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High-performance liquid chromatography of some alkaloids on unmodified silica gel with aqueous–organic solvent mixtures

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Abstract

The application of eluents composed of methanol–aqueous phosphoric buffer was investigated for the high-performance liquid chromatographic separation of alkaloids on a silica stationary phase. These eluents were shown to be very useful for preliminary analytical chromatography and allow the conditions for the preconcentration of some alkaloids from crude extracts to be established. The effects of different eluent pH values and methanol concentrations were examined.

1. Introduction

In most instances, the micropreparative isolation of alkaloids from plant material is carried out by liquid–liquid extraction. The content of alkaloids in plants is very low, so large volumes of organic solvents, usually chloroform, are necessary.

Preconcentration of alkaloids from aqueous extracts on columns filled with Ameberlite XAD or chemically bonded organic stationary phases is very expensive, because continued injection of plant extracts, with strongly retained solutes, degrades the column performance.

During the last decade, unmodified silica gel with aqueous solvent mixtures has been successfully applied to separate a large number of organic base mixtures on both analytical [1–6] and preparative scales [7–9].

The utility of silica gel with aqueous–organic buffered eluents in chromatographic systems for the separation of basic organic compounds has been demonstrated for basic pharmaceuticals [1,2], nucleosides [10], amino acids [11], biogenic amines [11,12], dipeptides [13], alkaloids [5,6,14,15], anaesthetics [3] and tricyclic antidepressants [3,6]. The main difference between the chromatographic systems used in these investigations [1–7,9–15] lies in the mobile phase composition; some eluents consisted of aqueous buffer and an organic solvent [1–4,6,7,10,14,15] and some also contain small amount of long-chain quaternary ammonium compounds [5,11,16,17] (e.g., cetyltrimethylammonium bromide).

The retention mechanism on unmodified silica gel with buffered aqueous–organic solvent

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eluents is complex. Organic amines at pH < 8 are at least partially protonated and are separated predominantly by ion-exchange interactions [1–3]. Bidlingmeyer and co-workers [3,4] pointed out that hydrophobic interactions must also be effective and that silica seems to be behaving in a reversed-phase mode. Cox and Stout [18] have also confirmed that the retention mechanism is a combination of ion exchange for compounds ionized under the conditions of elution and hydrophobic interaction for non-ionized materials. It was also confirmed [18] that the retention of organic bases is a complex function of the organic modifier, buffer concentration and pH.

Alkaloids usually occur in plants as salts of organic and inorganic acids with complex mixtures of water-soluble compounds, such as proteins, tannins or lipids [19]. It is often a great problem to remove these compounds from extracts during the isolation and purification of alkaloids.

It follows from TLC and HPLC investigations [14,15,24] that the retention of ternary and quaternary alkaloids in a system of silica gel with buffer–methanol increases with increasing water (buffer) content in the mobile phase. It should be stressed that quaternary alkaloids are more strongly retained than ternary alkaloids. Such differences in retention create the chance to preconcentrate quaternary alkaloids by frontal analysis of an alkaloid mixture contained in an extract or to separate the quaternary and ternary alkaloids contained in a crude extract into two groups, by using a very simple and cheap chromatographic system.

As mentioned, the retention of basis organic compounds depends on the composition and pH of the mobile phase, so it was necessary to check in detail how these parameters influence the retention of alkaloids. In this investigation, the retention behaviour of some alkaloids (mainly from *Chelidonium maius* L.) was studied as a function of the pH and concentration of the mobile phase. The results obtained will be of help in finding the optimum conditions for the micropreparative isolation of alkaloids from plant material.

2. Experimental

2.1. Apparatus

The experiments were carried out using a Type 302 liquid chromatograph (Institute of Physical Chemistry of the Polish Academy of Sciences, Warsaw, Poland), which consisted of syringe pump, a UV detector (fixed wavelength, 254 nm) and a four-port injection valve. A 100 × 4 mm I.D. stainless-steed column (ZOCh, Lublin, Poland) packed with 10-μm LiChrosorb Si 60 (Merck, Darmstadt, Germany) was used for measurements of capacity factors. The dead volume of the column was calculated after injection of a methanolic solution of benzene.

