Asian Journal of Chemistry Vol. 21, No. 1 (2009), 388-392

Use of Some Basic Dyes in the Extractive Spectrophotometric Determination of Tranexamic Acid

P. BABY SUDHA LAKSHMI, S.V.M. VARDHAN, D. RAMACHANDRAN and C. RAMBABU*
Department of Chemistry, Acharya Nagarjuna Universtiy
Nuzvid Campus, Nuzvid-521 201, India
E-mail: rbchintala1@yahoo.com

A simple and sensitive spectrophotometric method is developed for the determination of tranexamic acid. It is based on the formation of coloured ion-pair complex between the tranexamic acid and basic dyes (methylene blue, methylene violet and saframin-O) in the aqueous phase at suitable pH extractable into chloroform showing the characteristic absorption maximum at 650, 550 and 520 nm, respectively. The method has been applied to pharmaceutical formulations. The method has been statistically validated and is found to be precise and accurate.

Key Words: Spectrophotometric determination, Basic dyes, Tranexamic acid.

INTRODUCTION

Tranexamic acid[1] (TEC) is a *trans*-4-(amino methyl) cyclohexane carboxylic acid, which produces an antifibrinolytic effect by inhibiting the activation of plasminogen. Few methods appeared in the literature for the determination of tranexamic acid in analytical determinations[2-9] and pharmaceutical formulations. Some HPLC methods for biological samples have been reported[10-12]. The analytically important functional groups of TEC were not properly exploited for designing suitable spectrophotometric methods for the determination of TEC. In this paper, we report the results of our investigations on the utilization of basic dyes as analytical reagents for the spectrophotometric determination of tranexamic acid. The method is based on the formation of coloured ion-pair complex. The ion-pair complex is a special form of molecular complex resulting from two components extractable into organic solvents from aqueous phase at suitable pH. One component is a chromogen (dye or metal complex) possessing charge (cationic or anionic in nature) and so insoluble in organic solvents. The other is colourless, possessing opposite charge (anionic or cationic) to that of chromogen.

EXPERIMENTAL

Spectral and absorbance measurements were made with digital Elico UV-Vis spectrophotometer SL 159 and pH measurements were made with Digisun Electronics digital pH meter model DI-707.

Fresh solutions of 0.2% aqueous solutions of methylene blue (MTB), methylene violet (MTV) and saframin-O (SFN) (Fluka sample) were prepared in distilled water. The stock solution (mg/mL) of tranexamic acid was prepared by dissolving 100 mg of it in 100 mL of distilled water. A portion of stock solution was diluted stepwise with the distilled water to obtain the working standard tranexamic acid solution. 80 µg/mL (SFN-O) and 25 µg/mL (MTB and MTV) were also prepared. Buffer solution of pH-9.8 is prepared by mixing 7 g of ammonium chloride with 56.8 mL of liquor ammonia solution and diluted to 100 mL with distilled water and pH was adjusted to 9.8. All the chemicals and reagents were of analytical grade and the freshly prepared solutions were always used in the investigations.

Procedure: Aliquots of standard tranexamic acid solution (0.5-2.5 mL), 1 mL of pH-9.8 buffer solution were placed separately in a series of 125 mL separating funnels. A volume of 1.5 mL of saframin-O or 0.5 mL of methylene blue and methylene violet was added, respectively. The total volume of aqueous phase in each funnel was adjusted to 10 mL with distilled water and 10 mL of chloroform was added. The contents were shaken for 2 min and allowed to separate. The separated organic layer were made upto the mark with chloroform. The absorbances was measured at the appropriate λ_{max} (Table-1), against a similar reagent blank. The amount of tranexamic acid solution was obtained from the standard calibration curve.

The method has also been applied to pharmaceutical formulations. The tablet powder equivalent to 100 mg of tranexamic acid was taken and treated with 3 × 25 portions of chloroform. The combined chloroform extract was made upto 100 mL with the same solvent to get mg/mL stock solution. From one portion of chloroform extract, chloroform was gently evaporated. The residue was dissolved and diluted stepwise with distilled water as described under standard solution preparation to obtain working standard drug (TEC) solution.

