Conductometric Titration for Determination of Lomefloxacin Hydrochloride and Solubility Products of its Ion-Associated Complex Species

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ABSTRACT

This work describes development and validation of conductometric method for determination of lomefloxacin hydrochloride with two precipitating reagents; ammonium reineckate and sodium tetraphenylborate. The formed ion pair complexes were studied conductomterically for the determination of solubility products and other functions associated with the process of lomefloxacin hydrochloride precipitation. In this study, data processing was performed using numerical derivatization and Boltzmann algorithm. The described procedures allowed the determination of lomefloxacin hydrochloride within the range of 2-16 mg using both reagents. These methods were validated and successfully applied for the determination of lomefloxacin hydrochloride in Lomex® tablets. The obtained results werestatistically compared with those of the official method by applying *t*-test and *F*-value at 95% confidence level and no significant difference was observed regarding accuracy and precision.

INTRODUCTION

Lomefloxacin hydrochloride is chemically known as *(RS)*-1-ethyl-6,8-difluoro-1,4-dihydro-7-(3-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic acid hydrochloride, Figure 1. Lomefloxacin hydrochlorideis a white powder soluble in water and methanol with 387.81 molecular weigth[1]. It is a non-official drug which is known as a fluorinated 4-quinolone or fluoroquinolone antibacterial with a wider spectrum of activity than nalidixic acid and more favorable pharmacokinetics allowing its use in systemic infections. It has been used in the treatment of infections including bone and joint infections, gastro-enteritis (including travelers' diarrhea and campylobacter enteritis, cholera, salmonella enteritis, and shigellosis), gonorrhea, infections in immunocom promised patients (neutropenia), Q fever, lower respiratory-tract infections , typhoid and paratyphoid fever[2]. Literature survey reveals that many HPLC methods were reported for determination of lomefloxacin hydrochloride in pharmaceutical preparations and biological fluids [3-11]. Also atomic absorption [11], spectrophotometric [12-19], spectrofluorimetric [20-24] and electrochemical [25-30] methods were reported for determination of lomefloxacin hydrochloride alone or in presence of other fluoroquinolone antibiotics. The aim of the current studyis to use the differential conductivity methods and Boltzmann sigmoid
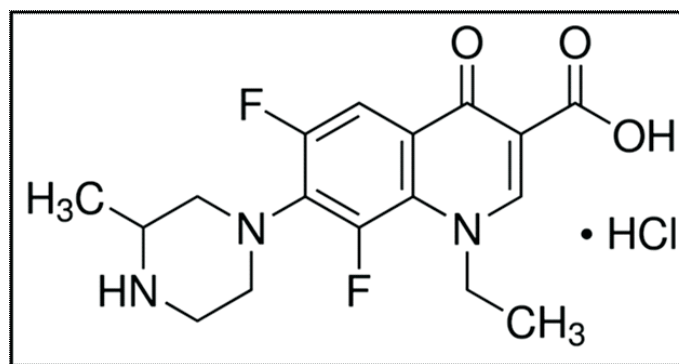

Fig. 1 : Structural formula of lomefloxacin hydrochloride.

function "without integration" for locating the equivalence point. The first derivative data were fitted to a built-in nonlinear regression model while approximations to Gaussians of the second derivative data were followed to locate the endpointand Boltzmann models with a perspective of avoiding the uncertainty resulting from locating the endpoint as the break in the conductance-volume curves. In addition, we depend on the data obtained from conductometric titration of lomefloxacin hydrochloride to calculate the solubility product of the formed ion associates and hence the equilibrium formation constant of the

investigated reactions.

MATERIALS AND METHODS

2.1. Instruments:

Jenway model 4510 Conductivity / TDS Meter (451001990) with a dip type conductivity cell of two platinum (non-polarized) electrodes of 1.0 cm^2 in area, rigidly fixed at 1.0 cm apart manufactured in the EU by Barloworld Scientific Ltd, Dunmow, Essex, CM6 3LB.

Elementar-Vario El (Germany) was used for elemental analysis of the ion pairs.

FT-IR, Nicolet IR 200 (Thermo electron corporation, USA).

Bandelin sonorox, Rx 510 S, magnetic stirrer (Hungarian).

