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Note

High-performance liquid chromatography on dynamically modified silica

VIII*. Gradient elution using eluents containing cetyltrimethylammonium bromide

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Recently the dynamically modified silica approach has proved to be a valuable technique in high-performance liquid chromatography (HPLC)[1]. In this technique the surface of bare silica is modified dynamically with long-chain quaternary ammonium compounds added to the eluent, *e.g.* cetyltrimethylammonium (CTMA) ions[2–3]. The influence on the retention of various test solutes of variations in the pH (ref. 4), the nature of the quaternary ammonium ion and the CTMA concentration[2], the ionic strength and buffer components[4], and the nature and concentration of the organic modifier[5] have been thoroughly investigated. From the data obtained it is obvious that all parameters have to be controlled carefully, but if this is done, reproducible systems with respect to retention and selectivity may be obtained[6–8]. This paper describes an investigation of three ways of performing gradient elution in the dynamically modified silica mode. The gradient is formed by increasing the concentration of the organic modifier in the eluent, or by increasing the ionic strength or by increasing the CTMA concentration above the critical micellar concentration.

EXPERIMENTAL

Apparatus

Chromatographic testing of the individual systems was performed on a Waters liquid chromatograph consisting of two 6000 A pumps, a 710 A WISP autoinjector, a 440 UV absorbance detector (254 nm), a 730 data module and a 720 system controller. The column system was thermostatted in a Kratos oven at 30°C.

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Chromatography

All experiments were performed on 120 × 4.6 mm I.D. columns from Knauer (Berlin, F.R.G.), packed with LiChrosorb Si 60 (5 μm) (E. Merck, Darmstadt, F.R.G.). The eluents in the three gradient experiments were as follows.

Elution strength gradient. Eluent A = acetonitrile–water–0.2 *M* phosphate buffer (pH 7.5) (10:85:5) with 0.25 m*M* CTMA added; eluent B = acetonitrile–water–0.2 *M* phosphate buffer (pH 7.5) (50:45:5) with 40 m*M* CTMA added. The gradient was linear from 0% to 100% B in 15 min at a flow-rate of 1 ml/min. The delay time was 10 min.

Ionic strength gradient. Eluent A = methanol–water–0.2 *M* phosphate buffer (pH 7.5) (35:63.75:1.25) with 2.5 m*M* CTMA added; eluent B = methanol–water–0.2 *M* phosphate buffer (pH 7.5) (35:40:25) with 2.5 m*M* CTMA added. The gradient was linear from 0% to 100% B in 10 min at a flow-rate of 1 ml/min.

Micellar concentration gradient. Eluent A = methanol–water–0.2 *M* phosphate buffer (pH 6.0) (30:65:5) with 5 m*M* CTMA added; eluent B = methanol–water–0.2 *M* phosphate buffer (pH 6.0) (30:65:5) with 50 m*M* CTMA added.

The buffer pH stated is that measured in the undiluted buffer and not in the final eluent. The buffer was prepared from 0.2 mol of potassium dihydrogenphosphate dissolved in 930 ml of water, titrated with 5 *M* potassium hydroxide to the appropriate pH value and finally diluted to 1000 ml. During chromatography the column was guarded by a silica saturation column (100 × 4.6 mm I.D.) situated between the pump and the injection device.

Chemicals

All chemicals were of analytical grade from E. Merck and were used as received from the manufacturer.

RESULTS AND DISCUSSION

Elution strength gradient

It is known that the elution strength of the eluent is increased in the reversed-phase mode when the concentration of the organic modifier is increased. In chromatography on dynamically modified silica this is also true, but the amount of stationary phase decreases as well since it is not chemically bonded to the surface[5].

This means that a smaller change in the concentration of the organic modifier is needed to obtain a certain effect relative to gradient elution on chemically bonded phases. It also means that the system will be totally out of equilibrium when a gradient is created by changing the concentration of the organic modifier. Following a gradient run, the next one should not be started until the initial amount of stationary phase on the silica surface in the first gradient run has been reestablished. This may require a rather long time (4–8 h), and an attempt to compensate for the loss of stationary phase during the gradient run by simultaneously increasing the amount of CTMA in the eluent was therefore made.

