ORIGINAL PAPER

Nig. J. Pharm. Res. 2018, 14 (2) pp 135-145
ISSN 0189-8434, e-ISSN 2635-3555 **Available online at http://www.nigjpharmres.com**

Spectrophotometric and Thermodynamic Studies of the Charge-transfer Complexation of Tranexamic Acid with Chloranilic Acid

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Tranexamic acid is a synthetic analogue of lysine that is clinically useful as an antifibrinolytic agent. Due to its lack of chromophores and aromaticity, chemical derivatization is necessitated and outcomes are often poor and/or associated with low sensitivity and poor stability.

Objective: To develop a colorimetric method for the determination of tranexamic acid (TXA) following the optimization and parameterization of the charge-transfer (CT) complexation of the drug with chloranilic acid (CAA).

Method: The method involved the utilization of TXA as n-electron donor and CAA as π-acceptor in methanol to generate a CT complex. Factors contributory to the formation and stabilization of the complex were optimized. The Benesi-Hilderbrand equation was used to estimate the molar absorptivity and formation constant of the CT band before its application to dosage form analysis.

Results: The CT band which absorbed maximally at 520 nm was associated with molar absorptivity of 807 $Lmol^{-1}cm^{-1}$ and a large formation constant ($1.14x10^4$). The calculated physicochemical properties including transition energy (2.303eV), oscillator strength (0.267), transition dipole moment (5.455 Debye), resonance energy (1.159 eV), ionization potential (8.679 eV) and dissociation energy (5.276 eV) as well as the thermodynamic parameters were indicative of a highly stable charge-transfer complex. Under optimal conditions, the assays of the drug were linear over the range 10-100 μg/mL and the method was accurate and reproducible (inter-day relative errors and standard deviations not greater than 2.92% and 3.40% respectively). When applied to dosage forms, there was no statistical difference in the mean recoveries of the new method when compared to reference method.

Conclusion: The new method is rapid, accurate and precise. It can serve as alternative to the routine analysis of tranexamic acid in bulk and dosage forms.

Keywords: Tranexamic acid, Chloranilic acid, Charge-transfer complexation, Physico-chemical studies, Thermodynamic studies

INTRODUCTION

Tranexamic acid, trans-4-(aminomethyl) cyclohexane carboxylic acid is an antifibrinolytic agent that competitively inhibits the binding of plasminogen and plasmin (by binding to their lysine residues) to fibrin thereby preventing the breakdown of a framework of blood clots (Martindale 2009). The drug is therefore useful in stemming excessive blood loss during surgery (especially prostatectomy, tonsillectomy), dentistry and conditions associated with fibrinolysis such as menorrhagia (Good et al., 2003). Tranexamic acid is a synthetic analogue of lysine which is 8-10 times more potent as an antifibrinolytic agent than the earlier analogue, aminocaproic acid. Chemically, the drug is a white, odourless, water-soluble amino acid with no chromophores except for the carbonyl of the carboxylic acid group (B.P. 2009). Various analytical methods have been developed for the analysis of tranexamic acid in bulk and dosage forms. These include fluorimetry (Duangrat et al., 2007), HPLC

(Patil et al., 2010), LC-mass spectrometry (Delyle et al., 2010), TLC-densitometry (Berniati et al., 2005) and UV spectrophotometry (El-Aroud et al., 2007). However, UV spectrophotometric analysis of the drug or its UV detection following liquid chromatographic separations often require chromophoric labeling of the analyte due to its minimal content of chromophores. The derivatizing agents that have been employed in reported spectrophotometric methods for pharmaceutical dosage forms include 7-chloro-4-nitrobenzofuran, vanillin, dimethylaminobenzaldehyde, acetylacetone, formaldehyde, ninhydrin, 2, 6-dichloroquinone-4-chlorimide, 2, 4-dinitrophenol (El-Aroud et al., 2007; Mohamed and Aboul-enein, 1984; Rind et al., 2009; Subramanian et al., 2011; Gadkariem et al., 2012; Sher et al., 2015), while sodium picrylsuphonate and 2,4,6-trinitrobenzenesulfonic acid (Atmaca, 1989; Duangrat et al., 2007) have been variously applied for post-column chemical modification in HPLC. Expectedly, the majority of the reported UV spectrophotometric methods are fraught with low sensitivity as evidenced by the low molar absorptivities, poor stability of the chromogenic product, narrow Beer's law limits and poor correlation of the calibration curve thus limiting their application. A number of the UV spectrophotometric methods also allow determination at analytical wavelengths lower than 400 nm with the attendant problems of potential interference by other components of the sample matrices leading to decreased selectivity. Charge transfer reactions are useful in chromophoric labeling. Chloranilic acid in particular has been variously employed as a π-electron acceptor in the visible spectrophotometric determination of several drugs (Adegoke et al., 2016; Sultana et al., 2013; Adegoke et al., 2010; Basavaiah et al., 2012; Basavaiah and Charan, 2002) The objective of this study was therefore to optimize the charge-transfer complexation of tranexamic acid with a suitable derivatizing agent (chloranilic acid) that will permit sufficient stability and bathochromic shifts into the visible region for assay. The physicochemical parameters of the charge-transfer complex (including ionization, transition, dissociation energies) as well the thermodynamics of its formation would be investigated before application to the assay of tranexamic acid in pharmaceutical dosage forms.

