Review

New synthetic ways for the preparation of high-performance liquid chromatography supports

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Abstract

The latest developments and in particular important synthetic aspects for the preparation of modern HPLC supports are reviewed. In this context, the chemistry of inorganic supports based on silica, zirconia, titania or aluminum oxide as well as of organic supports based on poly(styrene–divinylbenzene), acrylates, methacrylates and other, more specialized polymers is covered. Special consideration is given to modern approaches such as sol–gel technology, molecular imprinting, perfusion chromatography, the preparation of monolithic separation media as well as to organic HPLC supports prepared by new polymer technologies such as ring-opening metathesis polymerization. Synthetic particularities relevant for the corresponding applications are outlined.   © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Reviews; HPLC supports; Silica; Zirconia; Titania; Aluminum oxide; Poly(styrene–divinylbenzene); Acrylates; Methacrylates

Contents

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1. Introduction

During recent years, separation sciences faced important developments. Starting with solid-phase extraction (SPE) in the 1980s, other new techniques, e.g., supercritical fluid extraction, (SFE), supercritical fluid chromatography (SFC) and capillary electrochromatography (CEC) have been introduced. Besides of these new analytical tools, "classical" separation techniques such as high-performance liquid chromatography (HPLC) have been further developed and optimized. New inputs from synthetic chemistry and more efficient analytical tools for their characterization significantly enhanced the quality of LC supports in terms of stability, reproducibility, selectivity and efficiency.

This review intends to cover all main aspects of synthetic chemistry relevant for the preparation of new and highly efficient HPLC supports. The main emphasis is on the developments of the last 10 years. The principle synthetic concepts and key procedures for the preparation of functionalized supports as well as a few, selected applications will be presented. If necessary, cross-references for relevant applications in other separation techniques are given. In principle, surface-functionalization of HPLC supports may be accomplished by three approaches. One entails the chemical transformation of functional groups of the carrier material located at the surface. Consequently, any functional group, which may either be monomeric or polymer-bound, is attached to the surface via covalent bonding. In this review, the term "surface derivatized supports" will be used for the description of this type of stationary phase. The second approach for changing the surface of a carrier is the simple deposition/adsorption of other elements (e.g., carbon) or (macro)molecules. The term "sur-

face modified" will be used for the description of this type of stationary phase. Finally, special consideration will be given to a third approach that consists of the preparation of functional inorganic or organic polymer supports from functionalized precursors (monomers) by modern polymerization techniques.

2. Inorganic HPLC supports

2.1. Preparation of metal oxides suitable for separation techniques

Silica, zirconia, titania and aluminum oxide represent those metal oxides that are of particular importance in separation science. The chemical approach most commonly used for the preparation of metal oxides suitable for separation techniques entails the controlled hydrolysis of metal alkoxides. One of the first synthetic protocols was elaborated by Stöber et al. [1] and is based on the hydrolysis of alkylsilicates (=tetraalkoxysilanes) in alcoholic solution under basic (NH_4OH) conditions. Particles in the range of 0.05 to 2 μm may be prepared by this procedure. Based on this approach, similar base-promoted hydrolysis reactions have been reported for aluminum oxide-, titania- and zirconia-based stationary phases [2–4]. Modern and more convenient sol–gel techniques [5,6] consist of the controlled hydrolyses of certain metal alkoxides such as Al(*O-i*-propoxide)$_3$, Si(OEt)$_4$, Zr(*O-i*-propoxide)$_4$, Ti(*O-i*-propoxide)$_4$ [7], Ti(*O-n*-butoxide)$_4$ [8,9] under neutral conditions (Fig. 1).

These systems are now well investigated in terms of pH and temperature dependence as well as kinetics. Thus, even induction times for hydrolysis may be varied in the presence of β-diketones [10].

$$Si(OEt)_4 + EtOH + NH_4OH \longrightarrow SiO_2$$

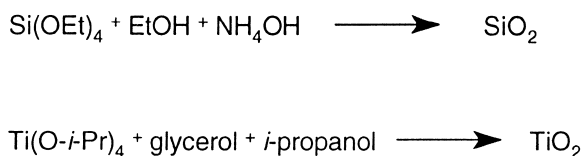$$Ti(O\text{-}i\text{-}Pr)_4 + glycerol + i\text{-}propanol \longrightarrow TiO_2$$

Fig. 1. Sol–gel process for the preparation of inorganic stationary phases.

The sol–gel process may further be modified [11] and extended to the preparation of metal oxides of particular shape. An example is the addition of tartaric acid under especially designed hydrolysis conditions, that allows the preparation of silica in form of nanotubes or worm-like materials. In cases where methyltrimethoxysilane is used instead of an tetraalkoxysilane, the resulting particles already bear a methylsilane group, which may easily be transformed into a chloromethylsilane via treatment with sulfurylchloride in CCl_4 under UV light [12]. If approximately one-third of the tetraalkoxysilane is replaced by methyltriethoxysilane, stationary phases that exhibit an enhanced pH stability (up to pH 12) are obtained. This hybrid-type of silica (XTerra) has been described as early as 1976 by Unger et al. [13] and possesses a homogeneous distribution of $Si\text{–}CH_3$ groups within the bulk material and at the surface, which significantly reduces surface dissolution. Typical values for the specific surface (σ) are around 175 m^2/g with pore diameters (d_p) of 125 Å. Surface modification of the residual surface Si–OH groups may be accomplished in the usual manner (see below). Pursch et al. [14] gave a detailed report on the use of a mixture of tetraalkoxysilanes and trialkylmonoalkoxysilanes for the preparation of reversed-phase (RP) materials. In this context, high ligand densities have been reported. A modification of the "Stöber-approach" has recently been reported by Schumacher et al. for the preparation of spherical mesoporous silica [15]. The resulting materials are a member of the MCM-48 molecular sieve family and are currently under investigation for their applicability in chromatography and related techniques. Boury et al. reported on the use of methylene-bridged bis(trimethylsilyl)diacetylenes as templates for the preparation of silica xerogels [16]. Removal of this organic template with methanolic ammonium fluoride results in the formation of micro- and mesoporous silica with narrow pore-size distributions. More recently, Imhof and Pine [17] described the preparation of titania foams from Ti($O\text{-}i$-propoxide)$_4$. The sol–gel process was carried out in a biphasic system using formamide as the polar and a mixture of i-octane and silicon oil as the apolar phase. A triblock copolymer poly(ethylene glycol$_{20}$-co-propylene glycol$_{70}$-co-ethylene glycol$_{20}$) was used as a surfactant. The resulting material was prepared with a specific surface area (σ) of up to 100 m^2/g. The use of other surfactants such as poly(ethylene oxide) for the preparation of mesoporous silica has also been reported by Sierra et al. [18].

Properties important to chromatographers are both the pore volume and the specific surface of the resulting material. In the case of silica, σ may be varied by pH. Thus, silica materials with high surface areas (>800 m^2/g) and low pore volumes (<0.5 ml/g) may be prepared at a pH<5.0 and vice versa. In contrast to silica, the total specific surface (σ) area of zirconia may best be varied by calcination temperature [4]. While values for σ of almost 400 m^2/g may be achieved at $T=200°C$, σ drops to <10 m^2/g at $T=800°C$ [19,20] (and literature cited therein). In course of this calcination, a major part of the micropores is lost while mesoporous structures are preserved. The pore structure itself may additionally be controlled by sodium chloride impregnation techniques during calcination [21].

From a separation scientist's point of view, metal oxides may either be used in form of irregularly shaped ("broken") particles or in form of spherical, uniformly sized ("pellicular") packings. Broken metal oxides are easily obtained by grinding and sieving of the corresponding hydrogels employing hammer, ball or jet mills. For commercial reasons, details for the preparation of spherical metal oxides are kept highly confidential. Nevertheless, several approaches for the synthesis of pellicular supports have been reported. They may either be prepared by spraying neutralized silicate solutions into fine droplets before gelling takes place. Subsequent drying of these droplets in hot air yields the desired materials. Another approach consists of passing stable (neutralized) silica sols through a non aqueous solvent in such a manner that droplets are formed. These droplets solidify, are filtered off and dried. Finally, a two-stage process for the preparation of spherical

silica that may in principle be applied to any other metal oxide, was developed by Unger and co-workers [22–24]. In a first step tetraethoxysilane (TEOS) is partially hydrolyzed to polyethoxysiloxane. This prepolymer is emulsified in an ethanol water mixture and subsequently catalytically hydrolyzed to the silica hydrogel. The xerogel is again obtained by thermal treatment. Carr and co-workers [25,26] developed oil emulsion and polymerization-induced colloidal aggregation (PICA) techniques [27], initially designed for the preparation of monosized zirconia particles. Alternatively, spherical mesoporous silica particles (MSU-X silica) with narrow pore size distributions may be prepared by the sodium fluoride-aided hydrolysis of tetraethoxysilane dissolved in dilute solutions of poly(ethylene) oxide-based surfactants [28]. For purposes of completeness, a sol–gel system involving the pyridine-catalyzed reaction of methyldichlorosilane with bis-(trimethylsilyl)carbodiimide shall be cited [29]. Finally, the applicability of sol–gel chemistry to the preparation of stationary phases for open tubular liquid chromatography (OTLC) [30,31], biosensors [32], electrodes [33], membranes and doped membranes [34] has to be mentioned. More information on the preparation of silica may be found in the literature ([22,35,36], and literature cited therein). While pellicular or even ''broken'' metal oxides nowadays represent standard materials in separation sciences, the usefulness of molecular sieve-, foam- or special shape-type materials so far rather lies in the area of materials science than in that of separation science. Nevertheless, in light of the many developments in membrane and monolithic materials chemistry, they may represent highly attractive stationary phases in the near future.

2.2. Properties of metal oxides suitable for separation techniques

Generally speaking, inorganic carriers such as silica, aluminum oxide, titania or zirconia are characterized by residual metal hydroxyl (M–OH, M= Si, Al, Ti, Zr) and metal–oxo–metal (M–O–M) groups and represent amphoteric materials with both anion- and cation-exchange properties. A comparative study about the retention behavior of the native materials for amines and polycyclic aromatic hydro-

carbons under identical conditions has been reported previously [37]. The total amount of M–OH and M–O–M groups, their accessibility and particular utility for surface-modifications strongly depends on the metal as well as on the ''history'' of the sorbent in terms of thermal and chemical treatment. The structure of the metal oxide and consequently its acidic/basic character is additionally strongly affected by the nature and amount of alkali metal and the calcination temperature. A detailed description for silica is given in Ref. [35]. While this amphoteric character is fully pronounced in silica-, alumina- as well as titania- and zirconia-based stationary phases, these phases have been reported to appear as non-acidic in the separation of certain anilines and pyridines [38]. At first glance, this might be attributed to the different isoelectric points (pI) of the M–OH groups in the corresponding metal oxides that increase in the order silica (3)>titania (5)> alumina (7)>zirconia {(8 [37]), (10–13 [38])}. Nevertheless, at this point it should be emphasized that the isoelectric point does not reflect the retention properties of the corresponding packing for basic analytes as pI is usually based on the pH-dependence of the ζ-potential of oxide particles. Since retention of analytes is mainly governed by the acidic and basic properties of the surface hydroxyl (M–OH) groups, chromatographic measurements provide more accurate data on the properties of these surface M–OH groups than measurements of the ζ-potential can do. Another important aspect in the debate over relative acidities is related to the fact, that pK_a values (e.g., roughly 7.1 for silica) represent an average of many different pK_a values, since hydroxyl groups may be (at least) classified into isolated, vicinal or geminal M–OH groups. Finally, these acidities appear to be strongly influenced by the presence of metal ions. A detailed description for this situation with silica and the problems related to free silanol groups is given in Refs. [36,39,40].

2.3. Stationary phases based on silica

2.3.1. Bonded silica phases
As already briefly mentioned, silica may nowadays be prepared in various forms with different particle diameters, surface areas and pore volumes [35,41,42]. The ease of derivatization using either

chloro- or alkoxysilanes finally led to the broad acceptance of this support in analytical chemistry and in particular in separation science and resulted in the synthesis of a large variety of ''bonded'' phases [11,35,43–48]. Nevertheless, the easy cleavage of the Si–O–C bond represents a severe impediment in the preparation of stable silica-based stationary phases. A survey over the preparation of such bonded phases as well as over certain physico–chemical aspects such as hydrolytic stability, shift of isoelectric point on storage, etc., is given in Ref. [49]. Despite the few reports [50] and many claims on the preparation of stable bonded phases, this type of surface functionalized silica must still be regarded as unstable at low and high pH, in particular under basic conditions at a pH>11.

