

Review

Modified aluminas as chromatographic supports for high-performance liquid chromatography

Joseph J. Pesek*, Maria T. Matyska

Department of Chemistry, San Jose State University, College of Science, One Washington Square, San Jose, CA 95192-0101, USA

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Abstract

This review begins by describing the relevant properties of alumina as a support material for chemically bonded stationary phases in HPLC. The most common chemical modification processes are summarized as well as the advantages and disadvantages of each method. In order to more fully understand the chemically modified alumina surface, some spectroscopic approaches are outlined for characterization of the bonded phases. Finally, a number of successful applications are described for a variety of chemically modified aluminas in order to illustrate their potential usefulness and to compare their chromatographic behavior to the more conventional silica-based materials. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Reviews; Stationary phases, LC; Surface modification; Supports; Alumina

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1. Introduction

Silica is used as the solid support for virtually all

of the commercially available inorganic oxide chemically bonded stationary phases in HPLC. Only polymer-based materials represent a significant fraction of any other type of stationary phase that are found in common use today. Despite the apparent

*Corresponding author. Fax: +1-408-924-4945.

dominance of silica as the solid support of choice, there has been considerable research and development into alternative materials. The main focus of this effort is to overcome the deficiencies that silica has under certain experimental conditions in HPLC. The most serious of these are the pH instability of silica and chemically modified silica, as well as its strong affinity for strongly basic compounds. For the vast majority of commercially available silica-based stationary phases, the typical operating range is between pH 2 and 8. At lower pH values the bonded material generally undergoes decomposition, while at higher pH conditions the silica matrix begins to dissolve. Higher operating pH values have been attained by saturating the mobile phase with silica or through the use of sterically protected [1] or bidentate [2] stationary phases that increase the hydrophobicity near the surface preventing access of the attacking base. Other approaches for high pH operation include densely bonded, extensively endcapped, longer chain column packings with certain organic buffers as part of the mobile phase [3–6]. One approach to overcoming the chromatographic problems associated with silica is to select a different type of oxide support. In order to improve the pH stability of the support material, oxides such as alumina, titania and zirconia have been tested. It is also possible that the surfaces of these oxides (principally the hydroxyl groups) might be less adsorptive than silica after chemical modification. This review describes some of the efforts made to modify and characterize alumina as a support for stationary phases in HPLC.

2. Alumina properties

The surface of alumina and its corresponding chemistry is complex, much more so than silica. The basic spinel structure often possesses defects that result in various arrangements of aluminum ions [7]. Therefore, hydration of the surface as well as the number of hydroxyl groups per unit area is determined by the specific three-dimensional structure of the oxide. This complexity is illustrated by comparing the results of various characterization

methods on silica and alumina. For example, the infrared spectrum of silica is characterized by a sharp band near 3750 cm^{-1} representing the isolated silanol groups on the surface and a broad band in the range of $\sim 3900\text{--}3200\text{ cm}^{-1}$ for the hydrogen-bonded silanols and the adsorbed water [8]. The existence of several types of hydroxyl groups on the surface of alumina was first determined by infrared spectroscopy [9]. For just the isolated hydroxyl groups, three bands were originally assigned to these OH stretches [10] and two more were identified in a later study [11]. While there is some variation in the overall hydroxyl content of silica, in most cases the value is assumed to be near $8\text{ }\mu\text{mol/m}^2$ for typical chromatographic grade, fully hydroxylated materials [12–14]. An early study on alumina reported the amount of hydroxyl on aluminum oxide between 16 and $25\text{ }\mu\text{mol/m}^2$ [11], while a more recent study on a chromatographic material determined a value of close to $40\text{ }\mu\text{mol/m}^2$ [15].

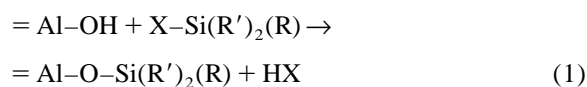
Further insight into the structure and properties of alumina can be gained from ^{27}Al nuclear magnetic resonance (NMR) studies. The spectra of various types of alumina are characterized by two peaks: one at 60 ppm that represents tetrahedral (T_d) coordinated aluminum while the other peak near 1 ppm is assigned to octahedral (O_h) coordinated atoms [16,17]. These early studies were obtained by magic angle spinning (MAS) which allows the probing of quadrupolar nuclei ($I \geq 1$). The MAS technique improves resolution so that the broad lines associated with quadrupolar nuclei in solids can be narrowed sufficiently in order to identify different chemical shift environments that may exist within a particular compound. It was determined from the ^{27}Al MAS NMR studies that the ratio of the tetrahedral (T_d) to octahedral (O_h) sites varied in different types of aluminas. For hydrated alumina, it was shown that by combining cross-polarization with MAS, sensitivity could be improved so that an ^{27}Al NMR spectrum could be obtained with a better S/N ratio in an equivalent period of time [15].

