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Study of spectrophotometric characteristics of the charge transfer complexation of epsilon aminocaproic acid with bromanil

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A spectrophotometric method has been studied for the determination of epsilon aminocaproic acid (EACA) through charge transfer (CT) complexation with bromanil (TBBQ). The CT reaction has been carried out at 70 °C of the water bath for 30 min in the borax buffer solution. The spectrum obtained for EACA/TBBQ system shows the maximum absorption band at wavelength of 352 nm. The CT complex is also confirmed by both FTIR and ^{1}H NMR measurements. The stoichiometry of the CT complex is found to be 1:1 ratio by Job and straight line methods between the donor and the acceptor. At the optimum reaction conditions, Beer's law is obeyed in a concentration limit of 2 ~ 8 μg mL^{-1}. The relative standard deviation is less than 1.5%. The apparent molar absorptivity is determined to be 1.16×10^4 L mol^{-1} cm^{-1} at 352 nm. The thermodynamic properties and reaction mechanism of the CT complexation have been discussed. The developed method could be applied successfully for the determination of the studied compound in its pharmaceutical dosage form with a good precision and accuracy compared to official method as revealed by *t*- and *F*-tests.

Keywords: Bromanil, Charge transfer complex, Epsilon aminocaproic acid, Spectrophotometry

Charge-transfer (CT) complexes are formed by interaction between electron donors and electron acceptors. CT complexation is important phenomenon in biochemical and bioelectrochemical energy transfer process. The CT reaction has been widely studied in recent years[1-4]. Many drugs are easy to determine by spectrophotometry based on stable CT complexes formed.

Epsilon aminocaproic acid (EACA) (also known as 6-aminohexanoic acid or epsilcapramin, marketed as Amicar) is attributed to synthetic amino acid derivative. It is an anti-fibrinolytic agent that acts by inhibiting plasminogen activators which have fibrinolytic properties. It binds reversibly to the kringle domain of plasminogen and blocks the binding of plasminogen to fibrin and its activation to plasmin, which is hydrophilic drug[5] used as to reduce blood loss and transfusion[6] after total hip and total knee arthroplastic surgery[7,8] and undergoing open heart surgery[9] and during cranial vault reconstruction surgery[10] and undergoing mitral valve replacement surgery[11]. It prevents high glucose and insulin induced-invasiveness in MDA-MB-231 breast cancer cells, modulating the plasminogen activator system[12]. It can be administered orally or intravenously and is excreted in the urine.

Several methods have been reported for quantitative determination of EACA by Rapid chromatographic technique[13] and high performance liquid chromatographic[14] and Gas-chromatographic[15] and reversed-phase chromatography with fluorescence detection[16] and liquid chromatography-tandem mass spectrometry[17] and High-Voltage Paper Electrophoresis[18] and sequential injection analysis[19] and titrimetric[5] methods. The titrimetric method is laborious, less sensitive and time consuming. The chromatographic methods require labor-intensive sample preparation procedure and personnel skilled in chromatographic techniques. The other methods generally require complicated equipment, provision for use and disposal of solvents.

A new spectrophotometric method for determination of studied drug was reported in this paper, through CT complexation with 2,3,5,6-tetrabromo-1,4-benzoquinone (bromanil, TBBQ) having been satisfactorily applied to the determination of this drug in injection pharmaceutical formulation.

Materials and Methods

Apparatus

A Cary 300 UV-visible spectrophotometer (Varian, USA) was used for the absorbance measurements,

using 10 mm path-length quartz cells. The pH was measured on a 210 precise acidometer (Hanna, Italy). An infrared (IR) spectrometer Nicolet iS5 FT-IR (Thermo Scientific, USA) was used to record the spectrum. All ^{1}H NMR experiments were carried out on a Bruker Avance III 600 MHz spectrometer (Bruker, Germany).

Reagents

All chemicals and solvents used were of analytical reagent grade. TBBQ (TCI Development Co. Ltd. Japan) was prepared as 1 mg mL^{-1} in ethanol. The drug standard sample of EACA was kindly provided by National Drug Group Chemical Reagents Co., Ltd. China. The standard drug solution of 100 µg mL^{-1} was prepared by dissolving studied standard drug sample in water. Borax buffer solution of 0.1 mol L^{-1} was prepared by dissolving borax in water.

Pharmaceutical formulation

The following available commercial preparations were analyzed: EACA injections (Xinyijinzhu Pharmaceutical Co. Ltd., Shanghai, China), labelled to contain 2 g EACA, 10 mL per injection.

