zirconia (CCZ)-C18 stationary phase. Unlike a C18-carbon clad zirconia two-dimensional liquid chromatographic system, which is orthogonal, the C18 and DiamondBond C18 columns combination exhibit correlations based upon the molecular weight of n-butyloligostyrenes. Using an alternative strategy to two-dimensional liquid chromatography, the molecular weight dependence displayed by both the C18 column and DiamondBond C18 has been used to increase throughput or decrease analysis time in the analysis of the n-butyloligostyrenes. However, this is at the expense of a portion of the two-dimensional peak capacity displayed by the C18-carbon clad zirconia system.

95. G. Srinivasan, A. Kyrlidis, C. McNeff, K. Müller, “Investigation on Conformational Order and Mobility of DiamondBond-C18 and C18-alkyl Modified Silica Gels by Fourier Transform Infrared and Solid-state NMR Spectroscopy,” *Journal of Chromatography A*, **1081**, 132-139 (2005).

    Abstract: The effect of surface coverage and solid supports on the conformational order of alkyl chains of commercially available carbon clad zirconia based supports and synthesized C18-alkyl modified silica based supports are probed in the dry state for the first time using variable temperature Fourier transform infrared (FT-IR) and solid-state (13)C NMR spectroscopy. From FT-IR spectroscopy, the conformational order of alkyl chains tethered to the substrates is examined by the analysis of CH(2) symmetric and anti-symmetric stretching bands. Through solid-state (13)C NMR spectroscopy, the order is inferred from the relative intensity of the main methylene carbon resonance assigned to trans and trans-gauche conformations. It is found that molecules tethered to the graphite layer experience a strongly diamagnetic component of the highly anisotropic magnetic susceptibility of the graphite lattice, which reflects upfield shift in the (13)C NMR spectra of commercially available octadecyl-modified carbon clad zirconia based column materials. The present results prove that temperature, surface coverage and solid supports have an influence on the conformational order and mobility of alkyl chains tethered to the carbon clad inorganic metal oxides.

96. R.A. Henry, C.V. McNeff, B. Yan, T.R. Hoye, “A Novel Chemical Route to Stable, Regenerable Zirconia-Based Chiral Stationary Phases for HPLC,” *American Laboratory (News Edition)*, **37(21)**, 22-24 (2005).

Abstract: A promising new route to preparing chiral stationary phases using a zirconia substrate has been developed. Chiral selectors that can be easily removed and replaced by simple, low-temperature reactions are targeted for commercialization.

97. H.K. Kweon and K. Håkansson, "Selective Zirconium Dioxide-Based Enrichment of Phosphorylated Peptides for Mass Spectrometric Analysis," *Analytical Chemistry*, **78(6)**, 1743 -1749 (2006).

    Abstract: Due to the dynamic nature and low stoichiometry of protein phosphorylation, enrichment of phosphorylated peptides from proteolytic mixtures is often necessary prior to their characterization by mass spectrometry. Several phosphopeptide isolation strategies have been presented in the literature, including immobilized metal ion affinity chromatography. However, that technique suffers from poor selectivity and reproducibility. Recently, titanium dioxide-based columns have been successfully employed for phosphopeptide enrichment by several research groups. Here, we present, to our knowledge, the first demonstration of the utility of zirconium dioxide microtips for phosphopeptide isolation prior to mass spectrometric analysis. These microtips display similar overall performance as TiO2 microtips. However, more selective isolation of singly phosphorylated peptides was observed with ZrO2 compared to TiO2 whereas TiO2 preferentially enriched multiply phosphorylated peptides. Thus, these two chromatographic materials possess complementary properties. For - and -casein, Glu-C digestion provided no evident advantage compared to trypsin digestion when combined with TiO2 or ZrO2 phosphopeptide enrichment.

Lewis Acid/Base Chemistry

5. J.A. Blackwell and P.W. Carr, "Study of the Fluoride Adsorption Characteristics of Porous Microparticulate Zirconium Oxide," *J. Chromatogr.* **549**, 43-57 (1991).

    Abstract: Lewis acid sites are present on the surface of metal oxide chromatographic supports and are responsible for the very strong adsorption of Lewis bases. Such sites must be masked or modified to elute solutes which contain Lewis base groups. Fluoride ion coordinates strongly with these sites on zirconium oxide and forms a surface whose composition is pH and ionic strength dependent. Coverages range from 13.7 μmol/m^2 fluoride at pH 4.8 to 0 μmol/m^2 fluoride without harming the underlying zirconium oxide particle. Readsorption of the fluoride can be accomplished by equilibrating the particles in a buffer of suitable fluoride concentration. Such fluoride modification has been found to occur on a time scale suitable for displacement chromatography on the Lewis acid sites.

8. J.A. Blackwell and P.W. Carr, "A Chromatographic Study of the Lewis Acid-Base Chemistry of Zirconia Surfaces," *J. Liq. Chromatogr.* **14(15),** 2875-89 (1991).

    Abstract: The chromatographic properties of porous microparticulate zirconium oxide surfaces in aqueous media are highly dependent upon the chemical composition of the eluent. In particular, retention is controlled by the type and concentration of "hard" Lewis bases when these species are present in the eluent. Ligand exchange is the dominant mechanism for the retention of solutes which are Lewis bases. Consequently, the capacity factor and plate height depend on both the thermodynamic and kinetic properties of whatever competing Lewis bases may be present in the eluent. These Lewis base eluent components act to control retention in two ways. They modify the net ligand exchange contribution to retention, and they serve as sites for secondary interactions, such as hydrogen bonding and hydrophobic interactions between solutes and the dynamic stationary phase.

9. W.A. Schafer, P.W. Carr, E.F. Funkenbusch, and K.A. Parson, "Physical and Chemical Characterization of Porous Phosphate-Modified Zirconia Substrate," *J. Chromatogr.* **587**, 137-47 (1991).

    Abstract: A phosphate modification of previously described porous zirconium oxide high-performance liquid chromatographic support particles has been developed. Modification of the surface with inorganic phosphate alleviates the irreversible adsorption of proteins on the native oxide surface and makes the surface more biocompatible. X-ray photoelectron spectroscopic, solid-state ^{31}P NMR, elemental analysis, pH stability and ^{32}P phosphate release studies that have been used to characterize physically the surface of these modified particles are reported.

11. J.A. Blackwell and P.W. Carr, "Ligand Exchange Chromatography of Free Amino Acids on Phosphated Zirconium Oxide Supports," *J. Liq. Chromatogr.* **15**, 727-51 (1992).

Abstract: Phosphated zirconium oxide particles loaded with copper ions are a mechanically superior alternative to resin based supports for ligand exchange separations of amino acids. They show good stability under acidic and alkaline conditions and afford different selectivity than resin based exchangers. Acidic amino acids are more strongly retained on this phase than on silica or resin based supports due to interactions with the underlying zirconia surface. These interactions occur despite rigorous phosphation of the zirconia particle. The operational aspects of amino acid separations on this material are examined and compared to conventional resin based models.

12. J.A. Blackwell and P.W. Carr, "Role of Lewis Acid-Base Processes in Ligand-Exchange Chromatography of Benzoic Acid Derivatives on Zirconium Oxide," *Anal. Chem*. **64**, 853-62 (1992).

    Abstract: Porous microparticulate zirconium oxide shows very different selectivities and pH dependencies for the separation of benzoic acid derivatives than do conventional bonded-phase anion-exchange supports. This results from a very significant ligand-exchange contribution to the retention of hard Lewis bases on the surface of transition-metal oxide supports. We have found that the capacity factors of a wide variety of derivatives of benzoic acid are closely correlated with their Bronsted acidities. The eluent pH is also a critical factor in determining the magnitude of the capacity factor, but it does not have much influence on chromatographic selectivity. The differential selectivity of this phase in comparison to conventional polymeric and bonded-phase anion exchangers can be attributed to complexation and steric effects which profoundly alter the elution patterns of certain solutes.

13. J.A. Blackwell and P.W. Carr, "Development of an Eluotropic Series for the Chromatography of Lewis Bases on Zirconium Oxide," *Anal. Chem*. **64**, 863-73 (1992).