Another approach to characterizing alumina, as well as other oxide materials, is to measure the distribution of adsorption energies for various molecules on the surface. An early study characterized acidic sites on alumina through the adsorption of

ammonia [18]. It was shown that acidic sites corresponded to the hydroxyl groups on catalytic surfaces that strongly held and isomerized olefins. More recently, gas chromatographic studies determined the surface heterogeneity of various ceramic alumina oxide powders as well as chromatographic alumina [19,20]. In this method the elution profiles for a number of test solutes are measured and used to derive the adsorption isotherm. Subsequently the identification and distribution of various energy sites on the surface is calculated from the isotherms. For chromatographic alumina [20], the energy distribution was either bimodal or trimodal depending on the probe (solute) used and the type (hydrated or dehydrated) of alumina tested.

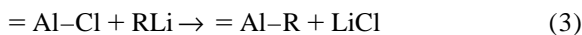
3. Chemical modification of alumina

Alumina, as is the case with silica, has been modified by a number of different chemical reactions. Because alumina was investigated as a possible substitute for silica, organosilanization was tested first for its potential to provide chemically modified surfaces, particularly materials for reversed-phase operation. Knox and Pryde were among the first to test this concept and proved that reversed-phase materials could be prepared on alumina by this



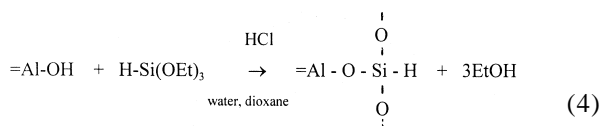
reaction [21]. However, further investigations showed that reaction (1) was not feasible for many applications since the Al–O–Si bonds formed in organosilanization are not stable under acidic conditions [22,23].

With a limited range of applications for phases prepared via organosilanization, other avenues for the chemical modification of alumina surfaces were explored. One approach involved first chlorinating the surface using thionyl chloride (Eq. 2) followed by reaction with an organolithium compound (Eq. 3) [24]. This two-step process was tested for the synthesis of a butyl-bonded reversed-phase material.

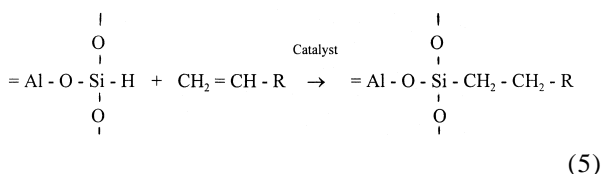


The process was shown to successfully bond the desired organic moiety but the two-step process involving the necessity of scrupulously dry conditions did not readily lend itself to commercial adaptation. Another synthetic method successfully adsorbed both octyl and octadecyl moieties on alumina through interactions between surface hydroxyls and either organophosphonic or carboxylic acids [25–28]. This same method has also been applied to the synthesis of perfluorooctyl and perfluorobutyl stationary phases on alumina [29]. The long-term stability of these phases has never been fully assessed and it has already been demonstrated that these phases degrade in the presence of certain buffers in the mobile phase, particularly phosphate.

One synthetic method that was developed for the modification of silica has been successfully adapted for use on alumina [14,30]. The two-step process involves silanization (Eq. 4 below) that first produces a hydride intermediate followed by hydrosilation (Eq. 5 below) that attaches the desired organic moiety [31]. On alumina the silanization reaction using triethoxysilane (TES) is described as follows:



Under ideal conditions the TES will crosslink with neighboring groups to produce a hydride monolayer on the surface. This intermediate can be further modified via hydrosilation that most often utilizes a terminal olefin in the presence of a suitable catalyst such as hexachloroplatinic acid (Speier's catalyst) as shown in the following reaction:



Other options that make the reaction versatile are the possibility of using other unsaturated functional groups (alkynes, cyano and nonterminal olefins) within the bonding moiety and a variety of catalysts, such as other transition metal complexes and free radical initiators. This synthetic route has not been utilized for any commercial phases to date.