Microcal Origin 8.0 (Microcal Software Inc., version 8E) computer program was applied in data treatment for graphical and statistical treatments and calculations.

2.2. Materials and reagents:

All reagents used were of analytical grade, water used throughout the procedure was freshly distilled and deionized.

Pure sample;lomefloxacin hydrochloride (99.65 %) was kindly provided by Sigma pharmaceutical industries - Quesna City Egypt, S.A.E.

Pharmaceutical preparation; Lomex® tablet : labeled to contain 442 mg lomefloxacin hydrochloride per tablet equivalent to 400 mg lomefloxacin, manufactured by Sigma pharmaceutical industries - Quesna City Egypt, S.A.E.; (batch number 40357), purchased from local market.

Ammonium reineckate $NH_4[Cr(NH_3)_2(SCN)_4]$ and sodium tetraphenylborate $NaB(C_6H_5)_4$, (Sigma-Aldrich, Germany), prepared as 1 mg/ml and (10^{-5} to 10^{-2} M) aqueous solutions.

Petroleum ether, (Sigma-Aldrich, Germany).

2.3. Standard solutions:

Standard solutions of 1mg/ml and 10^{-2} M of lomefloxacin hydrochloride were prepared by dissolving 100 mg and 387.8 mg of the drug powder, respectively in 80 ml of distilled water and complete to 100 ml with the same solvent. Different working solutions of varying strengths from (10^{-5} to 5×10^{-3} M) were prepared by suitable dilution from the standard solution with distilled water.

2.4. Procedures:

2.4.1. General procedure:

Aliquots (2-16 ml) of lomefloxacin hydrochloride standard solution (1mg/ml) containing 2-16 mg of the drug were transferred into the titration cell and the volume was made with water up to 50 ml. The conductivity cell was immersed in and the solution was titrated with (5×10^{-3} M) of the titrant. The conductance was measured 2 minutes subsequent to each addition of the reagent after thorough stirring.The measured values were corrected for volume change to eliminate the effect of dilution on the increase in conductance by means of the following equation, assuming that conductivity is a linear function of dilution:

$$k_{corr} = k_{obs}[(v_o + v_{added}) / v_o]$$

Where, k_{obs}, the observed specific conductivity, v_o, the initial volume, and v_{added}, the added volume. The corrected conductivity

was then plotted against the volume added of titrant and the second derivative or Boltzmann sigmoid method were used to estimate the end point and the stoichiometric ratios [31,32]. The nominal content of the compound under study was calculated using the following equation:

Amount of the drug (mg) = VMR / N

where V, volume (ml) of the titrant consumed in the titration, M, relative molecular mass of the analyte, R, molarity of the titrant, and N, number of moles of the titrant consumed per one mole of the analyte.

2.4.2. Procedure for stoichiometric ratios determination:

A definite volume (5 ml) of 5×10^{-3} M lomefloxacin hydrochloride solution was transferred to a 50 ml volumetric flask and made up to the mark with distilled water. The drug solution was placed in a suitable titrating vessel and the conductivity cell was immersed, then a titrant of 5×10^{-3} M was added from a burette. The solution was stirred for 1-2 min and allowed to attain equilibrium and the end point was determined as mentioned under general procedure.

2.4.3. Preparation of ion-associates:

Ion-associates synthesis protocol included addition of 50 ml of 10^{-2} M aqueous solution of ion pairing agents (ammonium reineckate and sodium tetraphenylborate) drop wise to 50 ml of 10^{-2} M lomefloxacin hydrochloride solution. The mixture was left to react for 60 min under stirring at room temperature. The resulting precipitate was then filtered off on Whatman filter paper and washed several times with distilled water. The compound was left to dry for 12 hours at 60°C, washed with petroleum ether to remove anyresidual moisture, and then ground to fine powder [33,34]. Small sample portions were sent to elemental analysis and IR.

