Paper Chromatographic Separation of Racemic Diphenylmethyl Alcohols Using Only Pure Water Surfactant Micellar Mobile Phase and Host–Guest Chromatography

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Abstract

This paper reports the separation of nine pairs of racemic diphenylmethyl alcohols with paper chromatography using hexadecyltrimethyl ammonium bromide pure water micellar solution as the mobile phase. The resulting resolution is excellent. The authors use a novel theory, host–guest chromatography, to describe the main driving force of chromatography on micellar paper and to discuss the retention behavior. The mechanism of micellar paper chromatography conforms to the Armstrong equation.

Introduction

Reports on surfactant micellar chromatography have increased substantially since Armstrong founded micellar chromatography in 1979 (1). Several papers have described the practical and theoretical aspects of micelles in separations (2–4). Unfortunately, micellar paper chromatographic (MPC) methods have lagged far behind others. Rawat and Singh first reported the separation of 28 phenol compounds on paper (5), but in their measuring system, the highest concentration of sodium dodecylsulfate (SDS) used was 0.008 mol/L. Because the critical micelle concentration of SDS is 0.0081 mol/L (25°C) (2), it could not have formed micelles, so the chromatography in these cases was not micellar. The separations of phenols, amino acids, and structural isomers of diphenylmethyl alcohol (DPMA) with MPC have been accomplished in this laboratory (6–8, Z.S. Fu and X.F. Wang. Micellar paper chromatographic separation of diphenylmethyl alcohols' structural isomers and their chromatographic behaviour. *Chinese J. Chromatogr.*, in press.), and their mechanism conformed to the Armstrong equation. The deviation from ideal retention behavior is due to the relationship between the molecular structures of solutes (guest) and the micellar shapes and sizes (host) (Z.S. Fu and X.F. Wang. Micellar paper chromatographic separation of diphenylmethyl alcohols' structural isomers and their chromatographic behaviour. *Chinese J. Chromatogr.*, in press.).

This paper reports using MPC to separate the racemic DPMAs. There are two general approaches for the direct liquid chromatographic (LC) separation of enantiomers. The first involves the use of chiral stationary phases, and the second involves the use of chiral mobile phase additives in conjunction with achiral stationary phases (9). Many articles using the two methods have been published (10–21). Hinze et al. reported the separations of optical isomers with chiral surfactant micellar mobile phases (22,23). However, to the authors' knowledge, no racemate has been resolved by paper chromatography using only an achiral surfactant micellar mobile phase with no other chiral additives.

In this work, the authors report the separation of nine pairs of DPMA racemic compounds by aqueous hexadecyltrimethyl ammonium bromide (CTAB) mobile phase; no other additives were used in the mobile phase, and the result was excellent. All nine pairs of racemates were separated. The authors present this work and discuss the retention behavior from a host–guest chromatographic point of view.

Experimental

Materials

Xinhua # 3 chromatographic papers (15 × 60 cm, 0.36-mm thickness) were obtained from Hangzhou Xinhua Filter Paper (Zhejiang, P.R. China). DPMA racemates were obtained from the laboratory of Professor J.T. Wang of the Nankai University Department of Chemistry (Tianjin, P.R. China). CTAB was obtained from Shanghai Chemical Purchasing and Supply Station (Shanghai, P.R. China). β-Cyclodextrin (β-CD) was obtained from Suzhou Gourmet Powder (Jiansu, P.R. China). All chemicals were used as received. Distilled water was used to make the stock surfactant solutions.

Methods

The different concentrations of CTAB in pure water were used as mobile phases and developed at room temperature (23°C) in

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a 20.0 × 10.0 × 20.0-cm glass chamber. The solutes' concentrations were approximately 20 mg/mL (acetone). The spotting was done with a glass capillary. It took approximately 1 h to saturate the glass chamber using CTAB aqueous solution vapor before development. The filter paper was cut into the size of 15 × 18 cm. The development distance was about 15 cm; approximately 0.5–4 h were required for complete development.