The technique that has been exploited most extensively for the chemical modification of alumina for HPLC involves coating chromatographic particles with polymers. Early studies demonstrated that alumina could be successfully coated with polybutadiene, polyoctadecylsilane and polystyrene–divinylbenzene to produce reversed-phase materials [32]. The coatings based on polybutadiene and polysilanes (octadecyl and propyl cyano) have proved the most popular and can be obtained commercially [33,34]. These materials possess excellent pH stability based on both the polymer coating and the underlying alumina support. However in common with other polymer chromatographic phases, these materials can display strong retention for certain solutes, create high backpressures due to swelling in some solvents and have poor efficiency for some solutes due to mass transfer effects [32].

4. Spectroscopic characterization of chemically modified alumina

In spite of their development over more than a decade, relatively little spectroscopic characterization exists for chromatographic aluminas, especially those which are chemically modified. As described above, there are a number of spectroscopic studies on various types of unmodified aluminas, particularly different types of ores. Infrared studies have mainly concentrated on the hydroxyl groups, particularly their number and variation as a function of the type of alumina and degree of hydration, as well as Al–O–Al linkages created during the dehydration process [9–11]. Similar studies by ^{27}Al NMR have been concerned with the relative distribution of the T_d and O_h sites as a function of the type of alumina [17]. Some scanning electron micrographs have been obtained for calcined alumina aerogel in order to

determine its overall morphology [11]. With respect to chromatographic alumina, scanning electron microscopy (SEM) has been used to characterize the unusual particle structure of the commercially available Unisphere[®] material [35]. This material has an overall spherical structure consisting of fused crystalline platelets possessing both megapores in the gaps between the platelets as well as internal micropores. Infrared studies have been used to characterize aluminas modified by adsorption. More extensive evaluations have been done concerning water [9–11] but studies involving carbon dioxide, carbon monoxide [11] and ammonia [18] have been used to characterize adsorption sites on alumina. The O–H, C–F and Al–O stretching bands have been identified for the perfluorinated phases prepared by the adsorption of organic acids on alumina [29].

More spectroscopic characterization exists for two other types of chemically modified aluminas: materials made by the chlorination/organolithium process [24] and by the silanization/hydrosilation method [15]. In the case of the former method, only diffuse reflectance infrared Fourier transform (DRIFT) spectra have been used to evaluate the final product since the intermediate (chlorinated surface) is not stable under normal conditions used to acquire IR data. In comparing the butyl alumina material made by the chlorination/organolithium process to bare alumina, there were strong peaks in the carbon–hydrogen stretching region for the product indicating successful modification of the surface. In the oxygen–hydrogen bending region, there was a noticeable decrease in the OH band intensity for the butyl alumina in comparison to the unmodified starting material. This result also indicated success of the modification process. In addition, DRIFT was used to characterize the butyl alumina after exposure to strongly acidic (pH 1) and strongly basic (pH 12) solutions. In both cases, strong carbon–hydrogen stretching bands were evident in the two spectra indicating that the modified material was stable in both acid and base.

For the silanization/hydrosilation process, it was possible to characterize both the hydride intermediate and the products (octyl and octadecyl modified alumina). Fig. 1 shows the DRIFT spectra for bare alumina, the hydride intermediate and the octadecyl product. In the case of bare alumina (Fig. 1A), the

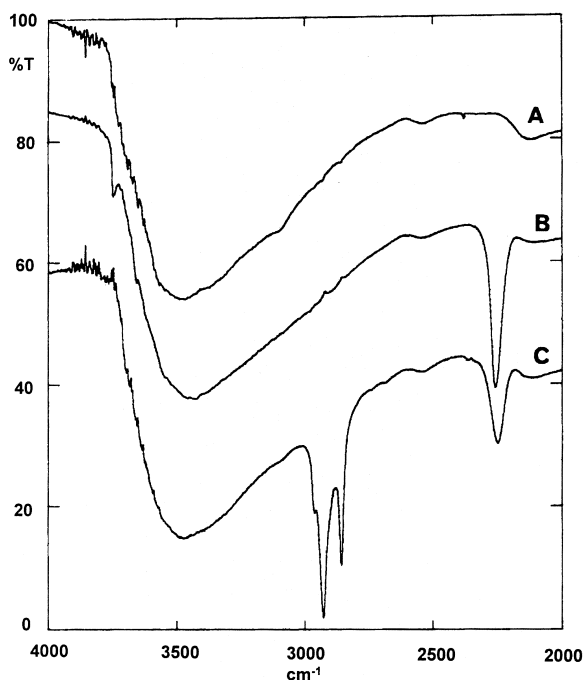


Fig. 1. DRIFT spectra of various alumina materials: (A) bare alumina; (B) hydride-modified alumina; and (C) octadecyl-modified alumina [15].

