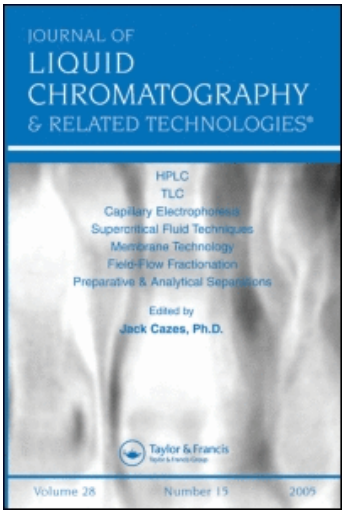

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Influence of Microemulsion Conditions on the Thin Layer Chromatographic Behavior of Amino Acids

Dating Tian[a]; Hong-Quan Xie[b]
[a] School of Chemical and Environmental Engineering, Hubei Institute for Nationalities, Enshi, P. R. China [b] Department of Chemistry, Huazhong University of Science and Technology, Wuhan, P. R. China

To cite this Article Tian, Dating and Xie, Hong-Quan(2008) 'Influence of Microemulsion Conditions on the Thin Layer Chromatographic Behavior of Amino Acids', Journal of Liquid Chromatography & Related Technologies, 31: 5, 763 — 771

To link to this Article: DOI: 10.1080/10826070701855961

URL: http://dx.doi.org/10.1080/10826070701855961

Journal of Liquid Chromatography & Related Technologies®, 31: 763–771, 2008

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070701855961

Influence of Microemulsion Conditions on the Thin Layer Chromatographic Behavior of Amino Acids

Dating Tian[1] and Hong-Quan Xie[2]

[1]School of Chemical and Environmental Engineering, Hubei Institute for Nationalities, Enshi, P. R. China
[2]Department of Chemistry, Huazhong University of Science and Technology, Wuhan, P. R. China

Abstract: The chromatographic behavior of 23 amino acids on the silica gel thin layers using cetyltrimethylammonium bromide(CTAB)/n-butyl alcohol/n-octane/water microemulsion as a new developer has been studied. The effects of the hydrous content of microemulsion and structures of amino acids on the flow rate (R_f) values were investigated. Several amino acid mixtures were separated and determined using a microemulsion with 40% hydrous content, which was compared with the traditional developer of ethanol/water/acetic acid mixture. The chromatographic mechanism of microemulsion was also discussed. The results showed that the water in oil (W/O) microemulsion and the bicontinuous (BC) microemulsion, composed of the mentioned components, appeared to be a suitable developer for thin layer chromatographic analysis for the amino acids.

Keywords: Thin layer chromatography, Amino acid, Developer, Microemulsion, Cetyltrimethylammonium bromide

INTRODUCTION

Microemulsion, composed of a certain amount of water, oil, surfactant, and cosurfactant, is a type of transparent stable system in thermodynamics with low viscosity. It has received considerable attention from a number of

Correspondence: Dating Tian, School of Chemical and Environmental Engineering, Hubei Institute for Nationalities, Enshi 44500, P. R. China. E-mail: tiandating@163.com

investigators due to its unusual structures and properties, and it has been extensively applied in areas of tertiary oil recovery, cosmetics, pharmacy, lubrication, enzyme catalysis, chemical synthesis, etc.[1–5] However, there are rare reports on the application of microemulsion in thin layer chromatographic analysis of organic compounds. As is well known, amino acids are important amphiphilic substances in biochemistry, pharmacology, and chemistry.

The most effective method of separation and identification of amino acids is chromatography. Although there are commercial automatic analyzers for amino acids, thin layer chromatography, which is simple and inexpensive, has still been extensively applied in the ordinary labs and/or factories. However, the developers used in traditional thin layer chromatography for amino acids have some defects, such as bad development, strong tailing, and bad reproducibility. Considering its unique solubilization and depression of surface tension, microemulsion may be used as a type of a good developer. In order to develop the application area of microemulsion, this article deals with the thin layer chromatographic behavior of 23 amino acids using cetyltrimethylammonium bromide(CTAB)/n-butyl alcohol/n-octane/water microemulsion as a new developer.

EXPERIMENTAL

Materials

Cetyltrimethylammonium bromide (CTAB), n-butyl alcohol, n-octane, and ninhydrin were analytical grade. Twenty-three amino acids, all chromatographic grade and all L-series except DL-β-phenylalanine were formulated into a aqueous concentration of 1 $g \cdot L^{-1}$ using deionized water, respectively. A series of microemulsions were obtained, using CTAB, n-butyl alcohol, n-octane, and deionized water as surfactant, cosurfactant, oil, and water, respectively, by changing the content of water in the case of the former 3 components being fixed.