    Abstract: Ligand-exchange interactions, which dominate the retention characteristics of Lewis base solutes on zirconium oxide, can be strongly attenuated by the addition of a competing Lewis base to the eluent. The chromatographic effects of these competing bases vary significantly in their structure and Lewis basicity. An eluotropic scale of mobile-phase strength for ligand-exchange/ion-exchange chromatography on zirconia has been developed which ranks the overall elution strength of a variety of Lewis bases in terms of their ability to elute a wide variety of benzoic acid derivatives. This series generally holds true for similar solutes; however, deviations are noted with some solutes and eluents where chelation and steric factors alter the kinetic and thermodynamic characteristics of the retention process.

14. J.A. Blackwell and P.W. Carr, "Ligand Exchange Chromatography of Free Amino Acids and Proteins on Porous Microparticulate Zirconium Oxide," *J. Liq. Chromatogr*. **15**(9), 1487-1506 (1992).

    Abstract: The Lewis acid sites present on the underlying zirconium oxide particles are responsible for the unusual elution sequence for amino acids on copper (II) loaded, phosphated zirconium oxide supports reported in an earlier study. To more thoroughly examine the effect of these strong Lewis acid sites, we have studied ligand exchange chromatography on copper (II) loaded zirconium oxide particles. It is shown here that carboxylate functional groups on amino acid solutes strongly interact with surface Lewis acid sites. Addition of competing hard Lewis bases to the eluent attenuates these specific interactions. The result is a chromatographic system with high selectivity which is also suitable for ligand exchange chromatography of proteins.

15. J.A. Blackwell and P.W. Carr, "Ion- and Ligand-Exchange Chromatography of Proteins Using Porous Zirconium Oxide Supports in Organic and Inorganic Lewis Base Eluents," *J. Chromatogr*. **596**, 27-41 (1992).

    Abstract: The applicability of an eluotropic scale pertaining to the desorption of low molecular weight Lewis base solutes from zirconium oxide is examined for its ability to rationalize the retention of proteins on this substrate. The strongest Lewis base eluents (phosphate and fluoride) are able to bring about elution of nearly all proteins provided that their initial mobile phase concentration almost saturates the eluent's adsorption isotherm. In contrast, weaker Lewis bases such as borate, sulfate and bromide are able to elute only those proteins which are retained primarily by ionic interactions. In weak eluents, proteins that contain a large number of accessible Lewis base sites are not eluted from the support. The effect of ionic strength and a variety of Lewis base eluents were also examined.

18. L. Sun, A.V. McCormick, and P.W. Carr, "Study of the Irreversible Adsorption of Proteins on Polybutadiene Coated Zirconia," *J. Chromatogr*. A **658**, 465-73 (1994).

Abstract: The cause of irreversible adsorption of proteins on polybutadiene-coated zirconia is investigated by comparing the chromatographic properties of polybutadiene-coated zirconia with that of other reverse-phase packing materials such as bonded phase silica, polybutadiene-coated alumina and polybutadiene-coated silica. We find that the polybutadiene-coated zirconia has a micropore size distribution similar to that of the polybutadiene-coated alumina, from which some proteins can be eluted. Thus, the irreversible adsorption of proteins on polybutadiene-coated zirconia is not caused by entrapment of proteins in the micropores of the packing. The high hydrophobicity of the polybutadiene coating and the strong Lewis acid sites on the zirconia surface cause strong interactions between proteins and the stationary phase, the combination of which lead to irreversible adsorption of proteins on polybutadiene-coated zirconia.

28a. D.A. Whitman, T.P. Weber, J.A. Blackwell, "Chemometric Characterization of Lewis-Base Modified Zirconia for Normal Phase Chromatography," *Journal of Chromatography A*, **691**, 205-212 (1995).

Abstract: Microporous zirconia was evaluated for its chromatographic properties as a normal phase support. Since previous studies in aqueous media had indicated a strong effect of eluent Lewis base on chromatographic properties, a number of Lewis base pretreatments of the support were evaluated as to their effect on normal phase selectivities. The retention characteristics of over thirty well characterized probe solutes were determined on eight different "acid"- or "base"-washed zirconia supports. These results were compared with those obtained for a silica column. Chemometric methodology was used to characterize the similarities and differences between the "acid"- and "base"-washed supports. The lack of chromatographic reproducibility previously observed under normal-phase conditions on zirconia appears to be a result of the lack of specific descriptions regarding the "acid" or "base" pretreatment.

47. A.M. Clausen and P.W. Carr, “Chromatographic Characterization of Phosphonate Analog EDTA-Modified Zirconia Support for Biochromatographic Applications,” *Anal. Chem.* **70,** 378-85 (1998).

Abstract: Zirconium dioxide (zirconia) has a great affinity for inorganic and organic phosphate. Previous work from is laboratory demonstrated the utility of phosphate-modified microparticulate zirconia as a support for protein separations. We have extended this investigation to include the study of ethylenediamine-N,N’-tetramethylphosphonic acid (EDTPA), a phosphonate analog of EDTA, as a surface modifier for zirconia. Our work explores the use of EDTPA-modified zirconia (PEZ) for its potential use as a high-performance inorganic cation-exchange support for the separation of proteins. The phosphate groups in EDTPA very effectively block the sites responsible for strong interactions of hard Lewis bases with zirconia’s surface. Modification of zirconia with EDTPA provides a “biocompatible” stationary phase, resulting in high mass recoveries of proteins. We compare PEZ with inorganic phosphate-modified zirconia to show increased efficiency, as well as unique selectivities for chromatography of proteins on the chelator-modified surface. Finally, the selectivity, efficiency, and separation mechanism are reported. The studies show the PEZ is a useful high-performance ion-exchange support for the separation of cationic proteins and for modulating the sites responsible for the high affinity of zirconia toward certain classes of anions.

62. Akio Yuchi, Yasuharu Mizuno, Tomonori Yonemoto, “Ligand-Exchange Chromatography at Zirconium(IV) Immobilized on IDA-Type Chelating Polymer Gel,” *Anal. Chem*., **72**, 15, 3642-3646 (2000).

Abstract: Ligand-exchange chromatography of organo-acidic compounds at Zr(IV) immobilized on IDA-type chelating polymer gel using an aqueous mobile phase was studied, to obtain some insight into their retention on zirconia. Zirconium(IV) has no contribution to the retention of phenols, while appreciably enhancing that of benzoic acids and strongly or occasionally irreversible adsorbing the potentially chelating substances. The presence of two stationary-phase species adsorbing benzoates was confirmed, and the retention over a wide pH range of 3.5-9.5 was quantitatively formulated. The correlation of the retention factor with the Bronsted acidity of samples and its dependence on pH were theoretically explained, and the performance of this chromatographic system was compared with that of zirconia.

68. Brain C. Trammell, Marc A. Hillmyer, Peter W. Carr, “A Study of the Lewis Acid-Base Interactions of Vinylphosphonic Acid-Modified Polybutadiene-Coated Zirconia,” *Anal. Chem*., **73**, 14, 3323-3331 (2001).

Abstract: Polybutadiene-coated zirconia (PBD-ZrO_2) is very useful for reversed-phase separation under a wide variety of conditions. Its excellent chemical (pH = 1-13) and thermal (up to 150°C) stability distinguish it from silica-based reversed phases. Just as with silica-based phases, zirconia’s surface chemistry significantly influences the chromatography of certain classes of analytes. Zirconia’s *hard* Lewis acid sites can be chromatographically problematic. Analytes such as carboxylic acids strongly interact with these sites on PBD-ZrO_2 and do not elute. Addition of phosphate or other strong, hard Lewis bases to the eluent brings about elution, but the resulting peak is often tailed and broad. Typically, cationic solutes are more retained in the presence of phosphate or fluoride due to adsorption of the Lewis base additives and the concomitant development of a negative charge on the surface. This Coulombic interaction can be used to optimize selectivity, but the reversed-phase-cation-exchange retention can produce broad peaks with excessive retention. As an alternative to adding Lewis bases to the eluent, we studied the effect of permanently modifying PBD-ZrO_2 by covalently attaching vinylphosphonic acid (VPA) to PBD which was predeposited in the pores of zirconia. We have investigated the chromatography of acids, bases, and small peptides on VPA-modified PBD-ZrO_2 (VPA-PBD-ZrO_2) and compared it to PBD-ZrO_2. VPA-PBD-ZrO_2 is a reversed-cation-exchange phase with properties quite different fromPBD-ZrO_2. The chemical stability of both phases led us to explore how low-pH (1.5-3), ultralow ph (0), and high-pH (12) eluents effect the retention properties of these mixed-mode phases. Ultralow-pH eluents effectively separate small peptides on both phases. This approach gives lower retention, without sacrificing resolution, and much higher efficiency for small peptides than previously reported.