Since the actual silanol concentration at silica surfaces is comparably low, hydroxylation is usually performed in order to increase this concentration which is limited to approximately 8 μmol/m^2. In principle, bonded phases are prepared by reaction of the corresponding trichloro- or trialkoxy derivatives with the (activated) support. Nevertheless, some general problems encountered with the chemistry of surface silanizations have to be addressed. Generally, surface silanizations have been shown to be strongly dependent on reaction time, solvent, silane reagent as well as on the type and surface area of silica [54]. Additional effects are encountered with the water content of the silica surface. Generally speaking any silanization does not convert all surface Si–OH groups. In this context, typical amounts of bonded ligands are found to be in the range of 4–4.5 μmol/m^2. While silanizations proceed cleanly in the case of the trichloro-compounds, the corresponding alkoxy-derivatives give raise to a series of side reactions. Evidence for the formation of differently bound silanes and the additional formation of alkoxy groups directly bound to the silica surface was obtained by ^{29}Si CP-MAS experiments [54,55]. An exact identification of the actual species bound to the surface may be achieved by solid-state nuclear magnetic resonance (NMR). The exact amount of vinyl groups may easily be determined by titration methods but definitely not by elemental (carbon) analysis. Volumetric microanalyses based on the addition of mercury(II)acetate [56,57], iodine [58] and bromine [35] have been reported for these purposes. Nevertheless,

due to the inactive character of the vinylsilane group, only the addition of bromine under catalytic conditions [35] leads to reproducible results. A comparison of the data for the total amounts of vinyl groups derived from elemental analysis (based on the assumption that $H_2C=CHSiO_3$ is the predominant group) with those obtained by titration clearly leads to considerable differences up to a factor of 10 (!) and underlines the importance of such investigations.

The problem of insufficient derivatization represents another aggravation in the preparation of high-performance silica-based materials. Despite sophisticated ''end-capping'' techniques including high-temperature end-capping [51], residual underivatized silanol (Si–OH) groups may be detected at the surface of silica. Daldrup and Kardel [52], Sander [53] as well as Engelhardt and co-workers [54–57] developed various tests in order to evaluate the separation properties of different RP columns. The remaining electrostatic interaction caused by residual silanol groups may sometimes even improve the selectivity of a RP column and resulted in the development of the ''third generation RP columns'' [58], which require the use of ultrapure silica materials [59]. Since such silanol interactions are completely unwanted in the analysis of biomolecules, Wirth and co-workers [60–64] reported on the preparation of mixed self-assembled monolayers, which actually overcome this problem. The synthetic concept involves the spontaneous self-assembly of functional and spacer groups at the surface to form a very densely packed and highly cross-linked monolayer structure (A) which – in contrast to standard vertically polymerized layers (B) – leaves no free surface silanol groups (Fig. 2).

The necessary cross-linking is achieved by using a mixture of trifunctional agents and a controlled amount of water. The additional implementation of C_3 or better C_1-spacers by preparing a mixed C_{18}/C_1 horizontally polymerized layer was shown to lead to silica-based RP materials with a pH stability of up to 10 [61,63]. Finally, Jordan et al. reported an interesting approach to polymer-supported alkyl monolayers on silica [65]. They used living [66] polymerization techniques for the preparation of α,ω-telechelic polymers. Thus, initiation of the polymerization of 2-ethyloxazoline with C_{16}- or C_{18}-triflates resulted in the formation of a living poly-oxazoline species,
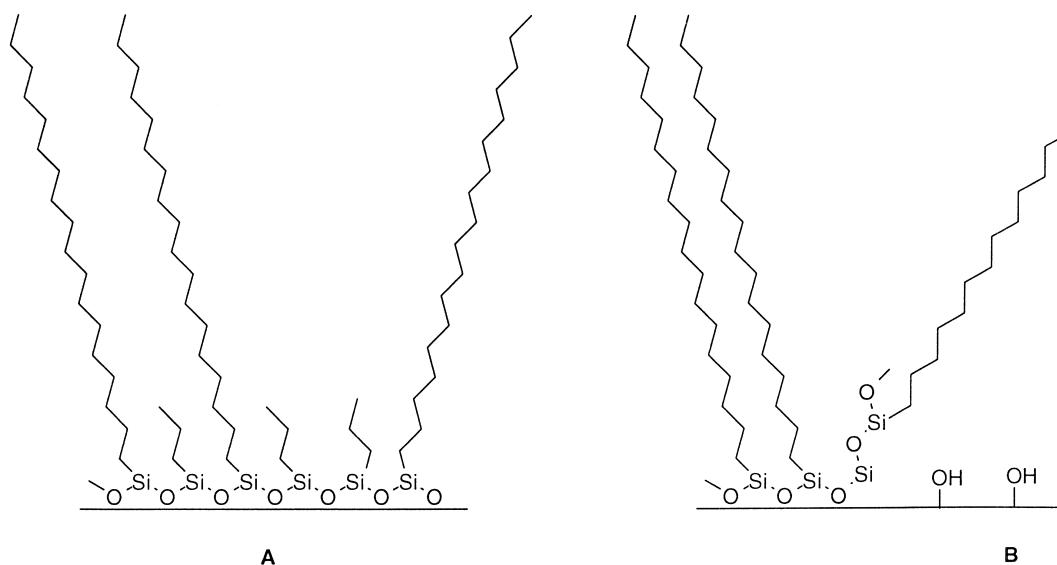
Fig. 2. Horizontally polymerized stationary phases (A), conventional approach (B).

which was terminated by reaction with 1-amino-butane-4-methoxydimethylsilane (Fig. 3).

Reaction of this polymer with the silica support yielded the corresponding bonded phase. The amphiphilic system has been proposed to be suitable for the preparation of polymer-supported membranes.

2.3.2. Hydrosilylation techniques

Hydrosilylation has been used for quite a long time to form covalent bonds between the chromatographically relevant functional groups and the support and consequently offer an attractive access to bonded phases. Pesek and co-workers [67–69], e.g., reported on the preparation and evaluation of ω-hydroxyalkyl-, ω-phenylalkyl- and perfluoroalkyl-derivatized silica supports. Another highly attractive approach that overcomes the problem of the Si–O–C bond cleavage has been reported by Sandoval and co-workers [70,71] and later by Buriak and Allen [72]. Taking advantage of the recent developments in hydrosilylation [73–77] and of the high stability of the carbon–silicon bond, a synthetic protocol employing the hydrosilylation of alkenes (A) and terminal alkynes (B) using either platinum or the soluble AlCl$_3$-analogue EtAlCl$_2$ as a catalyst was elaborated (Fig. 4).

The Si–H groups necessary for these types of reactions are readily available by the halogenation of silica followed by reduction with lithium tetrahydridoaluminate (LAH) [78]. The use of alkynes (B) results in the formation of vinylsilanes, which may further be derivatized, e.g., via hydroboration to yield the corresponding boronic acids. In-depth investigations conducted by Pesek et al. [79] using ^{13}C- and ^{29}Si-CP-MAS-NMR spectroscopy suggest the presence of more than one, if not all species shown in Fig. 5 at the silica surface.

Despite the originality of this approach, it should be emphasized, that careful endcapping of any residual surface silanol groups is still recommended. In terms of selectivity and retention characteristics, the resulting surface-modified silica materials were found to be comparable to standard octadecyl-functionalized silica-based columns [80]. The proposed advantage of the synthetic protocol based on hydrosilylation of Si–H-based supports lies in the enhanced pH stability of these bonded phases, since not Si–O–C bonds may be broken. Nevertheless, a hydrolytically stable bond is only a necessary, yet not sufficient requirement for a pH-stable phase. The problem with silica is related to the fact, that hydrolysis of silica may take place at any point of
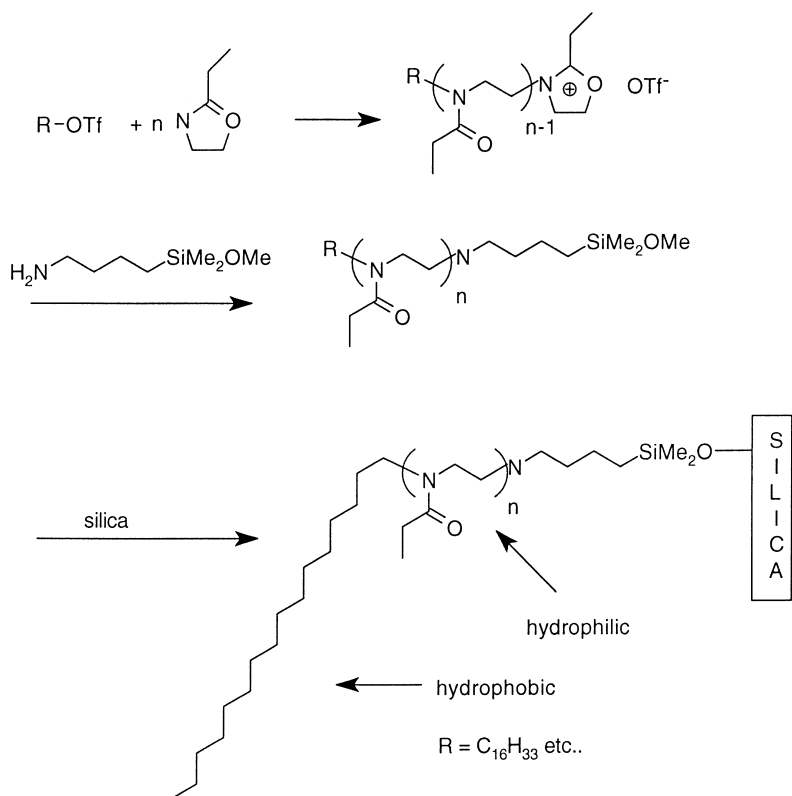
Fig. 3. Preparation of bonded phases via α,ω-functionalization of living polymers.

the support, including the polysiloxane-type silica surface. Once solvent reaches the surface, this process may start. This again underlines the necessity of careful and efficient end-capping. Hydrosilylations were also used for the build-up of organic monolayer
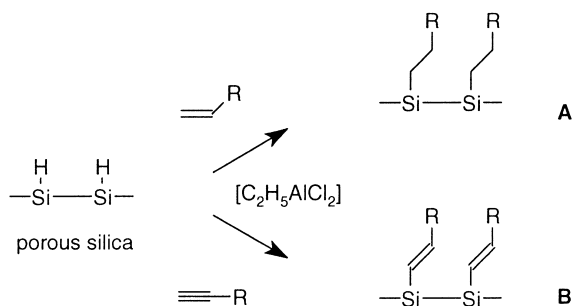

Fig. 4. Hydrosilylation of alkenes and alkynes using hydride-functionalized silica.

films as described by Mao et al. [81] The rather complex synthetic protocol involves the formation of surface-bonded ω-alkenylsilanes which are reacted with a polysiloxanehydride. A monolayer is formed and the residual Si–H groups are finally used for the further addition of functionalized ω-alkenes (Fig. 6).

Despite the rather time consuming approach, one advantage may be related to the high density of alkyl groups that may act as a protecting layer that reduces cleavage of the underlying Si–O–C bonds. Finally, synthetic protocols based on hydrosilylation have to be mentioned for the preparation of n-alkylfluoren-9-yl-bonded [82] phases. For a review on surface modification of silica surfaces – though not all applicable to chromatographic supports – refer to Ref. [83].

In summary it must be concluded that, despite some punctual ameliorations that may be achieved by all these highly sophisticated silanization, end-
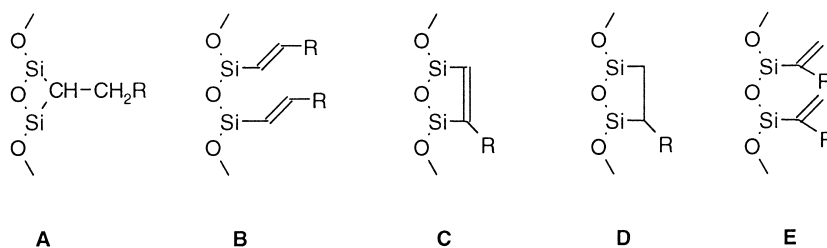
Fig. 5. Proposed structures of silica after reaction with ω-alkenes under hydrosilylation conditions.

capping and polymerization techniques, no substantial improvements in terms of pH stability may be reported.

2.3.3. Coating techniques

One way to overcome the problems related with bonded phases lies in the use of polymer-coated silica or polymer-grafted silica (see below). Besides of the use of dynamic coatings, e.g., described by Cassidy and Elchuk [84], Jun et al. [85], Lakhiari et al. [86] and Krokhin et al. [87], the preparation of permanently chemically coated HPLC supports has attracted major interest during recent years [88]. Due to the fact, that coatings are generally widely used, sufficient achievements for a profound analytical characterization have been made so far [89]. Besides a few reports about the use of graphitized carbon black as a coating for silica for applications in solid-phase microextraction (SPME) [90], the major part of coatings is of polymeric nature. In the following, a brief survey of the relevant coatings will be given.