spectrum contains a broad peak between 3800 and 2700 cm^{-1} as a result of adsorbed water on the surface. Also evident are several sharp peaks superimposed on the broad band between 3500 and 3700 cm^{-1} . These peaks are due to the various hydroxyl groups on the surface of alumina identified in previous studies [9–11]. Upon silanization (Fig. 1B), many of the alumina hydroxyl peaks disappear indicating the reaction was successful. This result is confirmed by the appearance of a strong Si–H stretching peak near 2250 cm^{-1} and a small peak near 3750 cm^{-1} representing the silanol (Si–OH) groups formed when not all of the adjacent TES moieties crosslink during silanization. The spectrum of the final product of the hydrosilation reaction with 1-octadecene is shown in Fig. 1C. Success of this reaction is indicated by a decrease in the Si–H band and the appearance of strong carbon–hydrogen stretching bands between 3000 and 2800 cm^{-1} . Additional characterization was also done by ^{29}Si cross-polarization (CP)-MAS NMR spectroscopy as shown in Fig. 2. Here the hydride intermediate (Fig.

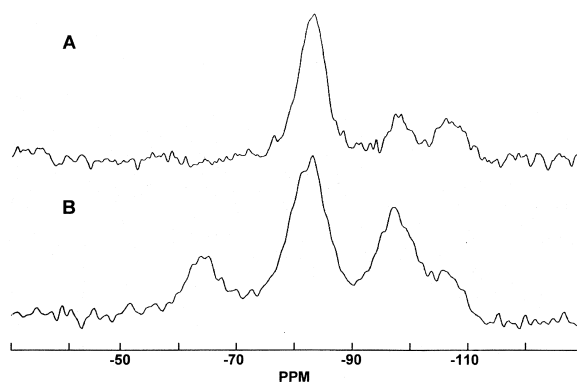


Fig. 2. ^{29}Si CP-MAS NMR of modified aluminas: (A) hydride-modified alumina; and (B) octadecyl-modified alumina [15].

2A) contains a strong peak for the Si–H group at -82 ppm and small peaks near -100 ppm for the silanols (also detected by DRIFT) and for a silicon surrounded by four oxygens (-110 ppm). The latter is the result of a small amount of polymerization of the TES beyond a monolayer coverage on the surface. The products were also characterized by ^{13}C CP-MAS NMR. The spectra obtained were identical to those of the octyl [36] and octadecyl [36] silica-based materials. Finally, ^{27}Al CP-MAS spectra were also obtained on the bare alumina, hydride intermediate and octadecyl product. From the integrated intensities of the two peaks, the bare alumina had approximately 20% of the aluminum atoms in the T_d geometry and 80% in the O_h geometry. A new peak appeared near the O_h chemical shift upon formation of the hydride intermediate that increased in intensity upon hydrosilation. No change in the T_d peak was seen in either the hydride intermediate or product spectra. These results confirm the success of the two reactions as well as establish that by this process all of the chemical modification is confined to aluminum atoms possessing octahedral geometry.

5. Chromatographic characterization and applications of chemically modified alumina

5.1. Materials made by chlorination/organolithium process

Only a limited amount of chromatographic charac-

terization was carried out on the butyl-bonded phase made by this process [24]. Two types of samples were tested. First, the retention of various alkyl aryl ketones was monitored as a function of the length of alkyl chain and mobile phase composition in order to establish reversed-phase behavior. Second, the short chain length of the bonded phase was conducive to separation of proteins. The peaks obtained with mobile phase compositions from 90:10 to 10:90 isopropanol–0.1% TFA were symmetrical with good efficiency indicating that neither significant adsorption or degradation of the samples occurred on the stationary phase.