73a. D. Xiang, L. Tang and J.A. Blackwell, "Evaluation of the Stability and Selectivity for Various Adjustable Stationary Phases Using Zirconium Oxide Supports in High-Performance Liquid Chromatography," *Journal of Chromatography A*, **953(1-2)**, 67-77 (2002).

<u>Abstract:</u> The selectivity and retention properties of a zirconia stationary phase were reversibly altered using various ligands containing Lewis base functional groups. A simple loading procedure allowed a variety of ligands to be attached to the zirconia surface via Lewis interactions. The resulting stationary phases were shown to be stable and produced different selectivity and retention properties from the native zirconia material. The metal oxide adsorbent was converted to a diol-type stationary phase using glucose-6-phosphate for use under normal-phase conditions. Reversed-phase supports were produced by loading either octyl- or octadecylphosphonic acid onto the native zirconia support. The properties of these new phases were then compared to commercially available bonded silica analogs. Ligands bound to the surface in this manner were effectively removed and the native zirconia was regenerated using a dilute base wash procedure.

74. Y. Hu, X. Yang and P.W. Carr, “Mixed-mode Reversed-phase and Ion-exchange Separations of Cationic Analytes on Polybutadiene-coated Zirconia,” *Journal of Chromatography A*, **968**, 17-29 (2002).

<u>Abstract:</u> The retention and selectivity of the chromatographic separation of basic (cationic) analytes on a polybutadiene-coated zirconia (PBD-ZrO2) stationary phase have been studied in greater detail than in previous studies. These separations are strongly influenced by the chemistry of the accessible surface of zirconia. In the presence of buffers which contain hard Lewis bases (e.g., phosphate, fluoride, carboxylic acids) zirconia's surface becomes negatively charged due to adsorption of the buffer anion at the hard Lewis acid sites. Consequently, under most conditions (e.g., neutral pH), cationic analytes undergo both hydrophobic and cation-exchange interactions. This mixed-mode retention process generally leads to greater retention factors for cations relative to those on silica-based reversed phases despite the lower surface areas of the zirconia phase, but, more importantly, adsorption of hard Lewis bases can be used to control the chromatographic selectivity for cationic analytes on these zirconia-based stationary phases. In contrast to our prior work, here we show that when mixed-mode retention takes place, both retention and selectivity are easily adjusted by changing the type of hard Lewis base buffer anion, the type of buffer counter-ion (e.g., sodium, potassium, ammonium), the pH, and the ionic strength of the eluent as well as the type and amount of organic modifier.

76. Y. Hu and P.W. Carr, “The Special Effect of Fluoride on the Chromatography of Acidic Analytes on Polybutadiene-Coated Zirconia,” *Chromatographia,* **56(7-8)**, 439-444 (2002).

<u>Abstract:</u> The special effect of fluoride as a Lewis base additive in suppressing the ligand-exchange interactions for acidic analytes on polybutadiene-coated zirconia (PBD-ZrO2) has been investigated. We found that fluoride is more effective than phosphate in improving the separation efficiency for strong acids. The improvement is attributed to that fluoride has a smaller size and a more flexible coordination chemistry towards zirconium centers than phosphate; consequently, fluoride can more effectively improve the kinetics of the ligand-exchange processes for strongly acidic analytes. We demonstrated that using a small amount of fluoride in combination with a larger quantity of phosphate is a practical way to improve the separation efficiency and resolution for acidic analytes. Some example separations are presented.

88. J. Nawrocki, C. Dunlap, J. Li, J. Zhao, C.V. McNeff, A. McCormick, P.W. Carr, “Part II. Chromatography Using Ultra-Stable Metal Oxide-Based Stationary Phases for HPLC,” *Journal of Chromatography A*, **1028**, 31-62 (2004).

    Abstract: In this part of the review authors discuss methods used for modification of metal oxide surfaces. On the basis of literature data it is shown, that silanization of the surfaces do not form stable supports for chromatography. On the other hand, the success of polymer modified surfaces such as polybutadiene (PBD) and polystyrene (PS) is emphasized. Permanent modification of metal oxide surfaces with Lewis bases is also widely discussed. Chromatographic properties of polymer-modified surfaces of zirconia are discussed in details. The perspectives of carbon-coated metal oxide surfaces in HPLC and high temperature separations are described.

96. R.A. Henry, C.V. McNeff, B. Yan, T.R. Hoye, “A Novel Chemical Route to Stable, Regenerable Zirconia-Based Chiral Stationary Phases for HPLC,” *American Laboratory (News Edition)*, **37(21)**, 22-24 (2005).

    Abstract: A promising new route to preparing chiral stationary phases using a zirconia substrate has been developed. Chiral selectors that can be easily removed and replaced by simple, low-temperature reactions are targeted for commercialization.

High Temperature LC

41. J.W. Li and P.W. Carr, “Effect of Temperature on the Thermodynamic Properties, Kinetic Performance, and Stability of Polybutadiene-Coated Zirconia,” *Anal. Chem.*, **69(5)**, 837-843 (1997).

    Abstract: This article describes the results of a study of the effect of temperature on the performance of a reversed-phase material prepared by coating polybutadiene (PBD) on porous zirconia. We examined the effect of temperature on retention, efficiency, and stability of this phase. The thermodynamic properties were evaluated via the separation of alkylbenzenes and a set a tricyclic antidepressant drug at different temperatures, while the intrinsic kinetic performance of the PBD phase at elevated temperatures was examined by using alkylbenzenes as probe solutes. Moreover, the thermal stability was determined by measuring the drift in *k’* while continuously pumping a mobile phase at 100°C. We found that enthalpy changes were between -2 and -4 kcal/mol and that changes in selectivity varied with the type of solute. High temperatures improved the column efficiency by 30%, mainly by accelerating the *solute diffusion rate* in the stationary phase. Finally, the PBD-coated zirconia phase was very stable at a temperature of 100°C for at least 7000 column volumes.

43. J.W. Li and P.W. Carr, “Evaluation of Temperature Effects on Selectivity in RPLC Separations Using Polybutadiene-Coated Zirconia,” *Anal. Chem.*, **69(11)**, 2202-2206 (1997).

    Abstract: The effect of temperature on selectivity on RPLC method development has been evaluated on polybutadiene-coated zirconia. We find that the influence of temperature on selectivity depends strongly on solute type. For solutes of similar structure such as polyaromatic hydrocarbons, temperature has almost no effect on selectivity; however, for solutes with very different functional groups such as chlorophenols, temperature changes did significantly affect selectivity. *We feel that simple mixtures with one dominant retention mechanism e.g., solvophobic retention – will not be helped appreciably by adjusting temperature. However, in complex mixtures with polar and ionizable solutes, optimization by varying the temperature may well be fruitful.*

44. J.W. Li, Y. Hu, and P.W. Carr, “Fast Separations at Elevated Temperatures on Polybutadiene-Coated Zirconia Reversed-Phase Material,” *Anal. Chem.* **69,** 3884-8 (1997).

