Journal of Chromatography, 202 (1980) 397–404
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,284

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF POLAR COMPOUNDS ON BUFFERED SILICA GEL

ROLF SCHWARZENBACH
Givaudan Research Company Ltd., CH-8600 Duebendorf (Switzerland)
(First received May 7th, 1980; revised manuscript received August 23rd, 1980)

SUMMARY

By treating the surface of silica gel with a crystalline salt or acid, an acidic environment is created which permits adsorption chromatographic separations and tailing-free elution of polar acidic solutes. For the chromatography of basic compounds the silica gel surface is coated with a basic salt. The nature of the salt and its concentration on the surface of the silica gel have no effect on the separation selectivity; instead it is the pH of the aqueous solution used to treat the silica gel, which has the main influence on peak symmetry and separation selectivity. Buffered silica gel may be used as an alternative to reversed-phase chromatography for polar solutes. It is shown that many separations so far possible only on C_{18}-columns can be achieved with adsorption chromatography.

INTRODUCTION

The chromatography of medium polar compounds on silica gel often results in strongly tailing peaks, which may lead to poor separations and low accuracies of determinations. Sometimes this problem can be solved by adding an acidic or basic reagent to the mobile phase. However, in most cases one had to change to reversed-phase partition chromatography. But what if the sample does not permit a change to an aqueous mobile phase and the presence of other reagents in the mobile phase has to be avoided? In such cases a buffered silica gel system may be of help.

The use of silica gel columns in combination with buffer and organic solvents for separation purposes is well established. Soon after the invention of partition chromatography on silica gel by Martin and Synge[1] in 1941, Boon[2] and Levi[3,4] used phosphate buffer solution instead of water as the sorbed stationary phase for the chromatography of penicillin acids. Isherwood[5] replaced the pure water of the partition system of Martin and Synge by 0.5 *N* H_2SO_4 for the separation of organic acids in fruits, and Moyle *et al.*[6] described the separation of saturated C_2–C_8 fatty acids on buffered partition columns.

All these separation systems have one thing in common: an aqueous buffer solution (50–75% w/w) is adsorbed by the silica gel and converts the solutes into the ionized form, where the partition is in favour of the stationary phase. In the

0021-9673/80/0000–0000/$02.25 © 1980 Elsevier Scientific Publishing Company

present case the silica gel is impregnated with an aqueous buffer solution, but the water is evaporated at a higher temperature under vacuum.

The term "buffered silica gel" is used to describe a silica gel the surface of which is coated with a crystalline salt or acid. Since the k' values of non-polar compounds are the same on buffered silica gel as on untreated silica gel, and since the separation power for *cis/trans* isomers is not affected by the buffer layer, the retention mechanism must be adsorption. The peak shape, and sometimes the separation selectivity, of polar compounds is determined by the pH of the aqueous solution of the buffer salt used to coat the silica gel. The stability of a buffered silica gel column is comparable with that of a normal silica gel column, providing the salt or the acid on the silica gel surface is not washed out by the mobile phase employed.

In this paper the preparation of buffered silica gel and the influence of the type, pH and concentration of the buffer solution used are described, and various applications are demonstrated.

EXPERIMENTAL

Apparatus

The liquid chromatographic system was constructed from commercially available components. The chromatographic column was a high precision glass tube (Duran 50; Jenaer Glaswerke, Mainz, G.F.R.). A high pressure slurry-packing technique was used to pack the column at a pressure of 450 bar. To avoid exposure of the glass tube to this high pressure, packing was done in a pressure vessel. This apparatus, the technique involved and the column assembly have been described previously[7].

Injections were made on-stream by syringe through a septum LC-inlet or a loop injector. All separations were made at room temperature and no attempt was made to control the temperature of the mobile phase or the column.

Materials

All reagents were of analytical reagent grade (E. Merck, Darmstadt, G.F.R.) and were used without further purification. The solvents used for the mobile phase were of HPLC-grade (Rathburn Chemicals Ltd., Walkerburn, Great Britain) and employed as supplied. For most of the work, LiChrosorb Si 60 and LiChrospher Si 100 (E. Merck) were used, but Partisil 5 (Whatman, Clifton, NJ, U.S.A.) also gave excellent results. All silica gels (mean particle diameter 5 μm, pore diameter 0.01–0.05 μm) were used as received.

