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CHREV. 193

ADSORPTION CHROMATOGRAPHIC SEPARATIONS ON BUFFERED SILICA GEL

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CONTENTS

1. INTRODUCTION

The buffered silica gel separation technique has been described previously[1,2]. It is used to reduce the tailing of polar compounds in adsorption chromatography with neutral organic mobile phases. No reagents have to be added to the mobile phase, which minimizes detection problems. The separation selectivity of adsorption chromatography for isomers is well known and has been used many times to separate non-polar solutes. With buffered silica gel it is now possible to separate polar isomers by adsorption chromatography.

The use of silica gel in combination with a buffer and organic solvents has already been described by Isherwood[3] and others An aqueous buffer solution was used to impregnate the surface of the silica gel. This layer formed a stationary phase, which converted the solutes into the ionized form where the partition favours the stationary phase. The separation mechanism is partition, because the water layer on the silica surface uses up most of the adsorption sites available and acts as a stationary phase.

By the term "buffered silica gel" we mean a silica gel that has been coated with a crystalline salt. The water used to bring this buffer salt into the pores and to distribute it evenly over the whole surface is evaporated and therefore has no influence on the chromatographic behaviour and the separation selectivity. The k' values of non-ionic compounds are the same as on a corresponding untreated silica gel system. The buffer layer affects only the peak symmetry of polar compounds and makes their elution on the adsorption chromatographic system possible. In some instances the pH of the aqueous solution of the buffer salt used to coat the silica gel can be an additional parameter for controlling the separation selectivity

This review was presented in part as a poster at the 8th International Symposium on Column Liquid Chromatography in New York, May 1984. It enlarges on a section of a previous review on carboxylic acid separations[4].

2 EXPERIMENTAL

2 1 Apparatus

Any commercial liquid chromatograph may be used for the application of buffered silica gel columns. We used an M-6000A high-pressure pump (Waters Assoc., Milford, MA, U.S.A.) to force the mobile phase through the column. The sample was introduced with a U6K sampling loop (Waters Assoc) and the eluent was monitored with a multi-wavelength UV detector (Pye Unicam, Cambridge, U.K.). The columns were made of glass, stainless steel or glass-lined steel and were packed in our laboratory. The technique used to slurry-pack the glass columns at 450 bar has been described previously[5]. For the preparation of the other columns a slurry of 2 g of buffered silica gel in 20 ml of *n*-hexane was packed into the column with *n*-hexane at 450 bar. During chromatography the columns were normally operated at room temperature and were not thermostabilized in any way.

2.2. Material

Different silica gels of high-performance liquid chromatographic (HPLC) quality were used as received without any further size classification of particles. All the reagents were of analytical-reagent grade (Fluka, Buchs, Switzerland) and were used without further purifications. The solvents used for packing the columns and as mobile phases were of HPLC grade (Rathburn Chemicals, Walkerburn, U.K.) and degassed with helium in an ultrasonic bath.

3 PREPARATION OF BUFFERED SILICA GEL

3.1. Batch procedure

A 150-ml volume of a 0.1 *M* aqueous solution of the buffer salt is prepared and to 50 ml of this buffer solution 5 g of silica gel are added to form a fluid slurry. The slurry is placed under vacuum (12 Torr) and immersed in an ultrasonic bath for 2 min to force the buffer solution into the pores. The slurry is then placed in a fritted disc funnel and filtered. The wet material is again added to 50 ml of buffer solution and the vacuum and ultrasonic treatment is repeated. After filtration in the fritted disc funnel the above step is repeated a third time with the final 50 ml of buffer solution.

The wet material from the last filtration is spread out on a crystallizing disc and dried at 80°C, under vacuum (12 Torr), for 20 h. The dry buffered silica gel is packed into the column as an *n*-hexane slurry, in order to prevent any of the buffer salt being washed off, under a pressure of 400–450 bar. The packed column may be equilibrated with the mobile phase or sealed and stored at room temperature for further use.

An *in situ* coating of the pre-packed silica gel columns is also possible, and permits the buffering of commercial columns. The batch procedure, however, is much more reliable and therefore preferred.

3.2. In situ preparation

The silica gel column, commercial or laboratory-packed, must first be rinsed with acetone or with any other water-miscible solvent. This intermediate solvent is then replaced completely with water. Attention to this step and the use of at least 50 column volumes of each solvent are essential for good performance of the buffered silica column. After the silica gel has been completely wetted with water, 80 column volumes of buffer solution are pumped through.