Spot visualization was performed with a fixed-wavelength (254 nm) ultraviolet (UV) lamp. Retardation factors (R_f) were calculated. There were four data points for every R_f value in two experimental developments; two pieces of paper were used each time. Final data were obtained from the average of four sets of data. If one of the data points was much different from the others, it was discarded, and the work was repeated until an acceptable value was obtained.

Results and Discussion

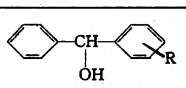
Resolution of racemic DPMAs

Table I shows the R_f data of racemic DPMAs using CTAB as a mobile phase in pure water. Each pair of spots in the chiral separation had the same brilliance and expected sizes (3 × 3 mm or diameters were 3–4 mm) under the UV lamp. Figure 1 shows one piece of paper developed. It illustrates that (±)-DPMAs can be much better separated without any additives (e.g., β-CD). These results were rather surprising. However, this phenomenon is reproducible; the authors present this work, and it is discussed in detail.

There were several reports about the resolution of racemic compounds by paper chromatography in the 1950's. But most of them used chiral stationary phases or chiral mobile phase additives. There were a few papers without the use of chiral stationary phase or mobile phase additives for a few special compounds, due in part to the crystallinity of cellulose (24). However, there was no chiral separation of DPMAs in an aqueous SDS mobile phase (Z.S. Fu and X.F. Wang. Micellar paper chromatographic separation of diphenylmethyl alcohols' structural isomers and their chromatographic behaviour. *Chinese J. Chromatogr.*, in press.); this result showed that the main driving force was the different mobile phase. Both CTAB and SDS are ionic surfactants with charges. Also, the strength of the electrostatic force and the formation of hydrogen bonds are greater in SDS than in CTAB. If those in CTAB were greater, it still would not explain the separation of racemates. The experimental results also showed that the structure of cellulose was not the reason for the chiral separation, or at least that the chiral separation abilities could be neglected in these separations. The key to the chiral separation lay in the

Table I. R_f Data of DPMA's Racemates with MPC Using CTAB Pure Water Solution*

R		CTAB (mol/L)									
		0.01	**0.02**	**0.03**	**0.04**	**0.05**	**0.06**	**0.07**	**0.08**	**0.09**	**0.10**
Cl	o†	0.42	0.67	0.65	0.17	0.35 0.64	0.05	0.47	0.38	0.52	0.63
	m†	0.48	0.42	0.48 0.62	0.33	0.58	0.49	0.22	0.55	0.57	0.64
	p†	0.28 0.48	0.22 0.58	0.47 0.57	0.41	0.70	0.77	0.44	0.64	0.45	0.61
CH_3	o	0.47	0.68	0.51	0.39	0.67 0.73	0.40	0.29	0.64	0.32	0.42
	m	0.33	0.50	0.62 0.82	0.34	0.31 0.48	0.27	0.63	0.57	0.55	0.56
	p	0.42	0.42	0.78	0.65	0.46 0.82	0.44	0.29	0.69	0.34	0.66
OCH_3	o	0.53	0.51	0.63	0.36	0.41 0.62	0.55	0.55	0.45	0.47	0.55
	m	0.51	0.44	0.63	0.42 0.59	0.42	0.31	0.50	0.49	0.32	0.71
	p	0.29 0.60	0.62	0.61	0.59	0.29 0.67	0.49	0.57	0.41	0.45	0.72

* DPMA structure:  R = Cl, CH_3, or OCH_3.

