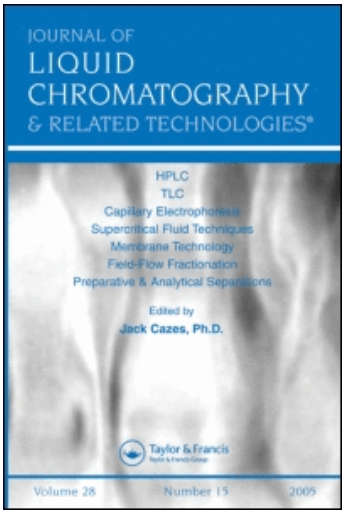

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Buffered Silicagel Systems - An Alternative Method for the Separation of Polar Compounds

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BUFFERED SILICAGEL SYSTEMS - AN ALTERNATIVE METHOD FOR THE SEPARATION OF POLAR COMPOUNDS *

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ABSTRACT

The adsorption chromatography of polar compounds on normal silicagel gives very often strongly tailing peaks and, therefore, poor resolution. By coating the surface of the silicagel with a buffer salt, the tailing disappears and all the advantages of adsorption chromatography can be used. On buffered silicagel, non-ionic compounds behave in the same way as on the untreated adsorbent. In this paper we describe the influence of type, pH and concentration of the buffer solution used to modify the silicagel on the performance of the separation system. The changes in separation selectivity and various applications are shown.

INTRODUCTION

While a separation of medium polar compounds on silicagel may be unproblematic in thin layer chromatography or in normal

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column chromatography, the use of the same chromatographic parameters in an HPLC adsorbtion system may lead to insufficient separation due to peak tailing. Sometimes this problem can be solved by changing to reverse phase partition chromatography, but often the solubilities of the compounds do not permit a change to an aqueous mobile phase. In such cases, a buffered silica system will be of great help.

With the term "buffered silicagel" we designate a silicagel whose surface is coated with a crystalline salt or acid. The pH of the aqueous salt or acid solution, used to coat the silicagel, determines the peak performance and the separation selectivity. A few years ago, when we had to separate hop acids, we found that a silicagel washed with methanolic hydrochloric acid gave tailing-free elution. The acid-treatment, however, was only effective for one or two injections. The acidic medium seemed to be washed out and therefore we tried to use a crystalline buffer salt instead of a liquid acid.

In this paper we describe the coating procedure and show the influence of type, pH and concentration of the buffer solution used on the peak performance and separation selectivity.

EXPERIMENTAL

Apparatus and Materials

The liquid chromatographic apparatus was assembled from commercially available components and described previously [1]. All the reagents and solvents used were of analytical-reagent grade (E. Merck, Darmstadt, G.F.R., Fluka, Buchs-SG, Switzerland, and Rathburn Chemicals, Walkerburn, Scotland G.B.) and were used without further purification. The silicagel used was Lichrospher SI 100 (E. Merck), a spherical silicagel with a particle diameter of 5 μm and an average pore diameter of 0.1 nm.

Preparation of the buffered Silicagel

The coating of the silica surface can be done batchwise in a fritted disc funnel or in-situ in a prepacked silicagel column. The batch procedure, however, is easier and more reliable, and is preferred in our laboratory.

Batchwise: A 0.1 M solution of the desired buffer salt or acid is added to the untreated silicagel to form a fluid slurry. Vacuum and/or an ultra-sonic bath is applied to ensure the penetration of the buffer solution into all pores of the silicagel. The slurry is then placed into a fritted disc funnel and filtered. The still wet material is spread out on a crystallizing dish and dried at 80°C under vacuum for 20 hours. The dry material is packed into a column as a hexane slurry.

In-situ: The silicagel column, commercial or home-packed, has to be rinsed first with acetone or any water-miscible solvent. This intermediate solvent is then replaced completely by water. The column is now ready for the buffer solution, of which at least 80 column volumes are pumped through. The column is then connected to a gentle stream of nitrogen at room temperature. When all the buffer solution has been blown out, the temperature is increased to 60 - 80°C and kept constant for at least 20 hours. The column is then cooled to room temperature and is now ready to be moistened directly with the mobile phase.