The column is then connected to a gentle stream of nitrogen and, after all the remaining buffer solution has been purged at room temperature, the column is heated at 80°C for 20 h. After cooling to room temperature it is then ready for use and can be wetted directly with the mobile phase

4 GENERAL PROBLEM OF ADSORPTION CHROMATOGRAPHY OF POLAR SOLUTES

The adsorption chromatography of polar and/or ionic compounds on silica gel often suffers from strongly tailing peaks, which leads not only to poor separations but also to insufficient accuracy of quantitative determinations The tailing of peaks due to a mis-match of solute polarity, adsorption activity and solvent strength can be minimized either by choosing a silica gel with appropriate properties, by adding reagents to the mobile phase to adjust its pH or by coating the silica gel surface with a buffer salt.

The pH of an aqueous silica gel slurry depends on the quantitative and qualitative distribution of hydroxyl groups on the surface. It is commonly accepted that several types of hydroxyl groups, differing in reactivity, exist on the surface of porous

TABLE 1

pH VALUES OF AQUEOUS SILICA GEL SLURRIES

1.0 g of silica gel was added to 15 ml of doubly distilled water (pH 5 1) and equilibrated at room temperature for 30 min

Silica gel	*Shape*	*Particle size (μm)*	*Pore size (nm)*	*Surface area (m^2/g)*	*pH of slurry*
Partisil 5	Angular	6	5	400	3 5
Grace silica gel	Angular	5	8	500	4.5
LiChrosorb SI 60	Angular	7	6	475	6 9
Hypersil	Spherical	5	10	200	6 9
Spherosil XOA-600	Spherical	5	3	600	6 4
Spherisorb	Spherical	5	10	180	8 9

silica gel[6]: free or isolated hydroxyl groups are assumed to be separated by a sufficient distance to prevent the formation of hydrogen bonds, and bound or paired hydroxyl groups, which can interact via hydrogen bonding. The paired hydroxyl groups are considered to be more reactive than the isolated groups and to play the dominant role in adsorption.

The pH of the aqueous silica slurry is a useful parameter in choosing the most suitable adsorbent for a given separation problem. The pHs of different silica gels

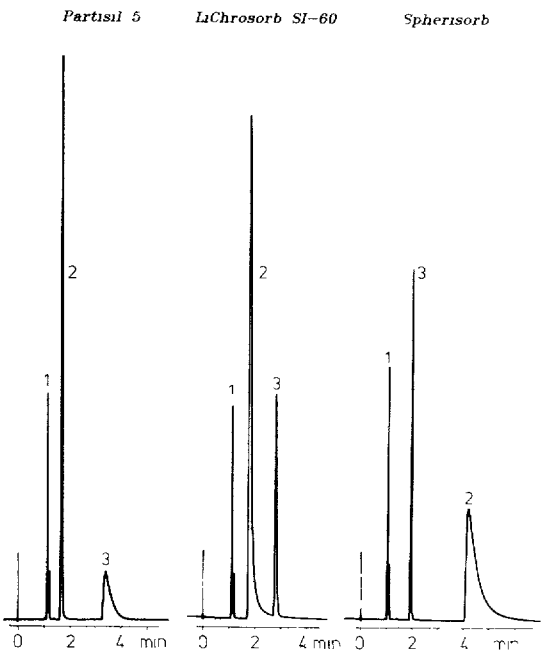

Fig 1 Mixture of methyl benzoate (peak 1), methyl 4-hydroxybenzoate (peak 2) and methyl 4-aminobenzoate (peak 3) chromatographed on various untreated silica gels Column 250 × 3 mm I D , operated at room temperature Mobile phase 35% *n*-hexane in diethyl ether Flow-rate: 1 5 ml/min. Detection: UV absorption at 254 nm