† o, m, and p, signify ortho, meta, and para substitution, respectively, on the benzene ring.

micelles that were formed. In general, the chiral separation resulted in a slight difference between R_{f1} and R_{f2}; the relative retention was approximately 1 (for example, R_{f1} = 0.30, R_{f2} = 0.35, and the relative retention was 1.17) (21). Also, the relative retention in paper chromatography was 1.1 (24). This is because the racemic compounds have the same molecular structures but different optical activities. In our experiment, however, there were great differences between R_{f1} and R_{f2} (see Figure 1 and Table I) and much larger relative retentions (Table II). This meant that a strong force had separated the racemic compounds. However, cellulose does not have this separation ability.

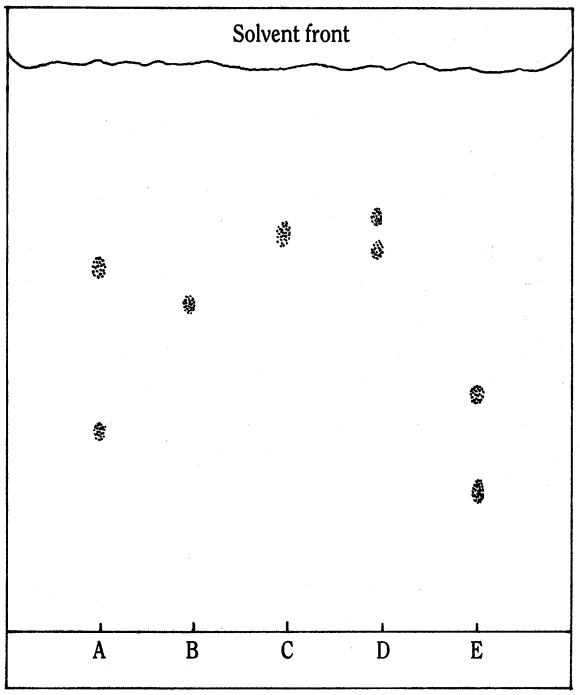


Figure 1. Paper chromatogram showing the resolution of racemates. A, B, and C are *o*-, *m*-, and *p*-chlorphenyl phenylmethyl alcohols, respectively. D and E are *o*- and *m*-methylphenyl phenylmethyl alcohols, respectively. The mobile phase was a 0.05 mol/L CTAB pure water solution.

The experimental results showed that the chiral separation had two characteristics of special selectivity for mobile phase and suddenly changing R_f values. The chiral separation could only be accomplished using certain concentrations (0.01–0.05 mol/L) of pure water–CTAB solutions as mobile phases. Under the other conditions (including in SDS, as previously described), it could not be accomplished at all (see Table III). Changing the pH also illustrated the impossibility of DPMAs' partial dissociation; no duplex spots formed. All of these illustrated that the main driving force in the chiral separation was the micelles whose shapes and sizes change under different conditions. If the reason for the chiral separation had been that cellulose had chiral separation abilities because of a certain amount of crystallinity in its structure, the separation would have had the properties of gradual change; for example, if the concentration of CTAB solution had changed from 0.04 mol/L to 0.06 mol/L, the separation of *o*-chlorphenyl phenylmethyl alcohol would have had a group of R_f values with some relationship. However, there was no evidence of this. The chromatogram maps showed two spots (R_{f1} = 0.35, R_{f2} = 0.64) in 0.05 mol/L solution and only one spot in 0.04 mol/L (R_f = 0.17) or in 0.06 mol/L (R_f = 0.05) solutions (Table I). This phenomenon emerged in all experiments.

β-CD has the ability to recognize chiral compounds. If cellulose also had this ability, the separation contribution of B-CD and cellulose together would have the additional property (each recognizing both *R*- and *S*-configuration) or subtractive property (one recognizing *R*, the other *S*). However, there was no such regularity (see Tables II and III). As previously mentioned, the main driving force of MPC was not the structure of cellulose, and the rest of the force was only due to surfactants and their micelles.

