Note

Lauroyl sarcosine: a weak cation-exchange reagent for on-column modification of reversed-phase material

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Ion-pairing reversed-phase chromatography is widely used to effect separation of cationic compounds including primary and secondary amines[1–6]. Compounds routinely used as ion-pair reagents are alkyl or aromatic sulfonic acids or sulfates and more rarely alkyl phosphonates[7]. These compounds are anionic across the working pH range for silica based reversed-phase chromatography, (pH *ca.* 2–7). As a result, pH manipulations have only minimal effects on ion-pair separations of amines[8,8]. Separations are generally governed by the concentration of sulfate or sulfonate, length of alkyl chain, concentration of organic modifier and concentration of counter ion in the mobile phase[8]. Sulfonate and sulfate reagents might be termed strong cation exchangers as these functional groups are present on strong cation-exchange materials and remain deprotonated in the working range of most reversed-phase materials. Weak ion-pair reagents analogous to weak cation-exchange functional groups have not been used in ion-pair reversed-phase high-performance liquid chromatography (HPLC). Long chain alkyl carboxylic acids are generally insoluble in water and are thus of little value in reversed-phase separations of polar molecules where mobile phases are predominantly aqueous.

In the present work, a water soluble long chain amino acid surfactant, lauroyl sarcosine (N-dodecyl, N-methylglycine, hamposyl L) was tested for its suitability as an ion-pair reagent in reversed-phase chromatography.

EXPERIMENTAL

Lauroyl arcosine was obtained from W. R. Grace & Co., (Lexington, MA, U.S.A.) as the free acid (hamposyl L). Amine standards, norepinephrine–HCl, epinephrine bitartrate, normetanephrine–HCl and dopamine (3-hydroxy tyramine)–HCl were obtained from Sigma (St. Louis, MI, U.S.A.).

Solvents (see figure legends) were prepared using distilled, deionized water. Other chemicals used were reagent grade.

Chromatographic separation was accomplished using a 10 cm × 1.1 mm I.D. column packed with 3-in ODS-Hypersil (Shandon) as previously described[9]. Constant flow-rate was maintained using a Milton Roy (Laboratory Data Control) mini pump. Separated amines were detected amperometrically at a glassy carbon electrode

at an applied potential of $+0.75$ V *vs.* Ag/AgCl using an EC/230 amperometric detector (IBM Instruments, Danbury, CO, U.S.A.). Sample was applied to the column using a Rheodyne 7413 injection valve with the 5-μl loop in place. Column dead volume (40 μl) was determined by observing the first deflection in the background signal following injection of the sample. This occurs when unretained ions elute, causing an ohmic current change due to a change in solution resistance, detected by the electrochemical detector.

RESULTS

Fig. 1 demonstrates the effect of column "loading"-time with lauroyl sarcosine on the capacity factors of a series of catecholamines. Equilibrium was attained in 2 h and no additional increase in capacity factor (k') was observed after that time. With lower concentrations of lauroyl sarcosine, equilibrium was established more slowly. Although not shown, loading time could be greatly reduced by using a much higher concentration of lauroyl sarcosine in the mobile phase, presumably by more rapidly saturating the C_{18} material. Once fully "loaded", appropriate conditions, ionic strength. pH and methanol content could be substituted. The order of elution for the catecholamines shown in Fig. 1 is the same as for the ion-pair separations of these same compounds using sulfate or sulfonic acid ion pair reagents[1,10]. At this concentration, 600 mg/ml (2.2 $\cdot$ 10^{-3} M), a much greater increase in k' would be observed when using lauryl sulfate as the ion-pair reagent under these same conditions.

The effect of counter ion concentration is demonstrated in Fig. 2. As with other ion-pair or ion-exchange reagents, increasing ionic strength decreases k'.