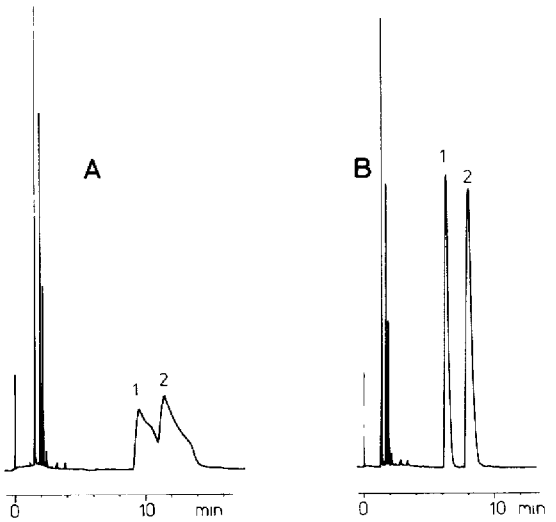

Fig 2 Comparison of the separations of *cis*- (peak 1) and *trans*-geranic acid (peak 2) on untreated (A) and buffered (B) silica gel Columns both 250 × 3 mm I D , operated at room temperature. Mobile phases both 10% diethyl ether in *n*-hexane Flow-rates both 1 0 ml/min. Detection UV absorption at 254 nm

are listed in Table 1. They vary between 3.5 and 8.9 owing to differences in the surface chemistry of the silica gel resulting from the manufacturing process. Fig. 1 shows a practical example: acidic solutes give the best results with acidic silica gels, whereas basic compounds elute without tailing on basic silica gels. Non-ionic compounds are not influenced in their behaviour by the nature of the silica gel.

Adjusting the pH of the mobile phase by adding appropriate reagents in small amounts may also reduce tailing. The reagents should be of approximately the same polarity as the mobile phase, otherwise they will be adsorbed on the silica gel and form a stationary phase that influences not only the polar and ionic solutes in their chromatographic behaviour, but also non-ionic and non-polar compounds. Reagents in the mobile phase may also react with the solutes and give misleading results. In semi-preparative and preparative separations they may hinder the complete evaporation of the mobile phase from the collected fraction.

5 GENERAL ADVANTAGES OF BUFFERED SILICA GEL

When the pH range of the commercially available silica gels is inadequate to give a tail-free elution and reagents in the mobile phase have to be avoided for other reasons, then the use of buffered silica gel is the only method for adsorption chromatographic separations of polar solutes. The crystalline buffer on the surface of the adsorbent does not influence the chromatographic behaviour of non-polar solutes, nor does it limit the adsorption mechanism As the buffer salt is virtually insoluble in the mobile phase, this separation system can be used for preparative separations like any normal silica gel system. The stability is comparable to that of untreated silica gel. The main advantage, however, is its potential to be used for the separation of polar solutes in the adsorption chromatographic mode, as shown in Fig. 2, with a neutral mobile phase.

6 THE BUFFER LAYER

6.1. Nature of the buffer

The term "buffered" may be somewhat misleading, as the modifying agent need not be a buffer salt in the usual sense. Both organic acids and inorganic salts have been used successfully. No change in retention behaviour of solutes can be observed on changing from one cation to another of the salt used to coat the silica gel.

The restriction on the choice of the buffer salt is its solubility in organic solvents, which should be as low as possible to prevent leaching from the column. The lower the solubility of the salt in the organic mobile phase, the better is the stability of the chromatographic system. Mostly we used citrate, phosphate and borate buffers to create pHs between 2 and 12.

6.2. Concentration of the buffer

The concentration of the buffer salt on the silica gel surface is controlled by its concentration in the aqueous solution used to coat the silica gel. The higher this concentration, the more salt will remain on the silica gel surface. A 0.1 *M* solution leads to a coating of approximately 1.5–2.5% by weight on silica gels with a specific surface area of about 400 m^2/g.

Concentrations of the buffer solution below 0.05 *M* lead to inadequate coverage of the surface. The tailing of peaks is not fully eliminated for very polar solutes. This lower limit of concentration is independent of the nature of the buffer salt.

Concentrations of buffer in the aqueous solution higher than 1 *M* lead to blocking of pores and thereby to a decrease in surface area. This results in a lower plate number of the column and in shorter retention times in comparison with normally treated columns. The chromatographic resolution of solutes is lower, although there are no tailing bands observable.

7. ROLE OF THE pH IN THE BUFFERED SILICA GEL SYSTEM

The only relationship between the buffer salt and the chromatographic behaviour of the solutes is in the pH of the aqueous solution of the salt used to coat the surface of the silica gel. All the pH values mentioned refer to the pH of this solution, as neither in the column nor in the mobile phase is there any means of characterizing the acidity of the silica gel. The pH of the aqueous solution has to be adjusted to a chosen value by mixing crystalline salts, acids or bases. Any liquids would easily be washed off and thereby alter the behaviour of the buffered silica gel system.