Proteins have a special ability to distinguish chiral compounds. Micelles are likened to proteins because they have cavities (namely cores) (25), although, to the authors' knowledge, there has been no evidence of the chiral separation until now. Micelle cavities have the ability to hold some structurally different molecules in different forms (25,26). Whether the micelles were bound or unbound on the surface of the cellulose is still an outstanding issue to the authors. If they were bound on the surface of cellulose, whose structure was the reason for separation, the force of the chiral separation would have come from the transmitting effect of the binding. This, however, still cannot be used to explain the chiral separation because of the experimental results described above (special selectivity, suddenly changing R_f values, and the effects of additives). However, the micelles bound on the surface of cellulose still had cavities, which would change their shapes and sizes along with the changing conditions. Thus, the chiral separation was only due to the relationship between the solutes (guest) and the cavities (host) of micelles.

Table II. Relative Retention of (±)-DPMAs under Different Conditions

R		CTAB (mol/L) 0.01	0.02	0.03	0.04	0.05	0.05 mol/L CTAB + 0.002 mol/L β-CD	0.05 mol/L CTAB + 0.007 mol/L β-CD
	o					1.83	4.00	2.80
Cl	m			1.29				
	p	1.71	2.64	1.21			1.93	
	o					1.09	1.93	2.15
CH_3	m			1.32		1.55	2.83	1.81
	p					1.78		3.50
	o					1.51	1.65	
OCH_3	m				1.40		2.02	1.55
	p	2.07				2.31	4.11	1.25

Host–guest chromatography and separation of DPMAs

Fu and Wang reported the separation of DPMA's structural isomers using aqueous SDS micellar mobile phase by paper chromatography (Z.S. Fu and X.F. Wang. Micellar paper chromatographic separation of diphenylmethyl alcohols' structural isomers and their chromatographic behaviour. *Chinese J. Chromatogr.*, in press.). Its mechanism conforms to the Armstrong equation. However, the curves of C_m (micellar concentration) versus $R_f/1 - R_f$ deviates considerably from the ideal curve. This curve is generally a straight line or shows little deviation (2,27). In Fu's work, the curves were not only nonlinear, but also indicated antibinding behavior. Fu and Wang explained the phenomenon using the theory that the solutes (guest) are suitable or unsuitable cavities (namely cores) of micelles (host) for each other. In this paper, the authors call this method host–guest chromatography.

CTAB is different from SDS, so the cavities formed are different also. The micelles are likened to proteins because they form cavities (25,26). The micelles of CTAB can distingush diphenylmethyl alcohols, whereas those of SDS cannot.

The shapes and sizes of micelles are in a continuous equilibrium system in an aqueous solution (25,26). In an aqueous CTAB micellar solution, there are several kinds of micelles with different shapes and sizes. These micelles are spherical, rod-shaped, disk-shaped, etc.; the cores (cavities) change with these shapes. The shapes and cavities of micelles are in dynamic equilibrium. At a certain concentration, one or several of these micelles assume a structure that can distinguish positively or negatively charged diphenylmethyl alcohols. At that time, the driving force of the structure is the main force separating (±)-DPMAS. Suitability between host and guest is the main force in separating racemates.

The separation of different (±)-DPMAs was achieved in different concentrations of CTAB micelles (Table I). When the concentration of CTAB surfactant was about 0.05 mol/L, all nine pairs of DPMAs were separated. However, the proper concentration for each pair of compounds was not the same. Thus the cavities of CTAB micelles around 0.05 mol/L could distinguish positively or negatively charged DPMAs, but each pair of compounds required somewhat different cavities for optimum resolution.

Table III shows the R_f values of (±)-DPMAs in different concentrations of CTAB surfactant and additives. It illustrates that the R_f values were not in direct proportion to the concentration of β-CD when different concentrations of β-CD were added to the same concentration of CTAB surfactant aqueous solution (0.05 mol/L CTAB) (Table II). This indicated that the main driving force was the cavities. Because the β-CD additive was in solution, it changed or influenced the shapes and sizes of the cavities. Thus, when the concentration of β-CD was increased or unchanged and CTAB concentration was 0.03 mol/L, 0.025 mol/L, or 0.0125 mol/L or SDS concentration was 0.01 mol/L, the compounds could not be separated. Also, when the pH was changed or butanol was added, the compounds could not be separated.