### 2.4.4. Procedure for solubility products and other constants determination:

Series of solutions of different concentrations (C = 10^{-5}-10^{-2} M) were prepared for each of lomefloxacin hydrochloride, ammonium reineckate and sodium tetraphenylborate. The conductivities of these solutions were measured at 25°C and the specific conductivities (k), corrected for the effect of dilution were calculated and used to obtain the equivalent conductivities (λ) of these solutions.

$$= 1000 \, k / C$$

(at a finite concentration) and λo (at infinite dilution) can be related by Onsanger equation [35]:

$$\lambda = \lambda o \ (a + b \, \lambda o) \, C^{1/2}$$

Where, (a) and (b) are constants related to the interionic forces (accounting for the electrophoretic and the time of relaxation effect, respectively). Kohlrausch's law of the square root of concentration predicts a nonlinear relation between conductivity and concentration at a lower concentration range. Straight line plots of λ versus $C^{1/2}$,were constructed and the equivalent conductance values at infinite dilution (λo) for lomefloxacin hydrochloride, ammonium reineckate and sodium tetraphenylborate were determined from the intercept of the respective line with the λ axis.The activity coefficients were taken as unity since the solutions were sufficiently dilute. The equivalent conductance values of the IPs under complete dissociation condition (λo) for ion pair were calculated from
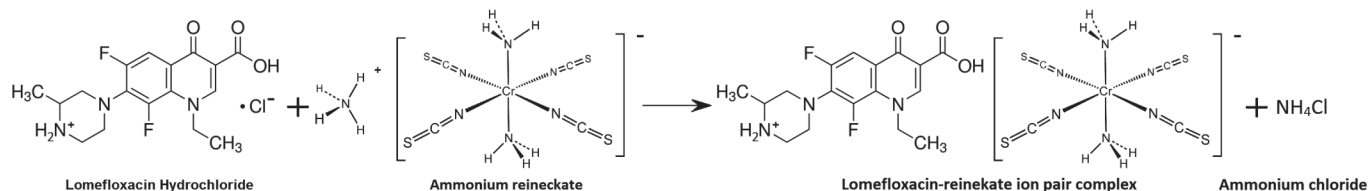
Fig. 2 : Proposed reaction pathway of lomefloxacin hydrochloride and ammonium reineckate.
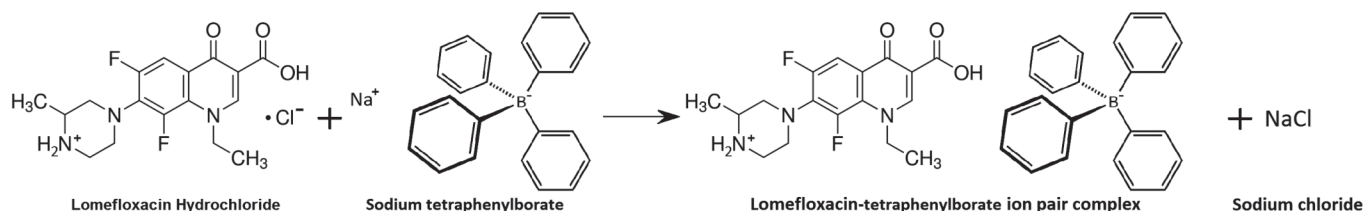
Fig. 3 : Proposed reaction pathway of lomefloxacin hydrochloride and sodium tetraphenylborate.

Kohlrausch's law of independent migration of the ions [35-37].

λo (Ion pair) = n λo (Drug) + λo (Ion pairing agent)

Where; n is the stoichiometric ratio.The solubility (S) and the solubility product (Ksp) of a particular ion associate were calculated using the following equations:

S = k$_s$ × 1000 / λo (Ion pair)

Ksp = S^2 for 1: 1 ion associate

Where k$_s$, is the specific conductivity of a saturated solution of the ion associate, at 25°C. The saturated ion pair solutions were prepared by stirring the ion pair suspensions in distilled water for 5 hours and then left for 24 hours before measurement [38].

2.4.5. Procedure for pharmaceutical preparation:

Ten Lomex®tablets (442 mg/tablet) were weighted and finely powdered. Appropriate weight of powder equivalent to 100 mg of lomefloxacin hydrochloride was accurately weighted, transferred to 100-ml volumetric flask and the volume was made up to 75 ml with distilled water. The solution was shaken vigorously for 15 min then sonicated for 30 min and then filtered. The volume was completed to 100 ml with water to obtain a concentration of 1 mg/ml. The content of the tablets was determined using aliquots covering the working concentration range by repeating the general procedure