An apolar surface that is comparable to standard bonded RP phases may in principle generated with apolar polymers such as polyethylene [91] or polystyrene [92]. The general advantage of any coating lies in the (not only claimed) higher pH stability of the resulting stationary phase. Especially in the case of hydrophobic polymer-coatings, the use of ≥5% of such a polymer usually ensures the formation of an impervious layer that basically prevents any contact of the analytes with the silica. In the case of polar coatings that allow solvent diffusion through the layer, thicker polymer layers may be necessary. At this point, it should be emphasized that there are some general problems associated with such coating techniques. Especially in the case of microporous materials valuable pore volume and specific surface
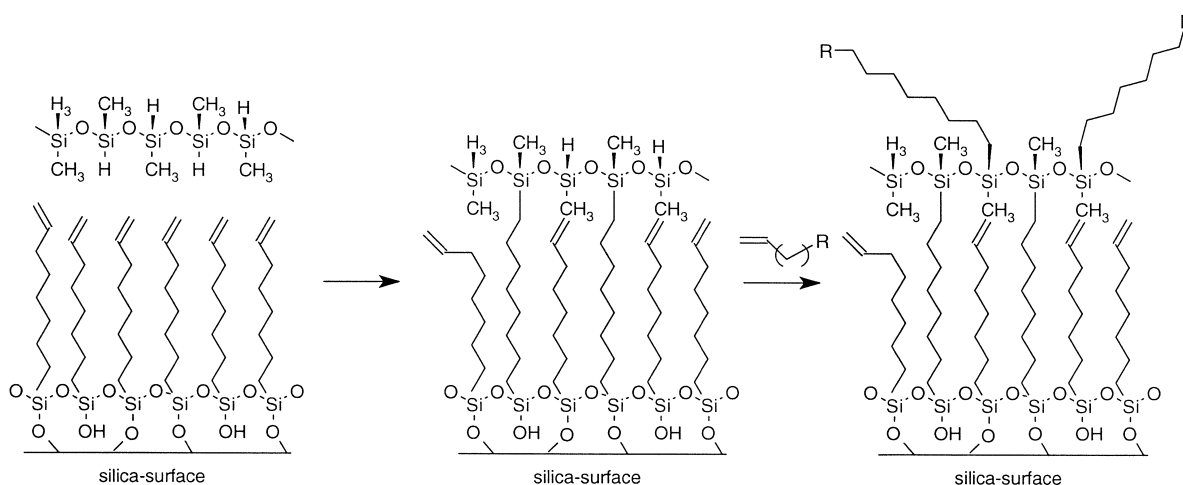

Fig. 6. Two-step hydrosilylation for the preparation of functional silica supports.

is lost due to pore clogging. This effect has been observed for homo- as well as copolymers, e.g., poly(styrene-*co*-methyldiethoxyvinylsilane) copolymers [92]. Hanson et al. presented a model that explains this loss of specific surface area during coatings. An inhomogeneous polymer distribution with high polymer loadings inside the pores was proposed [93]. Similar surface-modifications of silica with polymers that may further be cross-linked (e.g., poly-1,2-butadiene) have been described by Kurganov et al. [94]. Both reported on the use of poly(butadiene) (e.g., 44% 1,2- and 55% 1,4-*trans*-polybutadiene or 20% 1,2- and 80% 1,4- *trans*-polybutadiene) as a coating for non-porous silica. In contrast to porous materials, a loading of 1–3% was found sufficient in order to efficiently suppress any silanol interactions [95]. In general, such poly-(butadiene) coatings are deposited on the surface and subsequently cross-linked via the pendent vinyl-group. A schematic drawing is given in Fig. 7.

The first coatings with polar functional groups, e.g., suitable for ion exchange have already been described by Kolla et al. Thus, copolymers of butadiene and maleic acid were employed as coatings for the preparation of weak cation exchangers [96]. The initially prepared materials showed a very poor stability versus organic solvents and were later improved by Läuble and Kampus [97,98]. An inorganic coating of silica was accomplished using magnesium chloride hexahydrate [99]. The magnesium was precipitated onto the surface of silica under basic conditions. Subsequent calcination at 600–1000°C yielded magnesia-coated silica. Excellent separation abilities for neutral, basic and acidic compounds were reported. In contrast to poly-(butadiene) coatings, those based on polystyrene may easily be further derivatized. Kurganov et al. [100] reported on the successful transformation of PS-coated silica into the corresponding sulfonated and chloromethylated species. The latter served as a starting material for the preparation of the trimethyl-

ammonium derivative. The strong cation and anion exchangers obtained by this synthetic protocol were successfully employed in the separation of nucleotides and proteins. For the separation of a complete mixture of polypeptides, a zwitterionic-coated phase containing both functionalities was employed. Finally, the chromatographic properties of poly(siloxane) coatings using different types of silica have been investigated [101]. Functionalized poly(siloxane) coatings, e.g., those containing complexing ligands may conveniently be prepared by a route that consists of the reaction of polymethylhydrosiloxane with allylglycidylether [102]. This intermediate is further reacted with the complexing ligand to give access to stable, coated silica carrying a selective ligand for metal extraction and/or chromatography (Fig. 8).

A similar route may be used for the preparation of C_{18}-derivatized [103] or mixed-mode stationary phases for the analysis of drugs as described by Kanda et al. Thus, deposition of a poly(hydrosilane) onto porous silica and subsequent attachment of octadecyl and ω-allyloxy-poly(ethylene oxide) groups via hydrosilylation yields poly(siloxane)-coated silica bearing both apolar and polar groups [104].

Actual surface-derivatizations may be accomplished with a poly(vinylmethyldimethoxysilane)-*co*-(polystyrene) polymer [94]. Vice versa, a similar straightforward route to coated silica particles via grafting techniques represents the copolymerization of surface bonded vinylsiloxane groups with methylacrylamide and divinylbenzene as described by Wei et al. [105] (Fig. 9).

The resulting stationary phases may conveniently be used for protein separations. Zuo et al. reported on the use of vinylated silica for the preparation of a graft-type copolymer with divinylbenzene (DVB) [106]. The use of porogens (e.g., 1-hexanol) was claimed to enlarge the specific surface area. Another type of grafted, apolar coating is obtained by grafting
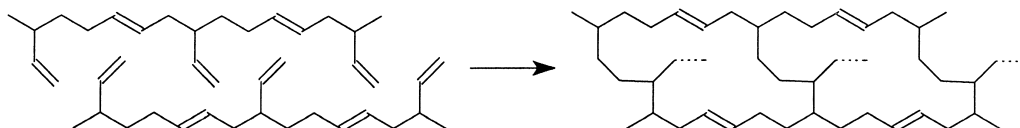

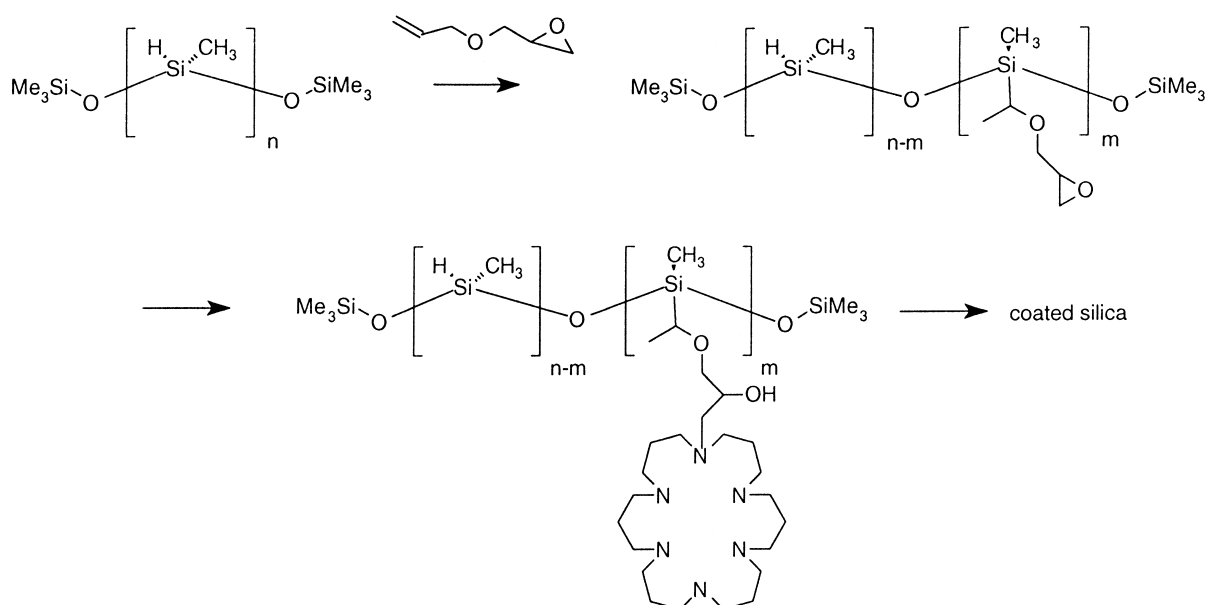Fig. 7. Cross-linking of polybutadiene via the pendent vinyl groups.

Fig. 8. Synthesis of crown-ether-functionalized poly(siloxanes) for coating.

of phenylmethylvinylpolysiloxane onto a vinyl-methyldiethoxysilane-derivatized silica [107].

In summary, coating techniques represent themselves as ambiguous synthetic approaches. Their major advantage lies in the convenient and reproducible generation of an impervious, pH-stable layer that may easily be derivatized for various purposes. Nevertheless, the significant loss of specific surface area that occurs in course of such coating procedures as well as the inhomogeneous distribution of polymer coating represent major drawbacks. Reeder et al. [108] and Sun and Carr [109] gave a quantitative

description for coating processes of zirconia and silica that underline the problems encountered with this technique.

2.4. Stationary phases based on zirconia

As already briefly mentioned, zirconia may be prepared by precipitation of zirconium and zirconyl salts or zirconium alkoxides. The method of preparation, the pH as well as the calcination conditions strongly influence the properties of the resulting material. A review describing the general physical
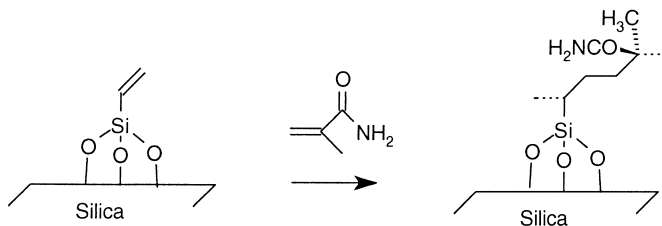
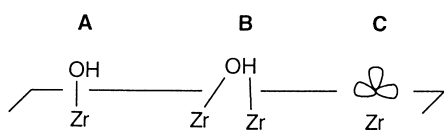Fig. 9. Preparation of poly(methylacrylamide)-functionalized silica.

Fig. 10. Types of zirconium sites present on the surface of zirconia.

properties and types of zirconia has been published recently [19]. Due to its particular chemistry, reports on a straightforward surface-modification of zirconia are rare. From IR studies [110–113], the surface of zirconia is known to consist of at least three different species. Thus, terminal (A) and bridging (B) hydroxyl groups as well as coordinately unsaturated zirconium(IV) species are present (Fig. 10).

Zr-OH surface concentrations are comparable to silica and are typically in the range of 10 μmol/m^2 [114]. This hydroxy-zirconia species may either be protonated or deprotonated, depending on the actual pH of the solute: $Zr\text{-}O^- + H^+ \leftrightarrow Zr\text{-}OH + H^+ \leftrightarrow Zr\text{-}OH_2^+$, thus giving rise to both anion- and cation-exchange properties [114]. One efficient method of derivatization of zirconia is its phosphatation. The synthetic protocol takes advantage of the high affinity of zirconium for phosphate and the low solubility of the corresponding zirconium phosphates in water. Alberti et al. described the use of trimethylphosphonoacetate for the preparation of a carboxylate-derivatized zirconia [115]. The surface of zirconia has been studied in detail and its phosphatation is well understood. Thus, reaction of phosphoric acid under mild conditions leads to, besides physisorbed

phosphates, the formation of zirconiumphosphates (D), while more rigorous conditions result in an additional formation of multi-layer zirconium phosphates (E) due to partial dissolution of the metal oxide [116] (Fig. 11).

^{13}P-MAS-NMR experiments allow a convenient and accurate monitoring of the different types of phosphates formed. As any undesired oxyanion interaction, e.g., of proteins with zirconia may effectively be suppressed either by phosphatation or even by simply using a phosphate buffer [117], such phosphate-derivatized zirconia-based carriers are in contrast to poly(butadiene)-coated zirconia [27] considered as promising ''bio-compatible'' stationary phases for the separation of proteins and other biologically important molecules. This biocompatibility has been demonstrated by a number of applications and may be used to overcome irreversible adsorptions of proteins on poly(butadiene)-coated zirconia [109].