    Abstract: Ion-exchange chromatography is a major method used for large-scale protein separations. New zirconia-based polymeric cation-exchange HPLC stationary phases have been developed for protein synthesis. In one method, polyethyleneimine (PEI) was adsorbed onto porous zirconia particles and cross-linked with 1,4-butanediol diglycidyl ether (BUDGE). Succinic anhydride was then reacted with the remaining primary and secondary amine groups on PEI to afford anionic functionalities. The second method utilizes poly(acrylic acid) anhydride as both the cross-linker and the stationary phase. The resulting stationary phases act to separate proteins by a weak cation-exchange mechanism with a slight contribution to retention from hydrophobic interactions. In the presence of 20 mM phosphate buffer, Lewis acid/base interactions between the zirconia support and the proteins, which can significantly broaden the peaks, are sufficiently suppressed. The effects of ionic strength, mobile phase pH, and salt type are discussed. Proteins mass recovery and loading capacity for protein separations on these phases have no limitation on the type of salt used as the eluent, and they exhibit unique selectivities. Therefore, they offer interesting alternatives for protein separations. To our knowledge, this work represents the first successful examples of protein separations using porous zirconia-based polymeric phases under normal chromatographic conditions, which will definitely help make zirconia-based supports more useful for bioseparations.

57a. T. Scott Kephart and Purnendu K. Dasgupta, "Hot Eluent Capillary Liquid Chromatography Using Zirconia and Titania Based Stationary Phases," *Analytica Chimica Acta* **414**, 71-78 (2000).

Abstract: High speed capillary liquid chromatographic separations using a simple home made system constructed from readily available inexpensive components have been studied. Using thermally stable zirconia and titania based packing, the separation of eight alkylbenzene derivatives in less than 2 min is shown. The system is capable of operating at temperatures as high as 200°C and pressures up to 10,000 psi and can, thus, take advantage of the reduction in eluent viscosity and enhanced mass transport at elevated temperatures to operate at very high linear velocities without sacrificing separation efficiency.

58. Y. Mao and P.W. Carr, “Adjusting Selectivity in Liquid Chromatography by Use of the Thermally Tuned Tandem Column Concept,” *Anal. Chem.* **72**, 110-118 (2000).

Abstract: In this study, we propose the novel “thermally tuned tandem column (T^3C)” concept for the optimization of selectivity in LC by continuous adjustment of the stationary phase. Two columns with distinctly different chromatographic selectivities (e.g., polybutadiene- and carbon-coated zirconia) are serially coupled and *independently* temperature-controlled. Selectivity is “tuned” by adjusting the individual temperatures of the two columns. The effect of changing column temperature is quite analogous to changing the relative column lengths, thereby altering the relative and absolute contribution each column makes to the overall retention time in T^3C. The distinct selectivity differences between polybutadiene- and carbon-coated zirconia as well as the extraordinary thermal stability of zirconia-based phases (thermally stable to 200º C) allow us to tune the overall chromatographic selectivity over a very substantial range. We have developed a simplified useful model, which characterizes retention and selectivity for the T^3C system as a function of the two column temperatures. The model is in good agreement with the experimental results. We also describe a simple computer-assisted optimization strategy based on the window diagram method, which facilitates the optimization of the T^3C system with only four or five initial runs.

60. B. Yan, J. Zhao, J.S. Brown, J. Blackwell and P.W. Carr, “High-Temperature Ultrafast Liquid Chromatography,” *Anal. Chem.* **72**, 1253-1262 (2000).

Abstract: A novel liquid chromatographic system which enables high temperature ultrafast liquid chromatography (HTU-FLC) has been designed through the careful consideration of heat transfer, band broadening, and pressure drop. Studies of the effect of linear velocity on the HETP show that column efficiency at high velocity, especially of well-retained solutes, dramatically improves at higher temperatures. At 150°C, at a flow rate of 15 mL/min with a 5 cm by 4.6mm (i.d.) column packed with 3 micron polystyrene-coated zirconia porous particles, long chain alkylphenones were completely resolved, and the analysis time could be decreased by a factor of 50 compared to that at room temperature (25°C) at a conventional flow rate (4 mL/min). In addition, using pure water as the mobile phase, five phenols were separated in less than 30 s.

61. Y. Mao and P.W. Carr, “Application of the Thermally Tuned Tandem Column Concept to the Separation of Several Families of Environmental Toxicants,” *Anal. Chem.*, **72**, 2788-2796 (2000).

Abstract: Separations of several families of environmental toxicants were optimized by means of the thermally tuned tandem column (T^3C) concept. We use a tandem combination of an octadecylsilane (ODS) and a carbon-coated zirconia (C-ZrO_2) column; and tune the selectivity by independently adjusting the isothermal temperatures of the two columns. This results in the change in the contribution that each column makes to the overall retention and selectivity. The separation was optimized by locating the optimum pair of column temperatures which give the best separation of the critical solute pair. For both triazine herbicides and carbamate pesticides samples, dramatically different selectivities and different critical pairs were observed for the two types of phases. Although neither individual phase gave adequate separation, the T^3C approach provided baseline separations using only four preliminary trial separations. We also showed that, for the triazine samples, the T^3C approach gave a better separation than did

conventional mobile phase optimization with an ODS column. The combination of superior selectivity of T^3C and high flow rate allows the baseline separation of complex mixtures in just a few minutes.

64. C.B. Castells, P.W. Carr, "A Study of Thermodynamics and Influence of Temperature of Chiral High-Performance Liquid Chromatographic Separations Using Cellulose *tris*(3,5-dimethylphenylcarbamate) Coated Zirconia Stationary Phases," *Chromatographia*, **52(9-10)**, 535-542 (2000).

    Abstract: In chiral HPLC, the separation is based on the differential interaction of a pair of enantiomeric molecules with a chiral selector. Temperature will affect such interactions. Most studies indicate that a decrease in temperature increase chromatographic selectivity. This is consistent with an enthalpy-controlled separation, but a more complete characterization of the physicochemical interactions is required to understand the driving forces for chiral recognition. In this work, we studied the separation of a number of enantiomers on cellulose *tris*(3,5-dimethylphenylcarbamate) supported on porous zirconia, over the temperature range of 0 to 55°C using *n*-hexanes/2-propanol mixtures as the eluent. The differences in the enthalpy (delta(deltaH°)) and entropy (delta(deltaS°)) of transfer of the enantiomers from the mobile to the chiral stationary phase were estimated from the van't Hoff plots. These relationships allow the study of the origin of the differences in interaction energies. The most interesting findings is that while most solutes show a negative delta(deltaH°) difference, the two most easily resolved enantiomeric pairs were separated by an entropy dominated process. Studies of the relationship between the thermodynamics of transfer of these two entropically controlled separations and the eluent composition showed a substantial change in the interaction energies of these two solutes with the chiral polymer when the alcohol was reduced by 2% (*v/v*). Finally, we show that there is virtually no correlation between delta(deltaG°) and overall retention, between delta(deltaH°) and deltaH°, and little or no enthalpy-entropy compensation. These findings indicate the extreme difficulty in predicting or even correlating chiral selectivity with overall intermolecular interactions.

65. C. McNeff, L. Zigan, K. Johnson, P.W. Carr, A. Wang, A.M. Weber-Main, "Analytical Advantages of Highly Stable Stationary Phases for Reversed-Phase LC," *LCGC*, **18(5)**, 514-529 (2000).

    Abstract: During the past five years, many manufacturers of high performance liquid chromatography (HPLC) columns have focused on improving stationary-phase stability and reproducibility. Improved column stability – both chemical and thermal – offers new advantages, such as decreased analysis time and new methods of selectivity optimization. More stable HPLC packing materials have been achieved through advances in silane chemistry; however, the greatest improvements in stability have resulted from the use of alternative nonsilica supports such as synthetic organic polymers, alumina, and zirconia. In this article, the authors describe their use of various test solutes to compare the efficiency, selectivity, and hydrophobic retention mechanisms of five commercially available HPLC columns based on silica, alumina, zirconia, and polystyrene cross-linked with divinlybenzene (PS-DVB).

67. Y. Mao and P.W. Carr, "Separation of Barbiturates and Phenylthiohydantoin Amino Acids Using the Thermally Tuned Tandem Column Concept," *Anal. Chem.*, **73**, 1821-1830 (2001).