METHODOLOGY

Materials

Methanol (Sigma Aldrich USA), chloranilic acid (Sigma-Aldrich USA), 25% ammonia solution (BDH England), glacial acetic acid (BDH UK), diethyl ether (Lobachemie), ninhydrin crystals (BDH England), Tranexamic acid secondary reference substance authenticated by IR spectrum matching.

Equipment used

Perkin Elmer Lambda 25 with UV WinLab V2.85 software (Perkin Elmer Singapore), Digital colorimeter (6051 Jenway, UK) Analytical balance (Mettler H80), precoated TLC aluminium plates (Merck, Germany), vortex mixer (Griffins and George, UK), thermostatic water bath (Uniscope).

Method

Preparation of stock solutions

A 1mg/mL (0.0064 M) solution of the analyte was prepared by dissolving 10 mg of tranexamic acid (TXA) in sufficient methanol:glacial acetic acid mixture (9:1) and then making up to 10 mL with the binary solvent mixture.

An equimolar concentration of the derivatizing agent (CAA) was prepared by dissolving 0.013 g of CAA in sufficient methanol to give 10 mL final solution.

Chemical derivatization of tranexamic acid (TXA) with chloranilic Acid (CAA)

Spot test

A 0.5 mL aliquot of the TXA stock solution was added to 0.5 mL of the CAA solution contained in a test tube. The reaction was left to proceed at 30ºC for 5 minutes and 20 minutes. The volume of the reaction mixtures were then made up to 5 mL with methanol.

Thin layer chromatography analysis

Thin layer chromatography analysis was carried out using precoated aluminium TLC plates. Samples of the methanolic solutions of TXA, CAA and adduct were spotted and the plates developed using glacial acetic acid: methanol (1:9) and methanol: ammonia: water (3:2:1). After air-drying, the developed plates were visualized under UV light of 254 nm and 365 nm and also following spraying with ninhydrin reagent (prepared by dissolving 0.2g of ninhydrin crystals in 100 mL of ethanol).

Optimization Studies

Selection of analytical wavelength

A 0.5 mL aliquot of CAA stock solution was added to a test tube containing 0.5 mL TXA stock solution. The mixture was vortex mixed and then maintained at 30 ℃ for 5 mins. The reaction was then quenched by making up the volume to 5 mL with methanol. The UV-visible spectrum, between 190-900 nm, of the complex was acquired using methanol as blank.

The UV-visible spectra of 0.5 mL aliquots of CAA and TXA stock solutions in methanol were similarly acquired.

Optimization of reaction temperature.
Optimization of temperature and time was done using the method of steepest ascent (Karnes and March, 1993). A 0.5 mL portion of CAA solution was added to 0.5 mL of the drug solution in a test tube and then incubated at 30 ℃ for 5 and 20 minutes. The procedure was repeated at 50, 60 and 70 ℃. At the end of the different times, the reaction was stopped by making up the volume to 5 mL with methanol. The absorbance was then determined at 520 nm against a reagent blank on a colorimeter. Each determination was carried out in duplicate.

Optimization of reaction time
A 0.5 mL aliquot of CAA solution was added to different test tubes containing 0.5 mL of TXA. The effect of time was then studied by incubating the different mixtures for 0, 2, 5, 10, 15, 20, 25, and 30 minutes at 30ºC. The reaction was stopped by the addition of methanol to make 5 mL volume. The absorbance values were taken at 520 nm against a reagent blank. Each determination was carried out in duplicates.

Determination of stoichiometric ratio of TXA with CAA
The Job's method of continuous variation (Rose, 1964) was employed to determine the optimum stoichiometric ratio for charge-transfer (CT) complexation. Into seven different test tubes, containing 0, 0.25, 0.33, 0.5, 0.67, 0.75 and 1.0 mL of the drug stock solution, appropriate quantities of the CAA solution were added to make up the volume to 1 mL. Each reaction mixture was then vortex mixed for 10 seconds at 30℃ and the solution made up to 5 mL with methanol. The absorbance values were determined at 520 nm against a reagent blank. Each determination was carried out in duplicates.