General procedure

A suitable amount of drug solution was pipetted into a 10 mL volumetric flask, then 1 mL of TBBQ solution and 1.5 mL of borax buffer solution was added, and the solution was diluted to volume with water and mixed thoroughly. The solution was thermostated at 70 °C of water bath for 30 min. After rapidly cooling, the absorbency of the CT complex of EACA with TBBQ was measured at 352 nm against a blank solution. The calibration graph was constructed in the same way with the standard drug solution of known concentrations. The amount of studied drug was computed from corresponding calibration graph.

Analysis of pharmaceutical formulation

0.5 mL of injection of EACA was transferred into a 100 mL calibrated flask, the solution was diluted to volume with water. The solution 10 mL was again diluted up to 100 mL with water. Then the same detection as mentioned in the section general procedure was used.

Preparation of the complex for IR measurement

5 mL of 2 mmol L^{-1} of the drug standard sample of EACA in water, as well as 5 mL of 2 mmol L^{-1} of TBBQ in ethanol, was added to a round bottom flask and thermostated at 70 °C in water bath for 30 min. The solvent was evaporated under reduced pressure and the resulting residues were dried over calcium chloride for IR measurement.

Solution for ^{1}H NMR measurements

5 mg of the drug standard sample of EACA was accurately weighed and dissolved in 1 mL of d_6-DMSO, and 1 mL of solution containing an equimolar amount of TBBQ in the same solvent was added. The solution was thermostated at 70 °C of water bath for 30 min and subjected directly to ^{1}H NMR measurement.

Results and Discussion

Absorption spectra

The absorption spectrum of the CT reaction product between TBBQ and studied drug is shown in Fig. 1. The spectrum shows the new maximum absorption band at wavelength of 352 nm, which is not due to the absorption of any of the reactants and considered to be result of CT complex formation between the investigated drug and TBBQ. The new, low energy absorption observed in solution containing both a donor and an acceptor has been described by Mulliken[20] as CT transition involving the excitation of an electron on the donor to an empty orbital on the acceptor. This drug can be determined colorimetrically by the formation of CT complex with TBBQ. The absorbance of the complex was then measured at its maximum wavelength. Investigations were carried out to establish the most favourable conditions for the CT formation. The influence of some variables on the reaction has been tested as follows.

Effect of solvent

Absorption spectral characteristics of the CT complex of studied drug with TBBQ in different solvents were evaluated. The studied solvents involved water, methanol, ethanol, acetone, acetonitrile and dimethylsulphoxide. Experimental results indicated that a mixed solvent of water–ethanol was suitable for studied drug as it gave the

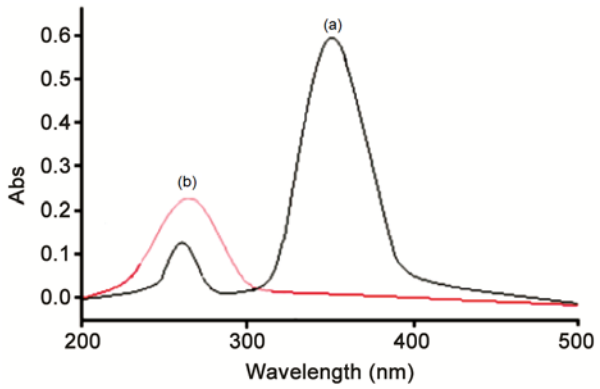

Fig. 1 — Absorption spectra of (a) EACA/TBBQ and (b) TBBQ [c(TBBQ) = 100 µg mL^{-1}; c(EACA) = 6 µg mL^{-1}]

maximum and stable absorbance at the ratio of water to ethanol being 9:1 (v/v).

Effect of reaction temperature and time

Effect of reaction temperature on the absorption of the CT complex was studied in the range of 20 to 80 °C on a water-bath for 0 to 60 min. The suitable temperature and time for obtaining maximum and stable absorbance were carried out at 70 °C for 30 min. The stable time of CT complex at room temperature is at least 3 h.

Effect of pH of working solution

The absorption intensity of the CT complex was dependent on pH. At pH<5.0 almost no reaction was observed, due to the low nucleophilicity of the protonated amino-group. At the pH range of 8.5 to 11.0, the absorption of the complex was most intensive in borax buffer solution. The suitable volume of borax buffer solution of 0.1 mol L^{-1} was 1.5 mL.