RESULTS

Lomefloxacin hydrochloride is a secondary amine cation having a high affinity towards the formation of water insoluble ion pair complexes with the oppositely charged anions such as ammonium reineckate or sodium tetraphenylborate. Elemental analysis and stoichiometric ratio determination revealed that lomefloxacin hydrochloride form ion association with ammonium reineckate and sodium tetraphenylborate in a stoichiometric ratio of 1: 1 (drug: titrant) as shown in Table 1. The following equation describes the reaction mechanism and the expected structure of the ion pair as shown in Figures2 &3:

Conductance measurements have been used successfully in quantitative titration systems where the conductance of the solution varied prior t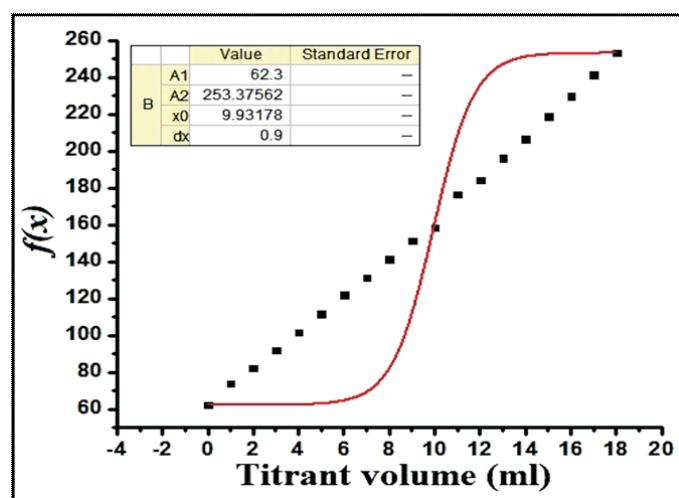o and after the equivalence point. The titration curve, obtained by plotting the change in conductance versus volume of titrant added, represented two straight lines intersecting at the end point.The first segment corresponds to the formed precipitate and the second segment represents the excess of ion pair agent as shown in Figure 4. In the present study, two proposals were considered. The first plan depended on numerical derivatization of the raw data, while in the second, experimental figures were well suited to a Boltzmann sigmoid model. The mathematical differentiation of the obtained conductivity data against the corresponding titrant volume was one of the suggested schemes as shown in Figures5 & 6. Applying the second derivative mode, the endpoint was located at the curve intersect with zero crossing point and as defined by the fitting parameters while in the first derivative, the endpoint was located at the curve maximum which is inaccurate in comparison to second derivative. Alternatively, the process of numerical handling of data creates unusual behavior represented in a noisy first derivative sigmoid. Noticeably, this problem arises from consolidating the instinctive experimental errors. These errors, in turn, are amplified after the numerical processing. The other proposal, Boltzmann paradigm is based on the fit of the experimental raw data to a simple nonlinear function obtained by direct integration of a Boltzmann type sigmoidal function. Besides, being a nonlinear curve fit in most of software, Boltzmann type sigmoid provides a straight forward and simple correlation between the function parameters and the conductivity-volume curve characters. This model has been described by the following equation [39]:

$$f(x) = \frac{A1 - A2}{1 + e^{(X - X_0)/dx}} + A2$$

The parameters A$_1$ and A$_2$ stand for the asymptotic value for small and large values of x respectively, x$_o$ represents the endpoint and expressed as the central point of transition and dx deals with the width of the transition. Figures 7 & 8 show the determination of equivalence point applying Boltzmann type sigmoid. The mathematical expression of Boltzmann shows the simplicity of this model where the value of x$_o$ is simply obtained as $f(x_o) = $ (A$_1$+A$_2$)/2 .

Table 1 : Elemental analysis data for lomefloxacin-reineckate and lomefloxacin-tetraphenylborate ion pairs:

Ion pair complex	M.Wt calculated	C%		H%		N%		S%		Molecular formula
		calculated	found	calculated	found	calculated	found	calculated	found	
Lomefloxacin-reineckate	705.19	37.60	38.29	3.91	4.03	18.79	18.54	19.12	18.92	$[C_{17}H_{20}F_2N_3O_3]$ $[Cr(NH_3)_2(SCN)_4]$
Lomefloxacin-tetraphenyl borate	706.03	73.33	72.65	6.01	6.22	6.26	5.97	-----	-----	$[C_{17}H_{20}F_2N_3O_3]$ $[B(C_6H_5)_4]$
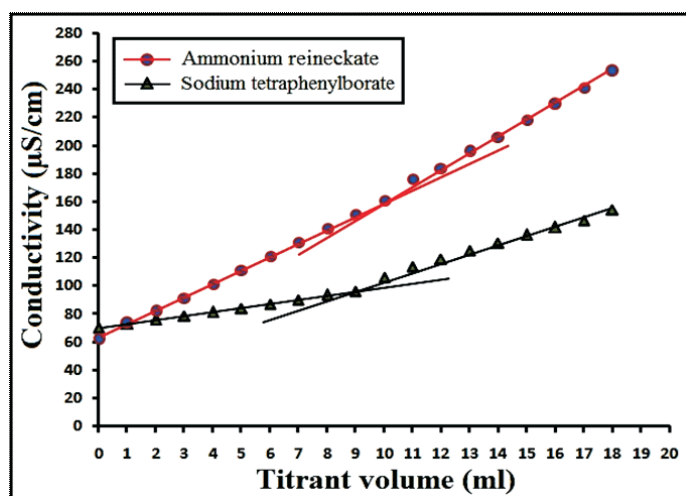
Fig. 4 : Conductometric titration curve of 10 mg lomefloxacin hydrochloride titrated with 1 mg/ml ammonium reineckate and sodium tetraphenyl borate by conventional procedure for locating the endpoint.
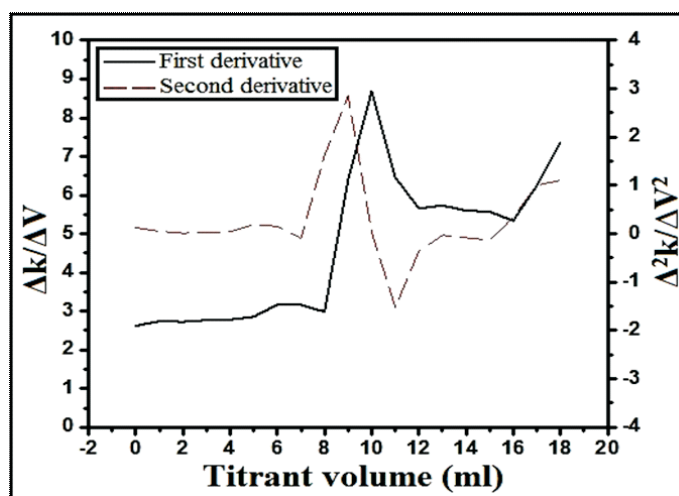
Fig. 6 : Conductometric titration of 10 mg lomefloxacin hydrochloride with 1 mg/ml sodium tetraphenylborate applying the numerical first derivative plot $(\Delta k/\Delta V)$ and numerical second derivative plot $(\Delta^2 k/\Delta V^2)$.
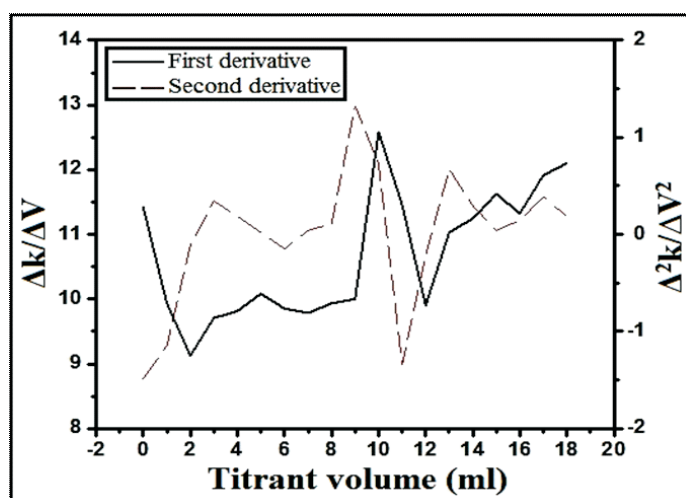
Fig. 5 : Conductometric titration of 10 mg lomefloxacin hydrochloride with 1 mg/ml ammonium reineckate applying the numerical first derivative plot $(\Delta k/\Delta V)$ and numerical second derivative plot $(\Delta^2 k/\Delta V^2)$.

