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Spectrophotometric Determination of Benzethonium Chloride Using Some Chromotropic Acid Azo Dyes

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FIVE simple, sensitive, rapid and accurate spectrophotometric methods were developed for the determination of benzethonium chloride in its pharmaceutical formulation. The proposed methods are based on ion-pairs formation of the drug with some chromotropic acid azo dyes such as chromotrope 2R (C2R), chromotrope 2B (C2B), arsenazo (I) ARZO (I), SPADNS, and arsenazo III ARZO (III) and subsequent extraction into methylene chloride. The extracts have maximum absorbance at 530, 520, 540, 520, and 520 nm, respectively. The method of solvent extraction showed good sensitivity and wide linearity over a concentration range of 8.96-89.62, 4.48- 44.81, 8.96-89.6, 4.48-44.81 and 4.48-44.8 µg/ mL. Limits of detection 0.62, .77, 0.52, 0.14, and 1.07 µg/mL for C2R, C2B, ARZO I, SPADNS, and ARZO III, respectively. They were also used for the analysis of the antibacterial spray Dermoplast® with excellent recovery (95.88-102.45%) and relative standard deviation values (0.49-2.68%). The statistical analysis of the obtained data was performed.

Keywords: Benzethonium Chloride; Chromotropic Acid Azo Dyes, Spectrophotometric Analysis, Extraction.

Introduction

Benzethonium chloride (BT.Cl) [($C_{27}H_{42}ClNO_2$) (CAS number: 121-54-0)] is Benzyldimethyl (2-{2-[4-(2,4,-trimethylpentan-2-yl)phenoxy} ethyl) azaium chloride [1], Scheme 1.

Benzethonium chloride (BT.Cl), is also known as hyamine, is a synthetic quaternary ammonium salt. It is an odorless white solid that is soluble in water, and has surfactant and antimicrobial properties [2]. It is used as topical antimicrobial agent in aid antiseptics [3]. BT.Cl is found in cosmetics and toiletries such as mouthwashes, and anti-itch ointments, antibacterial moist towelettes [4] and as a hard surface disinfectant [5,6]. It is also found in several grapefruit seed extract preparations and can be used as a preservative [7] such as in the anesthetic ketamine® [8]. It was identified as a novel specific anti-cancer agent by using a cell-based small molecule screen [9].

Several methods have been reported for BT.Cl determination. They include spectrophotometric [10-15] chromatographic [16-21], electrometric [22-25], and ion-selective sensor [26-29].

The aim of the present work is to propose new spectrophotometric methods for determination of BT.Cl in its pure form and pharmaceutical formulation based on the formation of new ion-pairs with C2R, C2B, ARZO (I), SPADNS, and ARZO III. The methods are based on solvent extraction of the formed product in methylene chloride. Analytical conditions and parameters have been investigated to choose the optimum analytical conditions for the application of the proposed methods.

At the end of the work, we suggested easy, fast, accurate and low-cost methods for the determination of the analyte under study in pure

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solutions and pharmaceutical formulations. The methods gave high recovery values and low LOD, LOQ and Sandell's sensitivity and %RSD values indicating the high accuracy and precision of the proposed methods.

Experimental

Apparatus

All the spectral measurements were carried out using a Jenway 6300 UV/Vis single beam spectrophotometer with a range of 200-1000 nm equipped with quartz cells of 1 cm optical path length. A micro-burette was used for measuring the solutions volumes and 60 mL separating funnels were used in case of organic solvents extraction. A Scientech SA210 digital balance was used for weighing throughout the study.

Material and Reagent

All reagents and chemicals used in this work are pure analytical grade reagents. Double distilled water was used to prepare all solutions. BT.Cl drug, the reagents C2R, C2B, ARZO I SPADNS and ARZO III were purchased from (Sigma-Aldrich), and the extracting solvent (methylene chloride), was provided by the El-Naser Pharmaceutical Chemical Company Egypt. Authentic samples of the pharmaceutical preparation Dermoplast®

Antibacterial spray (20% benzocaine+0.2% BT.Cl. were prepared in the laboratory.

benzethonium chloride (BT.Cl) stock solution1.0×10^{-2} mol L^{-1}) was prepared by dissolving an accurately weighed 0.448 g of the pure solid in double distilled water, the solution was transferred into 100 mL volumetric measuring flask and made up to the mark by using double distilled water.