New types of zirconia-based materials have been described by Clearfield and co-workers [118,119]. Treatment of zirconyl chloride with a mixture of phosphoric acid and diacetatoiminophosphonic acid [$(HO)_2(O)P\text{-}CH_2N(CH_2COOH)_2$] yields a mixed phosphate/phosphonate compound (Fig. 12). It represents a highly pH-stable sorbent with excellent chelating properties. Garcia et al. described the preparation of α-zirconium phosphate (α-Zr-P) intercalated with a chiral selector [120]. The synthetic protocol entails the preparation of α-Zr-P form zirconyl chloride octahydrate ($ZrOCl_2\cdot8H_2O$) and phosphoric acid and subsequent formation of a (S)-(+)-*N*-(3,5-dinitrobenzoyl)leucine-*N*-ethyltrimethyl
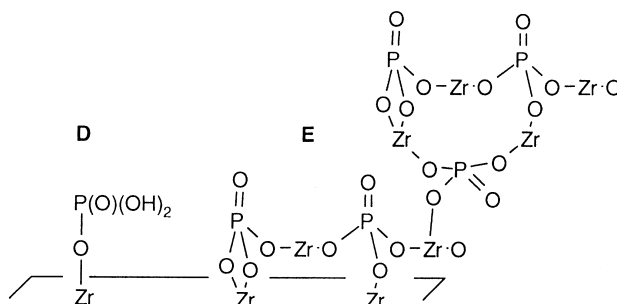

Fig. 11. Composition of moderately (D) and extensively (E) phosphatized zirconia.

ammonium iodide intercalate. Tetrabutylammonium hydroxide serves as a precursor that undergoes ion exchange with the inner surface hydroxyl groups of zirconia and that is subsequently replaced by the chiral ligand. As for titania (see below), octadecyl groups may be introduced by silanization using either octadecyldimethylchlorosilane or octa-decyltrichlorosilane [121,122]. While the former leads to "monomeric" C_{18} layers, the latter is known to form polymeric, surface-bound poly(siloxane) layers. As might be expected, these polymeric layers lead to a higher pH stability (2–12) of the stationary phase. Additionally, lower adsorptivities for acidic solutes are observed. Such poly(siloxane)-bonded zirconia phases allow the fast RP separation of polycyclic aromatic hydrocarbons or peptides in less than 4 min [122] as well as of dansyl-derivatized amino acids in less than 6 min [121]. In view of these results and in light of the significantly enhanced pH stability, such polymeric octadecyl-bonded zirconia must be regarded as comparable to standard silica materials. Composite materials consisting of silica and zirconia have been reported by Shalliker and Douglas [123]. The initially formed silica-coated zirconia is transformed into zirconium silicate (zirconia, $ZrSiO_3$) during the calcination process. The factors which influence the pore size distribution in these composite materials have been investigated intensively [124].

While an actual surface derivatization is hard to perform in the case of zirconia, surface modifications, e.g., those based on the chemical vapor deposition (CVD) of carbon, have been known for a long time. In general, the coating techniques described for the preparation of surface-derivatized silica [94] may also be applied to other inorganic carriers, e.g., zirconia or alumina. Identical to silica, a simple spin-coating of the inorganic support with an organic polymer may be performed with poly-(butadiene) (45% 1,2- and 55% 1,4-*trans*-poly-butadiene) [125,126]. Subsequent cross-linking of the remaining internal double bonds and pendent vinyl groups guarantees a sufficient chemical stability and inertness towards solvents. A schematic drawing of the latter reaction is shown in Fig. 7. HPLC investigations of the materials prepared by Sun and Carr revealed the additional demand for phosphate anions in the mobile phase for the quan-
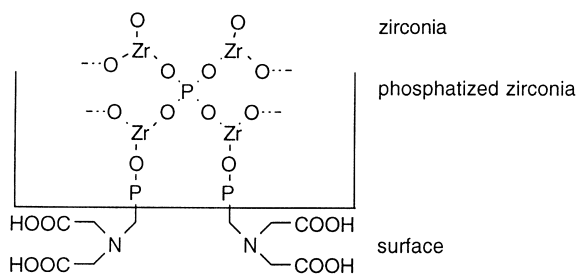

Fig. 12. Iminodiacetic acid (IDA)-derivatized zirconia.

titative desorption of proteins, indicating a non-quantitative coverage of the zirconia surface. In order to study these interactions in detail, a "mixed-mode" phosphate-derivatized-poly(butadiene)-coated zirconia was prepared. In the course of these investigations, both hydrophobic as well as cation-exchange sites were found in the analysis of peptides under acidic conditions [109]. By investigating the factors that influence the poly(butadiene) deposition onto porous zirconia [127], an inhomogeneous distribution, similar to silica (see above), was found. Thus, the deposition of the prepolymer seems mainly to be governed by its affinity for the carrier-surface, suggesting the demand for an apolar surface to achieve optimum poly(butadiene)-coatings. Interestingly, such poly(butadiene)-coated zirconia was reported to serve as an excellent stationary phase in gas chromatography (GC) using carbon dioxide as a mobile phase [128].

Finally, carboxymethyl dextran [129] and 1,4-butanediol-diglycidyl ether (BUDGE)-cross-linked poly(ethyleneimine) (PEI) [130,131] coatings have to be mentioned. The latter may further be derivatized following the procedure described by Hu and Carr [132]. Thus, the initially formed BUDGE-cross-linked PEI layer is reacted with either succinic anhydride or poly(acrylic anhydride) (Fig. 13).

The resulting weak cation exchangers were used for the separation of proteins. Interestingly enough, it should be mentioned that quasi vice versa, zirconia gels have been used as efficient and stable coatings for porous organic supports, e.g., based on PS–DVB [133]. Nevertheless, in view of the problems related with pure zirconia surfaces and despite its originality, this approach must be considered to be only of academic interest.
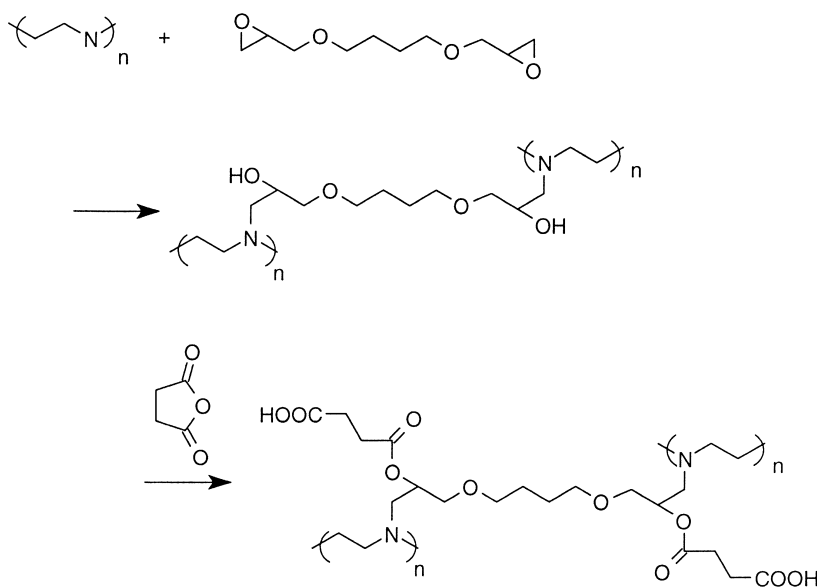
Fig. 13. Preparation of 1,4-butanediol-diglycidyl ether (BUDGE)-cross-linked and succinic acid-derivatized PEI.

2.5. Stationary phases based on titania

Introduced in the late 1980s and early 1990s by Kawahara et al. [134] and others, titania is still hardly ever used in routine HPLC. While the rather poor properties of silica in terms of pH stability initially accelerated the search for more pH-stable stationary phases such as zirconia and titania, researchers were rather quickly confronted with significant aggravations related to the surface-derivatization of the latter. So far, these surface derivatizations are limited to the preparation of octadecylsilyl derivatives. Thus, derivatizations are usually carried out using octadecyltriethoxysilane [135,136]. A carbon loading of up to 6.2% corresponding to roughly 0.2 mmol C_{18} was reported. Again, in light of the problems that are encountered with trialkoxysilanes

(see above), these numbers have to be examined critically.

Pesek et al. [137] reported on an alternative derivatization method. Reaction of titania with triethoxysilane yielded a surface-bound silane that was used for hydrosilylation reactions with ω-alkenes (Fig. 14). The resulting surface-derivatized titania was investigated by means of ^{13}C- and ^{29}Si-CP-MAS-NMR spectroscopy. These investigations confirmed both the presence of three-fold bound siloxane groups as well as the successful bonding of 1-octadecene to the silane precursor. Octadecyl titania prepared via these synthetic route shows properties that significantly differ from those of octadecyl titania prepared by the common approach based on octadecyltrichlorosilane [138]. Thus, the higher ligand density that may be achieved by the
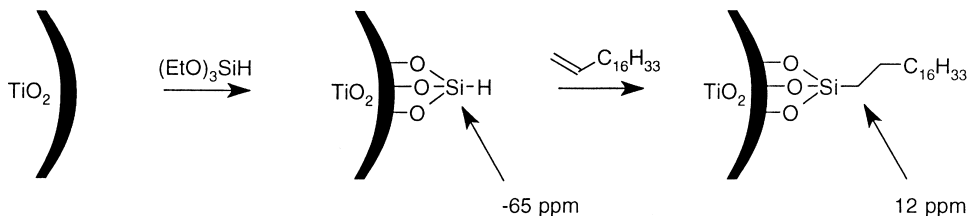

Fig. 14. Preparation of ''bonded'' titania-based stationary phases via silanization/hydrosilylation.

hydrosilylation method leads to significantly prolonged elution times. Another advantage lies in the higher pH stability of the entire phase. Nevertheless, since the accessibility and variability of pore volume, total porosity and specific surface area are much lower with titania than with silica, these phases never became really competitive alternatives. As for zirconia, the coating techniques initially elaborated by Kolla et al. [96] are also applicable to titania, e.g., those reported in Ref. [139]. Since any good coating basically isolates the underlying support from the mobile phase, silica still represents the preferred carrier due to more favorable surface and pore properties.

2.6. Stationary phases based on alumina or other inorganic carriers

Alumina is an attractive alternative to silica owing to its inherent tolerance towards wider pH variation ranging from 1 to 13. In contrast to silica, only few reports exist on its modification and derivatization, respectively, e.g., treatment with alkali halides [140] or transformation into a C_{18}-derivatized alumina as offered by ICN [141]. An interesting approach to RP-alumina represents the preparation of an anchored polymer coating reported by Mao and Fung [142], where maleic anhydride was adsorbed onto alumina (Fig. 15).

Subsequent addition of 1-octadecene and DVB as a cross-linker resulted in the formation of a C_{18}-derivatized alumina-based stationary phase. Despite poorer separation efficiencies compared to standard silica materials, such an approach must still be considered as relevant for separations that have to be conducted at low or high pH.

Other inorganic carriers such as cerium (IV) oxide-based stationary phases reported by Akama and Kanno for the separation of certain polycyclic aromatic hydrocarbons are of minor relevance [143]. Either the insufficient accessibility of narrow particle size distributions which are required for LC separations and/or their surface chemistry in terms of adsorption isotherms and derivatization basically prevent any broad use.

3. Organic HPLC supports

3.1. Polymerization techniques for the preparation of monosized organic HPLC supports

Organic beaded polymer supports based on PS–DVB, poly(acrylates) and poly(methacrylates) are almost exclusively prepared by emulsion, suspension, dispersion and precipitation polymerization techniques [144–146]. The basic differences between these techniques shall be outlined briefly.

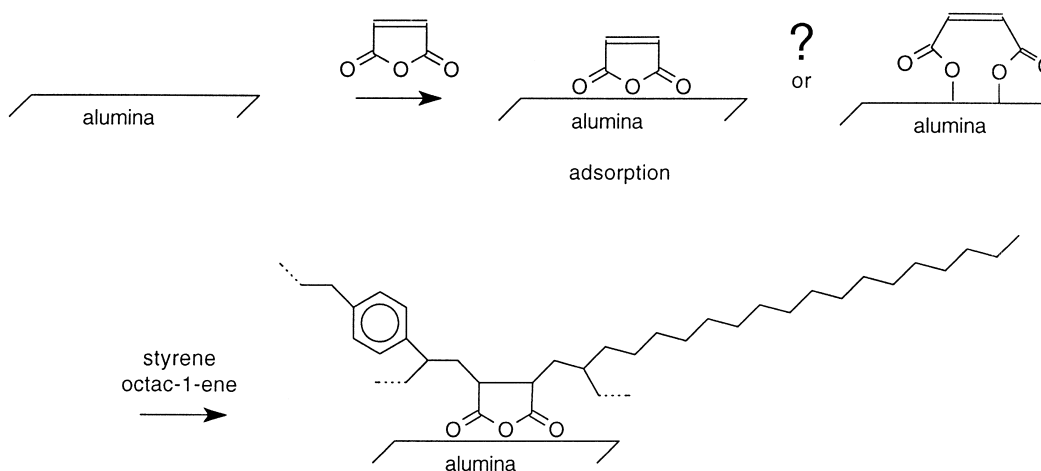The set-up of suspension polymerization [147] consists of monomer-cross-linker droplets that are

Fig. 15. Surface modification of silica.

suspended in a polymerization medium. The re-
sulting two-phase system may be a water-in-oil or
oil-in-water emulsion [148,149]. A fundamental re-
quirement for the initiator is its solubility in the
organic phase (droplets) and insolubility in the
suspension medium (e.g., water). Suspending agents
(surfactants) that stabilize the droplets and prevent
them from coagulation are usually added. The par-
ticle size is strongly dependent on the stirring
velocity as well as on the relative dimensions of the
reaction vessel as well as the agitator blade [150].
Beaded particles with diameters of approximately
15–20 μm up to a few millimeters may conveniently
be prepared by this technique. Nevertheless, particle
size distributions are significantly broadened com-
pared to those resulting from emulsion polymeri-
zation. Another disadvantage is related to the fact,
that some suspending agents may not be completely
removed from the beads. More recently [151], this
concept has been extended to microsuspension poly-
merization and was used for the preparation of
spherical, porous methacrylate-based resins with
high surface areas.