2.2. Chemicals

LiChrosorb Si 60, methanol and protopine were obtained from Merck, orthophosphoric acid and sodium hydroxide were supplied by the Polish Reagents Factor (Gliwice, Poland). The other compounds used came from various commercial sources or were obtained as gifts; they were used as received. Stock solutions of alkaloids were prepared in methanol at a concentration of 1 mg/ml. All solvents were filtered and degassed in vacuum before chromatography. The buffers were prepared from orthophosphoric acid by titration to the required pH with 5 *M* sodium hydroxide solution, followed by dilution to the final concentration of 0.2 *M*. Mobile phases of a given volume fraction were prepared by a mixing constant amount (volume) of phosphate buffer and various volumes of methanol and doubly distilled water. The pH of each portion of the mobile phase was checked with a calibrated pH meter; the stated pH of the solutions, measured after addition of modifier, refers to the organic–aqueous mobile phases.

Micropreparative preconcentration of alkaloids was carried out using a disposable, laboratory-made column (10 × 1 cm I.D.) packed with 40–60-μm Kieselgel 60 (Merck). A large volume (about 1 l) of dilute acidic (pH 6) aqueous plant extract was flushed through this

column and then the adsorbed alkaloids were eluted with 35 ml of methanol which contained 10% of 0.1 *M* HCl.

3. Results and discussion

3.1. Effect of co-solvent and pH of the mobile phase

It has been shown that the selectivity in ion-exchange chromatography on silica gel or alumina can be substantially improved by the addition of organic solvents to the aqueous mobile phase [5,6,14,15,21,22]; usually methanol or acetonitrile is used as the modifier. An explanation of this phenomenon can be formulated on the basis of changes in solvation of the competing cations and in solute ionization. In this work, improved selectivity is caused by changes in the methanol concentration in the mobile phase.

The capacity factors (k') of alkaloids were determined as a function of the methanol concentration of the mobile phase, keeping the buffer concentration and pH constant (Figs. 1 and 2). In all instances the capacity factor first decreases more or less sharply with increasing methanol content, passes through a minimum at ca. 50% of methanol in the mobile phase at lower pH and at ca. 70% at higher pH (7.4–8.5) and then increases moderately at higher methanol contents. The exact position of the minimum of the $k' = f(\varphi_{MeOH})$ depends on the degree of protonation of alkaloids and the polarity of the solute [5,21]. As the content of water increases, the retention of the alkaloids increases and for eluents rich in water, k' sometimes cannot be measured. The appearance of a retention minimum suggests that there are at least two contributing chromatographic mechanisms. Curves of similar shapes were reported previously for amino compounds and "pseudo-reversed-phase" conditions [5,14,18].

As can be seen from Figs. 1 and 2, the methanol content is a very valuable parameter for adjusting the retention and improving the selectivity. It should be noted that the content of