RESULTS AND DISCUSSION

The coating of silicagel with buffer salts is not new. This technique has already been used successfully in thin layer chromatography [2]. The separation mechanism is more closely related to adsorption than to partition chromatography. Ion-pair formation or ion-pair distribution has never been observed on these buffered systems. Non-ionic compounds behave in the same way as on untreated silicagel. The term "buffered" may be somewhat mis-

leading; the modifying agent has not necessarily to be a buffer salt in the common sense. Organic acids as well as inorganic salts have been successfully used. The pH-values given in this paper always refer to the aqueous solution used to coat the surface of the silicagel. The effect of the pH in a non-aqueous mobile phase on the separation selectivity is not fully understood yet. The humidity of the buffered silicagel, as well as the degree of water-saturation of the mobile phase have no influence on selectivity and peak performance.

The amount of buffer salt on the silicagel surface is controlled by the concentration of the buffer solution and can be varied over a wide range without influencing the performance of the separation system. The nature of the salt does not influence the column performance, either. The only limitation is its solubility in the mobile phase.

The only critical parameter is the pH of the aqueous solution used to buffer the silicagel. For acidic compounds this pH has to be lower than the pKa value of the compound to obtain a tailing-free elution.

Figure 1 demonstrates, for an acidic compound, the change of the peak form by using a buffered silicagel system instead of normal silicagel. The pKa of cohumulone is 4.7, the silicagel was buffered with a solution of pH 3.1. Not only the peak form can be changed, the selectivity as well is influenced by the pH of the buffer solution used. Figure 2 shows the separation of cohumulone from cis and trans isocohumulone on buffered systems prepared with solutions of different pHs. The change of the capacity factor, k', which describes the ratio of molecules adsorbed by the silicagel to molecules present in the mobile phase, may be due to pH-dependent enolisation of the cohumulone.

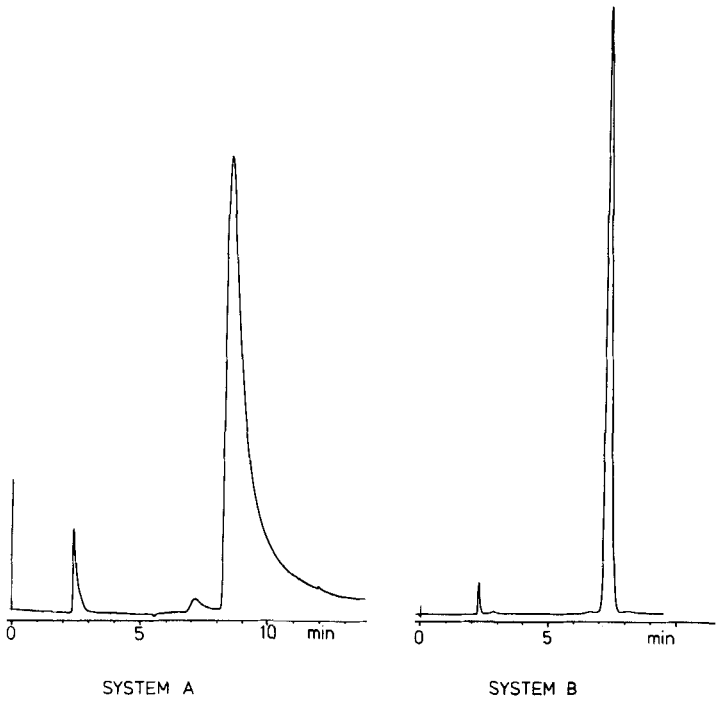

Figure 1. HPLC chromatograms of cohumulone on untreated and buffered silicagel.