5.2. Materials made by silanization/hydrosilation

This material also has undergone a limited amount of chromatographic testing [15]. A reversed-phase test mixture was run on the octadecyl material and the results are shown in Fig. 3. The elution is

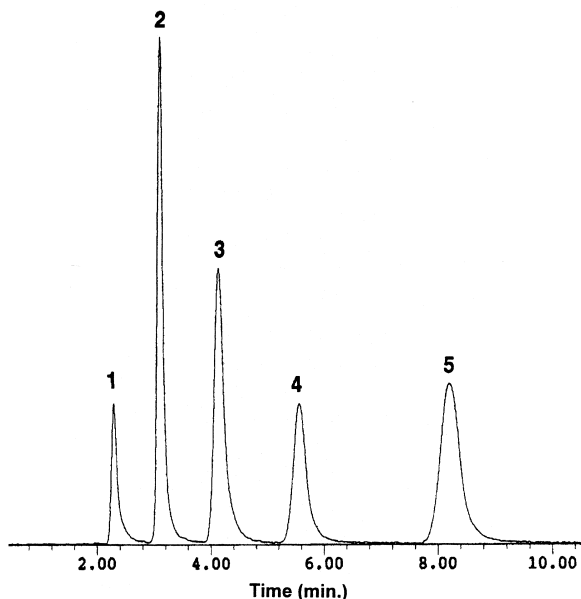


Fig. 3. Chromatogram of reversed-phase test mixture on octadecyl modified alumina. Mobile phase, 50:50 acetonitrile–water; particle size, 8 μm ; column, 150 \times 4.6 mm I.D.; flow-rate=1 ml/min at room temperature; detection at 254 nm. Solutes: (1) theophylline; (2) *p*-nitroaniline; (3) methylbenzoate; (4) phenetol; and (5) *o*-xylene [15].

consistent with reversed-phase retention and the peak symmetries and efficiencies obtained in this test were comparable to or better than those obtained on a commercially available octadecyl alumina column. A sample of anilines (aniline, *N*-methylaniline and *N,N*-dimethylaniline) was also tested on this column. All solutes were easily separated with symmetric peaks. Good pH stability ($\Delta k' < 10\%$ after more than 2000 column volumes) was obtained under both acidic (pH 2) and basic (pH 10) conditions. These results suggest that further testing of materials made by this method is warranted.

5.3. Materials made by adsorption of organic acids

The bonded phases produced by this method have been investigated chromatographically using a variety of test solutes with the focus being on making a comparison to similar bonded material on silica [19–22]. Since the predominant factors in retention are the length and bonding density of the alkyl group, it would be expected that capacity factors for the two materials would be similar. This was found in many cases but in some instances there were exceptions. In particular, aromatic compounds, especially those with multiple rings, were more strongly retained on octadecyl alumina (ODA) in comparison to octadecyl silica (ODS). It was also observed that hydrogen bonding interactions were stronger on the ODA phase than on the ODS material. These results indicate that there are some effects of the differences in chemical properties between the two solid supports that affect retention. An example of a separation of a drug mixture is shown in Fig. 4. This analysis was done using an alkaline mobile phase (0.10 *M* NaOH) illustrating the advantage of alumina-based stationary phases at high pH. The ODA phase can also be used to analyze proteins but in most cases an ODS column will give better resolution [27].

Similar characterization of the perfluoro-bonded materials made by this method was also undertaken in order to identify any advantages and/or differences with respect to the normal hydrogen-containing alkyl moiety as well as silica-based stationary