Validation Studies
Aliquots of 0.5 mL of CAA solution were added to different test tubes containing 0, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mL corresponding to 0, 10, 20, 40, 60, 80 and 100 µg/mL of drug stock solution respectively. The mixture was mixed for 10 seconds. The reaction was quenched by making up the volume to 5 mL with methanol before determining the absorbance values at 520 nm against a reagent blank. Each determination was done in triplicate for three consecutive days and the average used to generate a pooled calibration curve. The current International Conference on Harmonization (ICH) guidelines were adopted to determine the limit of detection (LOD) and limit of quantification (LOQ) estimated as the ratio of the 3.3 and 10 standard deviation of the blank signal (n=6) respectively, divided by the slope of the calibration line (ICH, 2011). The accuracies as well as the intra- and inter-day precisions of the method were assessed at three concentrations (24, 54 and 84 µg/mL) of tranexamic acid.

Estimation of molar absorptivities and formation constants
The molar absorptivities and formation constant of the CT complex between CAA and TXA were determined by using the calibration curve to successively construct Benesi-Hilderbrand, inverse Benesi-Hilderbrand, Scott, Seal-Sil-Mukherjee and the Foster Hammick Wadley plots (Srivastava et al., 2014). The effects of temperature changes on both parameters were investigated by obtaining the calibration curve at elevated temperatures and thereafter calculating the molar absorptivities and formation constants with each of the standard equations.

Physicochemical parameterization of the CT complex
The stability of the CT complex was investigated by the calculation of some of its physicochemical properties including molar transition energy, oscillator strength, resonance energy, ionisation energy and dissociation energy.

Thermodynamic study of the CT complex
The standard free energy, enthalpic and entropic changes associated with the formation of the CT complex between TXA and CAA were determined using calibration data obtained at temperatures of 30, 50, 70 and 80℃.

Interference studies
The selectivity of the new method for the analyte in the presence of commonly employed excipients was investigated by determining the recovery of spiked amounts of tranexamic acid in separate sample matrices containing starch, talc, gelatin, lactose, magnesium stearate and a mixture of these excipients. Four replicate determinations were done for each excipient.

Application to dosage form analysis
The new method was applied to the assay of the active pharmaceutical ingredient content of two brands of tranexamic acid. The weight uniformity test was determined on ten capsules and the amount of the contents equivalent to 10 mg of TXA acid was added to 5 mL of methanol:glacial acetic acid (9:1)

mixture in a 10 mL volumetric flask. The same binary solvent system was added to make up the volume and the mixture filtered after adequate equilibration. An aliquot of 0.3 mL of the filterate (equivalent to 60 µg/mL TXA) was added to 0.5 mL CAA and then mixed for 10 seconds at 30℃ before terminating the reaction by dilution with methanol. The absorbance was then determined at 520 nm against a reagent blank. The B.P. 2009 formol titrimetric method was adopted as reference method. This involved the use of sodium hydroxide as a titrant for the quantitative assay of aqueous solutions of the drug to which has been added neutralized 40% formaldehyde solution. Six replicate analyses of each of the brands of the drug were carried out using both methods.

RESULTS AND DISCUSSION

Evidence of charge-transfer complexation

On addition to the charge acceptor, TXA instantaneously formed a deep purple adduct which was distinct from the colourless and wine-coloured solutions of TXA and CAA respectively. This is an evidence of the formation of a charge transfer complex. Chloranilic acid is a well-known π acceptor which has been successfully employed for the assay of drugs which contain *n*-electron donors such as hydroxyl and amino groups (Adegoke et al., 2016; Basavaiah et al., 2012). The resultant CT complexes have been experimentally shown not to be due to the formation of a new bond but are rather stabilized by weak valence forces of the donor-acceptor type or hydrogen bonds. The transfer of part of its charge leaves the donor partially positively charged compared to the acceptor thus a resultant stabilizing weak electrostatic bond between the molecular pair ensues. This bond is encouraged by the close proximity and overlap of partially filled orbitals of the π-acceptor and the filled orbitals or non-bonding orbitals in the donor molecule (Adegoke et al., 2016). Thus steric factors play important roles in the formation of CT complexes (Alfred et al., 1993). The avidity of the CT complexation between CAA and TXA will therefore be encouraged by the presence of the free primary amino group in the drug, the positive inductive effect of the methyl cyclohexane group and its staggered chair conformation that minimally interferes with the axial amino group. In addition, the TLC analyses revealed marked differences in the R_f values of TXA, CAA and the complex in both mobile phases as depicted in Table 1. The formation of a new product different from the starting materials further corroborates the CT complexation. The thin layer chromatogram obtained with both mobile phases also revealed one spot with the CT complex indicating a 1:1 ratio in the combination of TXA and CAA. A comparison of the R_f values showed that the resultant CT complex is more polar than CAA and TXA. This is expected as the formation of the complex is associated with the transfer of part of the charge of the donor to the acceptor resulting in the formation of a weak electrostatic bond.