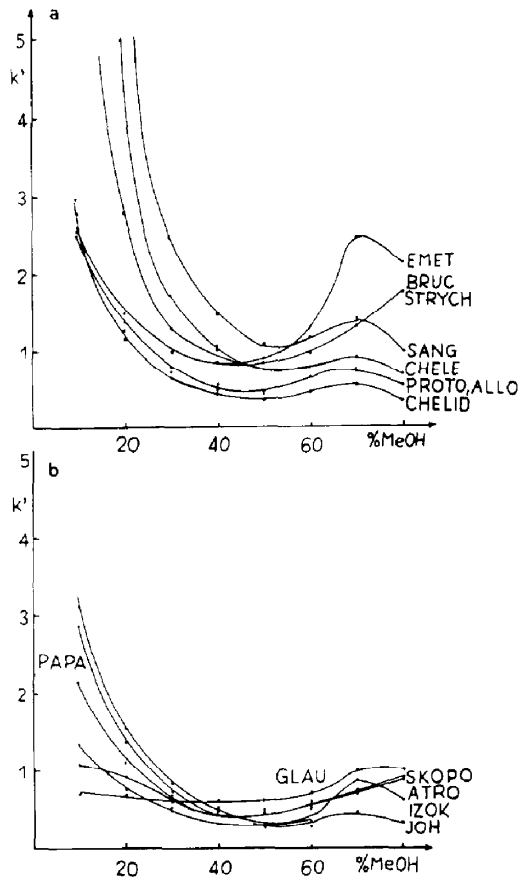

Fig. 1. Effect of the concentration of methanol on the capacity factors of alkaloids. Stationary phase: LiChrosorb Si 60, d_p = 10 μm. Mobile phase: methanol–water–phosphate buffer (pH 3.50). Constant ionic strength. Samples: BRVC = brucine; STRYCH = strychnine; EMET = emetine; SANG = snaguinarine, CHELE = chelerithrine; CHELID = chelidonine; PROTO = protopine; ALLO = allocrypropine; ATRO = atropine; GLAV = glaucine; SKOPO = scopolamine; IZOK = isocorydine; JOH = yohimbine; PAPA = papaverine.

methanol in the mobile phase also influences the column efficiency.

A final and more specific way to influence retention and selectivity is to vary the pH of the mobile phase (compare Figs. 1 and 2). For an ion-exchange mechanism to be operative, the solutes must be present in ionic form and silanol groups must be ionized. Silica gel can be used as a cation-exchange material at medium to high pH values [1,3]. The number of accessible ion-

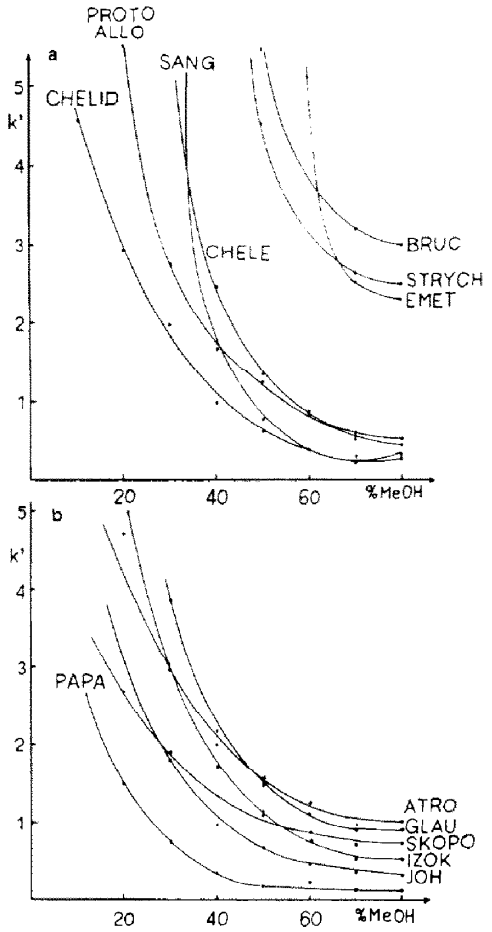

Fig. 2. Effect of the concentration of methanol on the capacity factors of alkaloids. pH of the mobile phase, 6.70. Other conditions and samples as in Fig. 1.

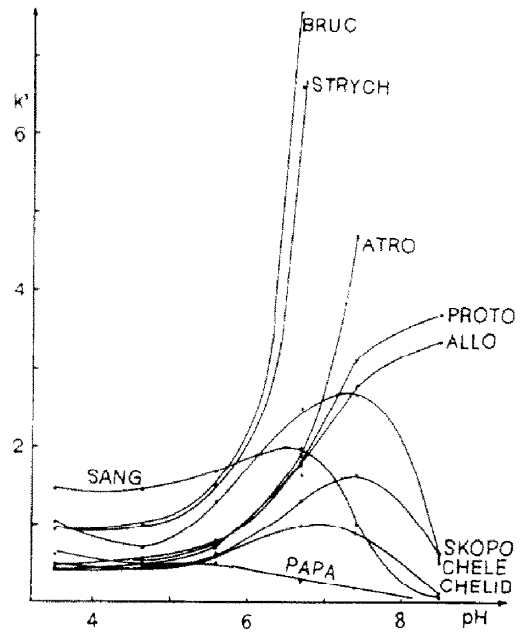

Fig. 3. Effect of pH of the mobile phase on the capacity factors of alkaloids. Constant concentration of methanol (40%, v/v). Other conditions and samples as in Fig. 1.