Fig. 7 : Conductometric titration of 10 mg lomefloxacin hydrochloride with 1 mg/ml ammonium reineckate applying the Boltzmann sigmoid method f(x). Value of x0 stands for the equivalence point determined using Boltzmann model.
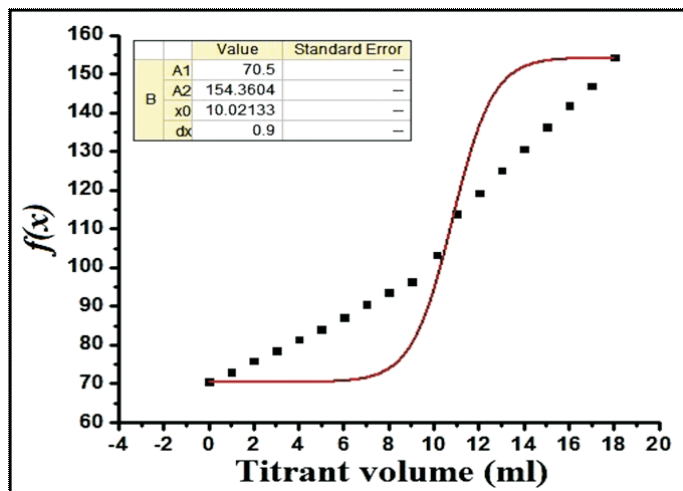
Fig. 8 : Conductometric titration of 10 mg lomefloxacin hydrochloride with 1 mg/ml sodium tetraphenylborate applying the Boltzmann sigmoid method f(x). Value of x0 stands for the equivalence point determined using Boltzmann model.

DISCUSSION:

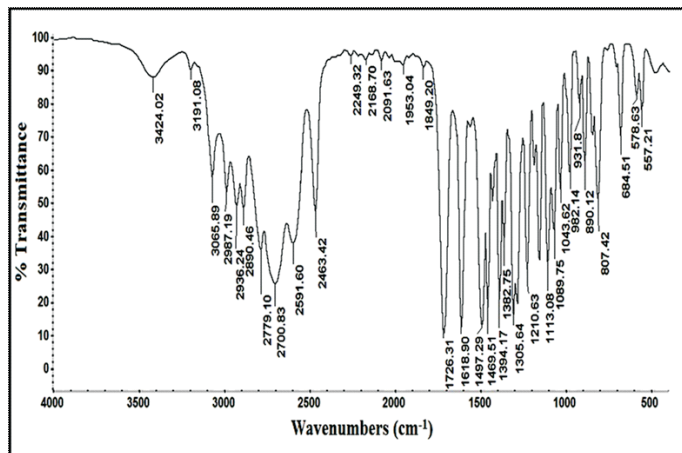### 4.1. IR spectra interpretation:

The IR spectrum of ammonium reineckate has a characteristic band at 2118 cm-1 due to v(CN) "in the Cr-NCS link" stretching vibration, a band at 703 cm^{-1} due to vsym (C-S) and at 495 cm^{-1} due to δ(NCS) deformation vibration [40].The IR spectrum of the formed ion associate shows a weak band corresponding to vCH (aliphatic) at 2997 cm^{-1}. The band corresponding to the stretching vibrations of C=O shifted to a lower frequency by ~ 35 cm^{-1}. In addition, the peak due to NCS is shifted to a lower frequency by 46 cm^{-1}. Peaks due to *vsym*(C-S) and *δ* (NCS) appear at 695 and 489 cm^{-1} respectively. The above IR interpretation indicates that an ion associate has been formed between lomefloxacin hydrochloride and ammonium reineckate. On the other hand, the band corresponding to the stretching vibrations of C=O in lomefloxacin hydrochloride was shifted to a higher frequency in ion associate complex between lomefloxacin hydrochloride and sodium tetraphenylborate by ~ 12 cm^{-1} and the strong band peak in sodium tetraphenylborate at 726 (four monosubstituted benzene rings attached to boron atom) was shifted to a higher frequency in ion associate complex by ~ 8 cm^{-1} and the band peak belongs to B-C at 1019 cm^{-1} was disappeared at an ion associate complex. The above IR interpretation indicates that an ion associate has been formed between lomefloxacin hydrochloride and sodium tetraphenylborate. Figures 9-13 show the IR spectra of the free ligands as well as the ion associate.