Standard stock solutions of 1.0×10^{-2} mol L^{-1} of C2R, C2B, ARZO I, SPADNS, and ARZO III were prepared by dissolving accurately weighed 0.5704, 0.4683, 0.6142, 0.5133, 0.8203 g, respectively, in 100 mL measuring flask using double distilled water.

Analytical Procedures

Aliquots of the working standard BT.Cl solutions 1×10^{-3} mol L^{-1} were added to the separating funnels and 5.0 mL of the same concentration of the reagents (1×10^{-3} mol L^{-1} for ARZO (I) and C2R while a concentration of 5×10^{-4} mol L^{-1} was used in case of the remaining reagents) 10 mL of Methylene chloride were added to the mixture and the solutions were shaked vigorously for 3 min. The organic phase was separated and transferred into 10 mL measuring flask and completed with methylene chloride. Subsequently, the absorbance values were measured at the selected absorption maxima of each reagent against a blank solution prepared in a similar manner.

Results and Discussion

Wavelength selection

The absorption spectrum of the BT.Cl solution does not display any absorption maxima in the visible region, the extracts show formation of ion-pairs between drug and some chromotropic acid azo dyes, the wavelengths of complexes were selected for subsequent measurement throughout the work: 530 (C2R), 520, (C2B) 520 (ARZO (I)) 520 ,SPADNS, and 540 ARZO (III)), Fig. 1

Effect of reagent concentration

In spectrophotometric titration, the reagent should be added in excess(5 ml of 1×10^{-3} mol L^{-1}) to make sure that all of the analyte in the sample solution reacts and gives out the measurable colored product. In this case, the amount of reagent may affect the final absorbance measurement. Therefore, the added reagent concentration should be optimized. Fig. 2 shows that the absorbance of the product increases with increasing the added amount of the reagent until it reaches a plateau. From this figure, we can notice that 5 mL of all reagents may be sufficient for complete reaction and color measurement with 5 mL of 1×10^{-3} mol L^{-1} concentration of the analyte.

Effect of time

Time is an important parameter in spectrophotometric analysis because of variations in the rates of chemical reactions. In this work, we investigated the effect of standing time on the intensity of color formation (i.e. concentration of the formed product. The obtained results, shown in Fig. 3, indicate that time has no significant effect on color formation because the absorbance of the product is almost constant with time. Accordingly, absorbance can be measured directly after solutions preparation and without a certain standing time.

Reaction stoichiometry

Job's method of continuous variation was used to investigate the reaction stoichiometry. In this method, the total concentration of the solution is kept constant while simultaneously varying the amounts of the analyte and the reagent. A plot

of the absorbance against the drug mole-fraction in the formed colored product gives information about the reaction stoichiometry. In addition, the molar ratio method was also used to confirm the results obtained from Job's method of continuous variation. In this method, one of the reactants is kept constant and the other is varied. The results in Fig. 4 show that all reactions proceed by 1:1 and 2:1 (reagent: drug) stoichiometry.

Benesi-Hildibrand method

Benesi-Hildebrand method [30] is a well-known spectroscopic method which is widely used for the determination of equilibrium constants K and Stoichiometry of non-bonding interactions. This method has been typically applied to reaction equilibrium that form one to one and one to two complexes. This method depends on the fact that one of the reactants (usually the colorless reactant) is put in a concentration which is about 10 times (or more) higher than that of the colored reactant. Moreover, one of the reactants is kept constant and the other is varied continuously. Generally the Job's method [31] which has been used to study the equilibrium in solution of complex compounds is more convenient. However, the equation used for calculating for formation constant could be carried out to the result

of other spectrophotometric methods specially the molar ratio [31] the following equation [32] was used

$$K_f = (A/Am) / [1 - (A/Am)]^{n+1}{}_C{}^n\, n^n$$

Where (A_m) is the maximum absorbance obtained from Job's method of continuous variation, curve (A) is the absorbance corresponding to the interaction of the two tangents of the continuous variation curve (C) is the concentration corresponding to the maximum absorbance and (n) is the amount of the drug in the reaction product.