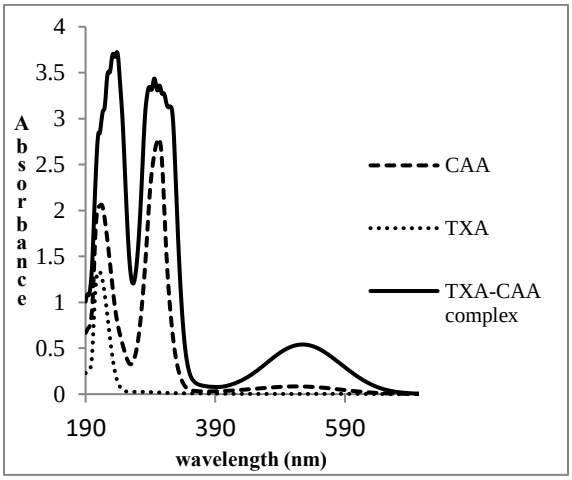


Figure 1: Overlaid spectra of tranexamic acid, chloranilic acid and their CT complex

Table 1: TLC analyses of the TXA, CAA and CT-complex solutions

Mobile phase	TXA	CAA	CT Complex
Glacial acetic acid: Methanol (1:9)	0.50	0.68	0.85
Methanol:Ammonia:Water (3:2:1)	0.67	0.76	0.82

Selection of analytical wavelength

The overlaid spectra of TXA, CAA and the CT complex are presented in Fig. 1. The results showed major absorption bands at 214 and 302 nm for CAA; around 211 nm for TXA while the CT complex showed a new low-energy band at 524 nm. This represents a marked bathochromic shift with respect to either of the starting materials and is further proof of the formation of a new chemical entity. The spectra of the complex at 520 nm revealed optimal differences in the absorptivities of the CT complex and the reagent blank when determined on the digital colorimeter and was therefore subsequently adopted as the analytical wavelength.

Optimization studies

The effect of reaction time on the formation of the CT complex was investigated by incubating 0.5 mL aliquots of TXA and CAA at 30, 50, 60 and 70 ℃ for 5 and 20 minutes at each temperature level. The results as depicted in Fig. 2 showed maximum absorbance values at 30 ℃ with a gradual decline in absorbance at elevated temperatures. This trend is anticipated as CT complexes are not products of actual bond formation but are held together by weak intermolecular forces of attraction which can be readily disrupted by elevated temperatures. Thus, 30℃ was selected as optimum and the reaction time was subsequently optimized at this temperature level.

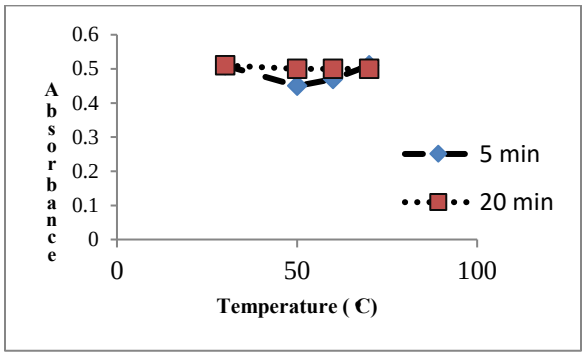


Figure 2: Optimisation of the complexation reaction temperature

The results of the reaction time optimisation, as shown in Fig. 3 showed that the complexation occurred instantaneously and longer reaction times conferred no additional improvement on absorbance. The reaction mixtures were therefore subsequently vortex-mixed for ten seconds before addition of dilution solvent.

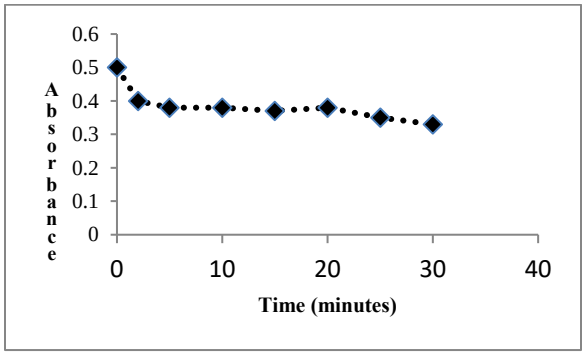


Figure 3: Optimisation of reaction time for complex formation

Methanol was found to be the optimal dilution solvent as it produced the highest absorbance values and most improved correlation coefficients when compared with ethanol and water. The dipolarity of methanol (0.6) is higher than that of ethanol (0.54). Thus the highly polar resultant CT complex will be more soluble in methanol resulting in the observed higher absorbance values. The 0.13%w/v CAA was optimal for colour development as found in previous optimization studies in method development involving the acceptor molecule (Adegoke et al., 2010; Basavaiah et al., 2012) while the order of addition of the reagents did not interfere with CT complexation.

Stoichiometric ratio determination and reaction mechanism

The Job's plot of continuous variation revealed the absorbance values of the CT complex varied with the stoichiometric ratio of the drug and the π-acceptor as shown in Fig. 4. Maximum signal response was observed at equimolar ratio of the donor and acceptor molecules (0.5:0.5) and was subsequently adopted for determinations.