Effect of TBBQ concentration

The influence of CT reagent concentration was studied in the range 50–300 μg mL^{-1}. Experiment indicated that 100 μg mL^{-1} of TBBQ concentration is enough for studied compound.

Investigations on the structure of the CT complex

TBBQ is attributed to quinone reagent, which is a strong π-acceptor, n–π or π–π CT complexes have been reported for determination of many compounds[21,22]. Studied drug, attributed to amino acid derivative agent, has one electron rich group of amino, which may form n–π CT complex with TBBQ at the same time. This drug is probably through the lone pair of electron donated by the N atom in the amino (n-electron donors) to bromanil (π-electron acceptor). So, CT complex can be formed with this drug. When TBBQ was added to the studied drug solution, the drug solution with TBBQ causes a change in the absorption spectrum with new characteristic band at 352 nm. The appearance of the new band was the evidence for the possible CT complex formation of the type n-π complex between the studied component and TBBQ.

The formation of the complex was also confirmed by IR measurement. The majority of infrared measurement on the CT complex has been concerned with the shifts in the vibrational frequencies of acceptor. Decrease in the vibration frequency of a particular band has been used as an evidence for a particular site of a CT interaction[23]. The IR spectra of TBBQ (Fig. 2) show strong bands at 1673, 1547 and

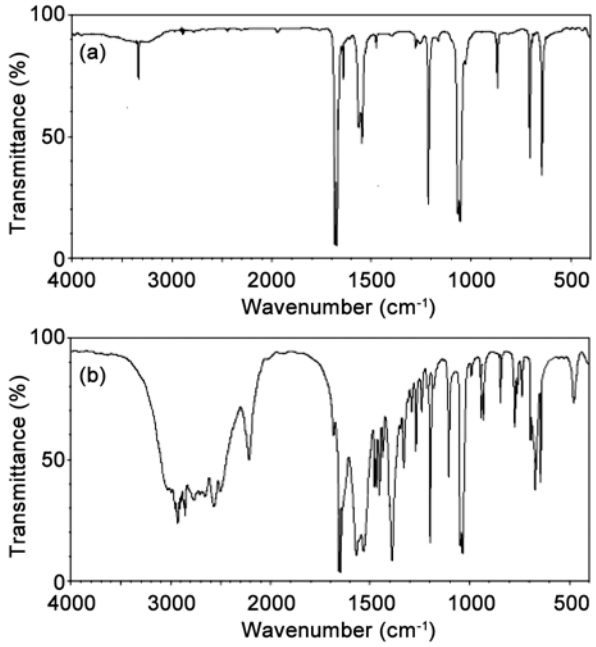

Fig. 2 — FTIR spectra of (a) TBBQ and (b) the CT complex

1055 cm^{-1} corresponding to $\nu_{C=O}$, aromatic $\nu_{C=C}$ and $\nu_{C\text{-}Br}$. These bands were shifted in the spectra of the complex with the investigated compound to 1652, 1534 and 1040 cm^{-1}, respectively.

In ^{1}H NMR, generally, the protons of the donor are shifted to a lower field (paramagnetic shift)[21,24]. Comparison between the chemical shifts in the ^{1}H NMR spectra (Fig. 3) before and after reaction helped in ascertaining the exact binding site of the studied drug. The ^{1}H NMR spectrum of the complex of the investigated compound with TBBQ was measured in d_6-DMSO together with the spectrum of the free compound. Experimental results indicated that in the ^{1}H NMR spectrum of the complexed EACA, since only the chemical shift of $–CH_2$ in $NH_2CH_2–$ of EACA molecule was shifted to a lower field ($\Delta\delta$ = 0.19), other protons of the complex showed no evident changes. This could be explained as follows: the unpaired electron on the nitrogen atom of the amino-group of EACA transferred to TBBQ, which induced a decrease of electron cloud density on the proton of $–CH_2$ in $NH_2CH_2–$ of EACA molecule, as a result, a lower field chemical shift occurred. This indicated that the complexation binding of TBBQ with the nitrogen atom of the amino-group of EACA gave rise to the CT complex.

Stoichiometry of the CT complex was determined by Job's method of continuous variations, in which master solutions of equimolar concentrations of

the donor and acceptor were used in this experiment, and it was found to be 1:1 for the drug with TBBQ. Further support has been observed in the straight line method which can be used as a qualitative mean for the determination of the stoichiometry ratio of the donor and acceptor in the complex. This ratio is likely to engender owing to the presence of the nitrogen atom acting as an electron drawing group in the molecule of studied drug. The nitrogen atom in amino has more electron density and less sterically hindered. So, *n*–*π* CT complex was formed (Fig. 4).