    Abstract: There are many more choices of column type than of eluent type for method development in reversed-phase liquid chromatography. It is common to switch between different column types or between the same type from different suppliers to achieve the desired separations. The key difficulty in modulation band spacing by adjusting the column type is that it is a discontinuous, "hit or miss" proposition. The thermally tuned tandem column (T^3C) concept effectively solves this problem by connecting two columns in series and independently controlling the two column temperatures. The columns are chosen to have distinctively different chromatographic selectivities (band spacing), so that the unresolved peaks on one column are separated by the other. The optimized separation in the T^3C is achieved by simultaneously tuning the two column temperatures. In this study, we used the T^3C combination of a carbon and a conventional bonded phase for separation of barbiturates and phenylthiohydantoin amino acids (PTH-amino acids). Good peak shapes and comparable retention times were observed on the two phases at room temperature. The selectivities on the two phases were quite different. Baseline separations were easily achieved with the T^3C set although neither column could individually resolve all the peaks. We further compared the separation of barbiturates optimized by the T^3C approach with that optimized by adjusting the mobile phase. We found that T^3C gave a better separation. We believe that the T^3C combination of a carbon phase and a bonded conventional reversed-phase material provides a powerful and general method to optimize the separation of various mixtures.

71. Christopher J. Dunlap, Peter W. Carr, Clayton V. McNeff, Dwight Stoll, "Zirconia Stationary Phases for Extreme Separations," *Anal. Chem.*, 598A-607A (2001).

Abstract: Composite materials surpass standard silicon and carbon for stability and robustness in complicated reversed-phase column applications. (General review paper)

71a. Yun Mao and Peter W. Carr, "Separation of Selected Basic Pharmaceuticals by Reversed-Phase and Ion-Exchange Chromatography Using Thermally Tuned Tandem Columns," *Anal. Chemistry*; **73**(18); 4478-4485 (2001).

Abstract: The separation of basic pharmaceuticals is usually performed on C8 or C18 bonded silica supports. Silanolphilic interactions between basic analytes and surface silanol groups often lead to tailed peaks, poor efficiency, and irreproducible retention times. To solve these problems, many new types of silica-, zirconia-, and polymer based columns, which provide unique selectivities, improved stability at high pH, or both, have been developed for the analysis of basic compounds. The essence of method development for the chromatographic analysis of basic compounds is to choose a system in which the band spacing can be varied dramatically, quickly, and conveniently while minimizing the tailing due to silanolphilic interactions. The thermally tuned tandem column (T3C) approach has been shown to provide an effective way to adjust stationary-phase selectivity for nonionic compounds. In this study, a tandem combination of an octadecylsilane (ODS) and a polybutadiene-coated zirconia (PBD-ZrO2) phase was used to separate nine antihistamines. Selectivity is tuned by independently adjusting the isothermal temperatures of the two columns. We found dramatic differences in the retention factors, elution sequences, and band spacing for the above set of basic drugs on the two types of columns. The T3C model has been used successfully to locate the optimal temperatures based on only four exploratory runs. The nine antihistamines were baseline separated on the tandem column combination even though they could not be separated on the individual phases. The effect of the buffer concentration on retention of the basic antihistamines was also studied. We conclude that the cation-exchange interactions predominate on the PBD-ZrO2 phase, while reversed phase interactions are more important on the ODS phase. Interestingly, an increase in column temperature causes a significant increase in the retention on the ODS column and a decrease of retention on the PBD-ZrO2 column. This can be explained by the change in the analyte's degree of ionization with temperature. The T3C combination of silica- and zirconia-based RPLC columns is demonstrated to be a powerful approach for the separation of the mixture of basic analytes.

71b. Steven M. Fields, Christine Q. Ye, Dee Dee Zhang, B. Russell Branch, X. Jason Zhang, Ngozi Okafo, "Superheated Water as Eluent in High-temperature High-performance Liquid Chromatographic Separations of Steroids on a Polymer-coated Zirconia Column," *Journal of Chromatography A*, **913**, 197 - 204 (2001).

Abstract: High-temperature liquid chromatography (HTLC), with superheated water mobile phase, has been shown to be a feasible replacement for medium-polarity acetonitrile-water mixtures as an eluent in reversed-phase HPLC. Instrumental parameters of flow-rate, injection volume and mobile phase preheating were shown to have significant effects on the quality of the chromatographic peaks. The selectivity and retention patterns of testosterone and several related compounds were investigated on a porous zirconia, polybutadiene-coated column at temperatures up to 200°C and compared with that of porous silica, octadecylsilane-coated column and the zirconia column under traditional reversed-phase conditions of an acetonitrile-water mobile phase at 40°C. The selectivity differences observed for testosterone and related compounds show that the separation mechanisms are complementary and unique selectivity is obtained with the zirconia column under HTLC conditions.

72. Jonathan D. Thompson and Peter W. Carr, "A Study of the Critical Criteria for Analyte Stability in High-Temperature Liquid Chromatography," *Anal. Chem.*, **74**, 1017-1023 (2002).

Abstract: There are three major impediments to the use of high-temperature ultrafast liquid chromatography. First, the stationary phase must be thermally stable. Over the past decade, a series of thermally stable, highly efficient stationary phases have been developed that can withstand temperatures exceeding 200ºC. Second, the temperature mismatch between the incoming eluent and the column must be minimized (<5ºC), because such a mismatch is a very serious cause of peak broadening, especially in ultrafast separations. The thermal mismatch problem can be significantly ameliorated at high column linear velocities by using narrow-bore columns (2.1-mm i.d.). Third, analytes that are exposed to high temperatures must be thermally stable *on the time scale of the chromatographic run*. We report here a study of the ability of several pharmaceuticals to withstand super ambient temperatures on the time scale of fast separations. We propose criteria by which a particular analyte may be rejected as a candidate for high-

temperature analysis, and we demonstrate that complex molecules are amenable to quantitation, even at temperatures in excess of 100ºC in an aqueous media. We also show that as the time an analyte spends on a hot column decreases, the extent of on-column reactions decreases for those analytes that do react. Although the seminal work of Antia and Horvath addresses these issues from a theoretical perspective, we hope to further alleviate fear of high temperatures in liquid chromatography through the empirical approach used here.

77a. T.S. Kephart, P.K. Dasgupta, "Superheated Water Eluent Capillary Liquid Chromatography," *Talanta*, **56**, 977-987 (2002).

Abstract: A capillary scale reverse phase liquid chromatography (LC) system using a super hot water eluent is described. The system, constructed in-house from readily available components, has been shown to operate at temperatures as high as 370 °C and pressures in excess of 10,000 psi. The capability of the system is demonstrated with the separation of a mixture of polar and non-polar benzene derivatives on polybutadiene and elemental carbon modified zirconia packings with or without temperature gradients. Six benzene derivatives can be separated in ~ 2 min.

78. Y. Xiang, B. Yan, B. Yue, C.V. McNeff, P.W. Carr, M.L. Lee, "Elevated-temperature Ultrahigh-pressure Liquid Chromatography Using Very Small Polybutadiene-coated Nonporous Zirconia Particles," *Journal of Chromatography A*, **983**, 83-89 (2003).

Abstract: Capillary columns packed with small diameter particles typically lead to low permeability and long separation times in high-performance liquid chromatography. Ultrahigh pressures (>10000 p.s.i.; 1 p.s.i. =6894.76 Pa) can be used to overcome the limitations that small particles impose. Ultrahigh-pressure liquid chromatography (UHPLC) has demonstrated great potential for high-speed and high-efficiency separations. Decreasing the viscosity of the mobile phase by elevating the temperature could additionally reduce the pressure drop and facilitate the use of longer columns or smaller particles to achieve even higher total plate numbers. For this reason, we investigated the use of elevated temperatures in UHPLC. Water-resistant, flexible heater tape covered with insulation was used to provide the desired heat to the column. Polybutadiene-coated 1 um nonporous zirconia particles were used because of their chemical stability at elevated temperature. A column efficiency as high as 420,000 plates m-1 was obtained. The effects of temperature and pressure on the separation of parabens were investigated. Separation of five herbicides was completed in 60 s using 26,000 p.s.i. and 90 °C.

79a. P. He, Yu Yang, "Studies on the Long-Term Thermal Stability of Stationary Phases in Subcritical Water Chromatography", *Journal of Chromatography A*, **989**, 55-63 (2003).