The Products summarized in table 1: indicates K-values in the table reflect the calculated equilibrium constants (K) for the formation of the colored stability of the formed products. It is obvious that the stability of the formed colored compounds lies in the order: C2R >

ARZO III > SPADNS > C2B >ARZO I.

Analytical parameters

Table 2 summarizes the experimental analytical parameters obtained from our study using the investigated reagents. It is worth noting that, the measurable colored products have high molar absorptivity and low LOD and LOQ values indicating high sensitivity of the proposed methods. In addition to the high sensitivity of the proposed methods, wide linear ranges were obtained enabling easy and fast application of the proposed methods to a wide range of samples. Sandell's sensitivity was also calculated to further confirm the high sensitivity of the proposed methods.

Recovery studies

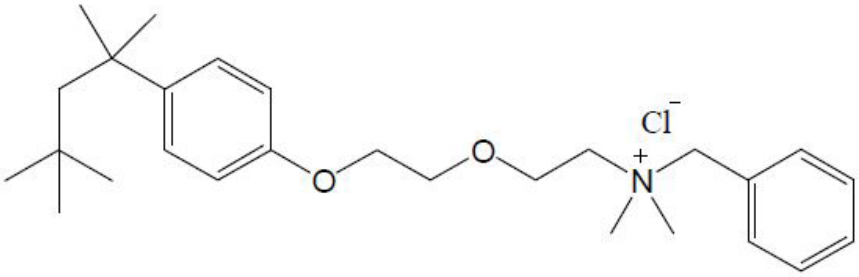
Calibration curves (Fig. 6) were constructed, as previously mentioned in the experimental section, which show that the suggested methods have wide linear ranges and low limits of detection. Ringbom plots (Fig. 7) were obtained from calibration graphs by plotting the % transmittance against the normal logarithm of BT.Cl concentration. They are used to determine the linear range of Beer's law which is free from instrumental errors. Our methods have wide Ringbom ranges (Table 1) that facilitate the accurate estimation of the analyte over a wide range of concentrations without experimental errors. Thereafter, the proposed methods were successfully applied for the determination of BT.Cl in pure solutions and Dermoplast® antibacterial spray (20% benzocaine + 0.2% BT.Cl). Different amounts of the formulation were used cover a wide concentration range. The obtained recovery values (Table 3: shows the recovery studies on Dermoplast® antibacterial spray) indicate that the proposed methods are very accurate (recovery range from 95.88 to 102.45 %) and precise (RSD values range from 0.4 to 2.68%). The statistical treatment of the analysis proposed methods were performed.

Statisticail Analysis

The proposed methods are successfully applied for the determination of BT.Cl in its dermoplast® antibacterial spray, the proposed methods were compared with the reference method [35], table 5 shows the results of t-test and F-test is less than critical value that indicates no significant difference between the proposed methods and the reference method. The proposed methods were more accurate with high recovery than the reference method, so the proposed method can be recommended for routine analysis of drug.