RESULTS AND DISCUSSION

The optimum conditions were established after a thorough systematic study of parameters such as concentration of dyes, pH, temperature, choice of organic solvent for the extraction of the coloured species, the order of addition of reagents for maximum colour developent and its stability. Least square analysis was carried out for the slope, intercept and correlation coefficient. Beer's law limits, molar absorptivity, sandell's sensitivity and

optimum photometric range for tranexamic acid with each of mentioned reagents were calculated. The optical characteristics are presented in Table-1. The per cent relative standard deviation and per cent range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods and are presented in Table-1. The accuracy of the methods was ascertained by comparing the results by the reference method (Table-2). This comparison shows that there is no significant difference between the results of studied methods and those of the reference one. To evaluate the validity and reproducibility of the method, known amounts of pure drug were added to previously analyzed samples and the mixtures were analyzed by the proposed method. There is no interference of other ingredients present in formulations. These results indicate that the method is accurate, precise and reproducible and is applicable to various formulations of tranexamic acid.

TABLE-1
OPTICAL CHARACTERISTICS, PRECISION, ACCURACY OF THE METHODS PROPOSED IN THE DETERMINATION OF TRANEXAMIC ACID

Optical characteristics	Methylene blue	Methylene violet	Saframin-O
λ_{max} (nm)	650	550	520
Beer's law limits (µg/mL)	0.5-2.5	0.8-2.0	1.6-8
Molar absorptivity (1 mol^{-1} cm^{-1})	4.15×10^{4}	3.31×10^{4}	1.05×10^{4}
Correlation coefficient (r)	0.9999	0.9999	0.9999
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.024	0.029	0.0603
Regression equation (y = a + bC)			
(i) Slope (b)	0.264	0.202	0.066
(ii) Standard deviation on slope (S_b)	4.62×10^{-4}	1.41×10^{-3}	3.59×10^{-3}
(iii) Intercept (a)	-0.0006	-0.0042	0.0037
(iv) Standard deviation on intercept (S_a)	7.66×10^{-4}	2.33×10^{-3}	1.91×10^{-3}
(v) Standard Error of Estimation (S_e)	7.30×10^{-4}	2.22×10^{-3}	1.82×10^{-3}
Optimum Photometric range (µg/mL)	1.0-2.0	0.8-2.0	2.8-6.4
Relative standard deviation*	1.062	0.622	0.578
% of range error (confidence limit)			
(i) 0.05 level	1.114	0.653	0.607
(ii) 0.01 level	1.748	1.024	0.952
% error in bulk sample**	0.253	0.332	-0.308

*Average of six determinations considered. **Average of three determinations.

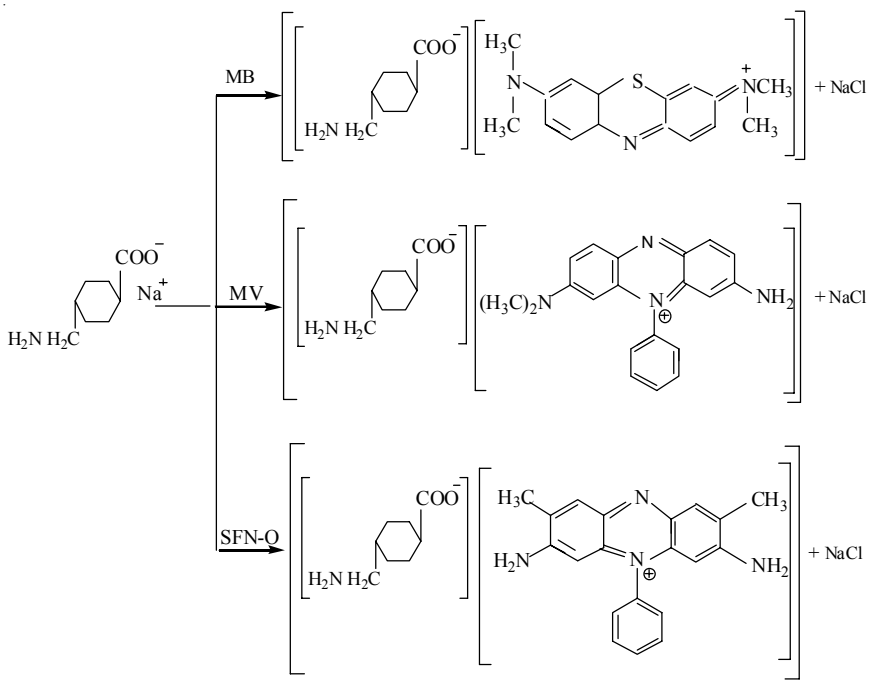
Chemistry of coloured species: Ion-association complex is a special form of molecular complex resulting from two components extractable into organic solvents from aqueous phase at suitable pH. One component is a chromogen (dye or metal complex) possessing charge (cationic or anionic in nature) and so insoluble in organic solvents. The other is colourless,