Preparation of the buffered silica gel

A 0.1 *M* solution of the buffer salt or acid was added to the untreated silica gel to form a fluid slurry, which was placed, under vacuum, into an ultrasonic bath for 2 min to force the buffer solution into the pores. The slurry was then placed into a fritted disc funnel and filtered. The wet material was spread out on a crystallizing disc and dried at 80°C, under vacuum, for 20 h. The dried material was packed into the column as a hexane-slurry.

An *in situ* coating of prepacked silica gel columns is also possible. Hexane or any other solvents immiscible with water must be exchanged for acetone before they

are replaced by water. Attention to this step and the use of at least 50 column volumes of each solvent were essential for good performance of the buffered separation system. After the silica gel had been adequately moistened with water, 80 column volumes of buffer solution were pumped through at a flow-rate of 0.5 ml/min. The remaining excess of buffer solution was then blown out of the column with the aid of nitrogen at room temperature. With the column connected to a gentle stream of nitrogen, the temperature was increased to 80°C and kept constant for at least 20 h. The column was then ready for use and could be moistened directly with the mobile phase.

This *in situ* procedure permits the buffering of commercial silica gel columns. However, the batch procedure is easier and more reliable and is preferred.

RESULTS AND DISCUSSION

The most obvious advantage of chromatography on buffered silica gel columns is the formation of sharp, symmetric peaks for polar compounds. Fig. 1 illustrates this. On normal silica gel (chromatogram A) oleic acid is eluted with very bad tailing and small amounts of linoleic acid cannot therefore be detected. However, on buffered silica gel (chromatogram B) the separation and the quantitation is readily achieved.

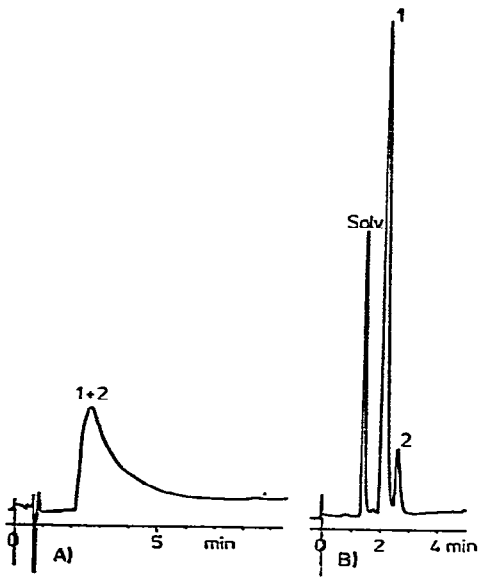

Fig. 1. Separation of oleic acid (peak 1) and linoleic acid (peak 2). Mobile phase: hexane–diethyl ether (4:1); flow-rate 1.0 ml/min. Columns: A, LiChrosorb Si 60, 5 μm, 250 × 3 mm, untreated; B, LiChrosorb Si 60, 5 μm, 250 × 3 mm, buffered with citric acid–sodium citrate, pH 3.0.

The coating of silica gel with a crystalline buffer salt has already been used successfully in thin-layer chromatography by Schur and Pfenninger[8] for the separation of hop acids.

A number of workers have washed silica gel with methanolic HCl prior to the chromatography of polar compounds, in order to remove metal ions from the surface of the silica gel which could lead to the formation of complexes with the compounds