Emulsion polymerization [152] is preferably car-
ried out with an initiator that is soluble in the
polymerization medium (e.g., $K_2S_2O_8$–water) as
well as with a monomer that is insoluble in the
polymerization medium. An emulsifier may be
added. It forms micelles with diameters of 4–10 nm
with monomer inside. Since the total surface of all
micelles (ca. $8 \cdot 10^{22} / nm^2$) is much larger than the
total surface of monomer droplets (ca. $3 \cdot 10^{20} / nm^2$),
initiator radicals start polymerization inside the
micelles. A permanent diffusion of monomer into
these growing micelles finally leads to the formation
of a polymer latex. The restricted space inside the
micelles leads to a very low radical concentration
which prevents any chain termination and leads to a
"quasi-living" system. Swelling of these latices may
be achieved by adding a suitable solvent (e.g., 1-
chlorodecane) that diffuses into these seeds. The
cross-linker and usually a porogene are then added in
a third step and again diffuse into these "activated"
polymer latices. Cross-linking is achieved by read-
ding a radical initiator. The fundamentals for this
"activated-swelling technique" [153–156] were
mainly elaborated by Ugelstadt and co-workers
[157,158] and allow the synthesis of monodisperse

polymer beads of up to approximately 2–5 μm. For
the preparation of larger beaded polymers, multi-step
swelling techniques are usually necessary. These
have been reported by Hosoya and Fréchet [159] and
Ellingsen et al. [158] for the preparation of monodis-
perse 5–6 μm and 10 μm sized PS–DVB particles
[160]. Further research focused on the investigation
of the parameters that control particle size and
porosity [160,161]. In this context, Cheng et al.
reported on the use of linear polystyrene as a diluent
during seeded emulsion polymerization [162]. In
general, the addition of prepolymers is known to
result in the formation of macroporous polymers
with low specific surfaces and large pores [163–
166]. Thus, the use of such prepolymers allows the
preparation of particles with pore diameters of 1000
Å and larger. More recently, physico–chemical and
theoretical considerations about emulsion polymeri-
zation have been reported [167–169]. Since multiple
activated-swelling techniques are rather time con-
suming, Ogino et al. reported on a single-step
swelling and polymerization method [170,171] that
allowed the synthesis of monosized polymer beads in
the range of 4.5–7.5 μm. Okubo et al. elaborated the
"dynamic swelling method" which may be used to
extend the size range of polymer particles [172].
Thus, polymer beads may conveniently be prepared
in the range of 0.05 to 15 μm and are characterized
by narrow particle size distributions. The experimen-
tal set-up for the preparation of miniemulsions [173]
leading to nanometer-sized particles relevant for the
coating of capillaries [174] has been reported by
Antonietti et al. [175] and Chern and Chen [176]. It
is worth noting, that especially for polymerizations
in microemulsions, initiation may be accomplished
using ultrasonic irradiation [177] or light [178]. The
chemistry and especially physics of such microemul-
sions have been studied extensively [179–181].
Emulsion polymerization may also be used for the
polymer-encapsulation of inorganic carriers such as
silica or alumina [182], thus offering an attractive
alternative to standard spin-coating techniques. Re-
cently, Hosoya et al. reported on a one-pot method
for the preparation of uniform-sized acrylate-based
chiral stationary phases [183]. The synthetic protocol
involved a two-step swelling method during which a
phenethylamine-based chiral methacryl amide mono-
mer was added. The CSPs prepared by this approach

allowed the successful separation of 2,2′-dihydroxy-binaphthyls. For purposes of completeness, dispersion polymerizations, that represent ''inverse'' emulsion polymerizations with an aqueous phase dispersed in an organic solvent, have to be mentioned.

In precipitation polymerization, the resulting polymer is insoluble in the polymerization medium. Nevertheless, diffusion of monomer to the propagating species (e.g., a macroradical) results in a continuous polymerization process that is governed by the flexibility of the polymer chains as well as by the stirring velocity [184,185].

All the above mentioned polymerization systems may conveniently be applied to styrene–divinylbenzene as well as to acrylates, methacrylates and acrylamides using ethylenedimethacrylate and vinylmethacrylate [186], trimethylolpropane trimethacrylate (TRIM) as cross-linkers. More recently, the search for alternative cross-linkers for divinylbenzene resulted in the use of other divinyl compounds such as bis(vinylphenyl)ethane, bis-(vinylbenzyloxy)hexane and bis(vinylbenzyloxy)-dodecane. With these new cross-linkers, higher mechanical strengths compared to DVB-based copolymers were observed for highly cross-linked polymers [187]. Nevertheless, since these cross-linkers are not commercially available, no extensive use is expected for economic reasons. Highly polar methacrylate-based monodisperse polymer beads may be prepared by hydrolysis of a poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) copolymer [188]. A comparison with non-modified silica revealed much stronger retention of the model compounds by the organic support. The hydroxyl groups present at the surface are chemically equivalent and significantly less acidic compared to the silanol groups. An advantage may stem from the findings that, unlike with silica phases, the properties of the polymeric phases are not affected by the presence of water in the mobile phase.

Since polymerizations in supercritical carbon dioxide have attracted increasing interest, its general advantages shall be outlined briefly. Besides the fact that CO_2 represents an environmentally friendly, inexpensive and non-toxic alternative to organic solvents, polymers prepared in supercritical CO_2 do not contain any other organic compound (except of unreacted monomer), since CO_2 rapidly evaporates

after pressure is released. The first applicationS in the preparation of monosized PS-beads that may further be used for activated-swelling techniques have been reported by Shiho and Desimone [189]. Taking advantage of the insolubility of PS in supercritical carbon dioxide, dispersion copolymerization of PS in the presence of fluorinated acrylates and methacrylates as polymeric stabilizers was performed. Despite any missing data on the use of such monosized, non-cross-linked PS-particles for further use in polymer support preparation, this method offers an attractive access to beaded polymers and can be expected to be used in the preparation of separation media in the near future. This also applies to the use of carbon dioxide as a solvent for the manufacture of molded rigid rods based on EDMA and TRIM [190].

3.2. Functionalized stationary phases based on poly(styrene–divinylbenzene)

For certain applications it is sufficient to adsorb (physisorb) the desired molecule onto the surface of a carrier (e.g., PS–DVB [191]). Nevertheless, for most HPLC applications, a covalently bound, yet well defined attachment of any functionality to the support is highly desirable. In general, derivatizations of PS–DVB are restricted to the chemistry of aromatic systems. Besides the already well known and established procedures for the derivatization of PS–DVB resins such as chloromethylation [192], amination, nitration [193], sulfonation, Friedel–Crafts alkylation and acylation [144,194,195] or the copolymerization of chloromethylstyrene and some simple derivatives [196], few reports exist on the introduction of more complex ligands or on more straightforward synthetic routes. A representative example is the preparation of PS–DVB-based chiral stationary phases from chloromethylstyrene and divinylbenzene by a staged-templated suspension polymerization step. Subsequent amination of the chloromethyl moieties and acylation of the benzylic amines leads to the desired CSPs. Liang et al. [197] reported on the synthesis of epoxide-functionalized PS–DVB beads via suspension polymerization starting from monosized PS–DVB. Access to further functionalization was achieved by swelling techniques and subsequent cross-linking with epoxypropyl vinyl-

phenyl ether and divinylbenzene. In a similar approach, vinylphenol-*co*-divinylbenzene-based mono-sized beads for use in size-exclusion chromatography and RP chromatography have been reported [198]. In analogy, "temperature-responsive" poly-(*N*-iso-propylacrylamide) (poly-NIPAM)-based polymer beads have been prepared starting from a monosized PS–DVB sorbent [199]. As a consequence of the temperature-dependent hydrodynamic volume of poly-NIPAM, pore size may be effectively varied by temperature.

Itsuno et al. reported on a straightforward and highly attractive halomethylation avoiding the highly toxic chloromethylmethyl ether. Instead, trioxane and tin tetrachloride were used as a source for the chloromethyl group [200]. In analogy, bromo-methylated species are accessible using tin tetra-bromide instead of tin tetrachloride. Seubert and Klingenberg [201] reported on the preparation of sulfoacylated resins. Starting with a Friedel–Crafts acylation employing ω-halogeneacylchlorides, the corresponding ω-halogene ketones were formed under $AlCl_3$ catalysis. Treatment with dimethyl sulfide (Me_2S) resulted in the corresponding sul-finium salt. Addition of aqueous sodium hydrogen-sulfite finally yields in an S_N^2-reaction the sul-foacylated species (Fig. 16).

The resulting materials are claimed to possess superior properties over standard sulfonated PS–DVB resins in ion chromatography. Li and Fritz [202] recently reported on the preparation of a novel polymeric PS–DVB-based resin by treating chloro-methylated PS–DVB with diethylene triamine. Inter-estingly, the charge of the resin may be varied from $+1$ to $+3$ by pH. Some of the first reports on the preparation of functionalized supports using func-tional monomers were by Buchmeiser and co-work-ers [203–208] (see below) and Frank et al. [185]. The latter group reported on the preparation of a carboxylic acid-derivatized DVB-based copolymer. Thus, precipitation polymerization [184] of maleic anhydride with DVB in MEK–heptane (60:40) yields porous monosized particles in the range of 0.5–1.8 μm. Similarly, precipitation copolymeriza-tion of chloromethylstyrene and DVB in acetoni-trile–toluene was found useful for the preparation of monodisperse chloromethyl-functionalized polymer beads of 4–6 μm. Despite this attractive approach, a restricted access to the functional groups (carboxylic acid and chloromethyl groups, respectively) must be assumed, as a major part of the functional monomer is located in the interior of the beads due to the continuous incorporation of functional monomer. This problem is generally solved by the use of "living" polymerization techniques (e.g., ring-open-ing metathesis polymerization, ROMP, see below). A general method for the controlled functionalization of PS–DVB particles has been reported by Loch-mann and Fréchet [209]. Metallation with a super-base (2-ethylhexyl lithium–potassium 1,1-di-methylpropoxide) results in the formation of the corresponding poly-potassium salt. Subsequent re-action with electrophiles yields the desired deriva-tives (Fig. 17).

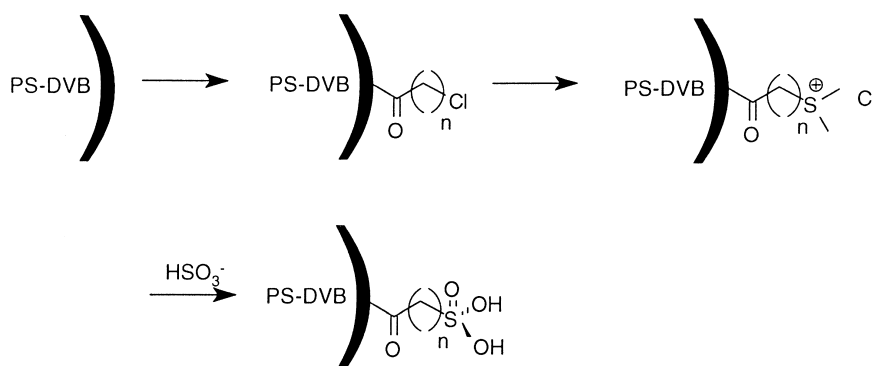It is worth noting that metallation occurs both at the aromatic ring system and the benzylic position.

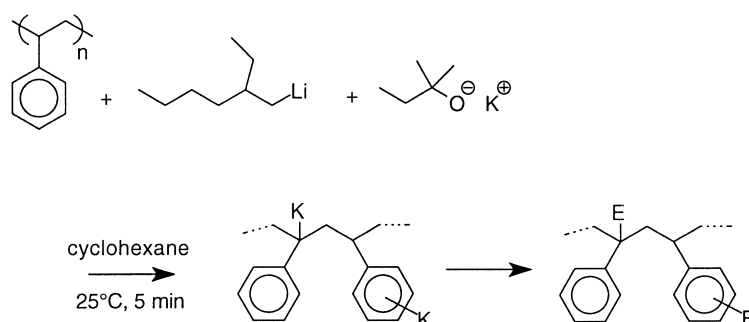Fig. 16. Sulfoacylation of PS–DVB resins.

Fig. 17. Metallation of PS and reaction with electrophiles.

The general advantage of this approach lies in the comparably high amounts of functional groups that may be attached onto the surface. Nevertheless, since alkyl lithium compounds and potassium *tert*-butylate represent rather hazardous compounds, this approach has not attracted much interest so far.

Pore-size specific functionalizations have been described for the separation of proteins and small hydrocarbons [210,211]. For that purpose, large polymer pores were provided with phenyl groups in the presence of hydrophilic groups. In contrast, small pores were provided with a much higher phenyl content. The entire synthesis was realized starting from porous glycidyl methacrylate–ethylene-dimethacrylate (60:40) polymer beads prepared by shape template swelling and polymerization [212,213]. The selective hydrolysis of the epoxide groups in the large pores was accomplished using an aqueous solution of PS–sulfonic acid. As a consequence of the large hydrodynamic volume of the polymeric PS–sulfonic acid, hydrolysis takes only place within the large pores. The selective hydrophobization of the small pores was achieved with a solution of phenol in KOH. Such separation media with bimodal chemistry may be used for the complete separation of complex samples containing analytes of different size, charge and polarity. Consequently, relevant applications have been reported in

the direct analysis of drugs from blood samples [214].