System A Column: Lichrospher SI 100, 5 µm, untreated, 250 x 3 mm
Mobile phase: hexane/ethyl ether 6:1, 1.0 ml/min
System B Column: Lichrospher SI 100, 5 µm, buffered pH 3.1 (citric acid/Na-citrate)
Mobile phase: hexane/ethyl ether 6:1, 1.0 ml/min

Similar results are obtained in the separation of cinnamic acid from its esters (Figure 3). There is no tailing of the acid on buffered silicagel at all. The separation of the three compounds is not affected by the salt layer on the silicagel. In both chromatograms the same mobile phase was used for elution.

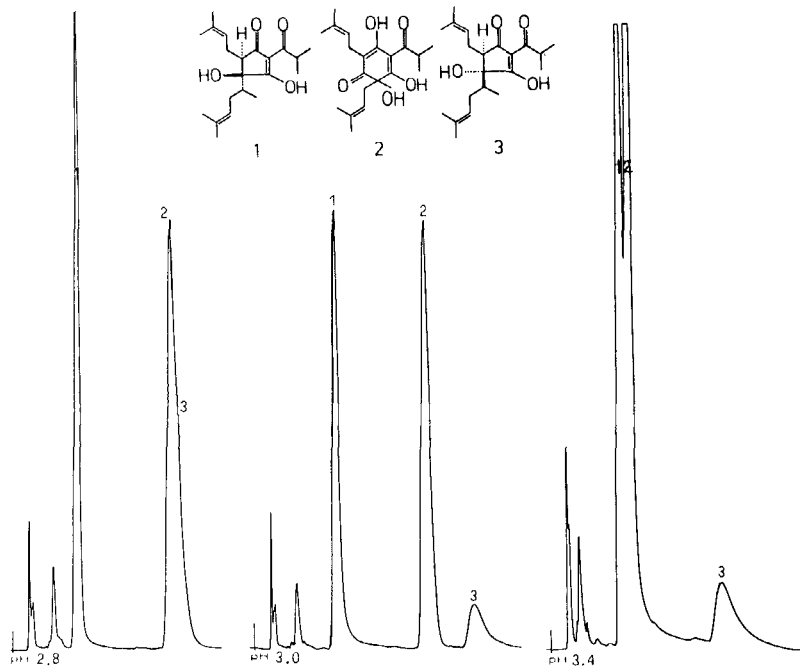

Figure 2. HPLC separation of cohumulone from cis and trans iso-cohumulone.

Column: Lichrospher SI 100, 5 µm, buffered, 250 x 3 mm
Mobile phase: hexane/ethyl ether 6:1, 1.0 ml/min
1 = cis iso-cohumulone, 2 = cohumulone, 3 = trans iso-cohumulone

Another example is the quantitative determination of bromo-undecane acid after esterification in the raw product (Figure 4). On normal silicagel the unreacted acid can hardly be detected and can not quantitatively be measured because of the strongly tailing peak of the acid. On a buffered column system (Figure 5) the detection and quantitative determination is possible.