7.1. Buffer for acidic solutes

The pH of the aqueous solution used to buffer the silica gel has to be low for acidic solutes. In general, the pH of the solution should not be higher than the pK_a value of the solute to be chromatographed (Fig 3A). Lower pHs will not influence the peak shape but may result in changes in the retention selectivity owing to hydro-

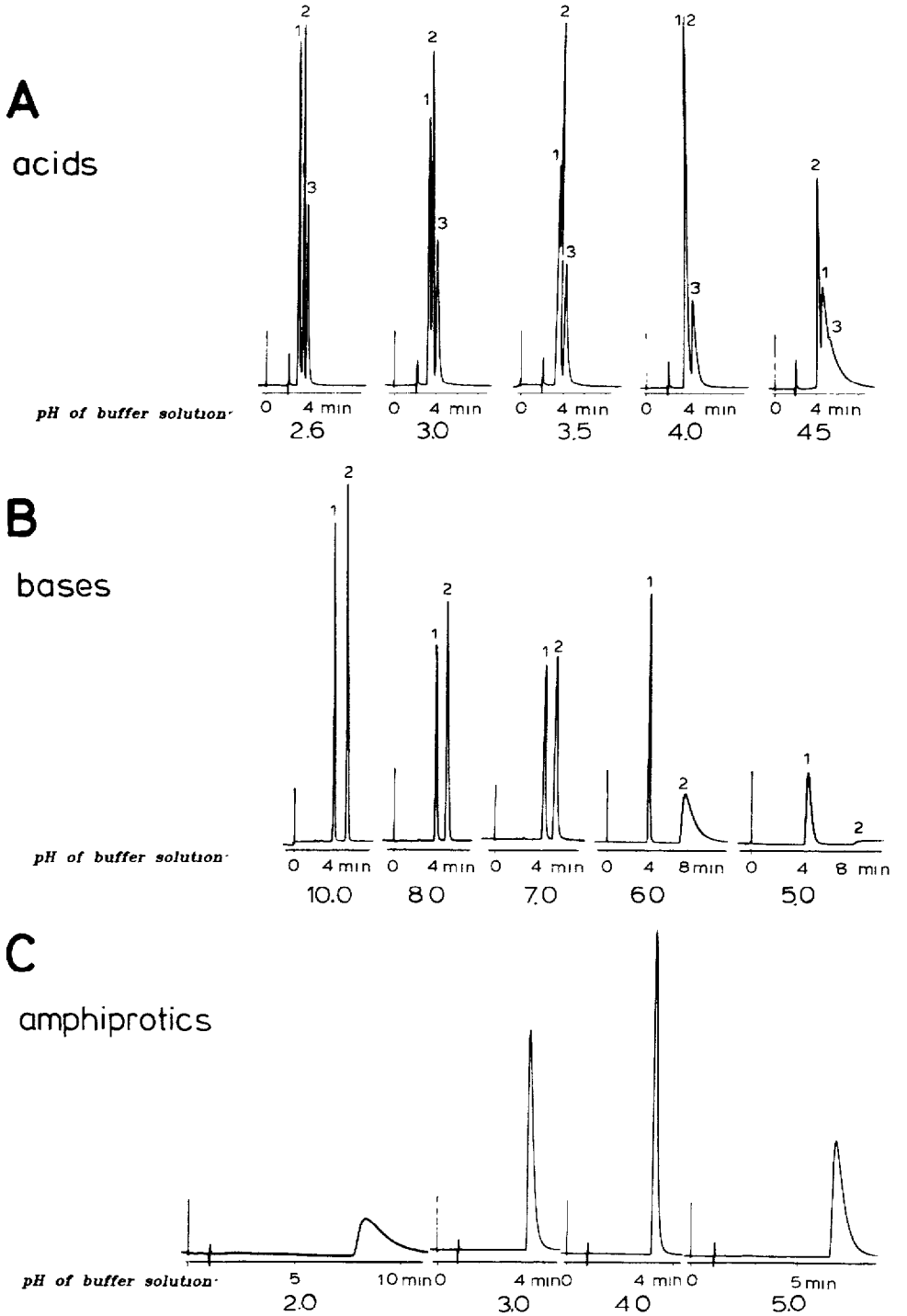

Fig 3. Role of pH in the buffered separation system Column: Grace silica gel, 80 Å, 5 μm, 0 1 *M* buffered, 250 × 3 mm I.D , operated at room temperature Mobile phase 30% *n*-hexane in diethyl ether Flow-rate: 1 5 ml/min Detection UV absorption at 280 nm. (A) Peaks 1 = 2,3-dihydroxybenzoic acid, 2 = 2,4-dihydroxybenzoic acid, 3 = 2,5-dihydroxybenzoic acid (B) Peaks 1 = aniline; 2 = quinoline (C) Solute 4-aminobenzoic acid, pK_a = (1) 2 3 and (2) 4.9

gen bonding. This possibility of using the pH to select the best separation conditions will be further investigated

The behaviour of acidic solutes on buffered silica gel cannot be influenced by the nature of the buffer salt It may be a fruit acid or an inorganic acidic salt such as sodium hydrogen sulphate We obtained very good results by using citrate–phosphate buffers. Citric acid and disodium hydrogen phosphate can be mixed to adjust any pH in the range 2.2–7.8 For pHs below 2.2 citric acid, oxalic acid and sodium hydrogen sulphate have been used successfully.

7.2 Buffer for basic solutes

For tail-free elution of polar basic compounds the silica gel has to be coated with a basic salt The pH of the buffer solution used should be higher than the pK_a of the solutes (Fig 3B). Usually we use a sodium borate buffer to adjust pHs between 8 and 12.

It is generally accepted that above pH 9 the solubility of amorphous silica increases expoonentially[6]. As the time of contact between the aqueous basic buffer solution and the silica gel during the preparation step is short, and the coating is carried out at room temperature, no dissolution of silica gel could be observed by negative influences on the chromatographic performance characteristics