Thin Layer Chromatographic Analysis of Amino Acids

A small amount of amino acid solution absorbed by a capillary tube was spotted on a silica gel G plate. Using microemulsion as developer, the sample was developed by the ascending method. After the sample was developed to a suitable distance, the plate was taken out and dried in air. Then the flow rate (R_f) value was calculated after the plate having been colored by ninhydrin.

RESULTS AND DISCUSSION

Results of Experiments

Table 1 shows the compositions and structures of microemulisons. We have known that a series of microemulisons can be prepared by changing the amount of water in the system when suitable amounts of CTAB, n-butyl alcohol, and n-octane were fixed, as shown in the pseudo-ternary phase diagrams from the literature.[6,7] Furthermore, the structures of series of microemulsions were monophasic regions from the water-in-oil (W/O) type, to the bicontinuous (BC) type, and then to the oil-in-water (O/W) type. The thin layer chromatographic analysis of 23 amino acids using these series of microemulsions as developers is shown in Table 2.

The Chromatographic Mechanism of Microemulsion

It is well known that the solute in the microemulsion chromatography is distributed between the immobile phase, continuous phase of oil or water, interior phase, and interphone, etc. During the process of chromatography, amino acids were distributed in the interior phase or continuous phase of microemulsion, and also could be interposed between barrier layers made of CTAB and n-butyl alcohol. On the other hand, amino acids could be continuously released from the enrichment phase due to the adsorption of silica gel G adsorbent. Influenced by the effects of adsorption, distribution, static electricity, hydrophobic force, steric barrier, extraction and back-extraction, the migration rate of each amino acid was different and the R_f values were also different.

Table 1. The compositions and structures of microemulsions

ω (Water)/%	m (Water)/g	Structure[7]
10	10.0	Water-in-oil
20	22.5	Water-in-oil
30	38.6	Water-in-oil
40	60.0	Bicontinuous
50	90.0	Bicontinuous
60	135.0	Bicontinuous
70	210.0	Oil-in-water
80	360.0	Oil-in-water
90	810.0	Oil-in-water

m(CTAB) = 36.9 g; m(n-butyl alcohol) = 44.0 g; m(n-octane) = 9.1 g.

Table 2. The R_f values of thin layer chromatographic behavior of 23 amino acids using miceoemulisons as developers with different hydrous content (n = 3)

		ω(Water)/%								
No.	Amino acid	10	20	30	40	50	60	70	80	90
1#	Glycine	0.10 ± 0.022	0.23 ± 0.020	0.28 ± 0.020	0.30 ± 0.010	0.32 ± 0.010	0.40 ± 0.022	0.54 ± 0.024	0.70 ± 0.045	0.85 ± 0.057 (ST)
2#	Alanine	0.15 ± 0.025	0.25 ± 0.025	0.30 ± 0.020	0.32 ± 0.012	0.36 ± 0.012	0.42 ± 0.020	0.58 ± 0.020	0.62 ± 0.050	0.88 ± 0.058 (Δ)
3#	Valine	0.18 ± 0.025	0.32 ± 0.024	0.35 ± 0.024	0.36 ± 0.020	0.40 ± 0.020	0.45 ± 0.011	0.60 ± 0.045 (T)	0.65 ± 0.057 (T)	0.90 ± 0.058 (ST)
4#	Leucine	0.20 ± 0.030	0.35 ± 0.030	0.38 ± 0.022	0.40 ± 0.013	0.42 ± 0.010	0.47 ± 0.020	0.61 ± 0.045	0.66 ± 0.045 (T)	0.92 ± 0.070 (Δ, T)
5#	Isoleucine	0.22 ± 0.030	0.36 ± 0.034	0.40 ± 0.030	0.44 ± 0.030	0.56 ± 0.012	0.50 ± 0.012	0.62 ± 0.045 (T)	0.65 ± 0.045 (T)	0.92 ± 0.072 (Δ, ST)
6#	Serine	0.08 ± 0.038	0.12 ± 0.034	0.24 ± 0.034	0.28 ± 0.032	0.35 ± 0.030	0.40 ± 0.030	0.50 ± 0.020	0.60 ± 0.034	0.78 ± 0.068 (Δ, T)
7#	Threonine	0.10 ± 0.037	0.15 ± 0.046	0.25 ± 0.046	0.30 ± 0.030	0.32 ± 0.030	0.38 ± 0.030	0.46 ± 0.032	0.50 ± 0.033	0.61 ± 0.073 (Δ, T)
8#	Lysine	0.10 ± 0.034	0.23 ± 0.034	0.28 ± 0.034	0.34 ± 0.031	0.38 ± 0.030	0.40 ± 0.030	0.47 ± 0.030	0.52 ± 0.047	0.71 ± 0.072 (Δ, ST)
9#	Arginine	0.20 ± 0.034	0.30 ± 0.034	0.34 ± 0.030	0.38 ± 0.030	0.45 ± 0.020	0.55 ± 0.020	0.65 ± 0.020	0.75 ± 0.055 (Δ, T)	0.85 ± 0.075 (Δ, ST)
10#	Histidine	0.01 ± 0.005	0.11 ± 0.035	0.20 ± 0.033	0.28 ± 0.030	0.35 ± 0.020	0.45 ± 0.011	0.60 ± 0.023	0.75 ± 0.047 (Δ, T)	0.83 ± 0.071 (Δ, ST)