Abstract: The long-term thermal stability of five commercially available reversed-phase columns has been evaluated under subcritical water conditions (100 and 150°C). The five columns included Zorbax RX-C8, Nucleosil C18 AB, Hypersil BDS C18, PRP-1 (poly(styrene-divinylbenzene)), and ZirChrom-PS (polystyrene) columns. Retention factors, plate numbers, and peak areas were monitored over a prolonged period of time. Comparing the three silica-based columns, the Zorbax RX-C8 column was the most stable followed by the Nucleosil C18 AB column. The Hypersil BDS C18 column was the least stable under subcritical water conditions. The ZirChrom-PS column was stable at 100 °C for at least 7600 column volumes. Of all five columns, the polymeric PRP-1 column was the most stable under subcritical water conditions.

80. Y. Mao and P.W. Carr, "The Thermally Tuned Tandem Column Approach to Optimizing Selectivity in HPLC," *LCGC North America*, **21**(**2**), 150-167 (2003).

Abstract: The authors describe the thermally tuned tandem column concept for the optimization of selectivity in liquid chromatography by effectively continuously adjusting the stationary phase. They serially connected two columns with distinctly different chromatographic selectivities (band spacings) and used independent temperature control for the columns. They tuned the overall selectivity of the tandem column set by individually adjusting the two column temperatures. The authors compare thermally tuned tandem column separations with some single-column optimization strategies, including the variation of temperature, eluent type, eluent composition, and simultaneously varying the temperature and eluent composition.

82. Y. Xiang, B. Yan, B. Yue, C.V. McNeff, P.W. Carr, M.L. Lee, "Synthesis of Micron Diameter Polybutadiene-encapsulated Non-porous Zirconia Particles for Ultrahigh Pressure Liquid Chromatography," *Journal of Chromatography A*, **1002**, 71-78 (2003).

    Abstract: In this study, 1-µm diameter polybutadiene-encapsulated non-porous zirconia particles were synthesized, slurry packed into 50-µm I.D. fused-silica capillary columns, and evaluated using ultrahigh pressure liquid chromatography. The dependencies of column efficiency and solute retention factor on pressure were investigated. Efficiencies as high as 280 000 plates per meter were obtained for the separation of anti-inflammatory drugs at a pressure of 1351 MPa. Comparing the reversed-phase behavior of the polybutadiene-encapsulated non-porous zirconia with octadecylsilane bonded non-porous silica, greater selectivity was found using the zirconia-based material for the applications reported in this study. The encapsulated non-porous zirconia particles demonstrated excellent thermal stability in the separation of polycyclic aromatic hydrocarbons at a temperature of 100 °C and a pressure of 1351 MPa.

84. T. Andersen, Quynh-Nhu T. Nguyen, R. Trones, T. Greibrokk, "Mesoporous Polybutadiene-Modified Zirconia for High-Temperature Packed Capillary Liquid Chromatography: Column Preparation and Temperature Programming Stability," *Journal of Chromatography A*, **1018**, 7-18 (2003).

    Abstract: In the present study, three different methods for packing of 3 µm PBD-ZrO2 particles in 0.5 mm i.d. glass-lined stainless steel columns have been examined. The two first methods were based on a traditional downstream high-pressure technique using tetrachloromethane (Method I) or aqueous Triton X-100 (Method II) as slurry solvents, while Method III was an upstream high-pressure flocculating method with stirring, using isopropanol both as the slurry and packing solvent. Method I was found to be superior in terms of efficiency, producing 0.5 mm i.d. x 10 cm columns with almost 90,000 plates m-1 for toluene (R.S.D.= 8.7%, n = 3), using a slurry concentration of 600 mg ml-1, ACN-water (50:50 (v/v)) as the packing solvent and a packing pressure of 650 bars. For Method I, the slurry concentration, column i.d., column length and initial packing pressure were found to have a significant effect on column efficiency. Finally, the long-term temperature stability of the prepared columns was investigated. In isothermal mode, using ACN-20 mM phosphate buffer, pH 7 (50:50 (v/v)) as the mobile phase, the columns were found to be stable for at least 3,000 void volumes at 100°C. At this temperature, the solute efficiencies changed about 5-18% and the retention factors changed about 6-8%. In temperature programming mode (not exceeding 100°C), on the other hand, a rapid decrease in both column efficiency and retention factors was observed. However, when the columns were packed as initially described, ramped up and down from 50 to 100°C for 48 h and refilled, fairly stable columns with acceptable efficiencies were obtained. Although not fully regaining their initial efficiency after refilling, the solute efficiencies changed about 19-28% (32-37%) and the retention factors changed about 4-5% (13-17%) after running 3,000 (25,000) void volumes or 500 (3,900) temperature programs.

84a. L.J. Lamm, Yu Yang, "Off-Line Coupling of Subcritical Water Extraction with Subcritical Water Chromatography via a Sorbent Trap and Thermal Desorption", *Analytical Chemistry*, **75**, 2237-2242 (2003).

    Abstract: In this study, the off-line coupling of subcritical water extraction (SBWE) with subcritical water chromatography (SBWC) was achieved using a sorbent trap and thermal desorption. The sorbent trap was employed to collect the extracted analytes during subcritical water extraction. After the extraction, the trap was connected to the sub-critical water chromatography system, and thermal desorption of the trapped analytes was performed before the SBWC run. The thermally desorbed analytes were then introduced into the subcritical water separation column and detected by a UV detector. Anilines and phenols were extracted from sand and analyzed using this off-line coupling technique. Subcritical water extraction of flavones from orange peel followed by subcritical water chromatographic separation was also investigated. The effects of water volume and extraction temperature on flavone recovery were determined. Because a sorbent trap was used to collect the extracted analytes, the sensitivity of this technique was greatly enhanced as compared to that of subcritical water extraction with solvent trapping. Since no organic solvent-water extractions were necessary prior to analysis, this technique eliminated any use of organic solvents in both extraction and chromatography processes.

88. J. Nawrocki, C. Dunlap, J. Li, J. Zhao, C.V. McNeff, A. McCormick, P.W. Carr, "Part II. Chromatography Using Ultra-Stable Metal Oxide-Based Stationary Phases for HPLC," *Journal of Chromatography A*, **1028**, 31-62 (2004).

    Abstract: In this part of the review authors discuss methods used for modification of metal oxide surfaces. On the basis of literature data it is shown, that silanization of the surfaces do not form stable supports for chromatography. On the other hand, the success of polymer modified surfaces such as polybutadiene (PBD) and polystyrene (PS) is emphasized. Permanent modification of metal oxide surfaces with Lewis bases is also widely discussed. Chromatographic properties of polymer-modified surfaces of zirconia are discussed in details. The perspectives of carbon-coated metal oxide surfaces in HPLC and high temperature separations are described.

89b. M.M Sanagi, H.H. See, W.A. Ibrahim, A.A. Naim, "High Temperature Liquid Chromatography of Triazole Fungicides on Polybutadiene-coated Zirconia Stationary Phase," *Journal of Chromatography A*, **1059**, 95-101 (2004).

> Abstract: High temperature liquid chromatography using water-rich and superheated water eluent is evaluated as a new approach for the separation of selected triazole fungicides, hexaconazole, tebuconazole, propiconazole, and difenoconazole. Using a polybutadiene-coated zirconia column at temperatures of 100-150 degrees C, clear separations were achieved when 100% purified water was utilized as organic-free eluent. Excellent limits of detection down to pg level were obtained for the separation of the triazole fungicides under optimum conditions. Van't Hoff plots for the separations were linear suggesting that no changes occurred in the retention mechanism over the temperature range studied.

89c. H.A. Claessens and M.A. van Straten, "Review on the Chemical and Thermal Stability of Stationary Phases for Reversed-phase Liquid Chromatography," *Journal of Chromatography A*, **1060(1-2)**, 23-41 (2004).