3.3. Functionalized stationary phases based on acrylates and methacrylates

In contrast to PS–DVB resins, poly(acrylate)- and poly(methacrylate)-based supports offer a straightforward access to high-capacity hydrophilic amine- and carboxylic acid-derivatized stationary phases by simple reaction sequences [215,216]. Generally speaking, methacrylate-based supports must be favored, since they are more stable under both acidic and basic conditions. Additionally, carboxylic acid and hydroxyl-modified resins may be prepared directly from *tert*-butylmethacrylate and 2-hydroxy-ethyl methacrylate [217]. Among the numerous polymer transformations that may be performed, the introduction of isocyano groups into copolymers of 2,4,5-trichlorophenyl acrylate with styrene that may be used for further derivatization, shall be mentioned [218] (Fig. 18).

Hirayama et al. [219] reported on the preparation of dimethylamino-functionalized beaded acrylates for the selective removal of endotoxin. The amino-group contents as well as the pore volumes were adjusted by the ratio of functional monomer, *N,N*-di-methylaminopropylacrylamide, and the cross-linker,
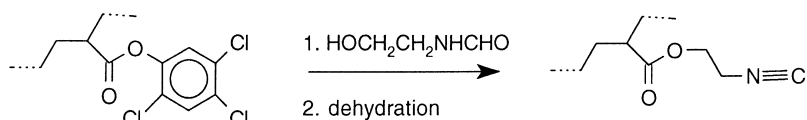

Fig. 18. Isocyano-derivatized poly(methacrylates).

N-allylacrylamide. The use of TRIM as a cross-linker in the synthesis of acrylates offers access to surface grafting. Thus, unreacted methacrylic groups of TRIM located at the surface may be copolymerized with other monomers, e.g., certain bisimidazole templates using thermally induced radical polymerization [220].

3.4. Other stationary phases

3.4.1. Stationary phases for perfusion chromatography

Perfusive stationary phases may be considered a ''missing link'' between regularly packed columns and monolithic separation media. Introduced by Afeyan et al. in 1990, the use of this type of packing material has always been restricted to the separation of larger molecules such as proteins and peptides [221–224]. A detailed description of the theory of perfusion chromatography which also underlines the importance of reproducible pore-sizes and pore volumes has been given by Liapis and co-workers [225–233]. A description of the importance of intraparticle convection that also applies to monolithic, rigid rod materials (see below) has been given by Rodriguez and co-workers [234–238].

The idea to synthesize perfusive materials may be regarded as a direct consequence of the results obtained by Unger et al. [239,240] and Kalghatgi and Horváth [241] They demonstrated that fast separations of macromolecules may be accomplished with small (1–3 μm), non-porous packing materials. The reason for these fast separations are based on the fact, that porous materials necessitate diffusion of the analytes into the micro- and mesopores, thus resulting in extensively prolonged analysis times and significantly elevated peak half-widths. If it was possible to synthesize materials with extraordinary large pores (usually in the range of 6000–8000 Å), diffusion should play a minor role and perfusion, in other words flow through the particle, should even further reduce analysis times. In this review, only the synthetic particularities shall be mentioned briefly. So far, perfusive materials are usually based on styrene–divinylbenzene (e.g., POROS R1, POROS R2, OLIGO R3, etc.) and are prepared in form of approximately 20 μm diameter particles via suspension polymerization [242]. Hydrophilization may be achieved by coating with polyvinylalcohol (PVA) and subsequent cross-linking with glutaraldehyde [243]. A detailed description of perfusive materials is given in Ref. [244].

Generally speaking, column efficiency turned out to be lower compared to standard HPLC packing materials. Consequently, perfusive packings are mainly used in preparative-scale LC separations, where the larger particle diameter and the low back-pressure allow higher flow-rates [245] as well as for the separation of large molecules such as proteins (Refs. [246–251] and literature cited therein), in particular for the purification of unstable proteins [252]. Thus, perfusion LC has also already been interfaced to electrospray ionization mass spectrometry (ESI-MS) [253] for that purpose.

3.4.2. Stationary phases based on membranes

Polymeric [254] as well as inorganic membranes [255] have attracted significant interest in separation science. Besides ion-selective membranes, the most prominent applications lie in the areas of gas-separation [256], haemodialyis, fluid and drug filtration [257]. Few reports exist on the use of membrane-type discs for separation purposes. In the following, a few representative examples shall illustrate the scope and limitations of these materials.

Macroporous polymeric membranes may be prepared from poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) and further derivatized by transformation of the epoxide group [258]. Thus, reaction with propane sulfone, diethylamine or water leads to the corresponding sulfonic acid, dimethylamino and diol-derivatized stationary phases. Since the membrane is produced in a mold and the resulting back-pressure was found to be lower than the one in packed columns by at least two-orders of magnitude in order to reach the same flow-rate, this set-up may be regarded as a ''precursor-approach'' to molded, rigid rods (see below). A similar system was obtained using stacked macroporous, diethylamino-ethyl-derivatized cellulose membranes for the preparative-scale separation of certain proteins such as α-chymotrypsinogen, cytochrome and lysozome [259]. Again, high protein loadings may be achieved. From a synthetic chemist's point of view, electron beam induced surface grafting of polyethylene membranes represent an interesting approach [260–262].

The electron beam generates radicals that may be used for subsequent radical graft polymerizations. Such membrane-grafting is often required in bio-separations, since it minimizes non-specific adsorptions of the target proteins. A general problem encountered with the surface modification of polymeric membranes lies in the reduced flow that is often a consequence of unfavorable swelling properties of the grafted polymer/compound. Such a reduced flow resulting from gel effects has been observed for membranes grafted with N-hydroxy-succinimide but may be avoided using glycidyl methacrylate (GMA) as the graft monomer. The epoxide group in poly(GMA) may further be reacted with phenol–water to produce a hydrophobic membrane or with diethylamine for the preparation of porous anion-exchange membranes.

In summary, applications of membranes have been limited to large biopolymers so far. Consequently, the main advantages of these materials are rather found in the areas of purification, enrichment or generally speaking, solid-phase extraction (SPE), than in the area of real high-performance separations.

3.4.3. Monolithic stationary phases

3.4.3.1. Rigid rods based on organic polymers

The preparation of monolithic supports and in particular of monolithic HPLC and micro-HPLC supports has gained significant interest during the last few years. Based on the principal intention to avoid any laborious polymerization techniques for the preparation of monosized particles and any subsequent column packing, Hjerten et al. [263] were the first to report on the preparation of monolithic materials. The column that was used by these authors consisted of a solvent–swollen poly(acrylic acid-co-methylenbisacrylamide) and was successfully used for the separation of proteins using an ion-exchange mechanism. Since this "rigid-rod" did not possess any permanent and well-defined pore structure, other polymeric systems that overcome these problems had been elaborated. The findings that a convective flow through pores could be achieved if these pores had diameters >600 Å [264] and that such sorbents allowed the fast separation of biomolecules due to a significantly enhanced mass-transfer, initiated intense research in this area. In particular, intraparticle

convection effects turned out to be crucial and have consequently been studied in detail [234–238]. While continuous stationary phases, e.g., consisting of yarns woven into a fabric [265] did not turn out to be useful for separations, "molded" rigid rod monolithic systems as reported in 1992 by Svec and Fréchet [266] showed promising properties in separation sciences. Since then, the field of monolithic materials faced a rapid development [267].

So far, monoliths are either based on PS–DVB, acrylates, or on methacrylates, the glycidyl methacrylate–EDMA system representing the most common one. The general set-up for the preparation of monolithic materials consists of the polymerization of a monomer and a cross-linker by an initiator in the presence of at least one, usually two porogenic solvents [268]. Together with the relative ratio of the monomer and cross-linker, these porogens are responsible for the diameter of the microglobules and especially for the pore structure [269]. In this context, the successful use of supercritical carbon dioxide as the porogenic solvent has been reported [190]. Alternatively, pore volumes may be controlled by temperature [270] or by temperature-sensitive polymers such as poly(N-isopropylacrylamide) (see above) [271]. Highly porous supports with pore sizes up to 1000 nm may be prepared from acrylamide and N,N'-methylenebisacrylamide [272]. Homolytic cleavage of the initiators for radical polymerization is usually induced thermally, nevertheless a photo-induced initiation has also been reported [273]. As a consequence of their pore structure and their hydrophobic nature that may even be enhanced by the copolymerization of highly hydrophobic monomers [274], they are mostly used in the separation of large molecules such as proteins [275–278] or other polymers [279]. Additionally, Maruska et al. reported on their use in normal-phase capillary chromatography of small analytes [280]. More recently, the preparation of monolithic material has been extended to large diameter (≥5 cm) materials with well-defined pore structure [281].

While defined microstructures may be generated with high reproducibilities [267], functionalization represents a problem that still remains to be solved. Another derivatization approach consists of the preparation of chloromethylstyrene-co-divinylbenzene rods that may be further derivatized, e.g., with
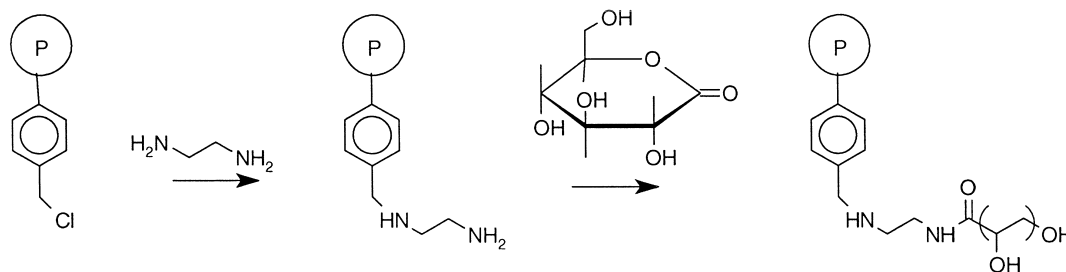
Fig. 19. Hydrophilic monolithic polymer support.

γ-gluconolactone using an ethylenediamine spacer (Fig. 19).

The resulting monolith possesses highly hydrophilic pores as was demonstrated by HPLC of small molecules. A similar system has been reported for micro-HPLC and CEC [282]. Unfortunately, any functionalization by this approach is limited to traditional chloromethylstyrene chemistry [283]. Hydroxy-functionalized rods that are additionally highly porous and may consequently be used for SPE are obtained from a system consisting of DVB and hydroxyethylmethacrylate [284]. The polymerization of functional monomers, e.g., 2-acrylamido-2-methyl-1-propanesulfonic acid, permits the use of the resulting sulfonic-acid derivatized monoliths in CEC [285,286]. Finally, molecular imprinting techniques for the separation of the positional isomers of diaminonaphthalene [287] and for drug screening [288] have been reported. Besides of standard glycidyl- and chloromethylstyrene chemistry, only a few reports exist so far on functionalized monoliths containing ''advanced'' ligands. In this context, the preparation of trypsin-functionalized poly(2-vinyl-4,4-dimethylazlactone-co-acrylamide-co-ethylene dimethacrylate)-based rigid rods and their use as high-flow bioreactors [289] has to be mentioned. A totally new and interesting approach for the functionalization represents the use of ''living'' polymerization systems. Peters et al. reported on the use of a TEMPO (2,2,6,6-tetramethyl-1-oxypiperidine) in the synthesis of PS–DVB-based rigid rods. The persistence of the TEMPO-capped radicals was used for the grafting of functional monomers (2-hydroxyethyl methacrylate, vinylbenzyl chloride) onto the surface [290]. So far, no chromatographic data that prove the utility of this concept are available. Another living

system, that allows the preparation of a large variety of functionalized monoliths was reported by Buchmeiser and Sinner [291]. Following the general set-up for the preparation of monolithic materials, a ROMP system (see below) based on norborn-2-ene, 1,4,4a,5,8,8a-hexahydro-1,4,5,8-exo-endo-dimethanonaphthalene and the porogenic solvents toluene and isopropanol was used for the synthesis of rigid rods. The size of microglobules, the specific surface area as well as the pore volumes are governed by the relative ratios of the monomer, cross-linker and solvents. An electron microscope picture of such a monolithic material is shown in Fig. 20.

Due to the living character of the polymerization system, the catalyst remains active after the polymerization is complete and may subsequently be fed with various functional monomers based on 5-substituted norborn-2-enes and norbornadienes including carboxylic acids, amines, phenols, amino acid-based chiral selectors and even cyclodextrins. Following this set-up, HPLC supports may be synthesized in a two-step procedure with high reproducibility and high variability in terms of capacity and functional groups, respectively (Fig. 21).

Examples of applications lie in the entire area of separation sciences. A representative example is the use of such a β-cyclodextrin-derivatized monolith for the separation of proglumide that was accomplished within less than 3 min [291a].