11#	Aspartic acid	0.02 ± 0.008	0.05 ± 0.016	0.10 ± 0.025	0.18 ± 0.030	0.28 ± 0.030	0.35 ± 0.011	0.40 ± 0.025	0.50 ± 0.036	0.60 ± 0.047 (Δ, T)
12#	Glutamic acid	0.04 ± 0.008	0.10 ± 0.015	0.15 ± 0.015	0.25 ± 0.023	0.35 ± 0.021	0.38 ± 0.011	0.45 ± 0.013	0.58 ± 0.035 (T)	0.70 ± 0.059 (Δ, T)
13#	Asparagine	0.08 ± 0.012	0.16 ± 0.025	0.20 ± 0.024	0.25 ± 0.014	0.30 ± 0.022	0.45 ± 0.012	0.50 ± 0.012	0.55 ± 0.034 (T)	0.65 ± 0.057 (Δ, T)
14#	Glutamine	0.10 ± 0.029	0.21 ± 0.027	0.25 ± 0.023	0.30 ± 0.022	0.35 ± 0.022	0.50 ± 0.011	0.52 ± 0.023	0.60 ± 0.035 (Δ, T)	0.70 ± 0.051 (Δ, T)
15#	Cysteine	0.12 ± 0.0025	0.16 ± 0.024	0.22 ± 0.022	0.25 ± 0.022	0.32 ± 0.011	0.36 ± 0.010	0.45 ± 0.023	0.55 ± 0.023	0.65 ± 0.045 (T)
16#	Methionine	0.18 ± 0.024	0.34 ± 0.022	0.36 ± 0.011	0.40 ± 0.010	0.45 ± 0.010	0.55 ± 0.020	0.60 ± 0.035 (T)	0.70 ± 0.037 (T)	0.85 ± 0.069 (Δ, T)
17#	Phenylalanine	0.38 ± 0.024	0.44 ± 0.023	0.50 ± 0.023	0.55 ± 0.020	0.60 ± 0.020	0.68 ± 0.020	0.70 ± 0.044 (T)	0.75 ± 0.047 (T)	0.85 ± 0.059 (Δ, T)
18#	Tyrosine	0.26 ± 0.025	0.35 ± 0.024	0.55 ± 0.020	0.65 ± 0.018	0.75 ± 0.020	0.80 ± 0.020	0.82 ± 0.045 (T)	0.85 ± 0.049 (T)	0.90 ± 0.059 (Δ, T)
19#	Tryptophane	0.40 ± 0.024	0.55 ± 0.022	0.66 ± 0.012	0.72 ± 0.018	0.75 ± 0.019	0.83 ± 0.023	0.86 ± 0.045 (T)	0.90 ± 0.059 (Δ, T)	0.92 ± 0.059 (Δ, T)
20#	Proline	0.12 ± 0.025	0.18 ± 0.023	0.24 ± 0.022	0.27 ± 0.022	0.32 ± 0.020	0.38 ± 0.020	0.55 ± 0.020	0.78 ± 0.048 (Δ, T)	0.82 ± 0.050 (Δ, ST)
21#	Hydroxyproline	0.10 ± 0.025	0.15 ± 0.020	0.25 ± 0.020	0.30 ± 0.020	0.36 ± 0.020	0.40 ± 0.020	0.57 ± 0.035	0.86 ± 0.047 (T)	0.90 ± 0.059 (Δ, ST)
22#	Ornithine hydrochloride	0.12 ± 0.037	0.13 ± 0.035	0.18 ± 0.034	0.25 ± 0.020	0.32 ± 0.020	0.34 ± 0.023	0.42 ± 0.023	0.46 ± 0.045 (T)	0.60 ± 0.049 (ST)
23#	Cysteine hydrochloride	0.10 ± 0.035	0.12 ± 0.033	0.25 ± 0.028	0.28 ± 0.020	0.34 ± 0.020	0.38 ± 0.020	0.48 ± 0.022	0.59 ± 0.037 (T)	0.70 ± 0.063 (Δ, ST)

T: Tailing; ST: Serious tailing; Δ: Irregular spot.