Unlike other ion-pair reagents for the separation of amines, however, pH has a marked effect on k'. This is shown in Fig. 3. One clearly observes a sigmoidal curve typical for titration of the carboxylic acid functional group. Below pH 3.5, no ion-exchange interaction is observed. Maximum ion-exchange interaction is observed at
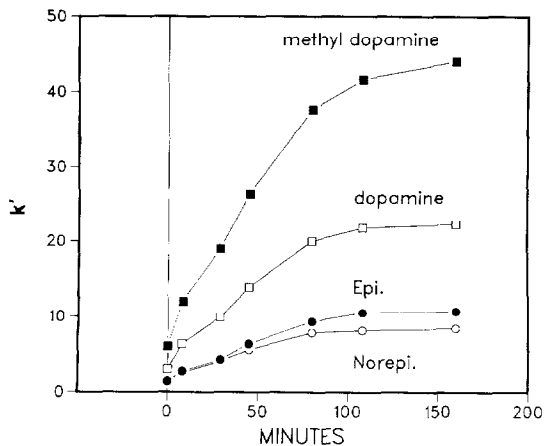

Fig. 1. Effects of column loading-time with lauroyl sarcosine on capacity factors of amines. Solvent conditions: 0.1 M NaH$_2$PO$_4$, 10% methanol, 600 mg/l lauroyl sarcosine, pH 5.8; flow-rate 200 μl/min. Column was 10 cm $\times$ 1.1 mm I.D. packed with 3 μm Shandon Hypersphere ODS. Epi. = Epinephrine; Norepi. = norepinephrine.
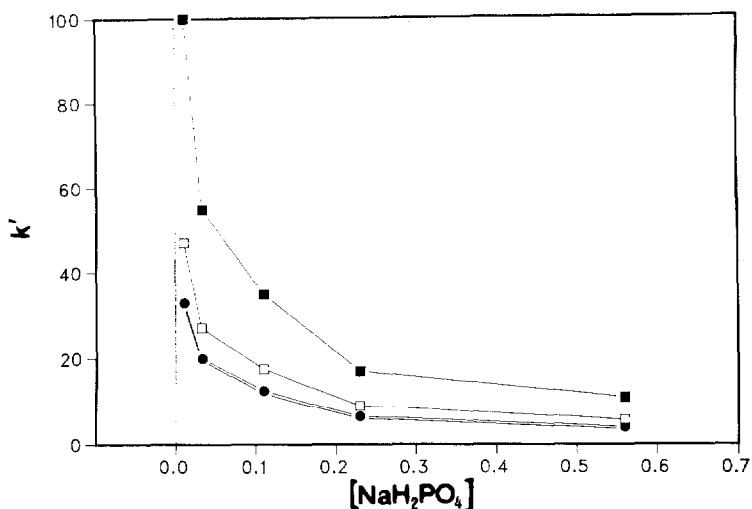
Fig. 2. Effects of mobile phase sodium ion concentration on capacity factors of amines. Na^+ was varied by altering the concentration of a phosphate buffer with fixed relative molar concentrations of NaH_2PO_4 Na_2HPO_4 of 5:1. Lauroyl sarcosine concentration was maintained at 600 mg/l. Other conditions were as in Fig. 1. Key to symbols: ■, dopamine; □, normetanephrine; ●, epinephrine; ○, norepinephrine.

pH 6 or greater. From these data, a pK_a^* of 4.6–4.65 can be obtained for lauroyl sarcosine in this solvent. In addition to the divergence from analogy to sulfate or sulfonate ion-pair reagents due to pH effects, other observations suggest that the separation is more ion-exchange than ion-pair reversed-phase. Fig. 4 shows the effect of varying the concentration of lauroyl sarcosine across two orders of magnitude.
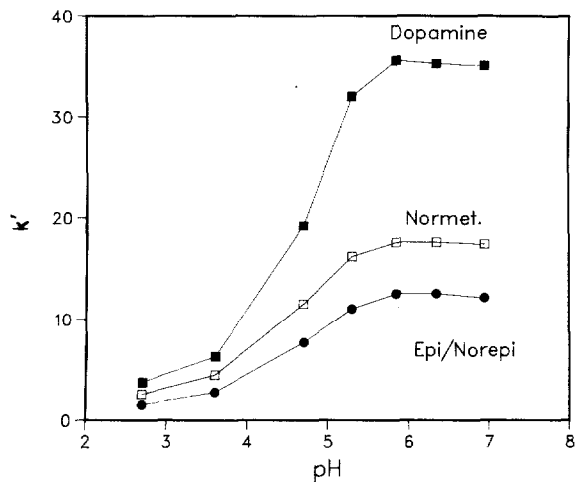