The separation of DPMAs in CTAB is well-described by host–guest chromatography. The authors plan to develop more work from this viewpoint and use MPC and the other chromatographic methods to develop more research in the field of host–guest chemistry. Hopefully this work will yield information not only on the solutes' molecular structures, but also on the shapes, sizes, or chemical and physical properties of micelles.

Host–guest chromatographic behavior of DPMAs

The curves of C_m versus $R_f/1 - R_f$ are shown in Figures 2–4. These curves are two irregular sine (cosine) curves that merge out of the chiral separation areas. They deviate greatly from the Armstrong equation. Fu and Wang discussed the deviation in detail (Z.S. Fu and X.F. Wang. Micellar paper chromatographic separation of diphenylmethyl alcohols' structural isomers anf their chromatographic behaviour. *Chinese J. Chromatogr.*, in press.). Armstrong (2) gave the correct

Table III. R_f Data of (±)-DPMAs with Different Concentrations of CTAB Surfactant and Additives in MPC

R		0.05 mol/L CTAB + 0.00 mol/L β-CD	0.05 mol/L CTAB + 0.002 mol/L β-CD	0.05 mol/L CTAB + 0.007 mol/L β-CD	0.03 mol/L CTAB + 0.0052 mol/L β-CD	Other conditions*
Cl	o	0.35 0.64	0.21 0.84	0.30 0.84	0.49	†
	m	0.58	0.81	0.84	0.51	†
	p	0.70	0.46 0.89	0.18	0.31	†
CH_3	o	0.67 0.73	0.44 0.85	0.33 0.71	0.58	†
	m	0.31 0.48	0.29 0.82	0.43 0.78	0.70	
	p	0.46 0.82	0.87	0.24 0.84	0.61	†
OCH_3	o	0.41 0.62	0.43 0.71	0.75	0.48	†
	m	0.42	0.43 0.87	0.49 0.76	0.63	†
	p	0.29 0.67	0.19 0.78	0.59 0.74	0.58	†

* 0.025 mol/L CTAB + 0.0035 mol/L β-CD; 0.0125 mol/L CTAB + 0.00175 mol/L β-CD; 0.05 mol/L CTAB, pH = 5; 0.05 mol/L CTAB, pH = 9; 0.05 mol/L CTAB + 5% butanol; 0.01 mol/L SDS + 0.002 mol/L β-CD.
† Racemates could not be separated at all.

pseudophase retention (for 2:1 complexes) as:

$$\frac{1}{k'} \text{ or } \frac{R_f}{1-R_f} = \frac{1}{k[A]\Phi} + \frac{k_1 c}{k[A]\Phi} + \frac{k_1 k_2 c^2}{k[A]\Phi} \quad \text{Eq 1}$$

where k, k_1, and k_2 are the binding constants to the stationary phase, first pseudophase, and second pseudophase, respectively. Fu and Wang considered that k_1 and k_2 could be more than or less than than zero, respectively or simultaneously. Equation 1 will produce third and fourth (and so on) constants in a solution that has a changing concentration (e.g., k_3, k_4, etc.).

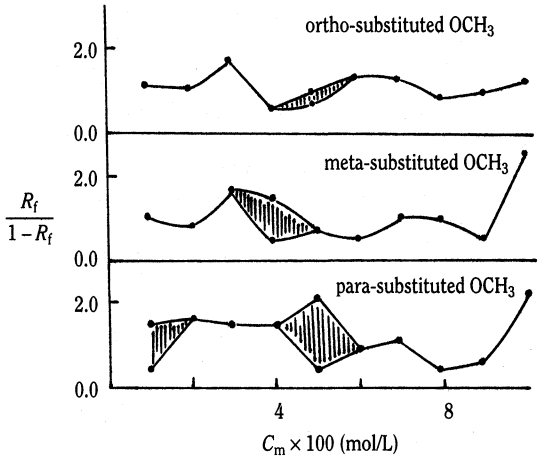


Figure 2. Curves of C_m versus $R_f/1 - R_f$ of (±)-DPMAs. C_m is the micellar concentration of CTAB (total surfactant concentration minus critical micelle concentration). Shaded areas represent chiral areas.