Effect of Hydrous Content of Microemulsions on the R_f Values

Table 2 indicated that the R_f values of samples increased with the increase of the hydrous content of microemulsions. It is well known that R_f values are influenced by the polarity of sample, activity of adsorbent, and polarity of developer. When the former two factors are fixed, the R_f values are controlled by the polarity of developer. In general, the higher the polarity of developer, the higher the R_f value of sample. Therefore, an appropriate development system is obtained by adjusting the hydrous content of microemulsions. However, due to an excess amount of hydrous content (e.g., above 60%), the polarity of the system was so high that many spots, with serious tailing and irregular shapes, migrated close to the front edge (wet line). So, an appropriate hydrous content of microemulsions was from 10 to 60%. In the W/O type microemulsions, free amino acids were enriched by entering the water phase (interior phase). But, in the BC type microemulsions, free amino acids were enriched suitably between two phases because water was both the solubilization phase and continuous phase. In this case, because the special stabilization of microemulsions, amino acids with specific structures, influenced by many effects mentioned above, were released at a certain rate and obtained good chromatographic behavior. Of course, when the hydrous content of microemulsions was high enough, i.e., in the O/W type microemulsions, amino acids mainly enter the continuous phase, in which the characteristics of microemulsions would be unable to be represented; then the developers were not different from the common polar developers.

Effects of Molecular Structures of Amino Acids on the R_f Values

The higher the polarity of the sample is, the stronger it is adsorbed by the adsorbent, and the lower the migration rate is under the same condition. In the case of the conditions of the main molecular structures of samples are the same (e.g., $6^{\#}$ and $2^{\#}$, $18^{\#}$ and $17^{\#}$, etc., as shown in Table 2), the R_f values of amino acids which contain -OH were lower than those which do not contain -OH.

Another phenomenon which should be paid attention to is that the R_f values of amino acids such as $15^{\#}$ and $6^{\#}$, $17^{\#}$ and $18^{\#}$, $20^{\#}$ and $21^{\#}$, $15^{\#}$ and $23^{\#}$, had something to do with not only their molecular structures but also the hydrous content of microemulsions. When the hydrous content was less than 30%, R_f $(15^{\#}) > R_f(6^{\#})$, R_f $(17^{\#}) > R_f$ $(18^{\#})$, R_f $(20^{\#}) > R_f$ $(21^{\#})$, R_f $(15^{\#}) > R_f(23^{\#})$. When the hydrous content was higher than 30%, the sequence of R_f is the opposite. In the comparison of $15^{\#}$ with $6^{\#}$, the former contains -SH and the latter contains -OH; in the comparison of $17^{\#}$ with $18^{\#}$, $20^{\#}$, with $21^{\#}$, the latter amino acid has one more -OH than the former, respectively. Moreover, in $15^{\#}$ with $23^{\#}$, the latter is the salt of hydrochloric acid for the former. In all, the polarity of the latter amino acids is stronger

than that of the former. This fact may be attributed to the following: On the one hand, in the case of the hydrous content, less than 30%, of the R_f values were controlled by the polarity of samples when the polarity of developers was low. On the other hand, in the case of the hydrous content higher than 30%, the R_f values not only were controlled by the polarity of samples and developers, but also influenced by effects such as static electricity, hydrophobic force, steric barrier, extraction, back-extraction, etc. As mentioned above, as the types of microemulsions began to convert from the W/O type to the BC type and to the O/W type under this condition, some of the R_f values of amino acids apparently changed, but some were only mild changes.

Separation of Amino Acid Mixtures and Comparison with Common Developers

Figure 1 showed a mixture containing several amino acids developed by a microemulsion with 40% hydrous content, compared with a single amino