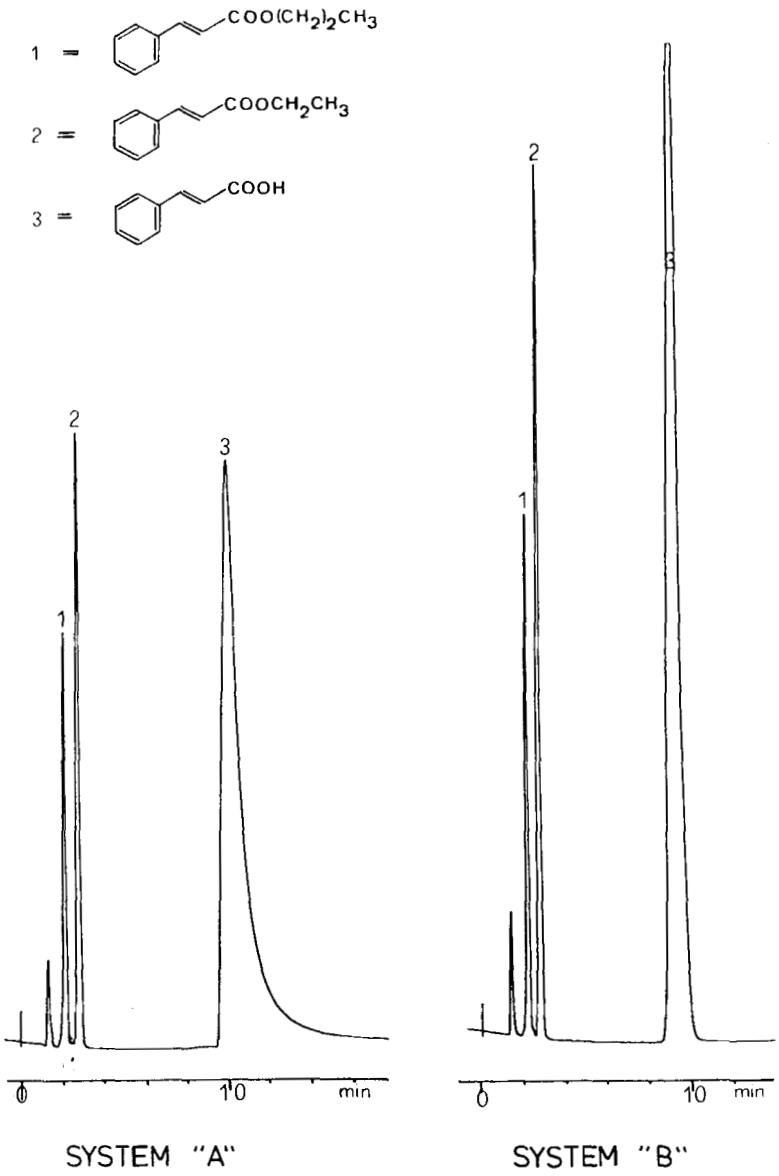

Figure 3. Separation of cinnamic acid from its esters on untreated and buffered silicagel.

System A Column: Lichrospher SI 100, 5 µm, untreated, 250 x 3 mm
Mobile phase: hexane/ethyl ether 9:1, 1.0 ml/min
System B Column: Lichrospher SI 100, 5 µm, buffered, 250 x 3 mm
Mobile phase: hexane/ethyl ether 9:1, 1.0 ml/min

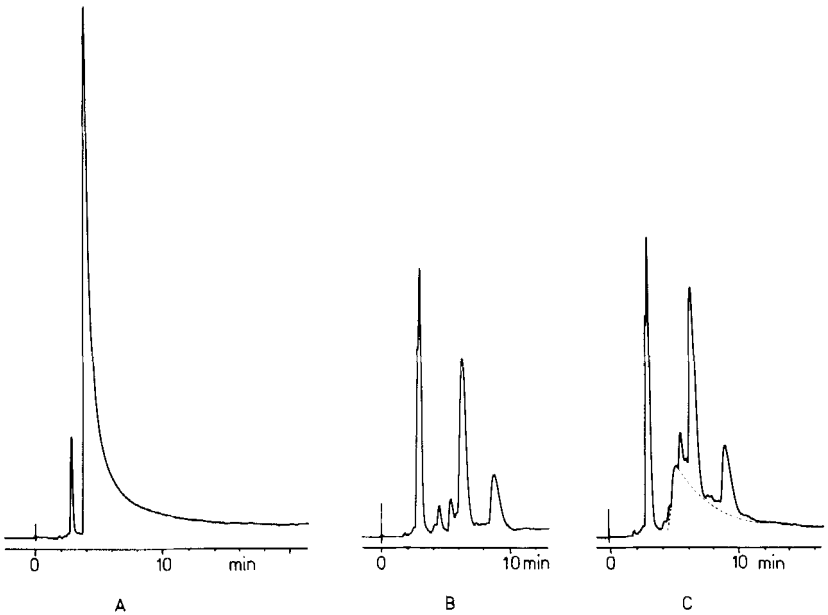

Figure 4. Chromatography of bromoundecane acid on untreated silicagel.