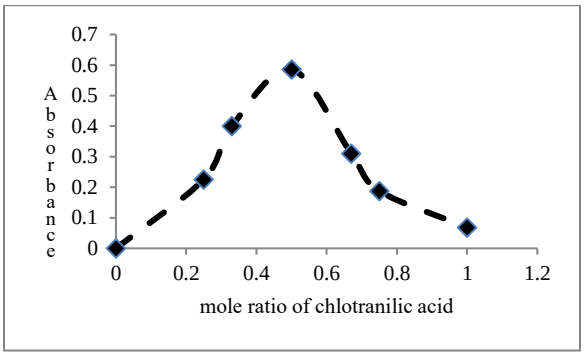
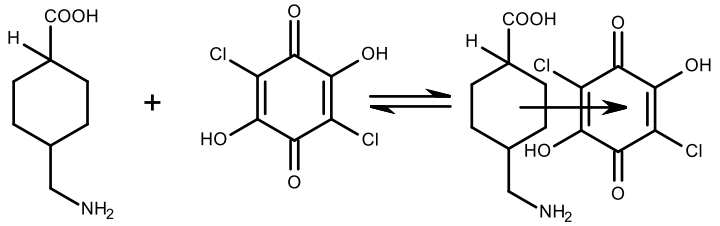


Figure 4: Job's plot for the charge-transfer complexation of TXA with CAA

This equimolar ratio and the formation of a single spot in the TLC analysis confirms that under optimal conditions one mole of TXA will combine with one mole of CAA to form a stable CT complex which absorbs maximally in the visible region. This charge-transfer absorption band has been attributed to the formation of the anionic radical of chloranilic acid following the transfer of charge from the primary amino group of the donor (TXA) as depicted in Scheme 1 (Abdel-Hamid et al., 1985). The CT complex is therefore held by weak electrostatic forces and expectedly the resultant charge of the complex will be better stabilized by the dipolar methanol resulting into higher absorbance and correlation in this solvent compared to ethanol.



TXA (donor) CAA (acceptor) TXA-CAA CT complex (donor-acceptor complex)

Scheme 1: Proposed mechanism of reaction between TXA and CAA

Validation studies

The 3-day calibration revealed that absorbance increased linearly with concentration over the range 10-100 µg/mL at 520 nm with a correlation coefficient of 0.998. Other analytical and validation parameters are presented in Table 2.

Table 2: Validation and analytical parameters for new method

Performance parameter	Value
Beer's law limit (µg/mL)	10 – 100 µg/mL
Correlation coefficient (r)	0.999
Coefficient of determination (r^2)	0.998
Slope ± 95% CI	0.0054 (±0.0003)
Intercept ± 95% CI	0.0348 (±0.0097)
Analytical wavelength	520 nm
Molar absorptivity (L mol^{-1} cm^{-1})	8.07×10^2
Sandell's sensitivity (µg cm^{-2} per 0.001 absorbance unit)	0.195
LOD (µg/mL)	2.372
LOQ (µg/mL)	7.188
Formation constant[b], K	1.14×10^4

[b]= *estimated from Benesi-Hilderbrand plot*

The method was also validated in terms of its accuracy and precision. The accuracy of the method was described in terms of percentage relative error (% RE). As shown in Table 3, the intra-day mean recovery of the new method was 101.44-102.46% while the relative error did not exceed 2.47% indicative of good accuracy. Similarly, the method showed excellent repeatability as the relative standard deviation was not greater than 3.36%. The inter-day precision for the new method was expressed as the percentage relative standard deviation which did not exceed 3.40% indicating good reproducibility. The limit of detection (2.372 µg/mL) and limit of quantification (7.188 µg/mL) were estimated as the ratio of the 3.3 and 10 standard deviation of the blank signal (n=6) respectively, divided by the slope of the calibration line.

Table 3: Accuracy and repeatability of new method

Amount taken µg/ml	Amount found µg/ml	Percent recovery	Relative Standard Deviation (%)	Relative Error (%)
Intra-day assessment[a]				
24	24.4	101.73	3.36	1.71
54	54.8	101.44	2.46	1.44
84	86.1	102.46	1.37	2.47
Inter-day assessment[b]				
24	23.3	97.10	3.27	2.92
54	54.21	100.39	2.02	0.39
84	85.88	102.23	3.40	2.23

[a] *Average of six determinations*
[b] *Average of twelve determinations*

The new spectrophotometric method like most of the previously reported ones is based on the chemical derivatization of TXA. However, the analytical performance parameters of this newly developed method showed one or more clear advantages over the majority of previously reported ones. As depicted in Table 4, most of the older methods are fraught with one of either low sensitivity as evidenced by the reported low molar absorptivities, poor stability of the chromogenic product, narrow Beer's law limits or

poor correlation of the calibration curve thus limiting their application. A number of the methods also allow determinations only at wavelengths lower than 400 nm with the attendant problems of interference by other components of the sample matrices leading to decreased selectivity.