Conclusion

In this work, five spectrophotometric methods were proposed for the accurate, precise, fast and



Scheme 1. Structural formula of BT.Cl

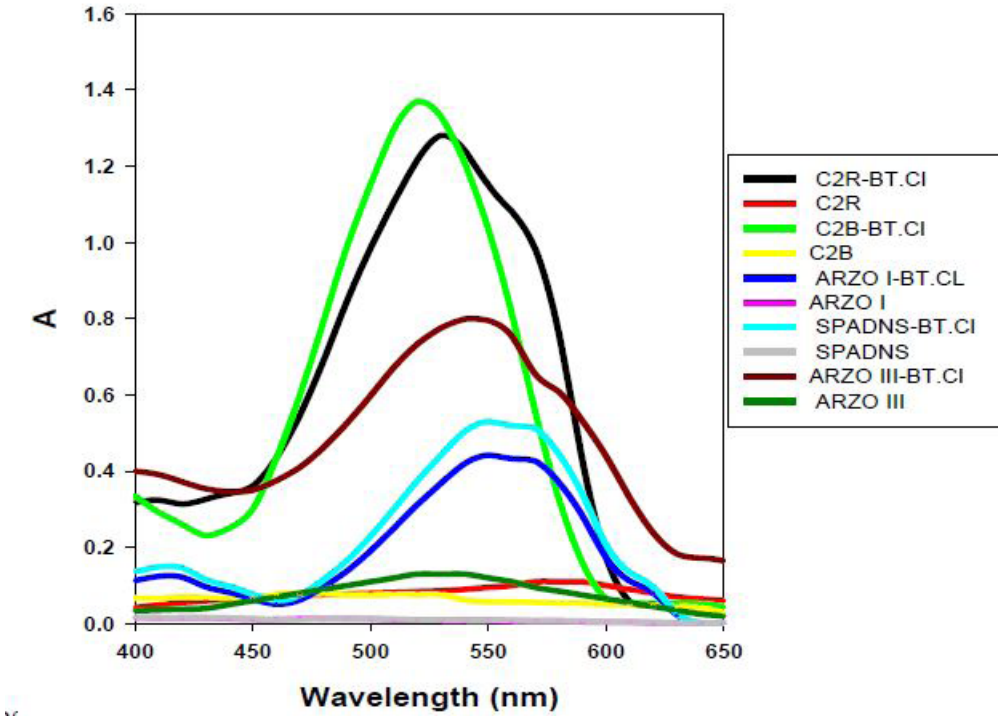
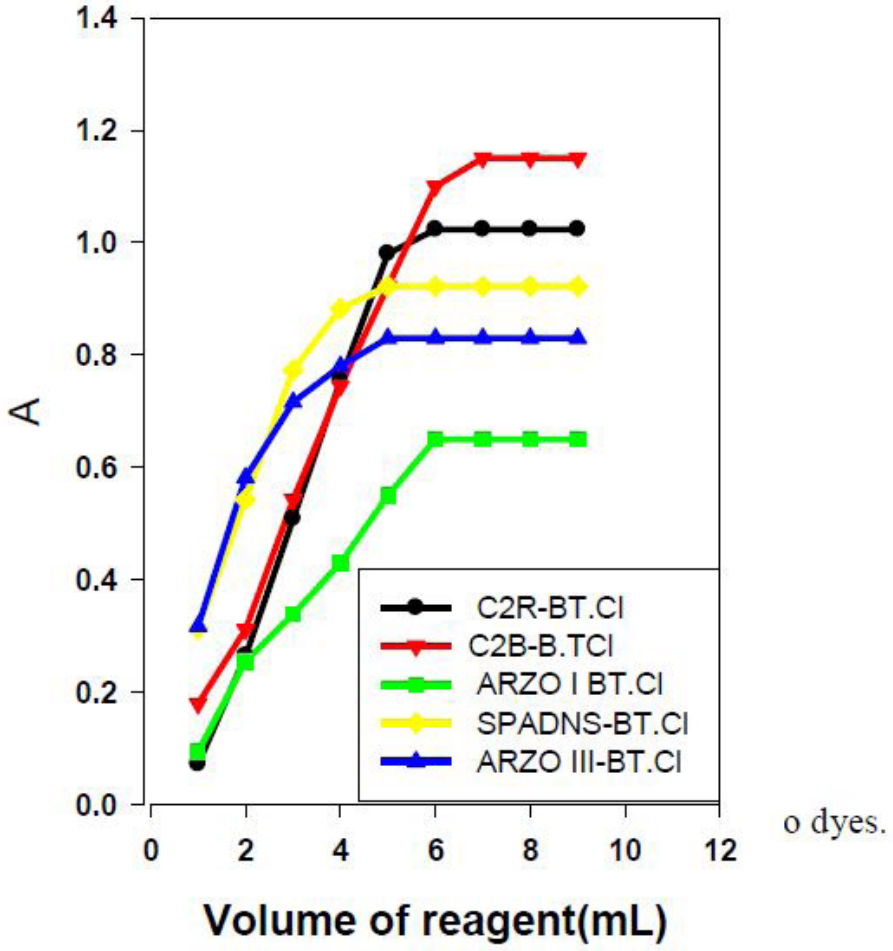


