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Aliphatic carboxylic acids and alcohols as efficiency and elution strength enhancers in micellar liquid chromatography

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ABSTRACT

Micellar liquid chromatography (MLC) uses surfactant solutions as mobile phases with added organic additives to enhance both the elution strength and the chromatographic efficiency. Two aliphatic carboxylic acids (1-butanoic and 1-pentanoic) were used as MLC additives and compared with the two corresponding alcohols (1-butanol, 1-pentanol) in terms of elution strength, efficiency and selectivity. A set of 11 phenol derivatives was used as probe compounds. All micellar mobile phases were prepared with sodium dodecylsulfate (SDS) with concentration ranging from 0.05 to 0.15 M and the modifier content within 1.0 and 5.0% (v/v). The elution strength of different mobile phases containing a constant amount of SDS and different amounts of modifiers; and mobile phases containing a constant amount of modifier and different SDS concentration were determined and discussed. The effect of the acid modifiers on efficiency was studied constructing van Deemter plots that showed no minimum within the 0.01–0.7 mL/min flow rate range studied. Temperature effects were also studied constructing the classical van't Hoff plots. The slight curvature of the plots in the 25–70 °C range may indicate some modification of the surfactant-bonded moiety layer on the stationary phase surface. Since no definitive advantage of the use of aliphatic acids were established compared to their alcohol counterpart, their terrible smell will probably preclude their use as MLC organic modifiers.

1. Introduction

Micellar liquid chromatography (MLC) uses the standard HPLC equipment. Simply, the mobile phases are aqueous solutions of surfactants, modified by small amounts of organic solvents. MLC was developed over the last three decades as a possible "green" and inexpensive alternative to classical RP-HPLC [1–3]. During last years, considerable progress was achieved in the understanding of MLC separations. It is now possible to control separations using different surfactants, organic modifiers, pHs, temperatures and column types. Robust, fast and validated MLC methods were developed for pharmaceutical, clinic, environmental analysis. Now, MLC is a powerful complementary tool in current analytical chemistry. Compared to classical HPLC, MLC has several advantages such as separation of complex mixtures of compounds with different hydrophobicity in isocratic mode and direct injection and simpler sample preparation in the analysis of biological fluids.

Since the beginning of the MLC development it was realized that MLC will never supplant classical HPLC. MLC is a valuable option especially when biological sample are treated. In 1983, Dorsey et al. explained that the low efficiency for MLC was due to a poor mass transfer on bonded stationary phase [4]. They showed that the addition of 3% (v/v) 1-propanol to sodium dodecyl sulfate (SDS) micellar mobile phases associated to a 40 °C column temperature improved the MLC efficiency significantly. As a result, most of MLC separations are conducted today using aqueous micellar solutions modified by small amounts of organic solvents [3]. The factors affecting the efficiency of MLC separations were thoroughly investigated [5–10]. Recently, the systematic investigation of a series of macroporous and specially bonded stationary phases in MLC mode has been completed [11–13]. It was found that acceptable efficiencies could be reached in MLC using stationary phases bonded with short chain alkyl chain or a fluorooctyl chain [13]. The first conclusions formulated by Dorsey et al. [4] were fully confirmed recently performing MLC with 12 different stationary phases [14].

The added organic modifiers improve efficiency and affect elution strength and selectivity [15–21]. Several empirical and mechanical retention models have been proposed to take into account the presence of modifiers in micellar mobile phases [22–25]. The models allowed to develop optimization procedures which can be used to find quickly the best surfactant concentration and modifier proportion in a micellar mobile phase

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that will give the optimal resolution in a particular situation [26–30].

A comprehensive study of 21 additives including short chain alcohols, alkane diols, dipolar aprotic solvents, and alkanes in SDS micellar mobile phases has been done, and recommendations for modifier selection were formulated [10]. However, many candidate modifiers for micellar mobile phases have not been fully evaluated yet. Hence, aliphatic carboxylic acids must also be tested as potential modifiers of SDS micellar eluents [31].

The aim of this work is to study the effect of small amounts of short chain organic acids on the chromatographic performance of micellar mobile phases. The effect of different amounts of aliphatic carboxylic acids added to SDS mobile phases is studied considering the mobile phase elution strength, selectivity of separation and efficiency of chromatographic peaks for several phenols. Similar amounts of the corresponding aliphatic alcohols were added to SDS mobile phases.

2. Experimental

2.1. Chemicals

Mobile phases were prepared with sodium dodecylsulfate (SDS) from Fluka (≥97% Buchs, Switzerland) or from Sigma–Aldrich (>98.5%, L'Isle d'Abeau Chesnes, France), 1-butanol (BuOH) (>99%) (SDS, Carlo Erba Reagents, Peypin, France), 1-butanoic acid (BA) (>99%, for synthesis) (Merck, Darmstadt, Germany), 1-pentanol (PtOH) (Aldrich), 1-pentanoic acid (PA) (99%) (Janssen Chimica, Geel, Belgium). The standard buffer solution was prepared from NaH_2PO_4 (Prolabo, Paris, France) and H_3PO_4 (Fluka, Buchs, Switzerland). The stock solutions of phenol (Ph) from Prolabo (Paris, France) and 2,5-dichlorophenol (25DCP) (98%), 4-chlorophenol (4CP) (99%), 2,3-dichlorophenol (23DCP) (98%), 3,4-dichlorophenol (34DCP) (99%), 3,5-dichlorophenol (35DCP) (97%), 3-nitrophenol (3NP) (99%), 2-nitrophenol (2NP) (98%), 4-nitrophenol (4NP) (99%) from Aldrich, anisole (An) (99%) from Janssen Chimica, benzene (99.5%) were prepared in methanol. The working solutions of test compounds were prepared by dilution of standard solutions with micellar mobile phase.