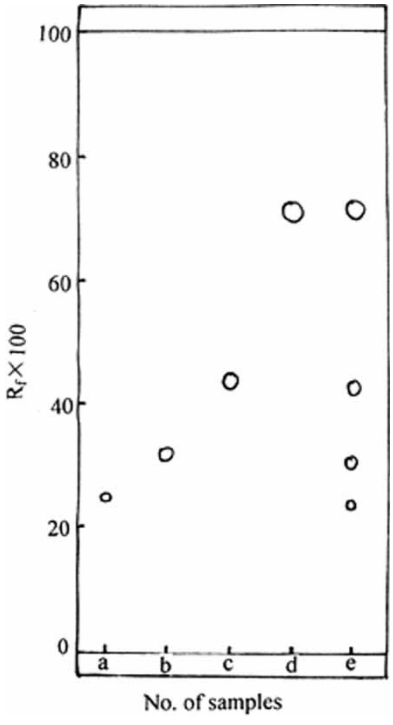

Figure 1. The thin chromatographic spectra of several amino acids developed by a microemulsion with 40% hydrous content (a = $12^{\#}$, b = $2^{\#}$, c = $5^{\#}$, d = $19^{\#}$, e = a mixture of $12^{\#}$, $2^{\#}$, $5^{\#}$, and $19^{\#}$).

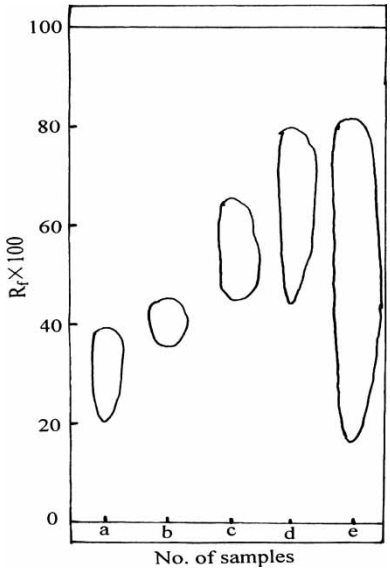

Figure 2. The thin chromatographic spectra of several amino acids developed by a mixed solution of ethyl alcohol, water and acetic acid (a = $12^{\#}$, b = $2^{\#}$, c = $5^{\#}$, d = $19^{\#}$, e = a mixture of $12^{\#}$, $2^{\#}$, $5^{\#}$, and $19^{\#}$).

acid. The results showed that the developer performed well in the separation of the mixture.

Moreover, the common developer, such as a mixed solution of ethyl alcohol, water, and acetic acid (volume ratio is 50:50:1) was compared with this microemulsion developer, as shown in Figure 2. The results indicated that there were many irregular spots with serious tailing in the former case, but few in the latter case. Therefore, a good performance of thin layer chromatographic analysis of amino acid can be obtained by using microemulsions as developers because of their unusual structures and properties.

CONCLUSION

Microemulsions containing cetyltrimethylammonium bromide (CTAB), n-butyl alcohol, n-octane, and water as a new developer were used in the thin layer chromatographic analysis of 23 amino acids. There existed different chromatographic results between our work and the commonly used developer because of the unusual structures and properties of microemulsions. These types of microemulsions exhibited a strong influence on the thin layer chromatographic behavior of amino acids. The W/O and BC types of microemulsions appeared to be suitable developers.

ACKNOWLEDGMENT

This work was supported by the Scientific Research Funds from Education Committee of Hubei Province, People's Republic of China (Grant No. D200629003)

REFERENCES

1. Beltrame, L.; Dantas, T.; Neto, A.; Neto, E.; Lima, F. Influence of cosurfactant in microemulsion systems for color removal from textile wastewater. J. Chem. Technol. Biotechnol. **2005**, *80* (1), 92–98.
2. Bernd, T. Polymerisation of styrene in microemulsion with catanionic surfactant-mixtures. Coll. Polym. Sci. **2005**, *283* (4), 421–430.
3. Mitra, R.K.; Paul, B.K. Physicochemical investigations of microemulsification of eucalyptus oil and water using mixed surfactants (AOT plus Brij-35) and butanol. J. Coll. Interf. Sci. **2005**, *283* (2), 565–577.
4. Ohtani, N.; Ohta, T.; Hosoda, Y.; Yamashita, T. Phase behavior and phase-transfer catalysis of tetrabutylammonium salts interface-mediated catalysis. Langmuir **2004**, *20* (2), 409–415.
5. Yaghmur, A.; Campo, L.; Sagalowicz, L.; Leser, M.E.; Glatteret, O. Emulsified microemulsions and oil-containing liquid crystalline phases. Langmuir **2005**, *21* (2), 569–577.
6. Kumar, S.; Din, K.U. Water-in-oil microemulsion: influence of co-surfactant chain-length and nature of emulsifier. J. Indian Chem. Soc. **2003**, *80* (4), 305–310.
7. Xu, J.; Li, G.Z.; Zhou, G.W.; Chen, W.J.; Yao, F.Y. Self-diffusion study of solvent molecules in CTAB inverse microemulsion. Acta Chim. Sinica **2001**, *59* (5), 707–712.

Received April 18, 2007
Accepted September 12, 2007
Manuscript 6108