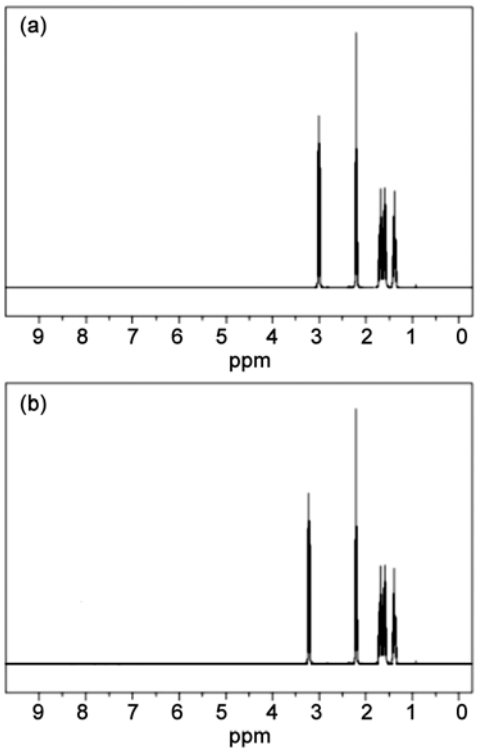

Fig. 3 — ^{1}H NMR spectra of (a) epsilon aminocaproic acid and (b) the CT complex

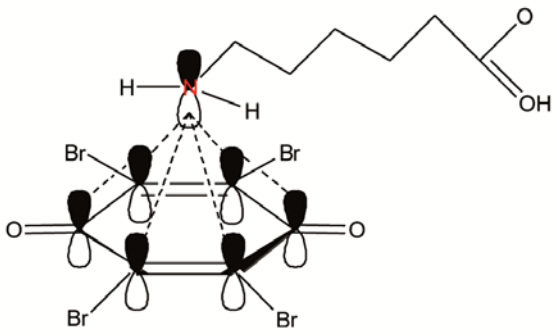

Fig. 4 — The structure of amino acid derivative EACA complex with TBBQ

Investigation of thermodynamic properties of CT reaction

The association constants for the interaction of the compound with TBBQ were estimated according to the Benesi-Hildebrand equation (Eqn 1) when the concentration of acceptor is excess enough to regard $[A_0] >> [D_0]$[25]:

$$\frac{[A_0]}{A^{AD}} = \frac{1}{\varepsilon^{AD}} + \frac{1}{K_{CT}\,\varepsilon^{AD}} \times \frac{1}{[D_0]} \quad \ldots (1)$$

where $[A_0]$ and $[D_0]$ are the total concentrations of the acceptor and donor, respectively, A^{AD} is the absorbance of the complex at the λ_{max}, ε^{AD} the molar absorptivity of the complex, and K_{CT} is the association constant of the complex (l mol^{-1}). From the previous equation, on plotting the values of $[A_0]/A^{AD}$ versus $1/[D_0]$, straight line was obtained (Fig. 5), from which the association constants and correlation coefficient were obtained (Table 1).

$\Delta G°$ value of the complex is calculated from Gibbs free energy of formation according to the relationship (Eqn 2):

$$\Delta G° = -RT \ln K_{CT} \quad \ldots (2)$$

where $\Delta G°$ is Gibbs free energy of the CT complex, R the gas constant (8.314 J mol^{-1} K^{-1}), T the

Table 1 — The association constant of the complex between donor and acceptor (70 °C)

Parameters	EACA-TBBQ
λ (nm)	352
K_{CT} Association constant (1/mol)	7827
Correlation coefficient (r)	0.9976
$\Delta G°$ Gibbs free energy (KJ/mol)	−25.57