Table 4: Comparison of performance parameters of new and older spectrophotometric methods

Method (derivatizing agent)	Anal. λ (nm)	ε_{max}	Beer-law's limit (µg/ml)	LOD	LOQ	References
Ninhydrin	571	NR	8-40	NR	NR	Subramanian et al., 2011
Acetylacetone	335	73210	5-20	NR	NR	El-Aroud et al., 2007
2,6-dichloroquinone -4-chlorimide	670	520	50-250	24.0	80.0	Gadkariem et al., 2012
2,4-dinitrophenol	418	1337	0.02-200	0.01	0.03	Sher et al., 2015
Vanillin	354	25160	0.5-2.5	NR	NR	Rind et al., 2009
N, N-dimethylamino benzaldehyde	394	16663	0.5-3	NR	NR	Mohamed and Aboul-enin 1984
Chloranilic acid	520	807	10-100	2.37	7.19	This work

LOD=limit of detection; LOQ=limit of quantification; NR=Not reported

In addition, the new method is more robust as it does not require strict pH control as is the case with Rind et al, (2009) method. It is also fast with instantaneous CT complex formation starkly contrasting with the 10 or 30-minute reaction times required for the Duangrat al (2007) or Sher et al., 2015 methods respectively. The instantaneous CT formation which does not require heating also makes the new method amenable to on-field testing applications.

Estimation of the molar absorptivity and formation constant

Based on the electronic spectra of the CT complex formed between varying concentrations of TXA with a fixed concentration of CAA, the molar absorptivity and formation constant K_{CT} were estimated using the Benesi-Hilderbrand, inverse Benesi-Hilderbrand, Scott, Seal-Sil-Mukherjee and the Foster Hammick Wadley equations (Srivastava et al., 2014) as shown in Figures 5-8. The obtained coefficients of determination with the various plots were 0.9964, 0.996, 0.6054, 0.6055 and 0.723 respectively. Thus, the Benesi-Hilderbrand and Foster Hammick Wadley plots best described the electronic spectra of the CT complexation between TXA and CAA. A plot of the absorbance values obtained in the calibration curve as a function of the ratio of the molar concentration of the donor: acceptor ($[D]_o$:$[A]_o$) according to the Benesi-Hilderbrand equation was therefore adopted (Benesi and Hilderbrand, 1949).

$$\frac{[A]_o}{A} = \frac{1}{K_{CT}\varepsilon_{CT}} \cdot \frac{1}{[D]_o} + \frac{1}{\varepsilon_{CT}}$$

where $[A]_o$ is the initial concentration of the acceptor CAA, A is the absorbance of the CT transfer band, $[D]_o$ is the initial concentration of the donor TXA, K_{CT} is the formation constant of the CT transfer band and ε_{CT} the molar absorptivity. A plot of the $\frac{[A]_o}{A}$ vs $\frac{1}{[D]_o}$ will therefore yield a straight line graph with the intercept being equal to $\frac{1}{\varepsilon}$ and slope equal to $\frac{1}{K\varepsilon}$ from which the molar absorptivity and formation constant of the CT transfer band can be calculated. The Benesi-Hilderbrand plot obtained at 30℃ is presented in Fig. 5.