ized silanol groups also depends on the pH of the mobile phase and the pK_a of the silica surface. Usually below pH ca. 4 the cation-exchange capacity is very limited and increases sharply with increase in pH. Further, basic compounds are largely ionized at pH values up to $pK_a - 1$ and then rapidly become neutral at $pK_a + 1$. Consequently, the retention of non-ionized amines, when an ion-exchange mechanism is involved, decreases rapidly when the pH is increased to one unit over the pK_a. Both statements are well illustrated in Fig. 3, where the effect of pH on retention at a constant concentration of methanol is shown. For all the alkaloids investigated, as the pH is increased the retention also increases to a maximum value, after it decreases.

It is well known that the pK values of acids and bases vary with the composition of mixtures of water and organic solvents [23]. This is demonstrated in Figs. 4 and 5, where the retention behaviours of two alkaloids at various compositions of buffer–MeOH mobile phases were measured as a function of pH. It is readily seen that the maximum retention of the k vs. pH relationship, for different compositions of buffer–MeOH, is shifted from the left (higher concentration of methanol) to the right (lower concentration of methanol). It should also be stressed that the retention of glaucine or chelerithrine, at a given pH, decrease with increasing of methanol content in the mobile phase. These variations in alkaloid retention can be interpreted by a change in ionization [5,22] and solvation [5,18] of the solutes. It is seen from Fig. 3 that a maximum separation factor α is obtained at a pH value intermediate between the two pK_A values, so even a 0.3–0.4 change in pK_A (e.g., when the concentration of methanol is

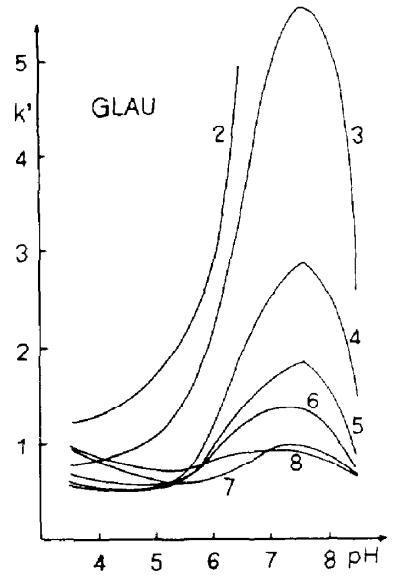

Fig. 4. Influence of pH (actual pH in mixed aqueous–organic solvent) and methanol concentration on the retention behaviour of glaucine. The concentration of the mobile phase is indicated by first digit of the methanol percentage, e.g., 1 = 10% of methanol.

changed from 20 to 40%) can result in significant changes in retention and selectivity, particularly when the pH of the aqueous–organic phase is near the pK_A [23].

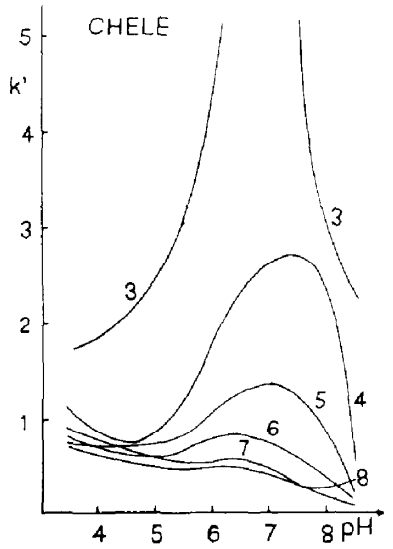

Fig. 5. Influence of pH and methanol concentration on the retention behaviour of chelerithrine. Conditions as in Fig. 4.