Silica gel columns buffered at pH 10 and 12 showed the same stability and lifetime as untreated silica gel columns The non-aqueous, non-polar mobile phase is not able to dissolve either buffer salt or silica gel and therefore the stability of the chromatographic performance is excellent, neither the separation selectivity nor the plate number change

Whereas acidic buffered silica gel could be replaced by a system containing an acidic mobile phase, the addition of a base to adjust the pH of the mobile phase to 10–12 is not feasible. It would slowly dissolve the silica packing of the column and

Fig 4 Non-ionic compounds on buffered silica gel Column Grace silica gel, 80 A, 5 μm, 250 × 3 mm I D , operated at room temperature. Mobile phase 25% diethyl ether in *n*-hexane. Flow-rate 1 0 ml/min. Detection. UV absorption at 215 nm Peaks 1 = lilial; 2 = lilial ketone, 3 = impurity, 4 = lilialic acid.

thereby alter the chromatographic performance of the system. Basic solutes therefore require the unique property of basic buffered silica gel

7.3. Buffer for amphiprotic solutes

Amphiprotic compounds, which contain both acidic and basic groups, are best chromatographed on silica gel buffered at a pH equal to the isoelectric point (Fig. 3C). At too low a pH the basic groups of the molecules will lead to tailing bands and at too high a pH the acidic groups become responsible for unacceptable peak shapes.

7.4. Non-ionic and non-polar compounds on buffered silica gel

Non-ionic solutes exhibit the same behaviour on buffered silica gel as on untreated material (Fig. 4) The separation selectivity and the retention are not affected by the buffer layer In the given example the aldehyde (lilial), the ketone and the impurities are separated as well on buffered silica gel as on the untreated adsorbent. Lilialic acid, however, is not eluted at all from normal silica gel, but can be detected as a symmetrical peak on buffered silica gel systems

A comparison of the chromatographic behaviour of non-ionic solutes on untreated and buffered silica gel indicates that all the adsorption sites on buffered silica gel are still available for the chromatography and are not occupied by the buffer salt.