> Abstract: At present, in high-performance liquid chromatography (HPLC) for the majority of analyses, reversed-phase liquid chromatography (RPLC) is the separation mode of choice. Faster method development procedures using aggressive eluents under elevated temperature conditions, the need for improved selectivities, efficiencies and resolution, the reduction of solvent consumption and also the decrease of analysis times require reversed-phase (RP) columns of high chemical and thermal stability. Until now, the majority of columns for RPLC separations are manufactured from silica substrates. Silica has many favorable properties making this material nearly ideal as a support for RP columns. However, its solubility, that increases considerably in eluents of pH above ±7, is a drawback preventing its widespread use over the entire pH range. In addition, also the thermal stability of silica is limited. Recently, however, substantial progress has been made in the synthesis of RPLC silica-based stationary phases showing satisfactory thermal and chemical stability under many different experimental conditions. Also, new substrates mainly based on other inorganic substrates like, e.g. alumina and zirconia have been developed now as a starting material for the preparation of RPLC stationary phases of improved chemical and thermal stability. In addition, for the same reasons, many efforts have also been made to synthesize polymer and also polymer-coated phases. These latter phases, more particularly those based on zirconia, but also polymer phases show a high degree of chemical and thermal stability compared to silica counterparts. In this paper, an overview will be given of the state-of-the-art of the thermal and chemical stability of the different available stationary phases for RPLC.

91. D.R. Stoll, P.W. Carr, "Fast, Comprehensive Two-Dimensional HPLC Separation of Tryptic Peptides Based on High-Temperature HPLC," *J. Am. Chem. Soc.*, **127**, 5034 (2005).

> Abstract: The dramatic improvement in the speed of reversed-phase gradient elution through the use of high-temperature HPLC and modest instrument modifications has led to a significant improvement in the performance of 2D-LC. Further optimization of this system will lead to improvements in the peak capacity productivity and make 2D high-temperature HPLC a practical approach for the analysis of complex biological mixtures.

93. M.M. Sanagi, H.H. See, W.A.W. Ibrahim, A.A. Naim, "High Temperature Liquid Chromatography of Tocol-Derivatives on Polybutadiene-Coated Zirconia Stationary Phases," *Chromatographia*, **61(11/12)**, 567-571 (2005).

> Abstract: High temperature Reversed-phase high performance liquid chromatography is evaluated as a new approach for the separation of vitamin E isomers (alpha-, gamma-, delta-tocopherol, and alpha-, gamma-,delta-tocotrienol) and alpa-tocopherol acetate. The separations of each analyte were examined by varying the eluent composition and column temperature. Using a polybutadiene-coated zirconia column at temperatures of 80 degrees C to 140 degrees C, complete separations were achieved within 10-20 min using organic modifier (acetonitrile) in the range of 40-50%. Irregularity of the Van't Hoff analysis was noted over the temperature studied. The plot tends to deviate from linearity at higher operating

temperatures (140 degrees C and 150 degrees C) with linear deviation errors of 12.7% and 41.6%, respectively, for the mobile phase examined.

95d. G. Vanhoenacker and P. Sandra, “High Temperature Liquid Chromatography and Liquid Chromatography–mass Spectroscopy Analysis of Octylphenol Ethoxylates on Different Stationary Phases,” *Journal of Chromatography A*, **1082(2)**, 193-202 (2005).

Abstract: Temperature was investigated as active parameter in the liquid chromatography (LC) analysis of octylphenol ethoxylates. Significant differences in selectivity were observed when the oligomers were analyzed by reversed phase LC (RPLC) on silica-, zirconia- and polystyrene/divinylbenzene based stationary phases at low (ambient), medium and elevated temperature with acetonitrile/water as mobile phase. As ascertained by LC–mass spectroscopy (MS), in most cases the elution order of the oligomers was completely reversed comparing ambient and high temperature separations. On a graphitized carbon type column, the selectivity remained unchanged, regardless the analysis temperature. Also in normal phase LC, the elution order remained unaffected by temperature variations both for acetonitrile/water and methanol/water mixtures as mobile phase. Surprisingly, when reversed phase LC on a octadecylsilicagel column at different temperatures was repeated with methanol instead of acetonitrile as mobile phase ingredient, the reversal of elution order did not take place. Results are evaluated in terms of thermodynamic parameters.

97a. T. Teutenberg, H.-J. Goetze, J. Tuerk, J. Ploeger, T.K. Kiffmeyer, K.G. Schmidt, W. gr. Kohorst, T. Rohe, H.-D. Jansen and H. Weber, “Development and Application of a Specially Designed Heating System for Temperature-programmed High-performance Liquid Chromatography Using Subcritical Water as the Mobile Phase,” *Journal of Chromatography A*, **1114(1)**, 89-96 (2006).

Abstract: A specially designed heating system for temperature-programmed HPLC was developed based on experimental measurements of eluent temperature inside a stainless steel capillary using a very thin thermocouple. The heating system can be operated at temperatures up to 225 °C and consists of a preheating, a column heating and a cooling unit. Fast cycle times after a temperature gradient can be realized by an internal silicone oil bath which cools down the preheating and column heating unit. Long-term thermal stability of a polybutadiene-coated zirconium dioxide column has been evaluated using a tubular oven in which the column was placed. The packing material was stable after 50 h of operation at 185 °C. A mixture containing four steroids was separated at ambient conditions using a mobile phase of 25% acetonitrile:75% deionized water and a mobile phase of pure deionized water at 185 °C using the specially designed heating system and the PBD column. Analysis time could be drastically reduced from 17 min at ambient conditions and a flow rate of 1 mL/min to only 1.2 min at 185 °C and a flow rate of 5 mL/min. At these extreme conditions, no thermal mismatch was observed and peaks were not distorted, thus underlining the performance of the developed heating system. Temperature programming was performed by separating cytostatic and antibiotic drugs with a temperature gradient using only water as the mobile phase. In contrast to an isocratic elution of this mixture at room temperature, overall analysis time could be reduced two-fold from 20 to 10 min.

98. D.R. Stoll, J.D. Cohen, P.W. Carr, "Fast, Comprehensive Online Two-dimensional High Performance Liquid Chromatography through the use of High Temperature Ultra-fast Gradient Elution Reversed-phase Liquid Chromatography," *Journal of Chromatography A*, **1122**, 123-137 (2006).

Abstract: A new approach to high speed, comprehensive online dual gradient elution 2DLC (LCxLC) based on the use of ultra-fast, high temperature gradient elution reversed phase chromatography is described. Entirely conventional gradient elution instrumentation and columns are assembled in a system which develops a total peak capacity of about 900 in 25min; this is equivalent to roughly one peak/2s. Each second dimension gradient is done in a cycle time of 21s and the peak retention times measured for a set of twenty six indole-3-acetic acid (IAA) derivatives are reproducible to 0.2s. Each peak eluting from the first dimension column is sampled at least twice across its width, as the corresponding peaks on the second dimension column appear in two or three consecutive second dimension chromatograms, clearly indicating that there is little loss in the resolution gained in the first dimension separation. Application to the separation of the low molecular weight components of wild-type and mutant maize seedlings indicates the presence of about 100 peaks on a timescale of 25min. Compelling illustrations of the analytical potential of fast, high temperature 2DLC are evident in the clear presence of nine distinct peaks in a single second dimension chromatogram from a single quite narrow first dimension peak, and the great power of 2DLC to solve the "analytic dynamic range" problem inherent in the measurement of small peaks that are neighbors to a gigantic peak.

98b. F. Cacciola, P. Jandera, E. Blahová, L. Mondello, "Development of Different Comprehensive Two Dimensional Systems for the Separation of Phenolic Antioxidants," *Journal of Separation Science*, **29(16)**, 2500-2513 (2006).

Abstract: Three different comprehensive 2-D HPLC systems for the separation of phenolic antioxidants have been developed on the basis of different selectivities of a PEG-silica column in the first dimension and a packed or monolithic C18 or a ZR-CARBON column, respectively, in the second dimension. Two-dimensional comprehensive liquid chromatography using a serially connected short PEG-silica column and a conventional C18-silica or a ZR-CARBON column in the second dimension was tested to improve the resolution of the earlier eluting compounds in the first dimension. Various types of interface were used to connect the columns in the first and in the second dimension: i) two injection sampling loops of 100 L in conventional arrangement; ii) a 10-port 2-position valve equipped with two trapping X-Terra columns instead of loops; and iii) two analytical D2 columns in parallel. The mobile phase in the first dimension has a lower elution strength than in the second dimension, allowing band compression of the solutes transferred from the first to the second dimension. This effect was enhanced using trapping columns instead of sampling loops as the interface between the two dimensions, thus allowing a decrease in the time of analysis. These systems were used for the analysis of beer samples. The relative location of the components in the 2-D retention plane varied in relation to their chemical structure in each instrumental set-up and allowed positive peak identification.