Fig. 3. Effects of pH on capacity factors of amines separated on a lauroyl sarcosine loaded reversed-phase column. The solvent was 0.1 M orthophosphoric acid, 10% methanol, 600 mg/l lauroyl sarcosine. pH was adjusted with 1 M sodium hydroxide. Column and flow-rate were as in Fig. 1.
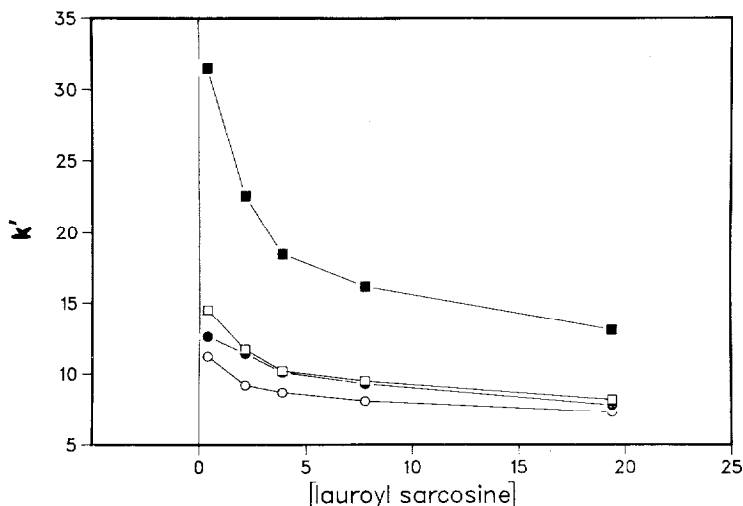
Fig. 4. Effect of increasing surfactant concentration on capacity factors of amines on a lauroyl sarcosine loaded C_{18} column. Solvent composition was 10% methanol, 0.10 M NaH$_2$PO$_4$, 0.02 M Na$_2$HPO$_4$, pH 5.8. Lauroyl sarcosine was initially equilibrated for several hours at 200 mg/l, (0.74 · 10^{-3} M). Concentration units are × 10^{-3} M. Flow-rate, 200 μl/min. For key to symbols see Fig. 2.

One observes a decrease in k' with increasing reagent concentration between 0.2 and 20 mM. The column was first equilibrated at 600 mg/l lauroyl sarcosine as in Fig. 1. Solutions of varying reagent concentration were then substituted as mobile phase and allowed to equilibrate. Several injections of standards were made over a 1–2 h period to establish that equilibrium had been obtained. As a result, the concentrations of lauroyl sarcosine shown in Fig. 4 represent the solution concentration. Increasing the concentration of organic modifier in the mobile phase, in this case methanol, has little effect on k' until greater than 10% (Fig. 5).

All these characteristics, Figs. 1–5, suggest that the loaded column performs much more like a weak cation-exchange column than ion-pair reversed-phase. It was also observed that after loading the column, using a solvent containing no lauroyl sarcosine (0.12 M phosphate buffer, pH 5.8) for several hours had no effect on k'. This shows quite clearly that under these conditions the lauroyl sarcosine is irreversibly partitioned onto/into the C_{18} material. Washing the column with methanol–water (50:50) for several hours, however, effectively removed the lauroyl sarcosine and reversed-phase characteristics were regained. The chromatographic separation achieved is shown in Fig. 6.