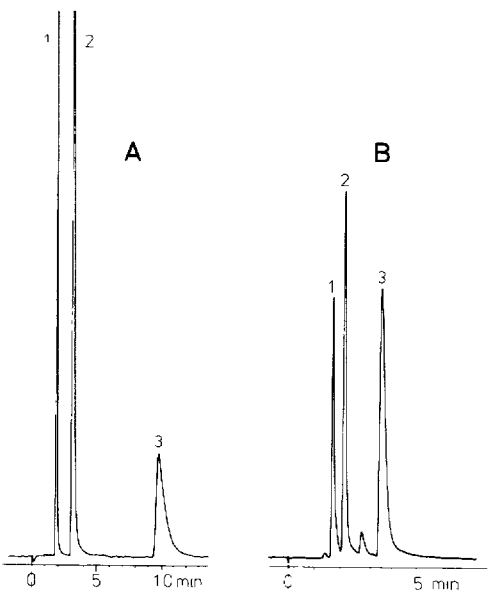

Fig 5. Separation of polar isomers Column Partisil 5. 7 μm, buffered to pH 1 1 (tartaric acid), 250 × 3 mm I D , operated at room temperature Mobile phase (A) 50% diethyl ether in *n*-hexane, (B) 100% diethyl ether Flow-rate 1 0 ml min Solutes (A) trihydroxybenzoic acids, 1 = 2,3,4-trihydroxybenzoic acid, 2 = 2,4,6-trihydroxybenzoic acid, 3 = 3.4,5-trihydroxybenzoic acid, (B) flavones, 1 = kaempferol, 2 = galangin, 3 = fustin

8 APPLICATIONS OF BUFFERED SILICA GEL SYSTEMS

The possible applications of buffered silica gel separation systems are numerous and therefore only a few examples can be given.

8.1. Separation of isomers

Adsorption chromatography is the best separation technique for isomers. In a previous paper[4] we showed the separation of dihydroxybenzoic acid isomers and compared it with the less successful results obtained with other types of separation systems. Another example is given in Fig. 5A. The complete separation of the dihydroxybenzoic acid isomers is possible on buffered silica gel with a neutral mobile phase. These examples show clearly that the separation power of adsorption chromatography is now available for polar compounds.

8.2. Separation of flavones

The solubility of some flavones in diethyl ether is good enough for them to be chromatographed with an organic mobile phase on buffered silica gel (Fig. 5B). The advantage of the buffered separation system is the compatibility with organic extracts from plants and fruits, and the possibility of a facilitated evaporation of the mobile phase for further identification of the components of the collected fractions. As flavone glucosides are insoluble in most organic solvents a pre-separation is achieved at the extraction stage. Aqueous separation systems for flavones, flavanones and flavanoids have been described by several workers[7–9].

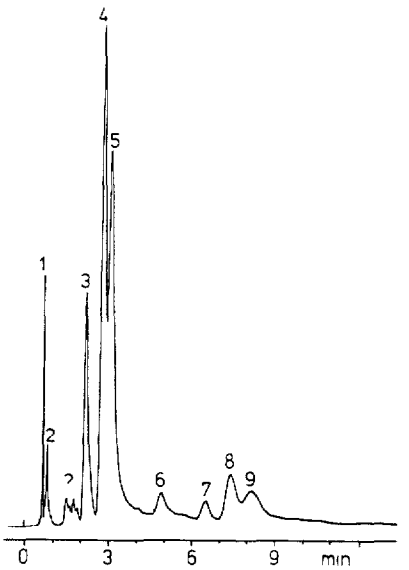

Fig 6 Separation of hop acids in a beer extract Column LiChrospher SI 100, 5 μm, buffered to pH 2 6 (citrate), 250 × 3 mm I D , operated at room temperature Mobile phase. 10% diethyl ether in *n*-hexane Flow-rate 1 0 ml/min Solutes. 1 = solvent, 2 = β-acids, 3 = *cis*-ad-iso-α-acid, 4 = *cis*-co-iso-α-acid, 5 = *cis*-*n*-iso-α-acid; 6 = *trans*-ad-iso-α-acid, 7 = α-acid, 8 = *trans*-co-iso-α-acid, 9 = *trans*-*n*-iso-α-acid