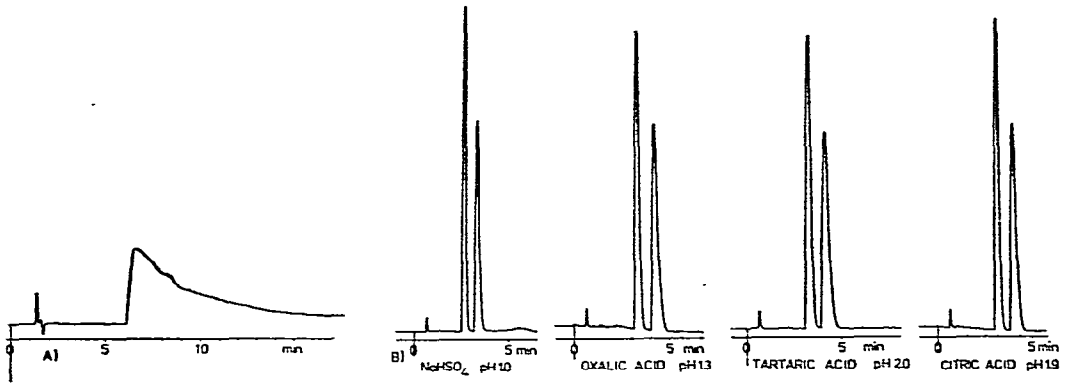

Fig. 2. Effect of the buffer salt, on the separation of *cis*- and *trans*-geranic acid. Mobile phase: hexane–diethyl ether (9:1); flow-rate 1.0 ml/min. Columns: A, as in Fig. 1; B, LiChrosorb Si 50, 5 μm, 250 × 3 mm, buffered with different salts and acids.

to be separated. In this study it was found that the tailing-free elution is not a result of the metal ion-free surface but of the acidic environment. Hydrochloric acid is not the appropriate reagent in high-performance liquid chromatography (HPLC) for this purpose; its effect is lost after two or three injections. A crystalline acid, however, will be effective as long as it is not washed out by the mobile phase. The term "buffered" may be somewhat misleading. Thus, the modifying agent need not be a

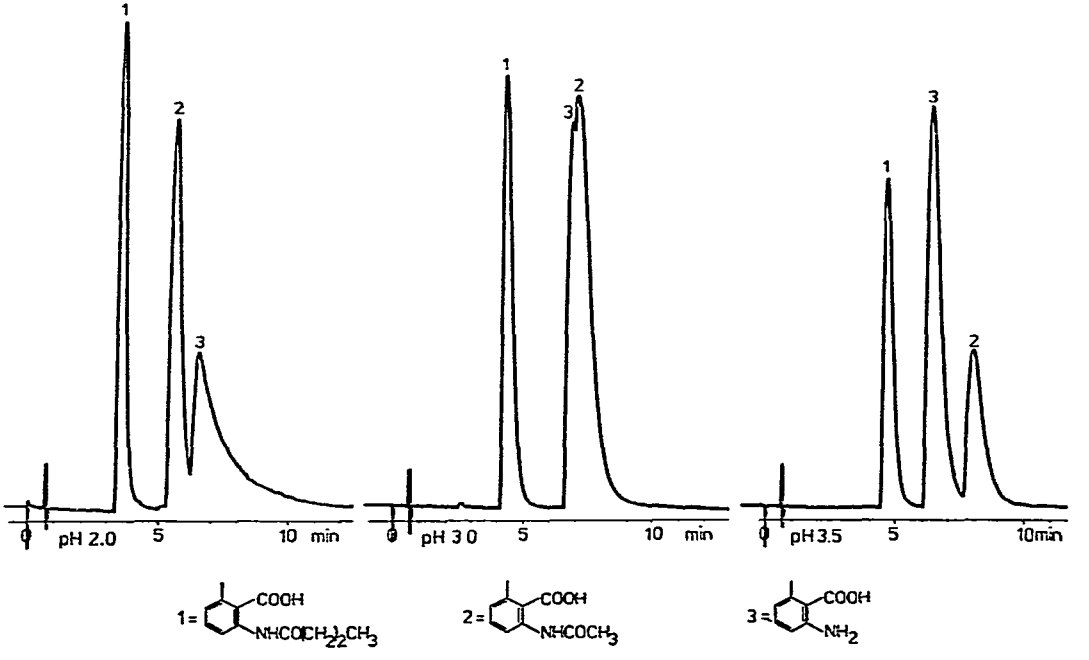

Fig. 3. Separation of methylanthranilic acid from its derivatives. Mobile phase: hexane–diethyl (2:1); flow-rate 1.5 ml/min. Column: Partisil 5, 5 μm, 250 × 3 mm, buffered with citric acid–Na_2HPO_4 of various pH.

buffer salt in the usual sense. Organic acids as well as inorganic salts have been successfully used. Fig. 2 demonstrates the separation of *cis*- and *trans*-geranic acid on untreated silica gel (chromatogram A) and on various buffered silica gels (chromatogram B).

The pH values given refer to the pH of the aqueous solution of the buffer salts used to coat the surface of the silica gel. The relation between the pH and the separation selectivity is not clear, but nevertheless it may be employed to select the most suitable separation conditions.