Although it is not really a safety concern, the extremely unpleasant and pungent odor of BA and PA must be mentioned. Working under a fume hood is required. The hot dirty foot odors of the modified micellar phases will always escape the fume hood and attract coworkers with unfriendly comments.

Distilled water was used throughout. Working solutions and mobile phases were filtered through Millipore 0.45 μm Nylon membranes (Magna, Osmonics Inc.).

2.2. Apparatus and protocols

The HPLC system was composed of a Shimadzu pump (model LC-10AS, Kyoto, Japan), a UV detector (model SPD-6A) and column oven (model CTO-6A), and in-line Rheodyne 7010 valve with a 20 μL sample loop. The retention data were obtained using isocratic conditions with the flow rate 0.5 mL/min for the Zorbax Extend-C18 (150 mm × 3.0 mm i.d., 5 μm particle size diameter) (Agilent, USA). The flow rates were in the range from 0.01 to 0.70 mL/min for van Deemter plots construction. After working with micellar mobile phase the columns were rinsed by water, water–acetonitrile (Prolabo, Paris, France) and water–methanol solutions, and with pure methanol before storage. Two identical columns from the same batch, which provide identical retention factors of test compounds, selectivity of separation and efficiencies, were used for collection of chromatographic data. The pH meter was a Mettler Toledo MP220 (Mettler, Virofly, France) equipped with a combined pH electrode, which was calibrated with pH 4.0 and pH 7.0 standard buffer solutions. An Elmasonic ultrasound bath (Elma Hans Schmidbauer GmbH & Co. KG, Stuttgart, Germany) was used for dissolution of samples.

2.3. Data processing and software

The UV detector signal was monitored at 275 nm and recorded through an A/D converter by a personal computer running the Azur 4.6 Data Acquisition Software (Datalys, Grenoble, France). The carboxylic acids have higher cut-off limit in comparison with corresponding alcohols: the rapid increasing of acids adsorption corresponds to 260 nm that can lead to problems with detection at 254 nm. The obtained experimental results were treated with Microsoft Excel (2002, Microsoft Corporation, http://office.microsoft.com/). The Statistica 6.0, data analysis software system (2004, http://www.statsoft.com) was used for retention modeling and contour maps constructing. The selectivity study was done using a home-made program for interpretive optimization in MLC, written in Matlab 7.0 (2004, The Mathworks, http://mathworks.com). The ACD/Log P 4.03 program (Advanced Chemistry Development, http://www.acdlabs.com/) was used for calculation of compound $\log K_{ow}$ (octanol/water distribution ratio) and the ACD/Log P 4.0 database was searched for experimental values of compound $\log K_{ow}$.

3. Results and discussion

3.1. Experimental design and selection of experimental conditions

SDS is the most commonly used surfactant in MLC when anionic micellar mobile phases are desired [2,3]. Its cost is low, it is available in very high purity and it has a low critical micelle concentration (CMC = 8×10^{-3} M) [2,32] and low Kraft point of 12 °C [33,34] associated to a high solubility in water. Moreover, the viscosities of aqueous SDS solutions with concentrations up to 0.3–0.4 M are appropriate for common HPLC pumps [5]. Also, SDS micellar solutions were extensively studied in colloid chemistry, and their physico-chemical properties are well documented [2,35,36]. The C18 bonded stationary phase was selected in this work because it is the most commonly used stationary phase in RPLC. Among the available short and middle chain aliphatic alcohols and carboxylic acids the 1-butanol, 1-butyric acid, 1-pentanol and 1-pentanoic acid were chosen as modifiers of micellar eluents. 1-butanol and 1-pentanol are often used as additives to micellar eluents for the separation of solutes with moderate ($1 < \log K_{ow} < 3$) and high ($\log K_{ow} > 3$) hydrophobicity [3]. The content of additives in all micellar eluents was within the range from 1.0% (v/v) to 5% (v/v) [Note: the content of modifier in micellar eluent is expressed as volume fraction percent in manuscript]. The SDS concentration was in the 0.05–0.15 M range (14–43 g/L). The most viscous mobile phase was the 0.15 M SDS, 5.0% 1-pentanol with a 20 °C viscosity close to three centipoises; it led to a backpressure at 0.5 mL/min for the Zorbax column (150 mm × 3 mm i.d.) of about 180 kg/cm^2.

The five point square experimental design has been used for investigation of 1-butanol and 1-butanoic acid additive effects on elution strength and selectivity. A similar nine point experimental design was set for 1-pentanol and 1-pentanoic acid. The mobile phase compositions are listed in Table 1. The pH of the alcohol containing micellar mobile phases was adjusted so that it became close to the pH of the corresponding carboxylic acid containing micellar mobile phases with the same volume fraction and SDS concentration. Phosphoric acid was used for pH adjustments. It is interesting to note that the two acids and the two corresponding alcohols have the same molar volumes (Table 2) so mobile phases with equal modifier volume fractions have also equal molar concentrations [31].