Fig. 1. Electronic absorption spectra of the formed ion-pairs .



o dyes.

Fig. 2. Effect of reagent concentration on the color change of the formed azo dyes.

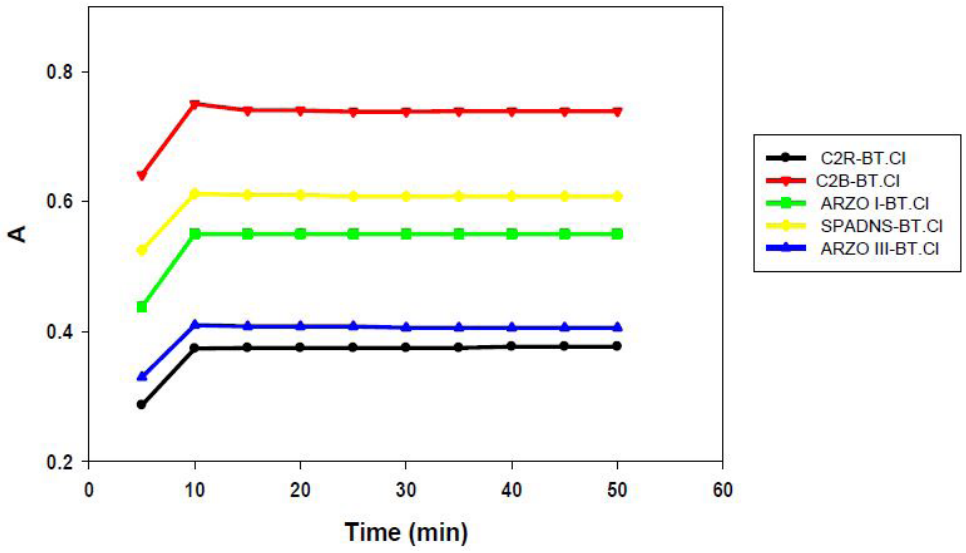


Fig. 3. Effect of standing time on the obtained color intensity.