3.4.3.2. Rigid rods based on inorganic polymers

RP-silica-based continuous column supports for HPLC have been prepared from potassium silicate/ formamide at 100°C and subsequent reaction with dimethyloctadecylchlorosilane (ODS) [292]. Alternatively, they may be prepared from tetramethoxy-
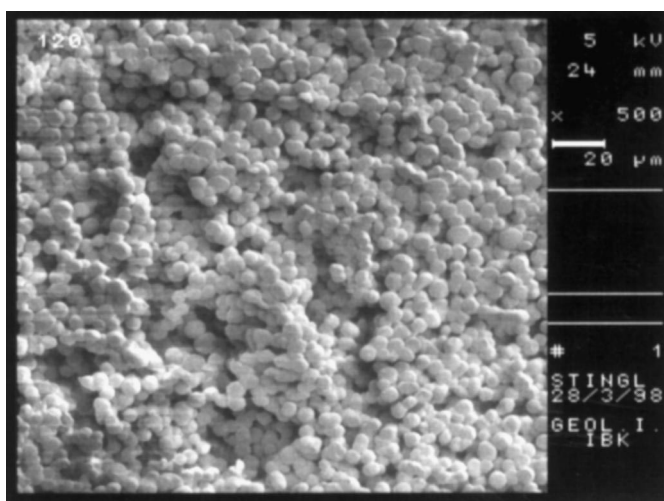
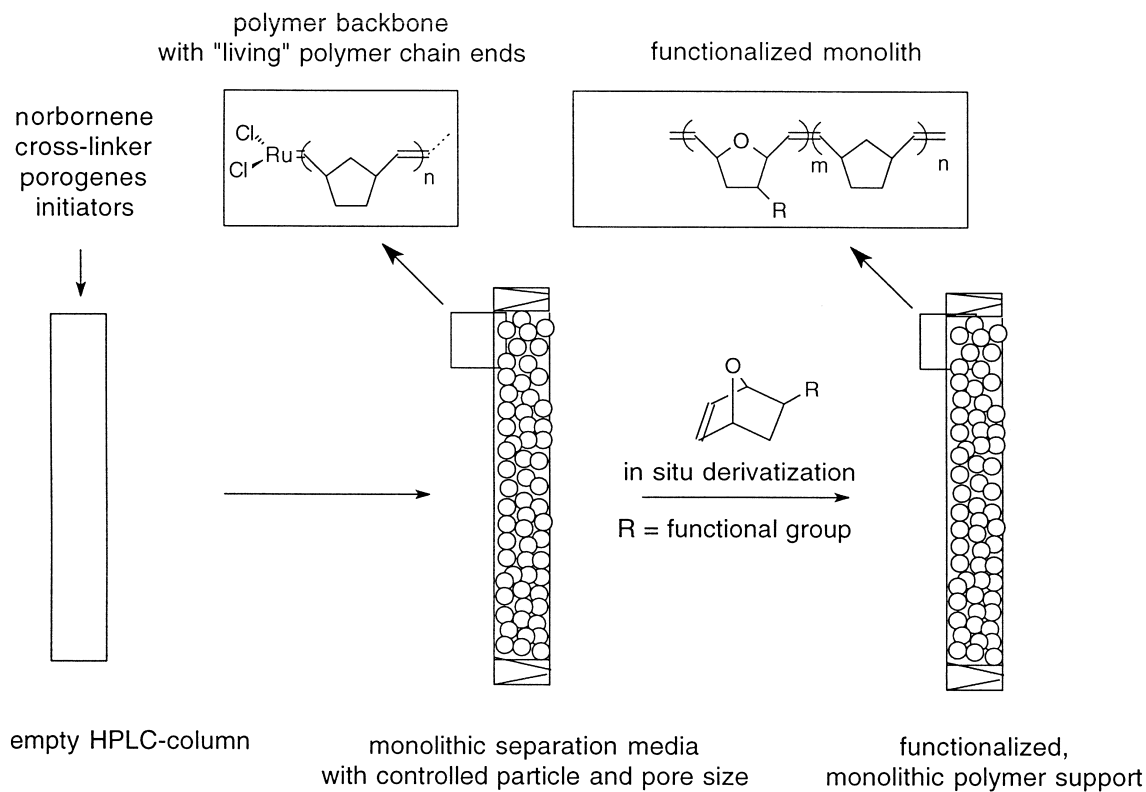Fig. 20. Electron microscope picture of a monolithic material prepared by ROMP.

Fig. 21. Preparation and in situ derivatization of a monolithic HPLC support.

silane–polyethyleneoxide–acetic acid and subsequent derivatization with octadecyldimethyl-(*N,N*-diethylamino)silane [293]. In both cases the pore size distribution was reported to be narrow and in the range of 1. 5 to 2 μm. Separations of alkylbenzenes [294] and polypeptides [295] under RP conditions were reported to proceed fast and with high efficiencies.

Fused-silica capillaries packed with 6 μm octadecyl-derivatized silica microspheres were subject to thermal treatment leading to a ''sintered'' rigid silica block. Subsequent octadecylation with ODS allowed its use in CEC for the separation of polycyclic aromatic hydrocarbons (PAHs) and phenylthiohydantoin (PTH)-protected amino acids. Alternatively [296], packed ODS columns were partially filled with a sol–gel mixture consisting of tetramethoxysilane and ethyltrimethoxysilane–methanol, TFA–water–formamide. Subsequent CEC separations of PAHs were reported successful.

Based on the present knowledge, rigid rods are unlikely to totally replace particulate supports. Nevertheless, they are believed to represent highly interesting alternatives, e.g., in capillary HPLC, CEC as well as SPE of biomolecules or large synthetic polymers. In particular, their use in hyphenated techniques, e.g., micro-HPLC–MS [297] gives rise to high expectations.

3.4.4. Stationary phases prepared by ring-opening metathesis polymerization (ROMP)

As already outlined in the previous sections, the reproducible preparation of well-defined stationary phases still encounters several problems. One approach to circumvent traditional impediments such as pH instability, poor reproducibility, poor and/or tedious surface-characterization lies in the use of organic supports by a polymer approach using polymerizable, functional monomers. If the entire synthesis could be conducted in a well-defined way, e.g., by ''living'' [66,298] polymerization methods, the synthetic protocol should result in an extraordinary high reproducibility. Unfortunately, classical polymerization methods do not allow the set-up of living systems in combination with functional monomers. Nevertheless, one rather ''young'' technique, ROMP [299–301], appeared to be a promising tool. ROMP has attracted significant interest during

the last decade. Based on the catalytic systems developed by Oskam et al. [302] and Schwab and co-workers [303,304], ROMP was the first polymerization technique which not only allows ''living'' polymerizations [66,298], but also exhibits a high tolerance versus a broad range of functionalities such as amines, esters, amides, anhydrides, sulfones, sulfoxides, chelating groups, nitriles, ethers and even alcohols or carboxylic acids, etc. While ROMP had already been used for many other high-tech applications (Ref. [305] and literature cited therein), its use for the preparation of new, well-defined stationary phases for SPE [203–205,208,306,307], ion-exchange [206,207,308] and HPLC [204,309–312] was elaborated in our laboratories.

Two different synthetic routes were chosen for the preparation of supports for high-performance ion chromatography and chiral HPLC. The first entails the preparation of functionalized prepolymers via living polymerization techniques and subsequent deposition on an inorganic carrier, e.g., silica, using standard spin-coating techniques [311]. The advantage of such an approach lies in the vast variety of functional groups including acids, bases, chelating or chiral groups which may be incorporated in a controlled and highly reproducible way. Preparation of block-copolymers is possible and ensures a high pH stability (0–12) of the resulting materials. Following this approach, highly hydrophilic cation-exchange resins have been prepared and were successfully applied to analytical as well as preparative-scale ion-chromatography [311] (Fig. 22).

Despite its advantage in terms of ease of the introduction of functional groups, the general problems that are related to spin-coating techniques remain unsolved. Consequently, a second approach that consists of a covalent surface-modification that may be conducted with a large variety of inorganic as well as organic polymer supports using ROMP [310,312], was elaborated. For that purpose, the corresponding carriers were surface-modified with polymerizable norborn-2-ene groups. Beaded polymer supports based on PS–DVB were bromomethylated, either via direct bromomethylation [200] or via transhalogenation of chloromethyl–PS–DVB [313] and subsequently transformed into the corresponding norborn-2-ene derivative by reaction with sodium norborn-2-ene-5-ylmethanolate (Fig. 23).
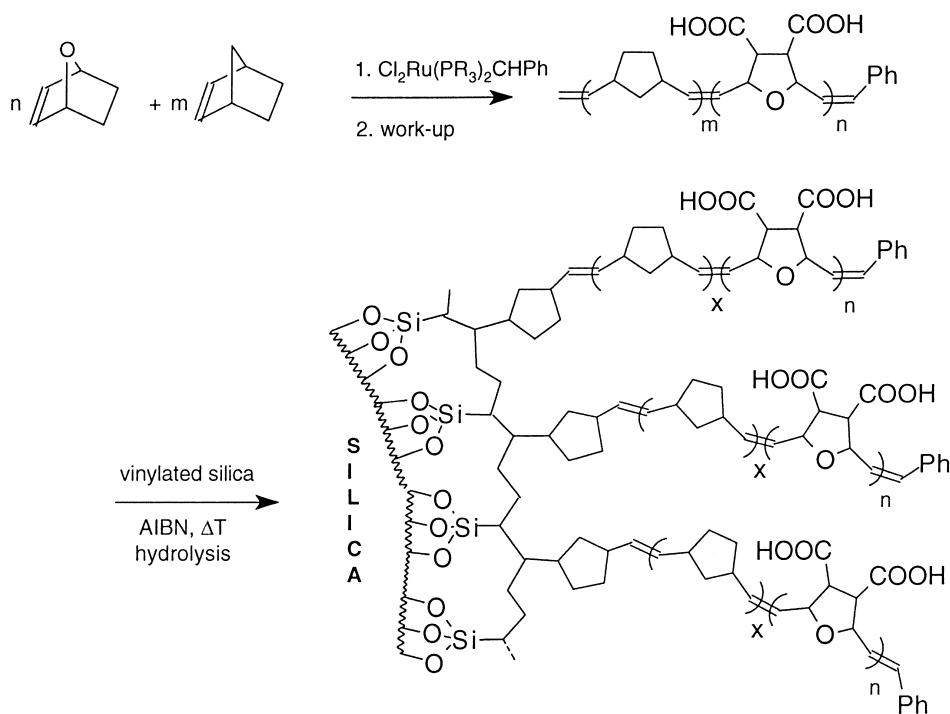
Fig. 22. Preparation of stationary phases for HPIC via ROMP.

In analogy, silica-based supports were prepared by silanization employing norborn-2-ene-5-yltrichlorosilane. A series of polymerizable chiral selectors based on L-valine, L-phenylalanine and β-cyclodextrin, respectively, were prepared starting from norborn-2-ene-5-carboxylic acid chloride (Figs. 24 and 25).

The resulting compounds, e.g., N-(norborn-2-ene-5-carboxyl)-L-valine, N-(norborn-2-ene-5-carboxyl)-L-phenylalanine and N-(norborn-2-ene-5-carboxyl)-L-phenylalanine ethylester as well as the norborn-2-ene-5-yl-derivatized β-cyclodextrins (R-β-CD, R= norborn-2-ene-5-carboxyl and norborn-2-ene-5-ylmethylsiloxyl) were attached to the support via ROMP-grafting (Fig. 26).

The resulting PS–DVB- and silica-based CSPs were used for chiral separations and proved to be highly chemically inert. Compared to commercially available CSPs, they showed comparable or even higher selectivities as well as separation efficiencies for β-blockers, N-dansyl- and N-2,4-dinitrobenzoyl (DNB)-protected amino acids. While the problems
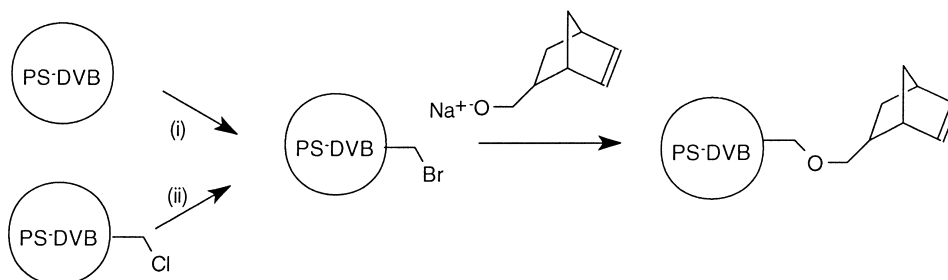