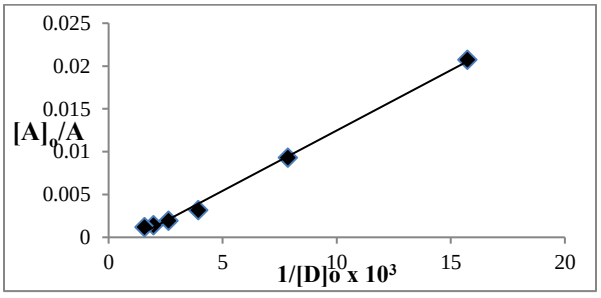


Figure 5: Benesi-Hilderbrand plot for the CT transfer band

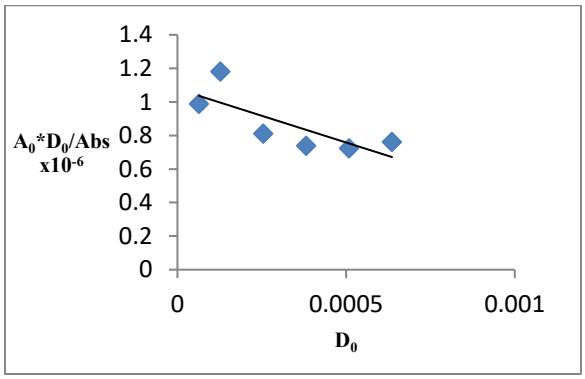


Figure 6: Scott plot for the CT transfer band

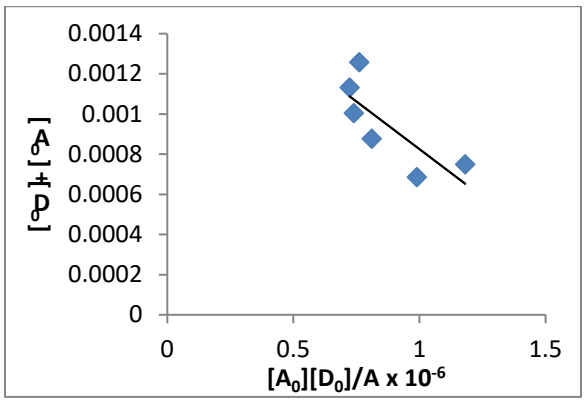


Figure 7: Seal-Sil-Mukherjee plot for the CT transfer band

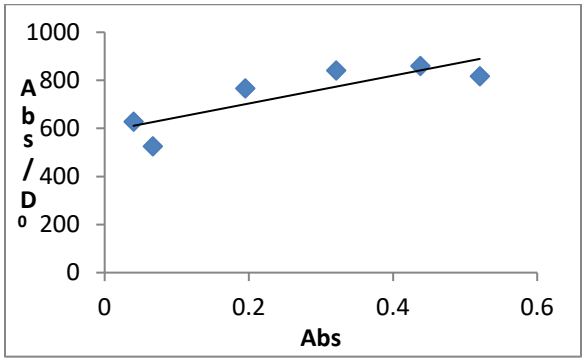


Figure 8: Foster Hammick Wadley plot for CT transfer band

The two physicochemical parameters were obtained from the Benesi-Hilderbrand plot and are depicted in Table 2. The values of K_{CT} and ε_{CT} obtained for an alicyclic molecule like TXA which is devoid of an extensive chromophoric system is indicative of the high propensity of the forward reaction to proceed and therefore explains the instantaneous CT complex formation observed with CAA. This might not be unconnected with the ability of the cyclohexane skeleton of TXA to adopt a strain-free conformation that minimises steric hindrance and favours the exposure of the primary amino group to the CAA molecule.

Physicochemical parameterization of the CT complex

Other physicochemical parameters that describe the energetics of formation and the stability of the resultant CT complex were also calculated. These include molar transition energy, oscillator strength, transition dipole, resonance energy, standard free energy and the ionization potential of the donor species.

The standard free energy changes associated with CT complex formation was calculated using the formation constant K_{CT} according to the Person (1962) equation: $\Delta G^o = 2.303\, RT\, logK_{CT}$ where ΔG^o is the free energy change in KJ Mol^{-1}, R is the gas constant (8.314 $JMol^{-1}K^{-1}$) and K is the absolute temperature.

The transition energy of the complex was obtained from the formula $hV_{CT}$where h is the Planck's constant and V_{CT} is the wavenumber of the absorption peak of the complex.

The oscillator strength (f) is a dimensionless quantity used to express the transition probability of the CT band and the transition dipole moment (μ_{EN}) of the CT complex. The two parameters are derived as follows:

$$f = 4.32x10^{-9}[\varepsilon\Delta V_{1/2}]$$

$$\mu_{EN} = 0.095\left[\frac{\varepsilon_{CT}\Delta V_{1/2}}{\Delta V_{1/2}}\right]$$

where $\Delta V_{1/2}$ is the width of the band at half the maximum absorption and ΔV is the wavenumber at absorption maximum.

The ionization potential, I_D, of TXA in the formation of the CT complex was derived from the Aloisi and Pignatoro (1973) equation:

$I_D(eV) = 5.76 + 1.53x10^{-4}v_{CT}$ where V_{CT} is the wavenumber of the CT band in cm^{-1}.

The resonance energy of the CT complex in the ground state was derived using the Brieglieb (1961) equation:

$\varepsilon_{CT} = 7.7x\,10^4/[hv_{CT}]\,/R_N - 3.5$

where ε_{CT} is the maximum molar absorptivity of the complex and hv_{CT} is the transition energy of the complex.

The dissociation energy (W) of the formed CT complex between TXA and CAA was calculated using the McConnell et al., (1953) equation:

$hv_{CT} = I_D - E_A - W$ where E_A is the electron affinity of CAA which is equal to 1.1.

A summary of the various physicochemical properties obtained are depicted in Table 5.