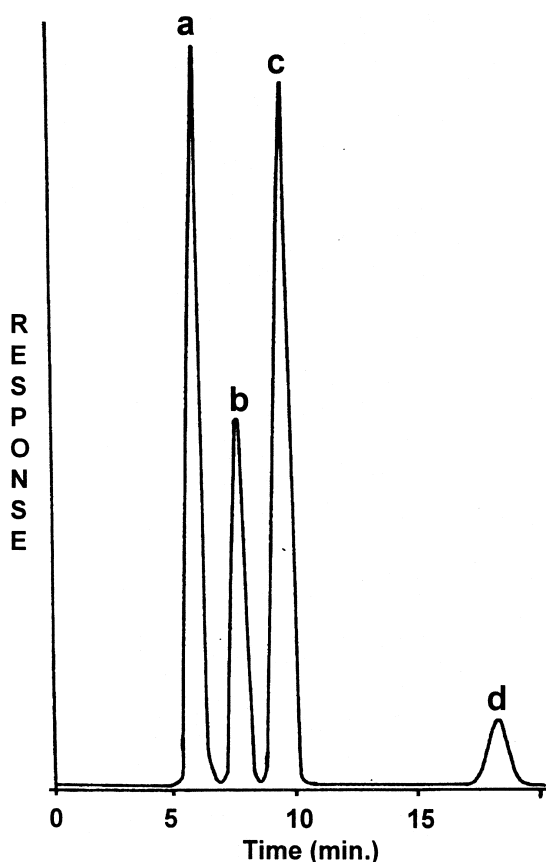


Fig. 4. Chromatogram of drug mixture on octadecyl-modified alumina. Mobile phase, 35:65 methanol–0.10 M NaOH; particle size, 8 μm ; column, 250 \times 4.6 mm; flow-rate=2 ml/min at room temperature; detection at 220 nm. Solutes: (a) codeine; (b) procaine; (c) thebaine; and (d) cocaine [25].

phases [29]. As expected, the perfluorinated phase of the same alkyl chain length had lower k' values for all solutes tested with respect to the same hydrogen-containing moiety on alumina and silica. While reasonable separation of a peptide mixture was obtained on the perfluorinated-bonded phase, the stability was not good (significant k' changes were observed after only a few injections) in the trifluoroacetic acid (TFA) mobile phase required for this analysis. However, stable separations of phenols were obtained on this phase. An example of such a separation is shown in Fig. 5.

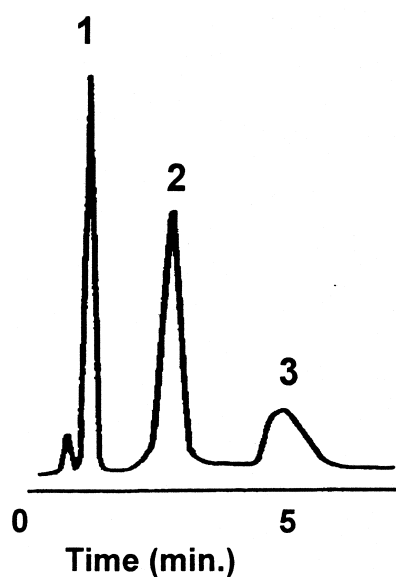


Fig. 5. Chromatogram of phenol mixture on perfluorobutyl-modified alumina. Mobile phase, 0.2% aqueous TFA; particle size, 8 μm ; column, 150 \times 4.6 mm; flow-rate=2 ml/min at room temperature; detection at 255 nm. Solutes: (1) *m*-cresol; (2) 2-naphthol; and (3) thymol [29].

5.4. Materials made by polymer coating

Except for the bare oxide, alumina materials made by this process have the most extensive chromatographic documentation in the literature. Even so, compared to reversed-phase silica, the characterization and application of these bonded materials is still quite limited. Because of commercial availability, the separations and retention data found in the literature are generally confined to three polymer-coated stationary phases: polybutadiene, polymeric octadecyl and polymeric cyano.

A variety of individual solutes as well as mixtures have been tested on polybutadiene-coated alumina (Al-PBD). It was shown that for most solutes the methylene selectivity (k'_2/k'_1 , where solutes 1 and 2 differ by only a methylene unit) was similar to standard octadecyl-bonded silica phases [33]. Similar findings were obtained as the number of solutes tested increased as well as with greater variation in the composition of the mobile phase [35]. In com-

parison to other polymer columns (polymer-coated silica and a styrene–divinylbenzene phase), the Al-PBD material gave similar but not identical separation to a mixture of theophylline, *p*-nitroaniline, methyl benzoate and *o*-xylene [37]. In addition to this test mixture, the Al-PBD was successful in the separation of samples containing purines and pyrimidines, PTH amino acids, peptides, catecholamines, fat soluble vitamins and analgesics (Fig. 6). These examples represent a broad range of different chemical structures and parallel the types of separations that have been achieved on reversed-phase silica materials. In another report, the separation of a