The separation of methylanthranilic acid from some derivatives (Fig. 3) depends strongly on the pH of the buffer solution used to modify the silica gel. This selective change of the capacity factor k', which describes the ratio of the number of molecules adsorbed by the silica gel to the number of molecules present in the mobile phase, may partly be due to pH-dependent deprotonation of the solute. Yet, the humidity of the buffered silica gel and the degree of water-saturation of the mobile phase have no influence on selectivity and peak performance. Methylanthranilic acid and its derivatives, a group of plant growth regulators, exhibit interesting behaviour on buffered silica gel systems. If the pH of the solution, chosen to modify the silica gel, is too low, the acid is eluted with a strong tailing caused by the basic NH_2 group. At high pH values the acid and the derivatives are elute with a tailing caused by the acidic COOH group. It was found that a silica gel buffered with a solution at a pH close to the isoelectric point gives the best results for amphiprotic substances.

The amount of buffer salt coated onto the silica gel surface is controlled by the concentration of the buffer solution used and can be varied over a wide range. At concentrations above 1 *M* the HETP increases drastically and below 0.01 *M* the effect of the coated surface is slowly lost.

Scanning electron microphotographs (Fig. 4) show no crystal formation of the buffer salt on the surface of the silica gel particle. The surface of the buffered silica gel is smoother than that of untreated silica gel. At too high concentrations the particles tend to cluster. Generally, a buffer concentration of 0.1 *M* was used which leads to an average coating of 2.5% by weight.

As already shown, the critical parameter is the pH of the aqueous solution of the buffer used to coat the surface of the silica gel. For acidic compounds this pH has to be lower than the pK_a value of the substances. For amphiprotic compounds, which contain both acidic and basic groups, the pH has to be around the isoelectric point. For basic compounds, a buffer solution of pH 8.0 gave good results, and higher pH values were not investigated since these may cause other changes in the surface of the silica gel.

Non-ionic compounds exhibit the same behaviour on buffered silica gel as on untreated material. In Fig. 5 the separation of *p*-hydroxybenzoic acid from its methyl, ethyl and propyl esters on untreated and on buffered silica gel is compared. On the untreated material the acid is not eluted at all, whereas on buffered silica gel the acid can be determined as a symmetrical peak. The separation of the esters, however, is not affected by the buffer. The k' value as well as the resolution is exactly the same on untreated as on buffered silica gel.

The separation of *p*-hydroxybenzoic acid and its esters can also be achieved in reversed-phase chromatography on C_{18}-silica gel, with methanol–water as mobile phase. The external circumstances, *e.g.*, the medium in which the compound has to