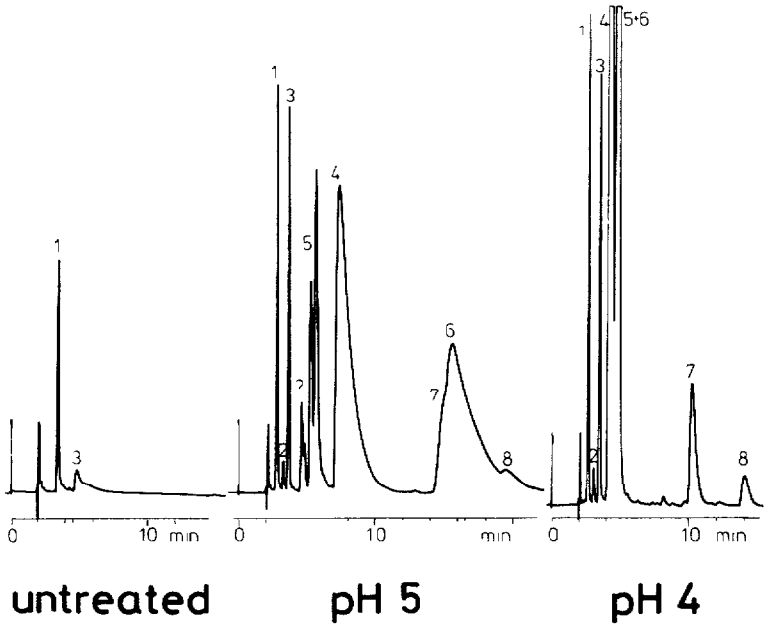

Fig 7 Separation of polar fraction of ylang-ylang oil. Column: Grace silica gel, 80 Å, 5 μm, 250 × 3 mm I D , operated at room temperature Mobile phase 34% diethyl ether in *n*-hexane Flow-rate 1.5 ml/min Detection UV absorption at 210 nm Peaks 1 = methyl salicylate, 2 = ?, 3 = isoeugenol, 4 = eugenol, 5 = *p*-cresol, 6 = benzoic acid, 7 = vanillin, 8 = acetic acid

8.3. Hop acids in beer and hop products

The bitter acids in hop and beer form a complex mixture of closely related compounds. Separations have been accomplished by counter-current distribution and by thin-layer, paper and ion-exchange chromatography. Partition chromatographic systems on C_{18} reversed-phase packings have been described by Verzele and co-workers[10,11]. The separation on buffered silica gel gives more information in a shorter time, because the homologues and their isomers are separated[12] (Fig. 6).

8.4. Polar fractions of natural oils

The analysis of flavour compounds in oils and extracts of plants, flowers and fruits is usually carried out by gas chromatography. The acid fractions of these plant extracts or oils, however, lend themselves better to HPLC for separation. Reversed-phase and other HPLC methods have been described[4,13]. The buffered silica gel system may be complementary to the others. Fig. 7 shows the possibilities: by choosing a suitable pH, the separation selectivity can be changed as required. At higher pHs some strongly acidic solutes do not elute and hence can be suppressed.