Table 5: Calculated physicochemical properties of the CT complex between TXA and CAA

solvent	λ_{max}(nm)	$hv_{CT}(eV)$	f	μ_{EN} (Debye)	$R_N(eV)$	ΔG^o KJMol^{-1}	$I_D(eV)$	W(eV)
methanol	520	2.303	0.267	5.455	1.159	-23.554	8.679	5.276

A close examination of the physicochemical parameters in Table 5 revealed trends that support the good stability of the CT complex formed between TXA as donor and CAA as the acceptor molecule. The high ionization potential of 8.679 indicated that TXA is a good *n*-electron donor. This is expected because of the primary amino group and conformation of the molecule that precludes steric hindrance. The ionization potential is also almost four times the transition energy and can thus be readily surmounted for the complex to be formed. The ionization potential of the CT complex is also greater than its dissociation energy. This will discourage the spontaneous break down of the complex especially at 30℃. The relatively high amount of externally applied energy (as evidenced by the high dissociation energy) that will be required to effect the decomposition of the CT complex explains the decrease in its absorptivity seen with elevated temperatures. The negative standard free energy changes associated with CT complexation reveals that the reaction is an exothermic one, which further explains the decreased absorptivity of the complex at elevated temperatures. The transition dipole moment is useful for determining if the transition from a bonding π orbital to an antibonding π* orbital is allowed. The high dipole moment observed is indicative of the existence of a good ion pair which was well solvated and stabilized by methanol used as dilution solvent.

Thermodynamic investigation of the CT complex formation

The changes in the standard free energy (ΔG^o), enthalpy (ΔH) and entropy (ΔS) associated with the CT complex formation were calculated using the following equations:

$$-\Delta G^o = 2.303\ RT\ logK_{CT}$$

$$\log K = \frac{-\Delta H}{2.303}\left(\frac{1}{T}\right) + constant$$

$$-\Delta G^o = \Delta H - T\Delta S$$

The enthalpy of the complexation was estimated from a Van't Hoff plot with log K on the y axis and reciprocal of absolute temperature on x axis. The Van't Hoff's plot is represented in Fig. 9 while the various thermodynamic parameters are depicted in Table 6.

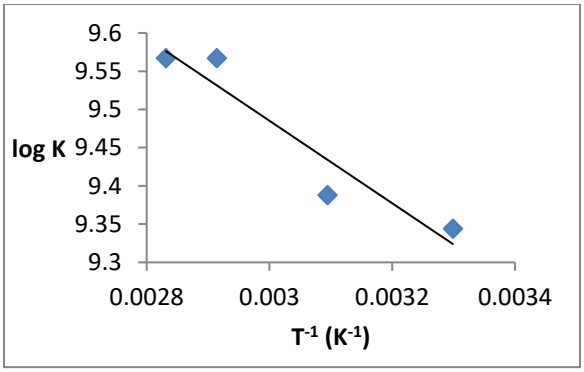


Figure 9: Van't Hoff plot for the CT complex between TXA and CAA

Table 6: Thermodynamic changes associated with CT complexation

Temperature (K)	Molar absorptivity (L $Mol^{-1}cm^{-1}$)	Formation constant, K (M^{-1})	ΔG KJ mol^{-1}	ΔH KJ mol^{-1}	ΔS KJ mol^{-1}
303.15	625.0	$1.14x10^4$	-23.554		0.062
323.15	625.0	1.19×10^4	-25.226		0.064
343.15	333.33	$1.42x\ 10^4$	-27.299	-4.49	0.066
353.15	333.33	$1.42x\ 10^4$	-28.094		0.067

The standard free energy changes at the four temperatures investigated gave negative values indicating the exothermic nature of the reaction. The standard Gibb's energy increased with temperature indicating that elevated temperatures will discourage the spontaneity of the CT complexation thus becoming increasingly more difficult to obtain the complex. Expectedly, the molar absorptivity of the complex decreased significantly at higher temperatures. The high Gibb's energy as well as the enthalpic change of -4.49 KJ/mol attests to the ease of formation of the CT complex.

Method selectivity

The mean recoveries of the analyte in the presence of excipients varied from 100.25±2.62 to 104.88±3.20% showing the selectivity of the method.

Dosage form analysis

The recoveries of the analyte from two commercial brands of tranexamic acid using the new method and the B.P. 2009 formol titrimetric method were carried out. The results are shown in Table 7.

Table 7: Comparative dosage form analyses using new CT and reference methods

Drug Formulation	New Method [a]		Reference Method [a]			Statistics (p-values)	
	%Recovery ± SD	RSD (%)	%Recovery ± SD	RSD (%)	Mean recovery* (%)	F-test	t-test
Razotran	96.13 ± 1.01	1.78	96.76 ± 1.24	1.78	99.35	0.65	0.64
Prexam	100.25 ± 1.91	2.52	101.93 ± 3.18	2.75	98.34	0.89	0.40

*[a]=Mean value, n is equal to 6; *ratio of results obtained with new to that of reference method*

No statistical difference between the mean recoveries obtained with the two methods using the Student's t test was seen. Similarly, there was no statistical difference in their variances as estimated by the F test. This establishes that the new method can serve as a suitable alternative to the reference method as there are no statistical differences in their accuracy and precision.

CONCLUSION

A new fast colorimetric method for the assay of tranexamic acid in dosage forms has been developed following the parameterization of the charge-transfer complex formed between the drug and chloranilic acid
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Conflict of Interest: None declared
Received: June 29, 2018
Accepted: October 10, 2018