TABLE-2
DETERMINATION OF TRANEXAMIC ACID IN PHARMACEUTICAL FORMULATIONS

Sample*	Labelled amount (mg)	Amount found by proposed method**			Ref. method†
		Methylene violet	Methylene blue	Saframin-O	
Tab I	500	498.89 ± 1.109 F = 2.06 t = 0.27	499.13 ± 0.997 F = 2.56 t = 0.82	497.64 ± 1.420 F = 1.30 t = 1.01	498.52 ± 1.594
Tab II	500	498.87 ± 1.120 F = 2.51 t = 0.90	497.96 ± 2.061 F = 1.35 t = 1.51	498.67 ± 1.361 F = 1.70 t = 1.06	499.63 ± 1.776
Tab III	500	499.66 ± 1.435 F = 1.34 t = 0.59	497.98 ± 2.536 F = 4.18 t = 1.96	499.77 ± 1.225 F = 1.03 t = 0.49	500.12 ± 1.240
Tab IV	500	498.28 ± 1.648 F = 3.95 t = 1.37	499.12 ± 0.906 F = 1.19 t = 0.28	500.12 ± 0.625 F = 1.76 t = 2.04	499.26 ± 0.829

†Reference was UV method developed in the laboratory.
*Tablets from four different pharmaceutical companies.
**Average ± standard deviation of six determinations, the t- and F-test values refer to comparison of the proposed method with the reference method.
Theoretical values at 95% confidence limit, F = 5.05, t = 2.228

possessing opposite charge (anionic or cationic) to that of chromogen. Ion-association complex extraction has been applied to the estimation of numerous compounds possessing acidic moeity (-COOH group) by using basic dye as a reagent and a chlorinated solvent as an extractant. The structure of the species formed may depend upon the experimental conditions (concentration of the components, pH of the aqueous phase). The selectivity of reaction may increase by using appropriate organic solvent as an extractant, which then depends upon parameters such as polarities of the carboxylic acid group and of the dye. Thus, in the present case, the tranexamic acid possesses carboxylic acid group (acidic) which exists as COO^- under neutral or weak alkaline conditions. It forms an ion association complex with basic dyes (SFN-O, MTB and MTV) which is extractable into chloroform from its aqueous phase. The carboxylic acid group in anionic form (-ve) of tranexamic acid is expected to attract the oppositely charged cationic charge (+ve) of the dye and behave as a single unit being held together by electrostatic interaction. The possible structure of the ion-association complex in each instance (**Scheme-I**) was established based on the analogy reports for similar types of molecules with basic dyes.



Scheme-I

REFERENCES

1. Drug Today, Ready Reckoner of Current Medical Formulations, April-June (2005).
2. K. Chitra, K. Sujatha, Ahmed, I. Rabia, K. Shalini, Priya and B. Lakshmi, *Indian J. Pharm. Sci.*, **66**, 836 (2004).
3. M.S. Rizk, S.S. Toubar, M.A. Sultan and S.H. Assaad, *Mikrochim. Acta*, **143**, 281 (2003).
4. S. Atmaca, *Acta Pharm. Turc.*, **31**, 115 (1989).
5. N. Buyuktimkin and S. Buyuktimkin, *Acta Pharm. Turc.*, **27**, 78 (1985).
6. T. Takeuchi, Y. Kabasawa, R. Horikawa and T. Tanimura, *Analyst (London)*, **113**, 1673 (1988).
7. G. Iskender and S. Atmaca, *Pharmazie*, **43**, 290 (1988).
8. M.Y. Khuhawar, F.M.A. Rind, *Chromatographia*, **53**, 709 (2001).
9. B. Li, J. Fan, *Guangpuxue Yu Guangpu Fenxi*, **22**, 461 (2002).
10. K. Matsubayashi, C. Kojima and H. Tachizawa, *J. Chromatogr.*, **433**, 225 (1988).
11. M. Abrahamsson, *J. Pharm. Biomed. Anal.*, **4**, 399 (1986).
12. P.M. Elworthy, S.A. Tsementzis, D. Westhead and E.R. Hitchcock, *J. Chromatogr.*, **343**, 109 (1985).

(*Received*: 20 December 2007; *Accepted*: 20 August 2008) AJC-6780