Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2010, 2(1): 281-286
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X

Application of Water-in-Oil Microemulsion for Chromatographic Study of Different Groups of Organic Compounds

Ali Mohammad*, Sameen Laeeq

Chromatography Research Laboratory, Department of Applied Chemistry, Faculty of Engineering and Technology, Aligarh Muslim University, Aligarh, U.P., India

---

Abstract

Thin-layer chromatographic procedures are described for the analysis and separation of a variety of organic molecules (amino acids, B-complex vitamins, purines and pyrimidines, neutral lipids, polar sugars and dyes) using water-in-oil-microemulsion containing N-cetyl-N, N, N trimethyl ammonium bromide (CTAB) as mobile phase. High performance thin layer chromatographic plates of silica gel G and cellulose were used as stationary phases. The proposed method is suitable for selective separation of bromocresole green from other dyes on cellulose HPTLC plates. Furthermore, vitamins (thiamine, pyridoxine and riboflavin in marketed formulations) were successfully resolved on silica HPTLC plates. Effect of other biomolecules as impurities was investigated on simultaneous separation of thiamine, pyridoxine riboflavin from their mixtures. The well intense colored compact spots for all compounds under study were realized.

Key words: HPTLC, CTAB, microemulsion, biomolecules, separation, vitamins

---

Introduction

The growing interest in microemulsion (swollen-micelles) as drug delivery vehicles arises mainly from their physicochemical properties such as transparency, low viscosity, and thermodynamic stability over a range of temperature interval and high solubilization capacity [1]. The possibility of increased solubility of sparingly soluble drugs in microemulsion is of great interest because of the improved therapeutic efficacy of the drug, reduction in the volume of the vehicle and minimization of the toxic side effects [2].

The original application of oil-in-water micro-emulsion in bioanalysis was reported by Berthod et al. when they separated a series of alkylbenzene and screened 11 drugs illegally used in sports [3]. A number of publications on the potential use of microemulsion (ME) as a separation media in HPLC [4], micellar liquid chromatography [5], capillary electrophoresis [6] and microemulsion electrokinetic chromatography (MEKC) have appeared recently [7]. In addition, other application of ME is tertiary oil recovery, cosmetic, lubrication; enzyme catalysis, chemical synthesis etc. were also reported in literature [8, 9].

MEs contain nanometer-sized surfactant coated droplets of oil suspended in water, referred to as oil-in-water microemulsion and vice-versa for water-in-oil microemulsion. In ME liquid chromatography, the surfactant is added in the excess of the critical micelle concentration (CMC) which resulted in large amount of micelles in the mobile phase in which the analyte partitions within the micelles rather than adsorbs onto the stationary phase.

The versatile nature of microemulsion generated renewed interest of chemists to utilize it in chromatographic procedures [10]. Literature suggests that MEs were used as mobile phase to investigate the retention behavior of nitroanilines, amino acids, herbal plant extracts, antibiotics etc. [11-14].

These micellar media seemed to offer another viable clean alternative to traditional organic solvents. Previous studies on thin layer chromatography (TLC) of sugar, amino acids, vitamins, lipids, dyes, purines and pyrimidines utilized hydro-organic, mixed-organic and aqueous surfactants with organic additives as mobile phases for their separation and identification.

So, far no work has been reported for the identification of common sugars by HTLC using water-in-oil ME as eluent. Furthermore, the present study also provides the general applicability of water-in-oil microemulsion in HTLC analysis of compounds belonging to different groups such as vitamins, amino acids, purines and pyrimidines, lipids and dyes.

Results and Discussion

Chromatography of six groups of organic compounds (amino acids, vitamins, sugars, lipids, dyes, purines and pyrimidines) was performed with water-in-oil microemulsion containing CTAB as eluent on two different stationary phases i.e. silica gel G and cellulose HPTLC plates. The following trends were noticed.

(a) All amino acids were strongly retained on both silica and cellulose plates and thus resolution of amino acids with microemulsion as mobile phase is not possible.
(b) All polar sugars, co-migrate without any possible separation on silica gel HPTLC plates showing low mobility (R_F = 0.29).
(c) Alike amino acids, polar purines and pyrimidines show high affinity towards both the adsorbents (silica gel and cellulose) and cannot be resolved from their mixtures.
(d) All lipids being hydrophobic well moved with elution window on silica gel G or cellulose showing significant mobility (R_F =0.98) and thus imposing the restriction on their mutual separation. However, they can be easily separated from other organic compounds (amino

acids, vitamins, sugars, dyes, purines and pyrimidines) having lower R_F value.