4.2. Determination of solubility products of the ion-associates:

The determination of solubility product of an ion-exchanger is very important since it is one of the main factors controlling the life span of the sensor incorporating it as electroactive material. The importance of solubility product determination lies in that its reciprocal is approximately equal to the formation constant, which in turns is tightly related to the degree of hydrophobicity of the ion pair. Since, as the hydrophobicity of the ion pair increases, the leaching rate into the aqueous bathing solution decreases [41-43].

According to Kohlrausch's law of independent migration of

the ions, the molar conductivity of an electrolyte equals the sum of the molar conductivities of the cations and the anions [44].The equivalent conductance (λ) of an ion is the conductance of a solution of unspecified volume containing one gram-equivalent and measured between electrodes 1 cm apart. Due to interionic effects, (λ) is concentration dependent, and the limiting ionic equivalent conductance (λo) at infinite dilution (no disturbing effect on the mobilities of ions other than solvent and temperature) reaches its maximum value and used for comparison purposes. The magnitude of (λo) is determined by the charge, the solvent viscosity, size and the magnitude of the applied potential. λo (Drug), λo (Ammonium reineckate) and λo (Sodium tetraphenylborate) can be determined from the intercept of the respective line with the λ axis from straight line plots of λ versus C$^{1/2}$ as shownin Figure 14.Hence, the equivalent conductance of the solvated ion pairs λo (Ion pair) at infinite dilution could be calculated as follow:

λo (Ion pair) = λo (Drug) + λo (Ammonium reineckate)

λo (Ion pair) = λo (Drug) + λo (Sodium tetraphenylborate)

The solubility products (Ksp) of the ion associates were determined conductometrically and found to be 7.19×10^{-12} and 3.82×10^{-12} for lomefloxacin-reineckate and lomefloxacin-tetraphenylborate, respectively as shown in Table 2. The very low solubility of lomefloxacin-tetraphenylborate ion pair (S = 1.96×10^{-6} M/L) and consequently, the high formation constant

Fig. 9 : IR spectrum of intact lomefloxacin hydrochloride on KBr disc.
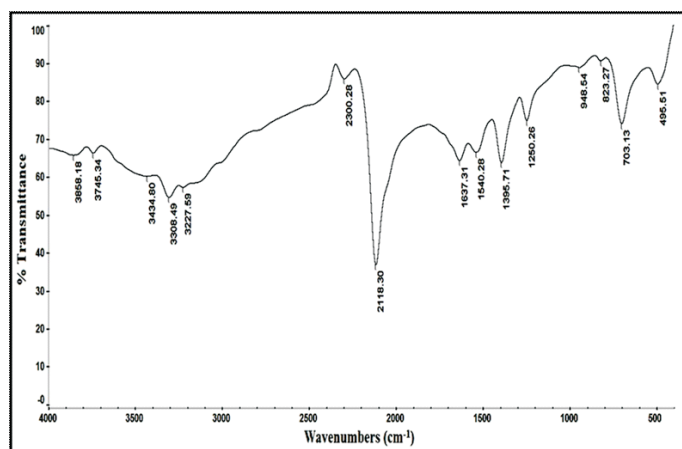
Fig. 10 : IR spectrum of ammonium reineckate on KBr disc.
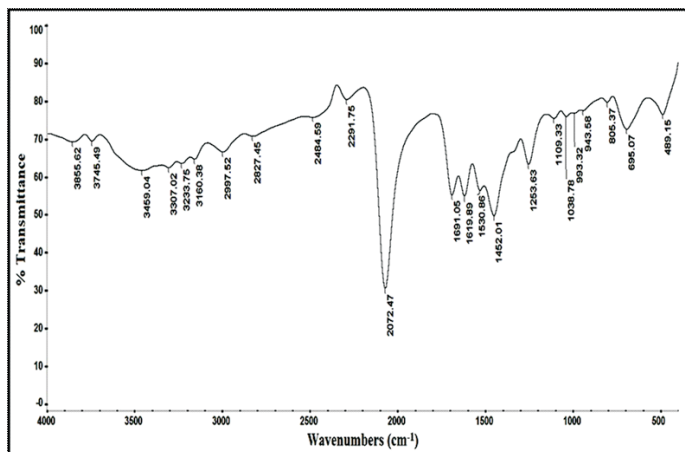
Fig. 11 : IR spectrum of lomefloxacin-reineckate ion pair complex on KBr disc.
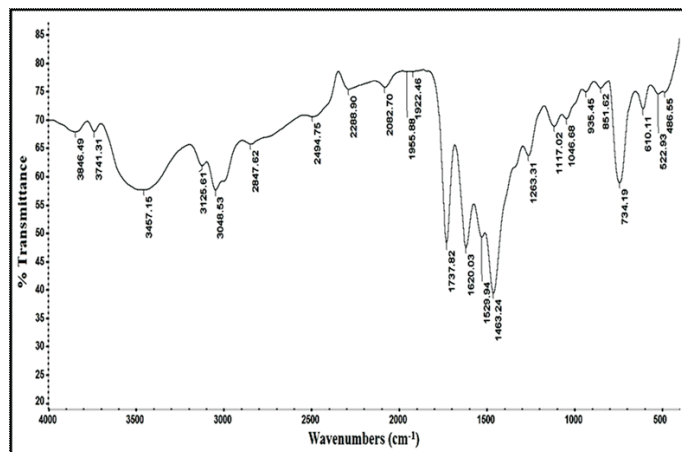
Fig. 13 : IR spectrum of lomefloxacin-tetraphenylborate ion pair complex on KBr disc.