3.2. Three-dimensional networks

In laboratory practice, optimization of the mobile phase is restricted to changing its composition or pH, keeping the other parameter constant. Combined optimization of the pH and concentration of the mobile phase can show additional selectivity effects and the region of strongest retention of alkaloids. For basic solutes there is also a second selectivity effect from the influence of the modifier on the pK_A values of the buffer and the solutes [22].

The dependence of the capacity factor, k', on the concentration and pH of the mobile phase may be represented three-dimensionally if sufficient data are available to construct a closely spaced network. In Figs. 6–8 the k' values of chelerithrine, sanguinarine and glaucine are plotted for various concentrations and pH values of the mobile phase. The data points become the intersections of a net, forming a three-dimensional surface. In consequence, during the optimization process, both the concentration of the

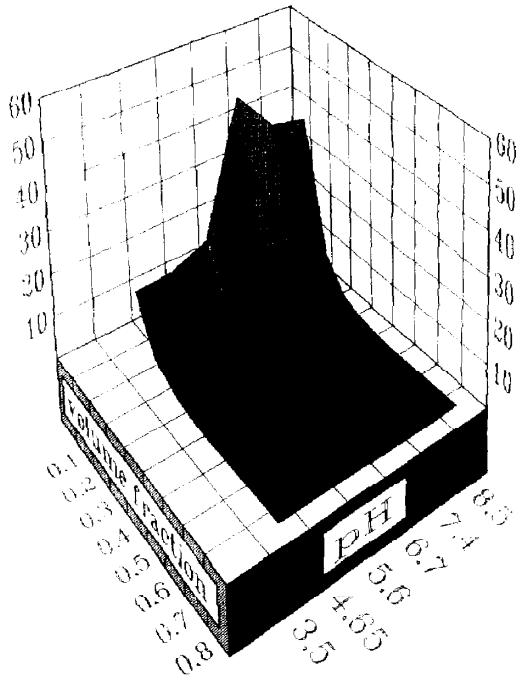

Fig. 6. Combined effects of pH and methanol concentration in the mobile phase on the chromatographic behaviour of chelerithrine. See text for discussion.

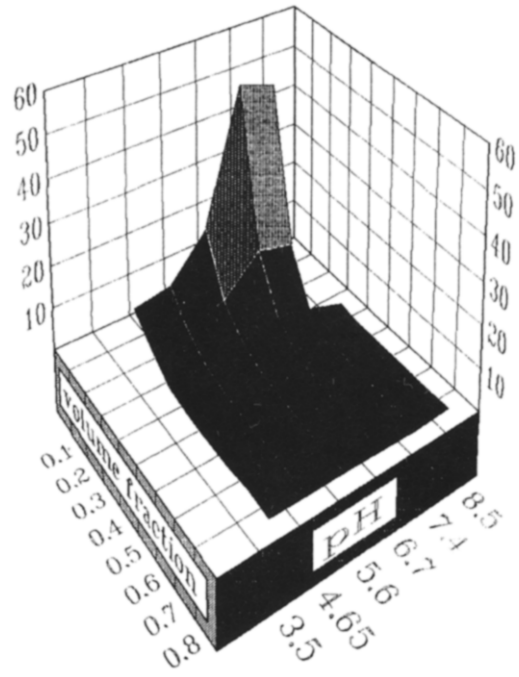

Fig. 7. Combined effects of pH and methanol concentration in the mobile phase on the chromatographic behaviour of sanguinarine. See text for discussion.

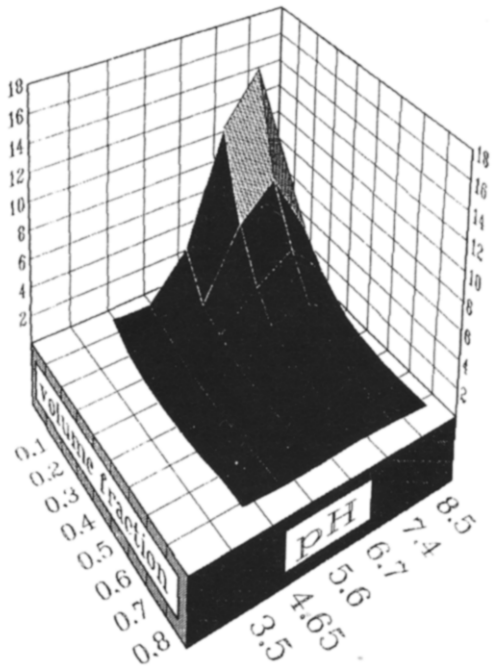

Fig. 8. Combined effects of pH and methanol concentration in the mobile phase on the chromatographic behaviour of glaucine. See text for discussion.