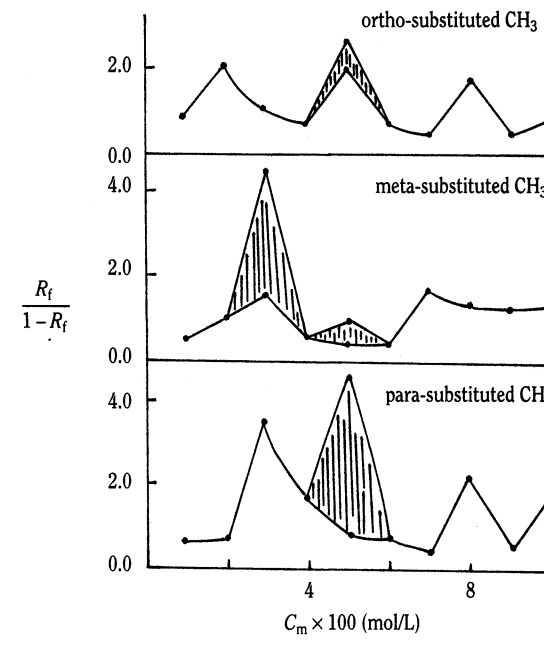


Figure 3. Curves of C_m versus $R_f/1 - R_f$ of (±)-DPMAs. C_m is the micellar concentration of CTAB (total surfactant concentration minus critical micelle concentration). Shaded areas represent chiral areas.

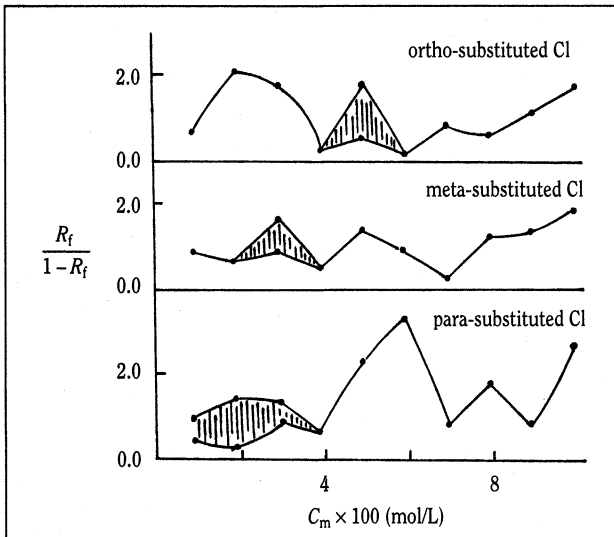


Figure 4. Curves of C_m versus $R_f/1 - R_f$ of (±)-DPMAs. C_m is the micellar concentration of CTAB (total surfactant concentration minus critical micelle concentration). Shaded areas represent chiral areas.

The MPC mechanism of DPMAs in CTAB still conforms to the Armstrong equation. In the MPCs discussed earlier (6–8), the curves that deviated less from the ideal curve were smooth like the Armstrong curve but were not sawtoothed.

Conclusion

The mechanism of MPC conforms to the Armstrong equation completely and can be used to describe host–guest chromatographic behavior. Host–guest chromatography can supply further information about solutes (guests) and micelles (hosts). Because of the complexity of surfactant micelles, more work must be developed on the use, mechanism, and theory of host–guest chromatography.

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