105.C.V. McNeff, B. Yan, D.R. Stoll, R.A. Henry, "Review: Practice and Theory of High Temperature Liquid Chromatography," *Journal of Separation Science*, **30**, 1672-1685 (2007).

Abstract: High temperature liquid chromatography (HTLC) exists in a temperature region beyond ambient (ca. 40 C) and below super critical temperatures. The promises of HTLC, such as increased analysis speed, enhanced separation productivity, "green" LC with pure water mobile phases coupled to universal FID detection, and fast analysis of complex samples by combination with fast 2-D techniques, have become an option for routine practice. The focus of this paper is to review the key developments that have made the application of HTLC a practical technique and draw attention to new developments in 2-D techniques that incorporate HTLC that offer an opportunity to vastly increase the usefulness of HPLC for the analysis of complex samples.

106. P. Dugo, K. Buonasera, M. Crupi, F. Cacciola, G. Dugo, L. Mondello, " Superheated Water as Chromatographic Eluent for Parabens Separation on Octadecyl Coated Zirconia Stationary Phase," *J. Sep Sci*, **30**, 1125-1130 (2007).

Abstract: In this study, the use of pure water at superheated temperatures, between 100 and 200°C, as a mobile phase for RP separation is explored. Instrumental parameters, such as temperature, flow rate, preheating and cooling, have shown significant effects on the quality of the chromatographic peaks. The properties of superheated water as an eluent were investigated by observing the chromatographic behaviour of four parabens on a carbon-clad zirconia (ZR) phase with covalently bonded octadecyl groups. Results were compared with those obtained at 308C on a silica-based phase with octadecyl groups, using water and ACN as mobile phase. The optimized method was finally applied to analyse parabens in a commercial body cream.

107. F. Cacciola, P. Jandera, L. Mondello, "Temperature Effects on Separation on Zirconia Columns: Applications to One- and Two-Dimensional LC Separations of Phenolic Antioxidants," *J. Sep. Sci.*, **30**, 462-474 (2007).

Abstract: The effects of temperature and mobile phase on LC chromatographic separation of phenolic antioxidants on zirconia-based columns were investigated. Unlike silicabased materials, zirconia columns show excellent thermal stability over a wide range of temperatures and enable high-temperature separations. Enthalpic and entropic contributions to the retention of phenolic compounds on ZR-Carbon and ZR-Carbon C18 columns were determined from retention versus temperature plots in order to elucidate the retention mechanism of sample compounds over the temperature range up to 1408C. High-temperature liquid chromatography on ZR-Carbon columns was used for comprehensive LC6LC two-dimensional separation systems based on the different selectivity of a Zorbax SB micro-column used in the first dimension and a ZR-Carbon column used in the second dimension. Two-dimensional

LC6LC systems employing a setup with two alternately operated parallel ZRCarbon columns in the second dimension were used for the analysis of phenolic antioxidants in beer and wine samples.

113. P. Skrdla, A. Bopra, T. Chasse, T. Wang, "A High-Temperature Liquid Chromatographic Reactor Approach for Investigating The Solvolytic Stability of a Pharmaceutical Compound and an Investigation of its Retention Behavior on a C18-Modified Zirconia Stationary Phase," *Journal of Pharmaceutical and Biomedical Analysis*, **47**, 312-319 (2008). DIAMONDBOND-C18 HIGH TEMP

> Abstract: The solvolysis kinetics of a developmental active pharmaceutical ingredient (API) were investigated using a high temperature (HT)-HPLC reactor approach to determine whether it might be possible to use the technique to efficiently screen the relative stabilities of typical APIs (particularly those that are stable at the column temperatures achievable on most HPLC systems and over durations of less than 60 min—a reasonable upper limit for typical method run time). It was discovered that the on-column API degradation kinetics better obeyed a second-order model than a first-order one. Employing a newly developed mathematical treatment, the apparent activation energy for the process was determined to be 85.7±1.6 kJ/mol; the apparent frequency factor was found to be (3.9±0.4)×104 s−1. The retention mechanism of the API on the C18-modified zirconia column (ZirChrom® DiamondbondTM-C18) was investigated using a van't Hoff analysis. It was discovered that the logarithm of the retention factor (following correction for the gradient elution of the assay method) exhibited a quadratic dependence on the reciprocal of the absolute temperature. While the retention was found to be predominantly enthalpically driven over the majority of temperatures investigated in this study (ranging from 40 to 200 ◦C), a regression fit of the curve predicted a maximum at □20 ◦C, indicative of a transition from predominantly enthalpically controlled retention to a mainly entropically driven mechanism. A table summarizing the thermodynamic retention parameters at each experimental column temperature is provided. Finally, the preliminary application of the HT-HPLC reactor approach to the study of degradation kinetics of other APIs is discussed in terms of some unexpected findings obtained using the zirconia column.

115. G. Vanhoenacker, P. Sandra, "High Temperature and Temperature Programmed HPLC: Possibilities and Limitations," *Anal Bioanal Chem*, 390, 245 – 258 (2008).

> Abstract: Temperature plays an important role in high-performance liquid chromatography (HPLC) since both the kinetics and thermodynamics of the chromatographic process are a function of temperature. To date, the possibilities of using temperature to improve LC separations have not been fully investigated. Most researchers mainly focus on column chemistry and dimensions, and on mobile phase composition and pH to optimize their separation. Temperature is rarely examined over a broad range and most separations are performed and/or optimized in the temperature region 20–50 °C. Moreover, currently a large number of liquid chromatographs are still operated at room temperature instead of using a column thermostat to control column temperature and thus retention time. We have recently reviewed the use of elevated temperature and temperature programming in HPLC [1].

117. B. Wenclawiak, S. Giegold, T. Teutenberg, "Mini-Review: High-Temperature Liquid Chromatography," *Analytical Letters*, 41, 1097-1105 (2008).

> Abstract: The influence of temperature on separation is discussed. Elevated temperature can significantly reduce the retention times in liquid chromatography and thus the throughput and running costs of a LC instrument. A decrease of viscosity at increasing temperatures allows the use of columns packed with very small particles and of higher flow rates as well. Modern reversed phase columns are stable up to (and >) 90_C. Metal oxide columns (Zirconium oxide, Titanium dioxide, Aluminium oxide) and polymer packed columns are temperature stable up to 200_C. The consumption of organic solvents can significantly be reduced and thus also the disposal costs.

126. R. Berta, M. Babjak, M. Gazdag, "A Study of Some Practical Aspects of High Temperature Liquid Chromatography in Pharmceutical Applications," *Journal of Pharmaceutical and Biomedical Analysis*, **54**, 458-462 (2011).

> Abstract: In the pharmaceutical industry fast and efficient separation techniques play an increasing role among analytical methods because the samples to be investigated grow both in complexity and number, and there is an increasing time pressure to complete the analysis. Reducing the analysis time without decreasing the efficiency is possible using higher pressures, elevated temperatures, smaller particle sizes, or a combination of these approaches. Recently developed chromatographic techniques such as the UHPLC

(ultra high performance liquid chromatography) and HTLC (high temperature liquid chromatography) are highly promising in meeting these demands. In this study, high temperature liquid chromatography (HTLC) with a zirconia-based column and temperatures elevated up to 150 ◦C was used. We investigated the chromatographic behaviour of a steroid active pharmaceutical ingredient (levonorgestrel) and its structurally related impurities as model compounds. The effect of the temperature in the range of 50–150 ◦C and the flow rate in the range of 0.5–3.0 ml/min, and using methanol as an organic modifier, were studied for optimisation of the separation method.