Fig. 23. Surface derivatization of bromomethylated PS–DVB for subsequent ROMP-grafting.
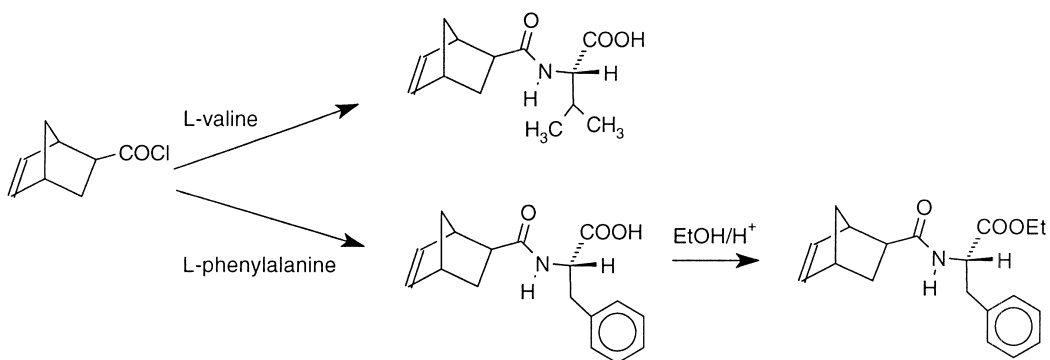
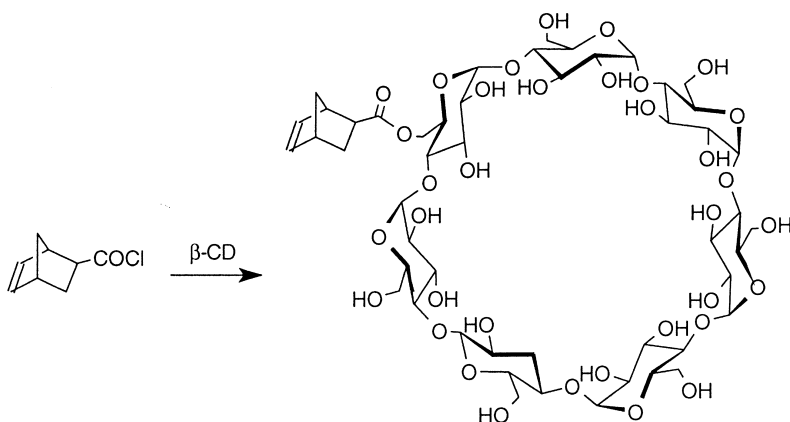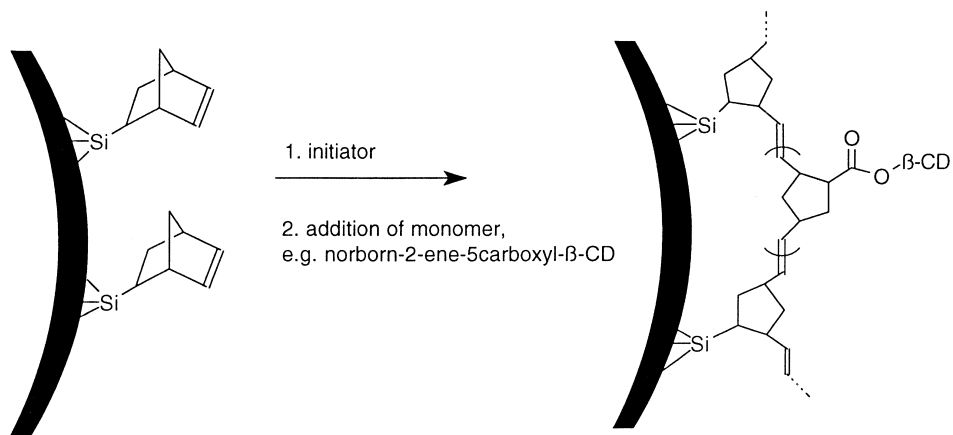Fig. 24. Synthesis of chiral monomers based on amino acids.

Fig. 25. Synthesis of polymerizable β-cyclodextrins (β-CDs).

1. initiator

2. addition of monomer,
e.g. norborn-2-ene-5carboxyl-ß-CD

Fig. 26. Surface grafting of silica using ROMP.

related to the pH instability of silica still remain, this approach offers an excellent access to pH-stable, functionalized organic supports. Finally, the applicability of the ROMP concept is further underlined by the fact that it may even be extended to the preparation of monolithic materials (see above) [291].

3.4.5. Stationary phases prepared by molecular imprinting

The first reports on molecular imprinting may be traced back to the early 1970s [314,315]. A system described in Ref. [316] may be regarded as typical. 4-Vinylphenylboronic acid adducts of 4-nitrophenyl mannopyranoside were copolymerized with ethylene dimethacrylate and methyl methacrylate to yield the corresponding imprinted polymer. In this context, it is worth mentioning that the nature of the cross-linker was found to play an important role [317] as it is regarded to be responsible for the "stiffness of the cavity". After cleavage of the template, a cavity with two free boronic acid groups pointing towards its interior was left and subsequently used for the selective removal of one enantiomer of α-mannopyranoside (Fig. 27).

As can easily be deduced therefore, ligands capable of providing covalent and ionic interactions [318–322] that also allowed the batch separation of racemic sugars [323] were mainly used for the preparation of imprinted stationary phases. In the

following years, numerous applications based in principle on the same concept yet using different linkers such as acetales [324] have been reported and the term "molecular recognition" was introduced (Ref. [325] and literature cited therein). Modern analytical tools were applied in order to elucidate the nature of recognition [326]. In the following, only those contributions relevant for the preparation of imprinted HPLC supports are summarized.

While imprinting techniques initially employed linkers that were capable of forming covalent bonds with the print molecule, modern imprinting techniques solely use the rigid skeleton of the imprinting polymer. Sellergen and Shea [327] gave a quantitative description of the requirements for polymer morphology which affect molecular recognition. One lies in the preparation of a stiff, highly cross-linked polymer with high T_g values, since only such rigid structures possess a shape persistence that is necessary for molecular recognition. Among the vast variety of polymeric systems, acrylates are known to fulfill these criteria in an optimum way. Consequently, many imprinted polymers are based on mixtures of methacrylic acid (MAA) and ethylene dimethacrylate (EDMA). In 1990, Mosbach and co-workers described the use of such an imprinted polymer for the separation of the enantiomers of phenylalanine anilide [328–330]. Dauwe and Sellergren [331] demonstrated that both the structural and electronic properties of the print molecule are rel-
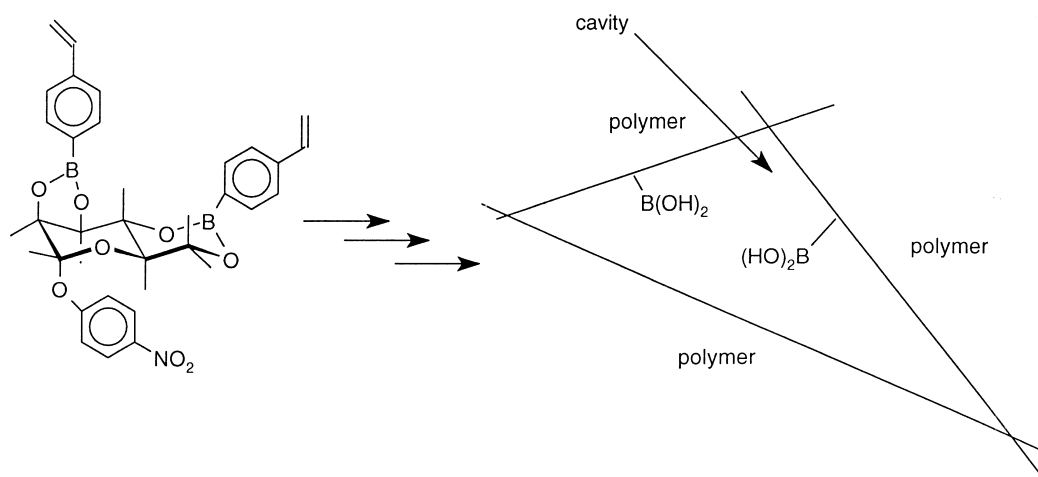

Fig. 27. Formation of a (sugar) imprinted cavity.

evant for the final binding selectivity. The finding that MAA did not only possess excellent structure forming properties but also form strong hydrogen bonds with a variety of polar functionalities such as carboxylic acids, carbamates, carboxylic esters and heteroatoms such as sulfur and phosphorus, further extended the applicability of molecular imprinting [329,332,333]. Thus, an EDMA–MAA system was used for the preparation of phenylalanine-imprinted stationary phases for CEC [334]. Imprinted polymers suitable for acidic analytes were additionally obtained by copolymerization of vinyl pyridine with EDMA and MAA [335]. Ion interactions have been proposed to represent the major binding. In case where chiral imprints (e.g., benzoyloxycarbonyl-L-tyrosine, dansyl-L-phenylalanine, Boc-L-phenylalanine, etc.) were used as the imprint molecules, highly efficient chiral separations – even in aqueous media – of the corresponding two enantiomers have been achieved [336,337]. The cavities formed by the polymerization process were found to be well defined and structure-stable. This even allowed their use as antibody mimics [338] and as selective (on-line)-SPE-materials [339–341]. The use of other chirally imprinted tri- and tetrafunctional cross-linkers such as pentaerythritol triacrylate and 2,2-bis(hydroxymethyl)butanol trimethacrylate leads even to comparably better enantiomeric resolutions and higher load capacities [342]. Surprisingly, molecular imprinting may even be applied to the selective binding of apolar compounds such as 2,3,7,8-tetrachlorodibenzodioxine (TCDD). This may be accomplished by taking advantage of weak interactions such as hydrogen-bonding to aromatic chlorine atoms or Π-stacking [343,344]. For the preparation of such imprints polymerizable TCDD analogues such as 2,8-bis(4-vinylphenylaminocarbamoylamino)-3,7-dichloro-benzodioxine have been used as an imprint molecule and copolymerized with divinylbenzene [345]. Removal of this template generated the hydrogen bonds necessary for molecular recognition. Similar hydrophilic interactions were reported in Ref. [346] for the separation of Boc-D,L-tryptophan.

Plunkett and Arnold described a combination of molecular imprinting and coating techniques [347]. For that purpose, trimethoxysilylpropyl-derivatized silica was surface-cross-linked with EDMA in the presence of the imprint molecule, Cu(II)-[*N*-(4-vinylbenzyl)-imino]diacetic acid (Cu-VBIDA). The resulting HPLC support was successfully used for ligand-exchange chromatography. In order to contribute to a maximum miniaturization, Yoshikawa and co-workers reported on the use imprinted polymeric membranes for the optical resolution of amino acids [348,349]. These membranes were prepared form THF solutions of an acrylonitrile–styrene copolymer (30% acrylonitrile) using oligopeptides as imprint molecules. 9-Ethyladenine imprinted polymer membranes prepared from methacrylic acid and EDMA [350] were found suitable for the selective transport of molecules similar to the imprint molecule through the membrane. This approach differs from traditional imprinting experiments in that it does not use the selective binding capabilities of the matrix for retention but for transport. Interestingly enough, highest selectivities were found at low imprint concentrations. The origin of selective transport was attributed to adenine selective binding sites in the membranes that only allow the transport of molecules that possess a purine structure similar to adenine.

Imprinted polymers are usually prepared by bulk polymerization resulting in the formation of monolithic materials using either thermal- or radiation-induced [351] radical polymerization. These monoliths are then ground or sieved prior to use. Mosbach and co-workers [352,353] reported on the preparation of spherical imprint particles via suspension polymerization in a perfluorocarbon liquid (perfluoromethylcyclohexane). Perfluorinated polymeric surfactants consisting of poly[*N*-acroyl-2-(*N*-perfluoroalkylsulfonamidoethanol) - *co* - *O* - acroylpolyethylenglycol-200-monomethylether] were used to create a biphasic system. The perfluorinated solvent is immiscible with other organic solvents and does – in contrast to water – not interfere with the non-covalent interactions involved in the formation of the molecular recognition sites. The particle diameter may be adjusted by the amount of fluorinated surfactant added to the system. The use of porogenic solvents such as toluene, chloroform, methanol, THF or acetone guarantees a sufficient porosity [353]. Using Boc-L-phenylalanine as the imprint molecule, the corresponding racemic mixture was successfully separated. Another interesting access to monosized

polymer-based imprinted stationary phases has been described by Hosoya et al. [354]. The two-step swelling protocol entails the activation of monosized PS–DVB particles with dibutylphtalate and subsequent swelling with the cross-linker (EDGMA), porogenic solvents and the template molecule. Using these imprinted materials, successful separations of positional isomers of diaminonaphtalene and racemates of DNB-Phe were reported. Alternatively, 1 µm uniform-sized PS seed particles prepared by emulsifier free emulsion polymerization (see above) have been used in the multi-step swelling and thermal/redox polymerization based on 4-vinyl pyridine and EDMA [355]. During the third and last swelling step S-naproxene was added as the imprint molecule. Nevertheless, separation performance for enantiomeric naproxene was rather low, a finding that was attributed to unfavorable pore size distributions and consequently poor mass-transfer due to low diffusion.

Modern aspects of molecular imprinting cover the aspects of selective materials for the enrichment and separation of drugs [356,357], herbicides and pesticides [358,359], for the preparation of biomimetic receptors [360] and enzymes [361,362] as well as for the preparation of sensors [363–365]. A review on the use of molecular imprinting for the preparation of stationary phases suitable for CEC has been given recently [366]. Based on the currently available reports and on the fact, that non-linear effects in enantioselectivity and adsorption have been observed with high analyte loadings [367], imprinted sorbents represent themselves as highly attractive materials rather for SPE than for HPLC.

4. Abbreviations

Boc	*tert*-Butoxycarbonyl
BUDGE	1,4-Butanediol-diglycidyl ether
CSP	Chiral stationary phase
CZE	Capillary zone electrophoresis
EDMA	Ethylene dimethacrylate
GMA	Glycidyl methacrylate
MAA	Methacrylic acid
MEK	Methylethylketone
NIPAM	*N*-Isopropylacrylamide
ODS	Octadecylsilane

PS	Polystyrene
PS–DVB	Polystyrene–divinylbenzene
RP	Reversed-phase
σ	Specific surface area
TRIM	Trimethylolpropane trimethacrylate
T_g	Glass transition temperature

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