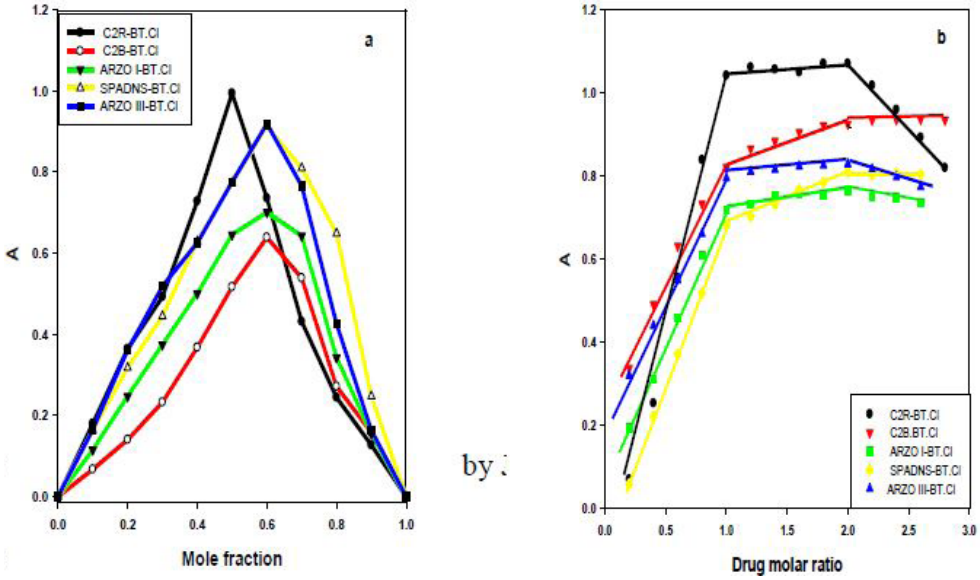


Fig. 4. Investigation of reaction stoichiometry by Job's method (a) and molar ratio method (b)