From data in ref. 5 it appears that in order to maintain a coverage of 0.65 mmol of CTMA per gram of silica when using a gradient from *e.g.* 40% to 70% of methanol, the CTMA concentration also has to be varied from 1 m*M* to 50 m*M* during the elution. Even then, complete equilibrium is not maintained, but repeating

the elution with a fixed delay-time between each run leads to a reproducible gradient within three or four runs[5]. It should also be noted that the saturation column in the system will contribute to the delay of the gradient.

Ionic strength gradient

From previous studies on the influence of changing the ionic strength on the retention of various solutes[4], it appears that non-ionic and cationic solutes are affected only to a minor extent by the addition of inorganic salts or buffers, whereas the retention times of the anionic compounds are markedly reduced. This is in good agreement with previous observations[4] that the amount of CTMA adsorbed on the silica surface is hardly affected by changes in the ionic strength, and thus it is possible to perform a selective gradient elution of anionic solutes (Fig. 1). Furthermore, this

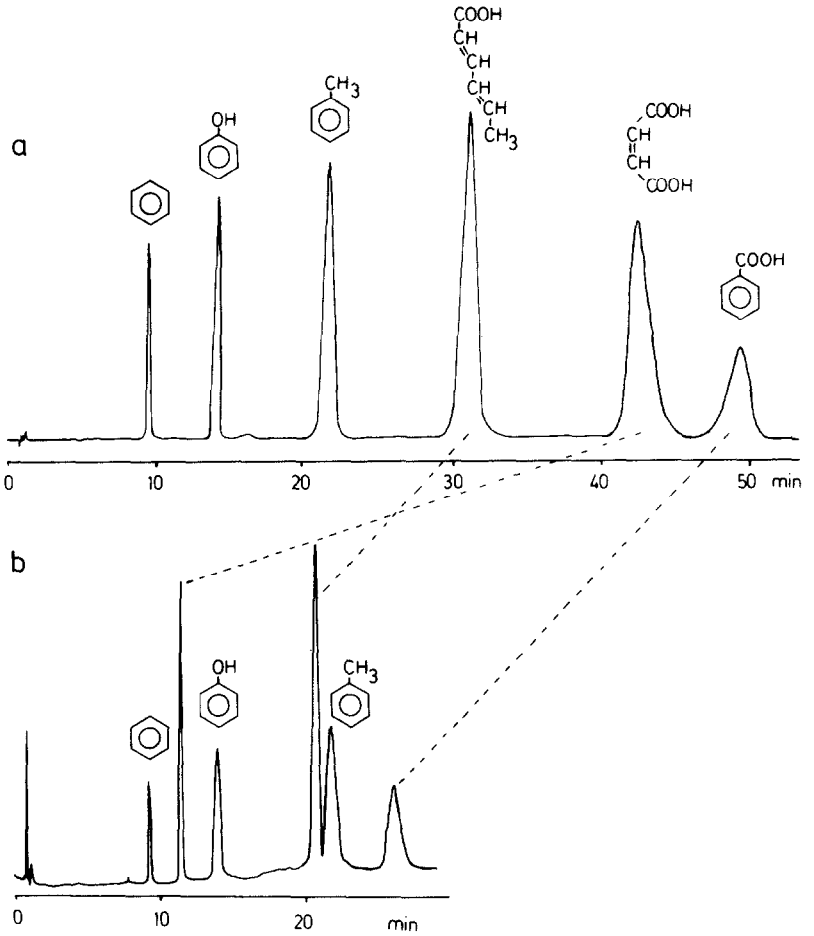

Fig. 1. Isocratic (a) and ionic strength gradient (b) elution of test solutes. Column, 120 × 4.6 mm I.D. LiChrosorb Si 60, 5 μm; eluent A, methanol–water–0.2 *M* phosphate buffer (pH 7.5) (35:63.75:1.25) with 2.5 m*M* CTMA added; eluent B, methanol–water–0.2 *M* phosphate buffer (pH 7.5) (35:40:25) with 2.5 m*M* CTMA added. (a) Isocratic elution with eluent A (1 ml/min); (b) gradient elution from 100% eluent A to 100% eluent B in 10 min at a flow-rate of 1 ml/min.

technique is able to provide information about the valency of the anionic species, since divalent anions are affected more than monovalent and trivalent anions even more. The reason for this selective effect on the anionic solutes is an inhibition of the formation of ion-pairs with the CTMA ions in the liquid phase. Consecutive gradient runs may be performed without intermittent delay-times, and the repeatability of the test solute retention times is better than 0.2% between runs. However, if more lipophilic organic anions are used to increase the ionic strength the effect will be more pronounced, and ion-pairs between CTMA ions and long-chain sulphonate ions, for example, will adsorb on the lipophilic surface thus increasing the amount of stationary phase[4], and rendering the column unsuitable for gradient elution.