Column: Lichrospher SI 100, 5 µm, untreated, 250 x 3 mm
Mobile phase: hexane/ethyl ether 2:3, 1.0 ml/min
A = acid, B = raw product without acid, C = raw product with acid

The use of buffered silicagel is not restricted to polar acidic compounds. Polar basic compounds require a basic buffered silicagel for tailing-free elution. Aniline and methoxyaniline, for example, elute on normal silicagel with an unacceptable tailing. (Figure 6). When the silica surface is coated with a buffer solution of pH 8.0 the tailing disappears (Figure 7).

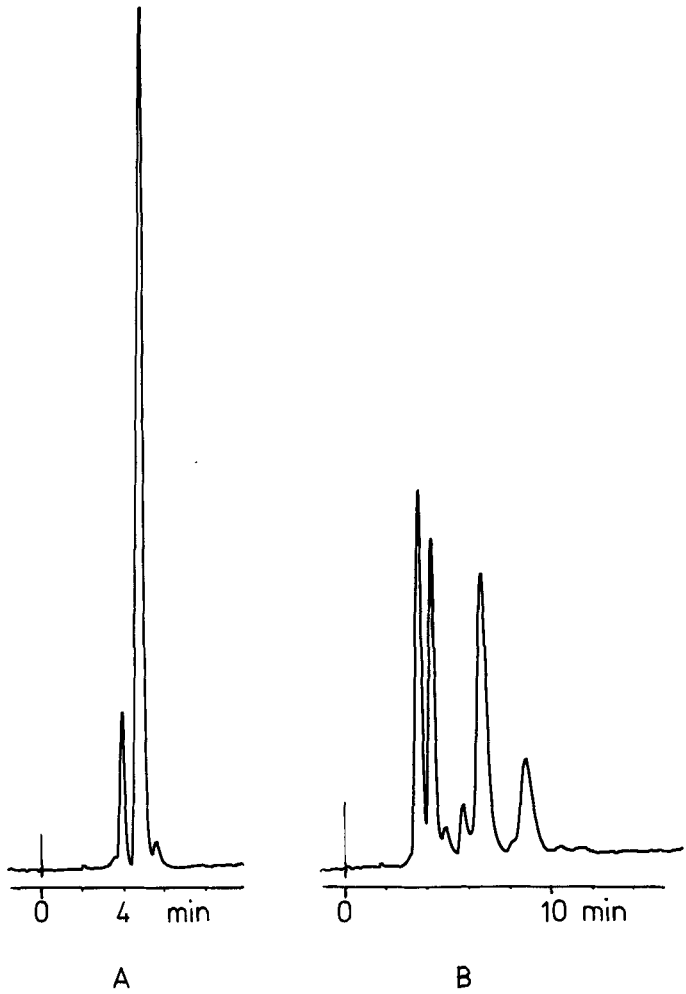

Figure 5. Chromatography of bromoundecane acid on buffered silicagel.

Column: Lichrospher SI 100, 5 µm, buffered pH 2.8 (citric acid) 250 x 3 mm
Mobile phase: hexane/ethyl ether 2:3, 1.0 ml/min
A = acid, B = raw product with acid