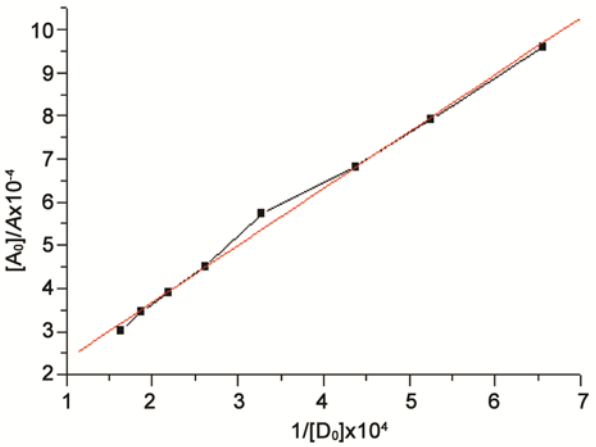

Fig. 5 — The curve of $[A_0]/A$ versus $1/[D_0]$ in EACA-TBBQ system

Table 2 — Quantitative parameters for the CT complex of studied drug with TBBQ

Parameters	EACA-TBBQ
λ_{max} (nm)	352
Beer's law limits (μg mL^{-1})	2−8
Intercept on the ordinate (a)	0.07259
Slope (b)	0.08838
Molar absorptivity (ε) (l mol^{-1} cm^{-1})	1. 16×10^4
Correlation coefficient (r)	0.99941
Sandell sensitivity (μg cm^{-2})	0.011

Table 3 — Determination of EACA drug in injection using TBBQ (n = 5)

EACA drug	Present method		Reference method[5]
	Equivalent nominal content (%) ± S.D.[a]	Recovery (%)	Equivalent nominal content (%) ± S.D.[a]
Injection	100.6 ± 2.9 (t, 2.47; F, 4.3)	99.8 ± 3.5	97.4 ± 1.4

The tabulated values of t and F at the 95% confidence limit are t = 2.78 and F = 6.39

[a] Average of five determinations with standard deviation

temperature in Kelvin, and K_{CT} (l mol^{-1}) is the formation constant of donor–acceptor complex. The parameters thus obtained are represented in Table 1, and these value show that complexation is thermodynamically favoured.

Calibration graph of EACA

Under the experimental conditions described, standard calibration curve of CT complex for the amino acid derivative was constructed by plotting absorbency intensity versus concentration. The linear regression equation is listed in Table 2. The correlation coefficient is 0.99941, indicating good linearity. The small value of variance confirmed the small degree of scattering of the experimental data points around the regression line.

Sample analysis

The proposed method was applied to the determination of EACA in injections. Satisfactory results were obtained for this drug. Moreover, to check the validity of the proposed method, the standard addition method was applied by adding the pure drug to the previously analyzed injections. The recovery of the drug was calculated by comparing the concentration obtained from the mixtures with those of the pure drug. Five replicate determinations were made (Table 3). The obtained mean values ± S.D. of the labelled amounts was 100.6 ± 2.9, the recoveries ranged from 97.3 ± 1.6 to 102.1 ± 2.6. In the t- and F-tests, no significant differences were found between the calculated and theoretical values (95% confidence) of both the proposed and official method[5], and this indicated similar precision and accuracy between proposed and official method.

Table 4 — Precision results of the proposed method (n = 10)

Drug	Concentration (μg mL^{-1})	Within-day R.S.D.[a] (%)	Between-day R.S.D.[a] (%)
EACA	3	1.9	2.5
	5	1.6	1.9
	7	2.5	2.8

[a] Average of ten determinations

Precision of the proposed method was determined in each concentration range, by ten measurements carried out on different days within a week of different solution of studied drug. Target concentrations corresponded to middle values of each range. Table 4 gives an R.S.D. (within-day and between-day) of solutions of certain concentrations determined by using the proposed procedure.

Effect of interfering substances

The assay result was unaffected by the presence of excipients as shown by the excellent recoveries obtained when analyzing the drug in presence of commonly encountered excipients. As samples containing a fixed amount of the studied drug (5 μg mL^{-1}) and excipients (50 μg mL^{-1}) were measured, no interference was observed from commonly used excipients such as starch, lactose, glucose, fructose, sucrose, and magnesium stearate. This fact indicates good selectivity of the method to determine the studied drug both in raw material and in their dosage forms.

Conclusions

The results obtained from the present study indicate that n–π charge transfer complex formation between the amino acid derivative and TBBQ was applied in the spectrophotometric assay of EACA in its dosage forms. The proposed method can be used for the routine quality control of the pure drug and in its dosage forms without fear of interference caused by the excipients expected to be present in its dosage forms. The method has been also applied successfully to the determination of the active constituent in a commercial pharmaceutical. The proposed method has the advantages of easy operation, high recovery,

speed, and minimal use of organic solvent. The investigation of real samples revealed the potential of the method in pharmaceutical analysis.

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