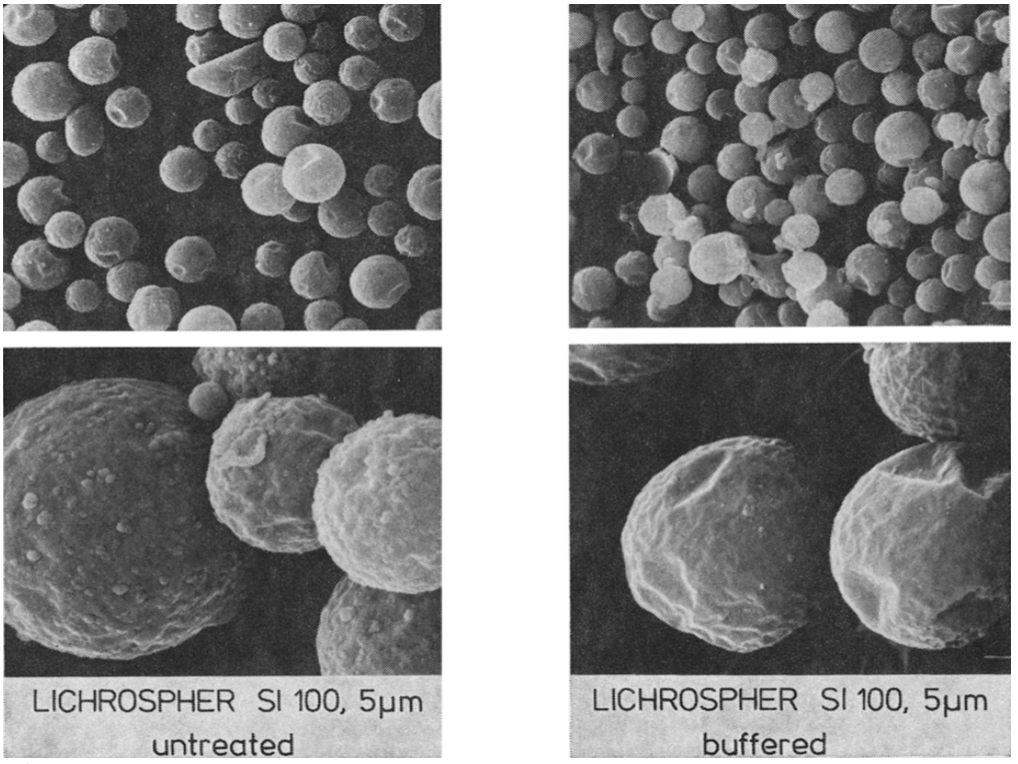

Fig. 4. Scanning electron microphotographs of untreated and buffered (0.1 *M* solution) LiChrospher Si 100.

be determined, will determine which separation system is chosen to solve the problem. The separation of polar phenylthiohydantoin (PTH) amino acids is illustrated in Fig. 6. On untreated silica gel PTH-Gln, PTH-Asn and PTH-His are eluted as strong

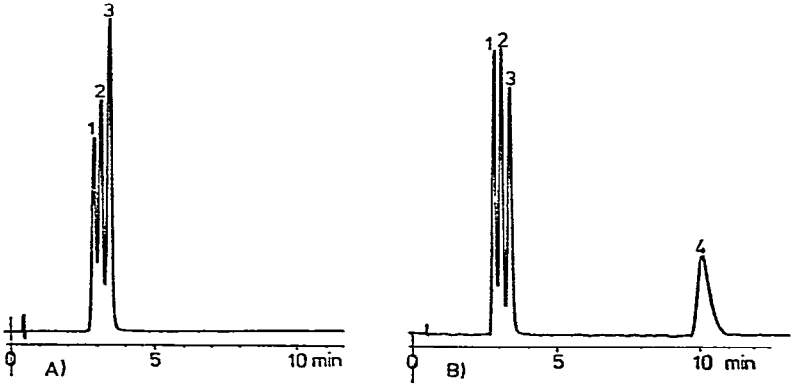

Fig. 5. Separation of *p*-hydroxybenzoic acid (4) from its esters. Peaks: 1 = *p*-hydroxybenzoic acid propyl ester; 2 = *p*-hydroxybenzoic acid ethyl ester; 3 = *p*-hydroxybenzoic acid methyl ester. Mobile phase: hexane–diethyl ether (6:1); flow-rate 2.0 ml/min. Columns: A, as in Fig. 1; B, LiChrosorb Si 60, 5 µm, 250 × 3 mm, buffered with citric acid–Na_2HPO_4, pH 2.4.