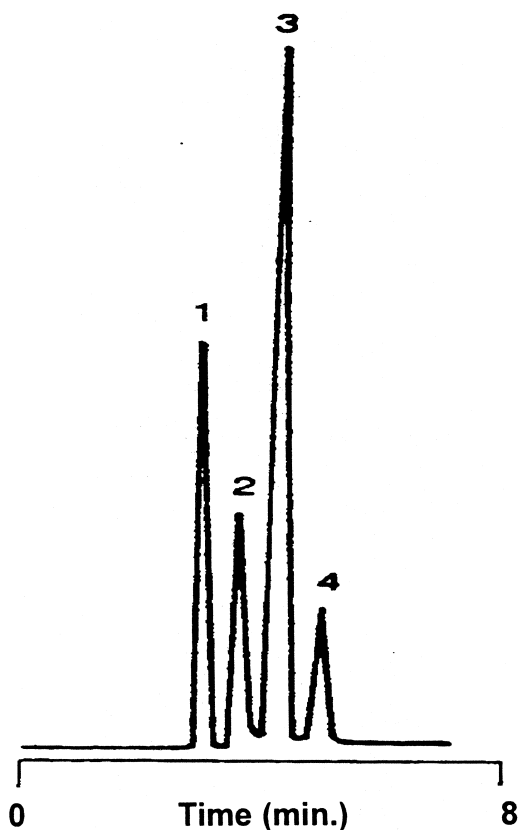


Fig. 6. Separation of a mixture of analgesics on polybutadiene-modified alumina. Mobile phase, 20:80 acetonitrile–water; particle size, 8 μm ; column, 250 \times 4.6 mm; flow-rate=1 ml/min at room temperature; detection at 254 nm. Solutes: (1) acetomenophen; (2) salicylamide; (3) phenacetin; and (4) acetylsalicylic acid [37].

series of anilines and opium alkaloids was presented [34]. The hydrophobicity of Al-PBD was evaluated under a wide range of eluent pH conditions and it was determined that solute interactions with this stationary phase were similar to those of typical silica-based reversed-phase materials [38,39]. The use of quantitative structure–activity relationships (QSAR) was tested on the Al-PBD column for a group of pharmacologically activeazole derivatives [40]. It was also demonstrated that polybutadiene alumina was not as effective as other normal polymer columns for the separation of charged and uncharged oligomer-like model compounds [41]. It was also shown for that for a protein mixture (ribonuclease A, cytochrome c, lysozyme and carbonic anhydrase) resolution and peak capacity were considerably less when compared to a commercially available ODS phase [42]. In addition, considerable adsorption of the proteins was observed on the Al-PBD column. Although most of the studies on the Al-PBD material and other modified aluminas have commented on and/or verified some degree of increased pH stability, one study [43] specifically addressed this point evaluating basic compounds and a wide pH range for the mobile phase.

A polymer-based octadecyl column might be expected to give a chromatographic performance similar to that of a standard C_{18} silica stationary phase. The methylene group selectivity study [35] confirmed similar retention characteristics between C_{18} silica and C_{18} alumina (Al- C_{18}) made from a polymer process. A number of applications of this material have also been reported [34]. The resolving power of this material was demonstrated by the separation of a mixture of five closely related peptides. This mixture developed for testing reversed-phase materials [44] includes five COOH-terminal decapeptides with only small differences in hydrophobicity among them. Another more complex mixture of peptides was separated on both Al- C_{18} and octadecyl silica columns. The two phases gave comparable resolution but there was an inversion of elution order for two components indicating that small differences exist between the two separation materials. When a protein mixture was tested on the two columns, the resolution was virtually the same when using identical gradient mobile phase con-

ditions. The separation obtained on the alumina-based column is shown in Fig. 7. However, when an Al-C₁₈ material was tested for the retention of a series of commercial pesticides, significant differences were observed when compared to ODS [45]. It was postulated that since elution was not directly correlated to hydrophobicity but different from bare alumina, that some adsorption sites on the surface were not completely covered by the bonded organic phase. These results contrast with the other reports cited above where little difference is observed between Al-C₁₈ and ODS. This discrepancy may simply be due to different sources of the bonded alumina material.