(e) A fair separation of B-complex vitamins (thiamine hydrochloride, riboflavin and pyridoxine hydrochloride), is always possible on silica HPTLC plate with water-in-oil ME system. For these vitamins, the order of R_F values, given in parenthesis, was thiamine (0.14) < pyridoxine (0.27) < riboflavin (0.44).

The effect of impurity of other bioorganic molecules (lipids, amino acids, sugars and purines & pyrimidines) on the separation of vitamins was also examined. In the presence of lipids, sugars purines and pyrimidines, the mutual separation is always possible but uracil is the exception which hampers the separation. Amongst amino acids, glutamic acid and tyrosine hurt the mutual separation by producing a singly tailed spot near the point of application as a result of co-migration of these vitamins (thiamine, pyridoxine, riboflavin) in the sample. However, in the presence of other amino acids, all the three vitamins are well resolved on single TLC plate. Thus, for vitamins silica gel (inorganic adsorbent) is more efficient stationary phase compared to cellulose (organic adsorbent) because on PEI cellulose HPTLC layers, the separation of these three vitamins could not be achieved. The developed method is applicable to identification of B-complex vitamins in marketed formulation as evident from results presented in Table 1.

Table 1: Identification of vitamins on silica HPTLC plates using water-in-oil micro - emulsion containing CTAB.

	R_F Value		
Sample	Thiamine	Riboflavin	Pyridoxine
B_1	0.08	0.27	0.40
B_2	0.08	0.27	0.41

(f) All dyes were found to stay near the point of application on silica HPTLC plates and hence they cannot be separated. However, a selective separation of bromocresol green (R_F = 0.89) is possible from all cationic **(**malachite green, methylene blue, brilliant green, rhodamine B and crystal violet) and anionic (xylenol orange, bromocresol green**,** alizarin red S, congo red, pyrocatechol violet) dyes ($R_F \approx 0.01$) on cellulose HPTLC plate.

Almost all the analytes produce well compact spots near or at the point of application on silica HPTLC, which shows a strong affinity of polar analytes with polar silica gel. Low mobility of polar analytes may be attributed to their strong attraction to negative silanol groups on the silica gel surface [15].

Materials and methods

Experimental

Chemicals and materials

Silica gel 60 F_{254} HPTLC plates (1.05715.001) and PEI Cellulose F_{254} (1.05579.0001) were procured from Merck, Germany. n-Pentanol (Acros organics, New Jersey, USA), heptane and

CTAB were of CDH, India. Amino acids (D- glutamic acid, L–serine, D–alanine, L–tyrosine, L–histidine, L–lysine, L–arginine and phenylalanine), sugars (dextrose, D-fructose, D-lactose, D-galactose, D-ribose and D-xylose), lipids (lauric acid, palmitic acid, stearic acid, n-butyric acid and oelic acid), vitamins (thiamine, riboflavin and pyridoxine) and dyes (xylenol orange, bromocresol green**,** alizarin red S, congo red, pyrocatechol violet, malachite green, methylene blue, brilliant green, rhodamine B and crystal violet) were from CDH, India. Purines (alanine and guanine) and pyrimidines (cytosine, thymine and uracil) were obtained from Himeda (Mumbai), India. All chemicals were of analytical grade. All experiments were performed at 25±2^{o}C.

Preparation of test solutions

1% (w/v) of amino acids, sugars and B-complex vitamins were prepared in double distilled water. In case of pyridoxine or riboflavin few drops of NaOH were added to make clear solution. Purines and pyrimidines (1% w/v) were prepared in water-methanol mixture (60:40, v/v) and few drops of ammonia were added when necessary. Lipids (1% w/v or v/v) in chloroform : ethanol (1:1) were prepared. Dyes **(**0.1%**)** were prepared in mixture of double distilled water plus ethanol in 1:1 ratio by volume.

Marketed formulations

Becosules* capsules (B-complex Forte with vitamin C), Pfizer and Becozinc syrup, Dr. Reddy's, Hyderabad, India, analysed by the proposed method.

1.2.Preparation of formulation solutions

Becosules *capsule was finely powdered and dissolved in 10 ml of double distilled water (B_1 sample) and 1 % solution of Becozinc syrup was prepared in double distilled water(B_2 sample).

Detection reagent

Ethanolic solution (0.3%) of ninhydrin was sprayed on the HPTLC plates to locate the position of amino acids. Purple spots appeared on heating the HPTLC plates at 60^{o}C for few minutes.
Sugars were detected by spraying ethanolic orcinol solution on silica HPTLC plates and heating at 110^{o}C for 5-10 minutes. The detected spots appeared as brown- purple for all disaccharides and hexoses except D-fructose (appeared as orange) and blue for pentoses.

All vitamins, purines, pyrimidines and lipids were UV active and can be easily visualized under short UV lamp. Dyes were detected visually according to their original color.