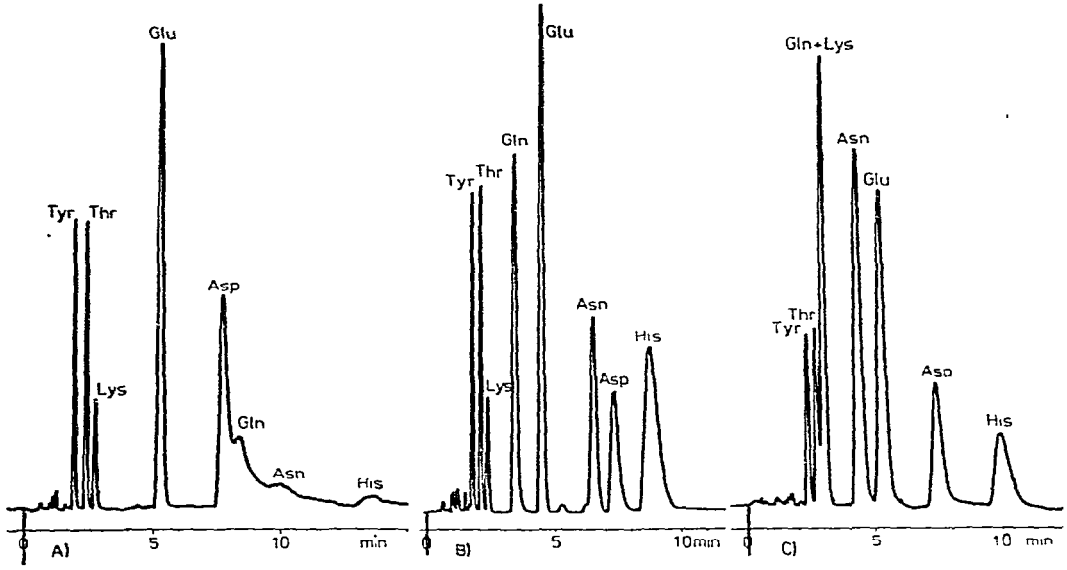

Fig. 6. Separation of polar PTH-amino acids. Mobile phase: diethyl ether–ethanol–dimethyl sulphoxide (1000:125:30); flow-rate 1.5 ml/min. Columns: A, Partisil 5, 5 μm, 250 × 3 nmm, untreated; B, Partisil 5, 5 μm, 250 × 3 mm, untreated, with 1% acetic acid in the mobile phase; C, Partisil 5, 5 μm, 250 × 3 mm, buffered with citric acid–Na_2HPO_4, pH 3.0.

Fig. 7. Separation of hop acids in a beer extract. Peaks: 1 = β-acids; 2 = *cis*-isoadhumulone; 3 = *cis*-isocohumulone; 4 = *cis*-isohumulone; 5 = *trans*-isoadhumulone; 6 = α-a-cids; 7 = *trans*-isocohumulone; 8 = *trans*-isohumulone. Mobile phase: hexane–diethyl ether (9:1); flow-rate 1.0 ml/min. Column: LiChrospher Si 100, 5 μm, 250 × 3 mm, buffered with citric acid–sodium citrate, pH 2.6.

Fig. 8. Separation of organic acids. Peaks: 1 = ferulic acid; 2 = gallic acid; 3 = ascorbic acid. Mobile phase: diethyl ether–ethanol–dimethyl sulphoxide (1000:125:30); flow-rate 1.5 ml/min. Column: LiChrosorb Si 60, 5 μm, 250 × 3 mm, buffered with citric acid–sodium citrate, pH 2.3.

tailing peaks. This can be avoided either by adding small amounts of acetic acid to the mobile phase or by changing to a buffered silica gel column.

The possible applications for buffered silica gel separation systems are numerous[9]. The separation and determination of the different bitter acids present in hop, hop products and beer on buffered silica gel (Fig. 7) is ten times faster than the traditional ion-exchange chromatography[10].

The only disadvantage of the buffered silica gel system is in the limited choice of the mobile phase. An extreme example of this is shown in Fig. 8. A mobile phase containing alcohol and dimethyl sulphoxide will shorten the lifetime of the buffered column, but makes possible adsorption chromatography for very polar compounds. Otherwise the stability of a buffered silica gel system is comparable with that of a normal silica gel system. By using less than 50% diethyl ether in hexane, most buffered columns can be used for several months before any loss of performance can be observed. Flow and gradient programs can be used as with normal silica gel columns. 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.

ACKNOWLEDGEMENTS

The author thanks Miss R. Schneider for careful technical assistance and Dr. V. E. Colombo (Hoffmann-La Roche, Basle, Switzerland) for the scanning electron microphotographic experiments.

REFERENCES

1 A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, 35 (1941) 1358.
2 W. R. Boon, *Analyst (London)*, 73 (1948) 202.
3 A. A. Levi, *B. Pat.*, 569, 844 (1943).
4 A. A. Levi, *Biochem. Soc. Symp.*, 3 (1950) 83.
5 F. A. Isherwood, *Biochem. J.*, 40 (1946) 688.
6 V. Moyle, E. Baldwin and R. Scarisbrick, *Biochem. J.*, 43 (1948) 308.
7 R. Schwarzenbach, *J. Chromatogr.*, 129 (1976) 31.
8 F. Schur and H. Pfenninger, *Brauwissenschaft*, 24 (1971) 151.
9 R. Schwarzenbach, *J. Liquid Chromatogr.*, 2 (1979) 205.
10 R. Schwarzenbach, *J. Amer. Brew. Chem.*, 37 (1979) 180.