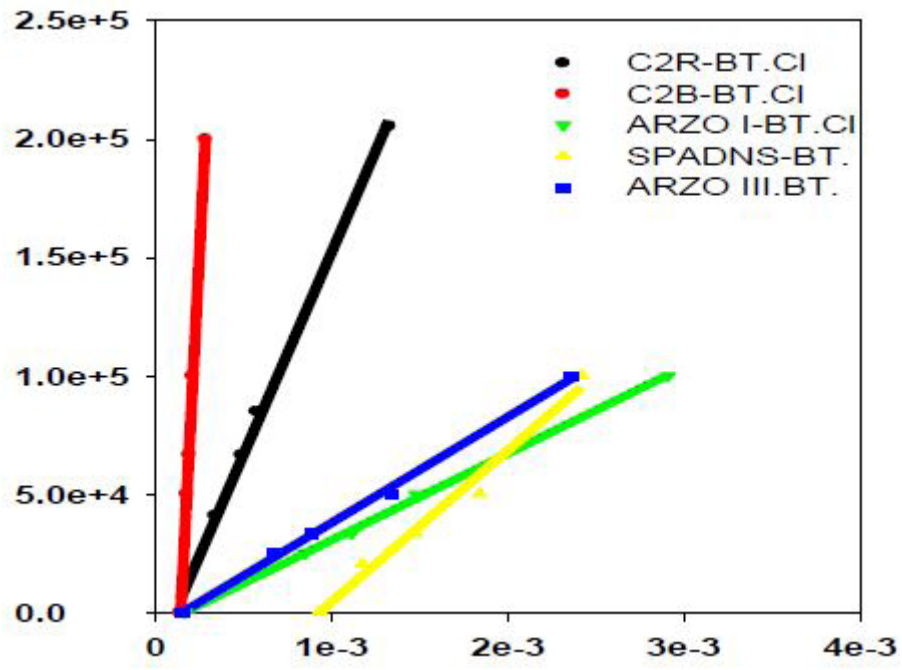


Fig. 5. Benesi-Hildebrand plots for the five azo dyes under investigation

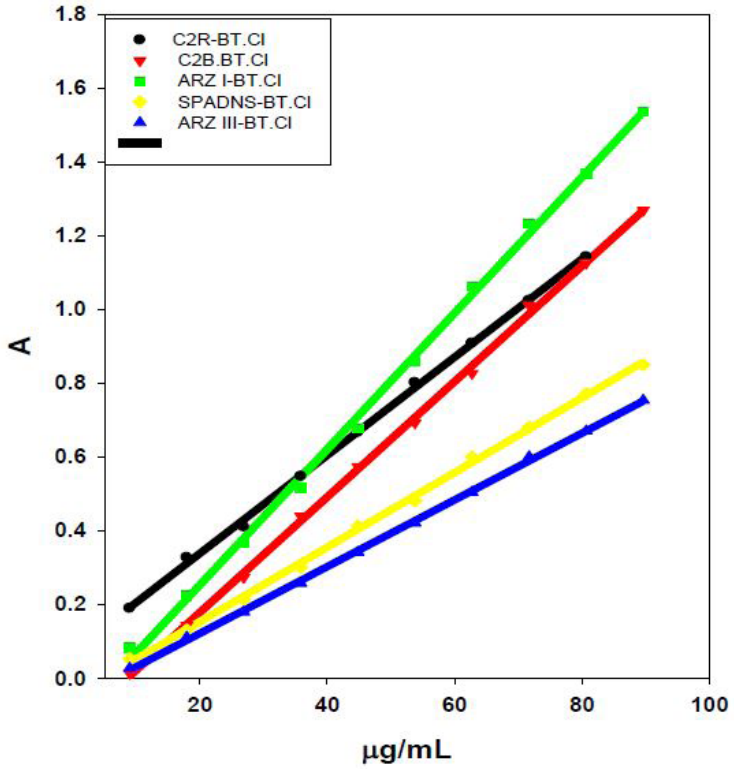


Fig. 6. Calibration curve of ion-pairs complexes

1.

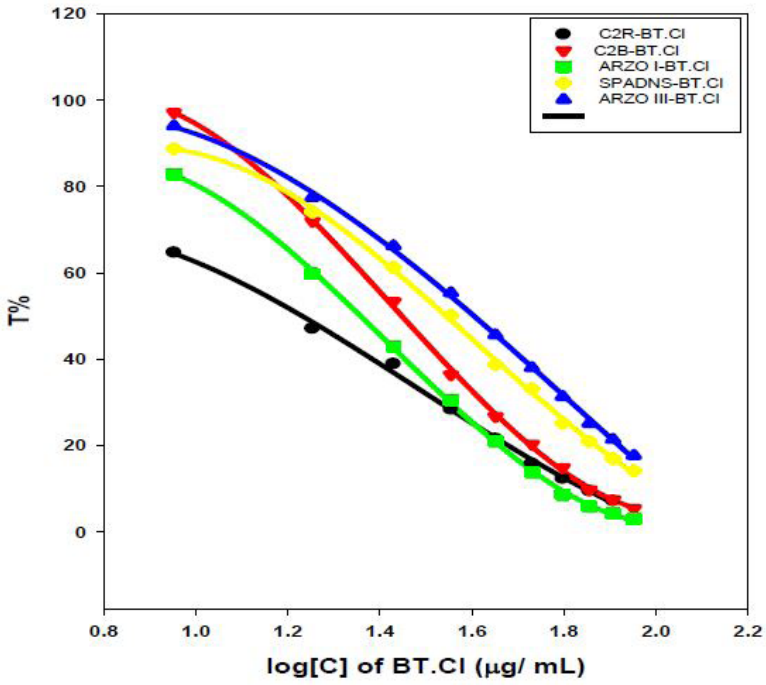


Fig. 7. Ringbom curve plots [34]

TABLE 1. Associate, formation Constant and free energy change for BT.CL-ion-pairs.

#

Parameter	Methods				
	C2R	C2B	ARZO I	SPADNS	ARZO III
K_c^{AD}(L mol^{-1})	10^3×0.71	10^3×2.07	10^3×6.12	10^3×6.22	10^3×5.74
K_f(L mol^{-1})	10^6×3.34	10^6×1.43	10^5×2.63	10^6×1.55	10^6×1.84
ΔG^o (kcal. $mo1^{-1}$)	-8896.33	-8393.82	-7391.01	-8441.54	-8543.12

TABLE 2. Analytical parameters of the proposed methods

Parameters	Methods				
	C2R	C2B	ARZO I	SPADNS	ARZO III
λ_{max}(nm)	530	520	520	520	540
Beer's Law limit (µg mL)	8.96-89.62	4.48-44.8	8.96-89.6	4.48-44.8	4.48-44.8
Ringbom (µg mL^{-1})	11.58-81.7	6.9-35.8	13.0-53.7	8.96-44.8	13.44-44.7
Molar absorpt-ivity (L.mol/ cm)	10^3×5.7	10^4×1.2	10^3×7.9	10^3×8.11	10^3×7.2
Sandell sensiti-vity (µg cm^{-2})	10^{-5}×1.3	10^{-5}×2.9	10^{-5}×1.7	10^{-5}×1.9	10^{-5}×1.7
LOD (µg m^{-1})	0.62	0.77	0.52	0.14	1.07
LOQ (µg mL^{-1})	2.09	2.58	1.74	0.45	3..58
Intercept	0.013	-0092	-0.078	-0.034	-0.040
Slope (b)*	0.013	0.029	0.017	0.019	0.017
Correlation coefficient (r^2)	0.996	0.992	0.995	0.996	0.995