8.5. Separation of 1,3-diketones

Fig. 8 shows the separation of the impurities from Parsol 1789 (a sun-screen

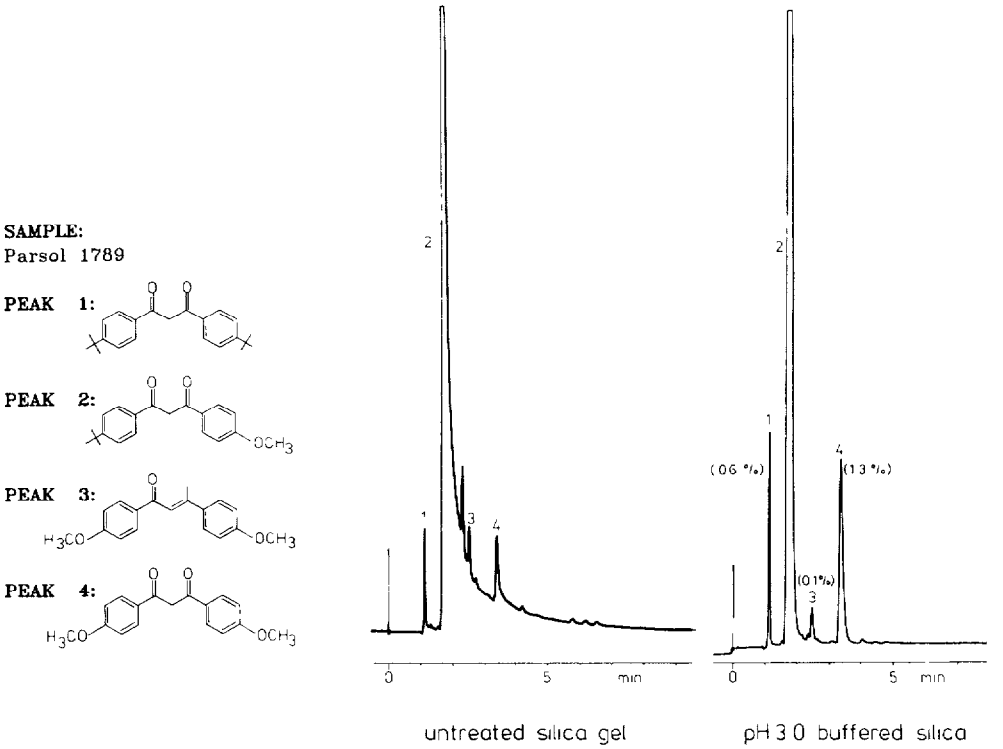

Fig. 8 Determination of small amounts of impurities of 1,3-diketone Column Grace silica gel, 80 Å, 5 μm, 250 × 3 mm I.D., room temperature Mobile phase: 20% tetrahydrofuran in *n*-hexane Flow-rate: 1.5 ml/min Detection UV absorption at 280 nm Sample Parsol 1789.

product). These 1,3-diketones tend to tail owing to the formation of hydrogen bonds, even when using apolar, neutral mobile phases. On buffered silica gel the tailing can be reduced and the determination of small amounts of the impurities is possible.

8.6. Preparative and semi-preparative separations

For preparative separations of polar compounds, the buffered silica gel systems are preferred to reversed-phase systems, because the mobile phase can easily be removed from the collected fraction as it is only a mixture of low-boiling organic solvents and free of any reagents (buffers, acids, etc.). The collection of peaks from the analytical column for further identification purposes is also facilitated.

8.7. HPLC–MS coupling

In direct liquid interfacing of HPLC with MS the flow-rate of the mobile phase has to be adjusted in order to reduce the amount of gas evolved from the complete vaporization of the solvent to the maximum allowed level for operation of the mass spectrometer under high vacuum. A flow-rate of 1 ml/min of water will give about 1200 ml/min of water vapour. By using the buffered silica gel system for LC–MS

coupling of polar compounds, with *n*-hexane and diethyl ether as the mobile phase, the flow-rate may be five times the maximum allowed value for a reversed-phase system, because 1 ml/min of *n*-hexane will give only about 180 ml/min of *n*-hexane vapour.

9 LIMITATIONS

The only disadvantages of the buffered silica gel system is the limited choice of the mobile phase composition. The buffer salt should not dissolve in the mobile phase. However, we successfully used diethyl ether–ethanol–dimethyl sulphoxide (1000:125:30) on citric acid-coated silica gel to separate highly polar compounds such as polar PTH-amino acids, gallic acids, ascorbic acid, tartaric acid and malic acid. Under these conditions very small amounts of buffer could be found in the eluent and the lifetime of the column was reduced to only about 3–4 weeks. By using *n*-hexane–diethyl ether as the mobile phase the stability and the column lifetime are comparable to those of normal silica gel columns.

10 CONCLUSIONS

The buffered silica gel separation systems allow tail-free elution of polar solutes with neutral, organic mobile phases. They have the separation power of adsorption chromatography and show the same chromatographic behaviour as untreated silica gel for non-polar solutes The stability of buffered systems is comparable to that of untreated silica gel systems provided that the buffer salt is virtually insoluble in the mobile phase. Gradient elution and flow programme can be used as with normal silica gel systems. The buffered silica gel facilitates the semi-preparative and preparative isolation of polar solutes and the direct liquid inlet coupling of HPLC with mass spectrometric detectors.

11 ACKNOWLEDGEMENTS

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12 SUMMARY

HPLC separations of polar solutes, by adsorption chromatography on silica gel, is often hindered by tailing of the bands. By treating the silica gel with a buffer salt, insoluble in the mobile phase, an environment is created on the surface of the adsorbent that permits tail-free elution of very polar compounds.

The influence of the nature of the buffer salt and of its concentration and pH has been demonstrated. Buffered silica gel is not only an alternative to reversed-phase chromatography but may also be superior to it for the separation of isomers or for semi-preparative separations.

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