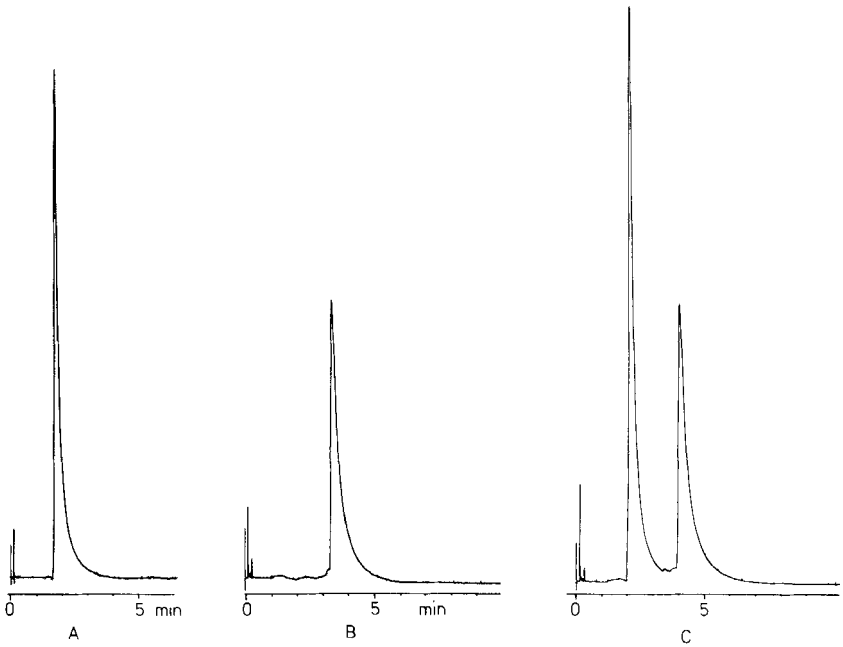

Figure 6. Chromatograms of aniline and methoxyaniline on untreated silicagel.

Column: Lichrospher SI 100, 5 µm, untreated, 250 x 3 mm
Mobile phase: hexane/ethyl ether 9:1, 1.0 ml/min
A = aniline, B = methoxyaniline, C = mixture

The stability of such buffered columns is comparable with that of normal silicagel columns. It depends largely on the sample. The main problem is the solubility of the buffer salt in the mobile phase, by using less than 20 % ethyl ether in hexane, most of the buffered columns can be used for several months without loss of performance. Gradient and flow programs can be applied to such systems as to untreated silicagel columns and the buffered silicagel can be used for preparative separations as well. A loss in capacity after coating the surface with the buffer was not observed.

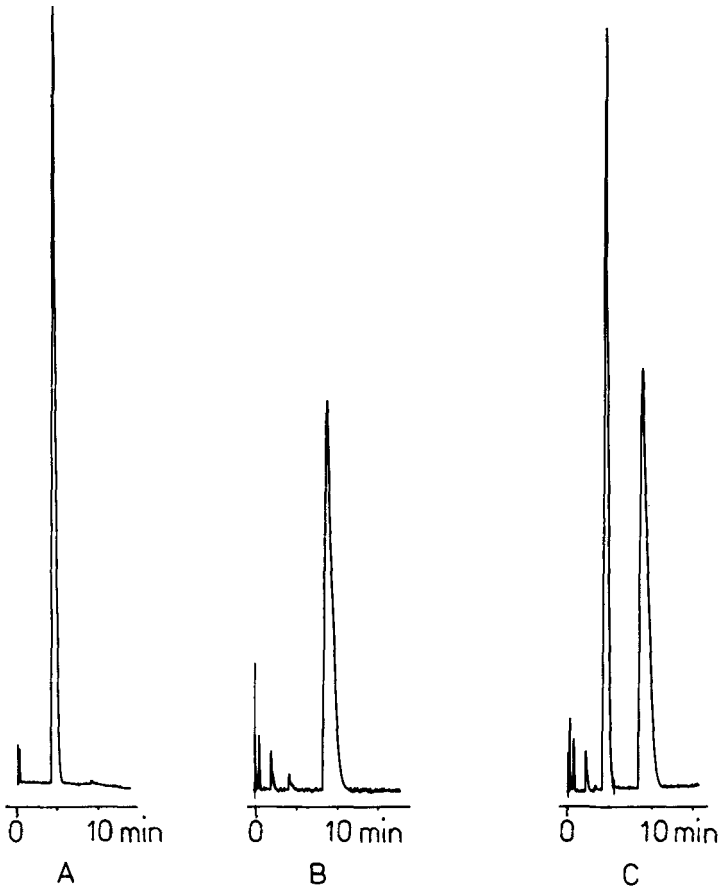

Figure 7. Chromatograms of aniline and methoxyaniline on buffered silicagel.

Column: Lichrospher SI 100, 5 µm, buffered pH 8.0 (boric acid/Na-borate), 250 x 3 mm
Mobile phase: hexane/ethyl ether 9:1, 1.0 ml/min
A = aniline, B = methoxyaniline, C = mixture

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