DISCUSSION

A variety of sulfate and sulfonate ion-pairing agents are available for use in separation of amines by reversed-phase ion-pair chromatography[7]. Weak cation-exchange properties are obtained by decreases in chain length or concentration of ion-pair reagent in the mobile phase. pH adjustments have little effect on k' as sulfonic acids are anionic across the entire useful pH range of silica based reversed-phase
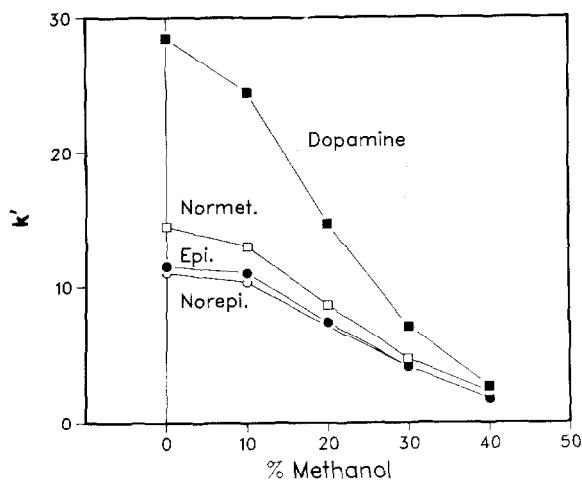
Fig. 5. Effect of altering mobile phase methanol content on capacity factors of amines separated on a lauroyl sarcosine loaded reversed-phase column. Solvent composition was maintained at 600 mg/l lauroyl sarcosine and 0.12 M phosphate buffer, pH 5.8 while methanol content was varied. Column was 10 cm × 1.1 mm I.D. packed with 3-μm Shandon Hypersphere ODS.
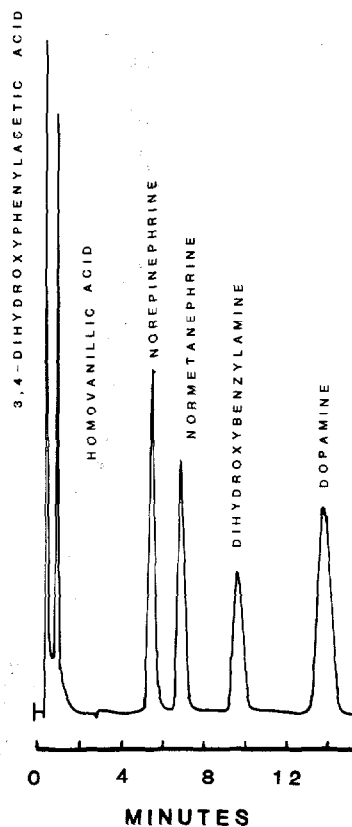
Fig. 6. Chromatographic resolution of biogenic amines by reversed-phase ion-pair–ion-exchange chromatography using lauroyl sarcosine as the ion-exchange reagent. Column was 10 cm × 1.1 mm I.D. packed with 3-μm Shandon Hypersphere ODS. Solvent: 0.12 M phosphate buffer, pH 5.8, 600 mg/l lauroyl sarcosine, 10% methanol.

materials. The amines used in the present work are protonated across this pH range. Long chain carboxylic acids are generally too insoluble in aqueous solution to make them viable choices for ion-pair reversed-phase separations. Lauroyl sarcosine is one of the few water soluble surfactants with a carboxylic acid as the polar functional group. Several interesting properties may be exploited when this compound is used as an ion-pair reagent for separation of amines. When lauroyl sarcosine is included in the mobile phase, a reversed-phase material will become saturated with the reagent such that the absolute concentration of lauroyl sarcosine included in a solution of otherwise fixed composition makes little difference. Once the column is fully loaded, addition of more lauroyl sarcosine to the mobile phase will decrease k', presumably through increased counter ion (amine) concentration as the reagent is an amino acid

and would be protonated at pH 4–7 or possibly through the formation of micelles. Establishment of variable on-column equilibrium concentrations can be accomplished by varying the organic content of the "loading" mobile phase. Once equilibrium has been established, a mobile phase containing no organic modifier may be used. Apparently the partition coefficient for lauroyl sarcosine is so large that negligible leeching occurs and retention times are stable. Modification of k' can then be accomplished using pH or counter ion manipulations. Lauroyl sarcosine is not irreversibly bound to the C_{18} material. Washing the column with methanol–distilled water (50:50) for several hours will effectively remove the surfactant from the stationary phase. However, as noted above, a strictly aqueous eluent will not cause a gradual leeching of the surfactant. It is when this aqueous solvent is used that this reagent clearly acts by a weak cation-exchange mechanism. Both pH and ionic strength manipulations are effective approaches to altering capacity factors of amines. Decreasing pH decreases k' by protonating the carboxylic acid of the lauroyl sarcosine. In fact, below pH 3, this reagent is nearly insoluble in aqueous media. When using ion-pair reversed-phase chromatography to separate amines, ion supression is frequently used to obtain simultaneous separation of carboxylic acid[10]. When lauroyl sarcosine is loaded onto the reversed-phase material, these carboxylic acids are unretained (see Fig. 6) indicating that most reversed-phase sites are inaccessible or absent and the separation quite resembles cation exchange.

Perhaps more utility can be found for this reagent and similar carboxylic acid derived surfactants for selective extraction of amines from physiological samples. pH can quite easily be used to exclude simultaneous extraction of endogenous carboxylic acids while back stripping into low pH, high ionic strength should be quite efficient. Studies of this type are presently under way. This and similar surfactants can be used to extend the versatility of reversed-phase HPLC in separation of polar amines. *In situ* conversion of reversed-phase materials to cation exchange offers the possibility of obtaining much higher efficiency for cation-exchange separations than that available using conventional cation-exchange stationary phases.

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