Preparation of mobile phase (Microemulsion)

Water-in-oil ME used as mobile phase was prepared at 25^{o}C by titrating a coarse emulsion of heptane (160 mL), water (8 mL) and CTAB (8 gm) with n-pentanol (25 mL). The microemulsion system was transparent optically clear and remained stable at 25±2^{o}C for several weeks. This was used as mobile phase for six groups of organic compounds.

HPTLC method

Test solutions (1µL) of all analytes were applied on (6 x 6 cm) silica gel 60 F_{254} and PEI cellulose high-performance thin-layer plates with the help of micropipette at about 1 cm above

the lower edge of the plates. The solvent ascent was fixed to 5 cm in all cases for the determination of R_F value of individual analyte. Linear ascending development was carried out in vapor equilibrated Camag TLC twin-trough chamber. The optimized chamber saturation time for the mobile phase was 10 min at room temperature (25±2^{o}C). Subsequent to the development, TLC plates were dried at room temperature. The plates were detected by using appropriate detector for desired analyte. The R_L (R_F of leading front) and R_T (R_F of trailing front) values of each spot were determined and the R_F value was calculated

For the separation of vitamins or dyes from their mixtures, equal volumes of vitamins or dyes were mixed and 1 µL of the resultant mixture was applied on TLC silica gel or cellulose plate. The plate was developed with mobile phase, the spots were detected and the R_F values of the separated spots of vitamins and dyes were calculated. Similarly dyes were also separated on cellulose plates.

For investigating the interference of other bioorganic molecules (amino acid, sugars, lipids, purines and pyrimidines) on the mobility and mutual separation of vitamins, aqueous solutions (as prepared earlier) were used for interference studies. The effect of biomolecules as impurities on the resolution of vitamins from their mixture, 1 µL of the standard test mixture of vitamins was spotted on the plate followed by the spotting of 1 µL of biomolecules considered as impurities. The plates were developed with microemulsion containing CTAB mobile phase, spots were detected under UV lamp and R_F values of the separated vitamins were calculated.

Conclusion

Oil-in-water microemulsion proved to be useful surfactant-mediated system for ternary separation of vitamins (thiamine, riboflavin and pyridoxine) in pharmaceutical preparations. On cellulose layers, bromocresol green was selectively separated from other anionic as well as cationic dyes.

Acknowledgment

The authors are grateful to the Chairman, Department of Applied Chemistry, Aligarh Muslim University, Aligarh, India and University Grant Commission (UGC), New Delhi, India, for providing research facilities and financial assistance respectively.

References

[1] P.K. Ghosh, R.S.R. Murthy, *Drug. Del. C.*, **2006,** 3, 167.
[2] R. Ryan, S. Donegan, J. Power, E. McEvoy, K. Altria, *Electrophoresis*, **2009,** 30, 65.
[3] A. Berthod, J.J. Lasesna, I. Carretero, *J. Liq. Chromatogr. Relat. Technol.*, **1992,** 15, 3115.
[4] K. Altria, M. Broderick, S. Donegan, J. Power, *Chromatographia*, **2005,** 62, 341.
[5] M.B. Simon, D.A. Kevin, *J. Sep. Sci.*, **2004,** 27, 1498.
[6] A. Marsh, M. Broderick, K. Altria, J. Power, S. Donegan, B. Clark, *Methods Mol. Biol.*, **2008,** 384, 205.
[7] M. Murillo–Arbizu, E. Gonzatez–Penas, S.H. Hansen, S. Amezqueta, J. Ostergaard, *Food*

Chem. Toxicol., **2008,** 46, 2251.
[8] B.K. Paul, S.P. Moulik, *Curr. Sci.*, **2001,** 80, 990.
[9] K.D. Altria, *J. Chromatogr. A*, **2000,** 892, 171.
[10] G.M. Eccleston, Emulsion and Microemulsions, (IInd Ed., J. Swarbrick, J.C. Boylan, Marcel Dekker, Inc., New York, 2002) 1080.
[11] A. Mohammad, S. Hina, *Acta Chromatographica*, **2005,** 15, 238.
[12] A. Mohammad, V. Agrawal, S. Kumar, *J. Planar Chromatogr.*, **2003,** 16, 220.
[13] S.F. Cui, B.Q. Fu, F.S.C. Lee, X.R. Wang, *J. chromatogr. B*, **2005,** 828, 33.
[14] A. Malenovic, D. Ivanovic, M. Medenica, B. Jancic, S. Markovic, *J Sep. Sci.* **2004,** 13, 1087.
[15] B.A. Olsen, *J. Chromatogr. A*, **2001,** 913, 113.