Micellar concentration gradient

Reversed-phase HPLC systems using micellar elution were first described by Armstrong and Henry[9], and this technique has since been utilized by several workers *e.g.* refs. 10 and 11, and also in the gradient elution mode[12,13]. In the dynamically modified silica mode, micellar gradient elution may be performed provided that the concentration of the long-chain quaternary ammonium ion (*e.g.* CTMA) in the initial eluent is above the critical micellar concentration (CMC). The CMC in the eluent

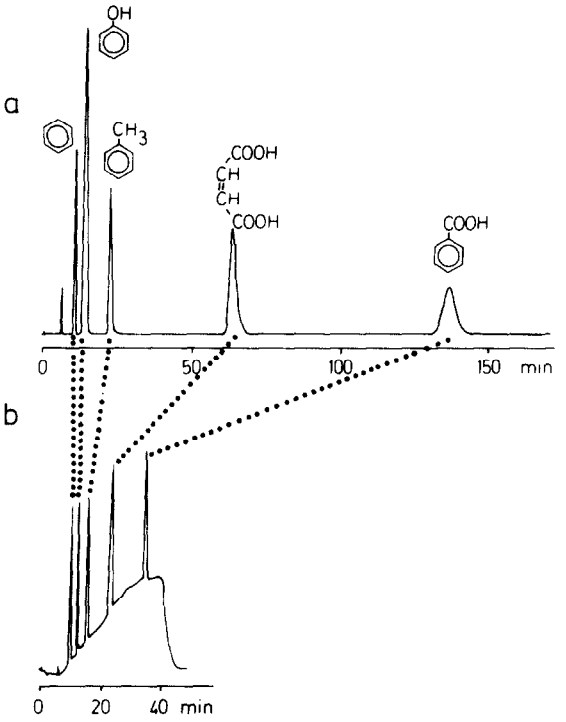

Fig. 2. Isocratic (a) and micellar gradient (b) elution of some test solutes. Column, 120 × 4.6 mm I.D. LiChrosorb Si 60, 5 μm; eluent A, methanol–water–0.2 *M* phosphate buffer (pH 6.0) (30:65:5) with 5 m*M* CTMA added; eluent B, methanol–water–0.2 *M* phosphate buffer (pH 6.0) (30:65:5) with 50 m*M* CTMA added. (a) Isocratic elution with eluent A (1 ml/min); (b) gradient elution from 100% eluent A to 100% eluent B in 30 min at a flow-rate of 1 ml/min.

may be determined as described previously[2]. When the CMC is exceeded the concentration of the free quaternary ammonium ions in the liquid phase remains constant and thereby the amount of stationary phase as well. However the number and size of the micelles in the liquid phase will increase thus causing the elution strength of the eluent to increase also. The presence of micelles in the eluent may be described as an extra lipophilic phase, migrating together with the polar eluent. Using the micellar approach, a reversed-phase gradient elution may be performed in a reproducible way without using fixed delay-times. The repeatability of retention times between runs is within 0.1–0.2%. An example of this type of gradient elution is shown in Fig. 2. The increased baseline signal is due to the increase in bromide concentration. This may be avoided by using CTMA salts with a non-UV-absorbing anion.

CONCLUSION

Three ways of performing gradient elution in the dynamically modified silica mode are presented.

When using a micellar concentration gradient it is possible to obtain results similar to those seen when using gradient elution on chemically bonded phases with increasing amounts of the organic modifier.

When increasing the ionic strength during a chromatographic run, a selective gradient elution of the anionic solutes is obtained.

Increasing the amount of organic modifier in the dynamically modified silica approach will, apart from the increase in elution strength, cause a decrease in the amount of stationary phase. Gradient elution by this technique should, therefore, only be performed under strictly controlled conditions, including fixed delay-times between runs.

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