TABLE 3. Evaluation of accuracy of the formulation dosage

Taken (µg)	Found (µg)	Recovery±SD Dermoplast antibacterial spary	RSD%
C2R			
19.98	19.27	96.42±1.23	1.28
39.97	39.71	99.35±0.88	0.89
49.97	50.15	100.37±0.89	0.90
C2B			
9.99	9.870	98.77±1.83	1.85
14.99	14.75	98.39±1.19	1.20
24.98	24.67	98.76±1.15	1.16
ARZO I			
19.98	19.87	99.44±1.02	1.91
39.97	39.03	97.66±1.46	1.64
49.96	34.86	98.47±2.5	2.27
SPADNS			
14.99	14.16	97.47±1.73	1.77
24.98	24.47	97.97±0.98	1.00
34.97	34.86	99.74±0.77	1.93
ARZO III			
19.98	19.16	95.88±1.70	1.77
24.98	24.19	96.82±1.77	1.82
29.98	29.60	98.75±2.63	2.68

Table 4. Statistical treatment of results

Method	Recovery± SD %	t- value	F-value	Recovery ±SD% for the reference method
C2R	100.37± 0.89	3.03	0.09	98.54± 1.34
C2B	98.77 ± 1.15	1.58	0.25	98.31± 0.80
ARZO I	99.44 ± 0.96	0.8	0.5	98.54 ± 1.34
SPADNS	99.74 ± 0.77	2.58	0.12	98.31 ± 0.80
ARZO III	98.75±2.63	0.15	0.89	98.81 ± 1.58

*Mean ± standard deviation three replicate analysis

TABLE 5. Some spectrophotometric determination of BT.Cl

Methods	Reagent	Solvent	λ_{max} (nm)	concentration Range	Ref.
Vis/Spectro (Extraction)	Tetrabromophenolphthaline Ethyl Ester	Ethanol	615	10^{-6} M×4	10
Vis/Spectro (Extaction)	Bromocresol green	Ethanol	630	1-5 ×10^{-6} M	11
Vis/ Spectro (Extraction)	Tetrabromophenolphthaline Ethyl Ester	Ethanol	610	10^{-6} –×0.5 3.0×10^{-6} mol. dm^{-3}	12
Vis/ Spectro (Extraction)	Bromophenol blue	Ethanol	610	0.5-20 micro M.	13
Direct Spectrophotometric assay	Bromothymol blue	Ethanol	615	0-300 µg/mL	14
Vis/Spectro (Extraction)	Chromotrope 2B	Double distilled water	524	-----	15

affordable determination of the antibacterial compound benzethonium chloride in pure form and pharmaceutical formulations based on the formation of chromotropic azo dyes C2R, C2B, ARZO I, ARZO III, and SPADNS. Different experimental conditions such as the effect of reagent concentration, reaction stoichiometry and the effect of standing time were investigated. In addition, the equilibrium constants for the formed azo dyes ion-pairs were calculated using Benesi-Hildebrand method. The formed ion-pairs have high formation constants indicating their stability over a long period of time enabling flexible measurement of the formed color. The suggested methods have wide Beer's law and Ringbom ranges, low LOD (0.14-1.07 µg/mL) and LOQ (0.45-3.58 µg/mL) values and low RSD values (0.49-2.68%). The methods were successfully applied for the determination of different concentrations of BT.Cl in Dermoplast® antibacterial spray with excellent recovery (95.23-102.45%) were the developed methods used for routine quantitation of BT.Cl in pharmaceutical and formulation.

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