The last of the polymeric materials that is com-

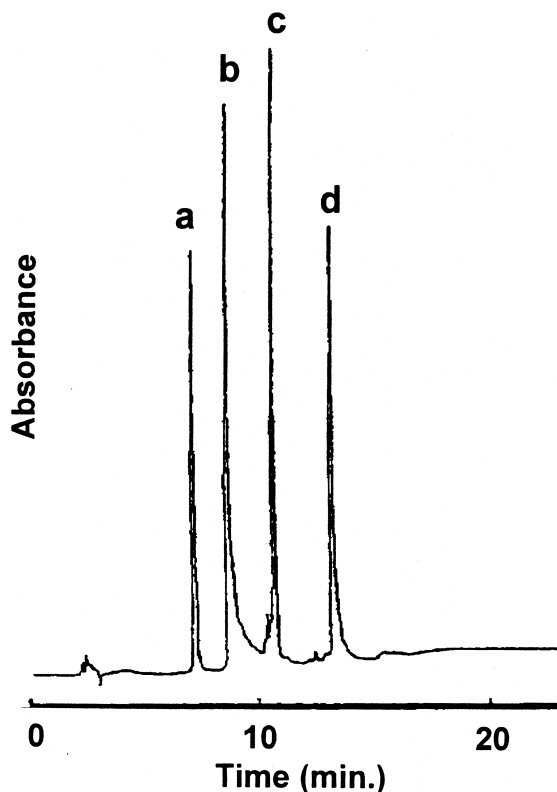


Fig. 7. Separation of a protein mixture on polymeric octadecyl-modified alumina. Mobile phase, (A) 0.2% TFA in water and (B) 0.2% TFA in acetonitrile. Linear gradient of 25% B to 100% B in 30 min. Solutes: (a) ribonuclease; (b) insulin; (c) lysozyme; and (d) myoglobin [34].

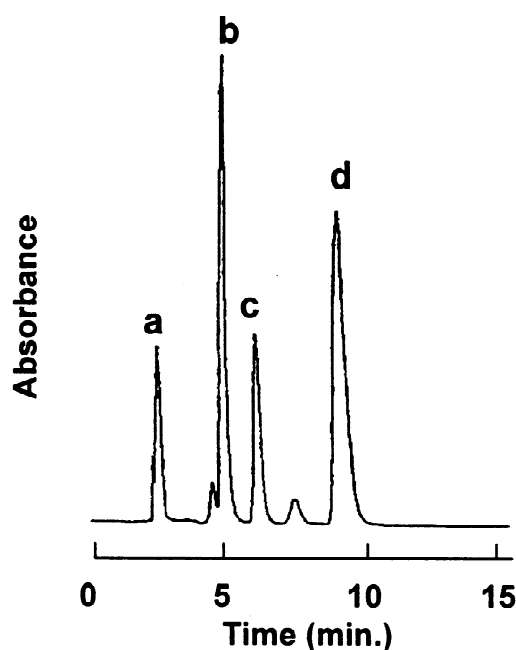


Fig. 8. Separation of a mixture of penicillins on polymeric cyano modified alumina. Mobile phase, acetonitrile–aqueous phosphoric acid (pH 3.0) (28:72). Solutes: (a) ampicillin; (b) oxacillin; (c) cloxacillin; and (d) dicloxacillin [34].

mercially available is the cyano phase. Its methylene selectivity was similar to the other polymeric alumina phases and to silica-based reversed-phase material indicating that the polymer backbone provides the basic hydrophobic character and that the cyano group has a small effect for the solutes tested under RP conditions [35]. However, the presence of the cyano group can be exploited for improved selectivity when molecules possess chemical properties in addition to hydrophobicity. Some examples of mixtures where good resolution of components has been obtained on the polymeric cyano alumina phase include penicillins (Fig. 8), antibiotics, and tricyclic antidepressants [34].

6. Conclusions

The chemical modification of alumina for use as a stationary phase in HPLC can be accomplished by a

number of synthetic approaches. Organosilanization appears to be the least effective. Chlorination/organolithium modification is feasible but the synthetic restrictions make this method less attractive commercially. Adsorption of organic acids is simple and produces usable materials but appears to have limitations with respect to buffers in the mobile phase. Silanization/hydrosilation appears to produce a viable material but has not been extensively tested. Polymerization processes have been the most extensively tested and are commercially available. In many cases, their retention properties are not significantly different than comparable silica-based materials. However, it has been shown that alumina-based stationary phases do have enhanced pH stability in comparison to similar silica materials. To date, there have been relatively few commercial products available and not many applications reported in the literature. This is probably due to the fact that in reality there are few applications where a pH extreme is necessary and when such a mobile phase is required methods have already been developed on alternate materials such as polymers [46]. Also, new synthetic methods have expanded the viable pH range of silica-based stationary phases further narrowing the window of opportunity for other supports like alumina. This is likely to be the situation until some new distinct advantage or application is identified for alumina-based stationary phases.

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