organic solvent and the pH of the mobile phase should be carefully established.

All data collected so far for a mobile phase composed of methanol and buffer, alkaloids as solutes and unmodified silica gel as the stationary phase are similar to the general behaviour of k' in Figs. 6–8. At low pH (<4.5), k' for the alkaloids is essentially independent on the concentration of methanol and at a high concentration of methanol ($\varphi > 0.5$) k' in practice is independent of both pH and concentration of methanol. Such plots are useful in predicting of the conditions for the chromatography and preconcentration of alkaloids from crude aqueous extracts.

The applicability of the investigated chromatographic system to the separation of some quaternary alkaloids, obtained from *Chelidonium maius* L. roots, is demonstrated in Fig. 9. The resolution between chelirubine–sanguinarine and chelilutine–chelerithrine is even better than in a normal-phase system [24]. One of the disadvantage of the present chromatographic system is the low column efficiency. It is well known that in ion exchange the kinetic processes are slow, especially when a mobile phase rich in water is used. For the column used in this work, the efficiency was measured for real samples of berberine and chelerithrine. The number of theoretical plates depends strongly on the concentration of methanol and for 25×0.4 cm I.D. column packed with 10-μm particles of silica gel it is about 2500 for a 50% methanol concentration [25]. It should be kept in mind that the proposed chromatographic system will be used for the preliminary separation and preconcentration of alkaloids present in crude plant extracts.

4. Conclusions

This study has demonstrated that the chromatographic system described can be successfully applied to the analytical chromatography or

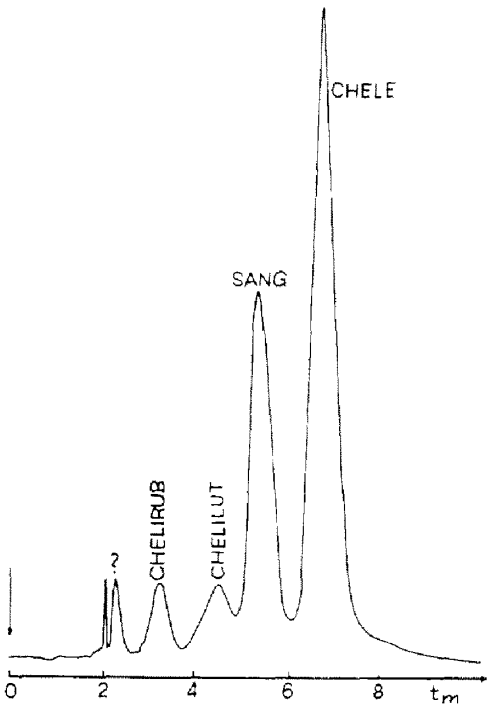

Fig. 9. Chromatogram of some quaternary alkaloids obtained from *Chelidonium majus* L. Stationary phase, LiChrosob Si 60, d_p = 10 μm; mobile phase, methanol–water–phosphate buffer (40:40:20, v/v/v); pH of the mobile phase, 6.9. Peaks: 1 = unknown; 2 = chelirubine; 3 = chelilutine; 4 = sanguinarine; 5 = chelerithrine.

preconcentration of alkaloids. These enriched and cleaner samples can subsequently be re-chromatographed using normal- or reversed-phase chromatography.

The concentration of methanol and pH or a combination of these parameters can be used to adjust the order and degree of retention of alkaloids. The described system is simple and cheap and the alkaloids are chromatographed as salts so they are more stable than in normal-phase systems, where they are chromatographed as free bases.

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