Fig. 12 : IR spectrum of sodium tetraphenylborate on KBr disc.

Fig. 14 : Molar conductance against the square root of concentration C0.5 for lomefloxacin hydrochloride, ammonium reineckate and sodium tetraphenylborate.

value (k = 2.62×10^{11}), revealed that the degree of completeness of the reactionwas more than 99.9%. At equilibrium, the solubility product of the undissoci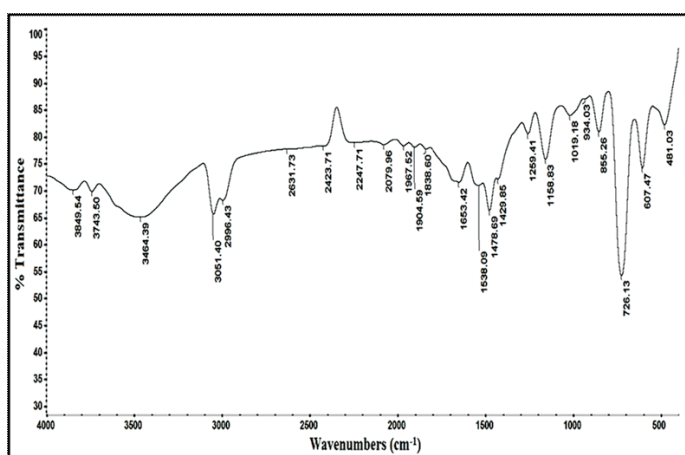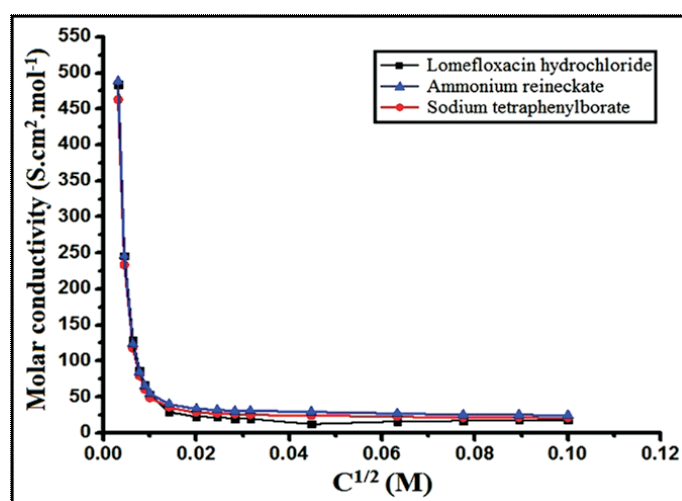ated ion pair in water (the intrinsic solubility) was omitted as this term makes a negligible contribution to the total solubility because the ion pairs were sparingly soluble in water and their saturated solutions were, therefore, very dilute [45].

METHODS VALIDATION

Validations of the proposed methodswere assessed as per the ICH guidelines [46].

Linearity and range:

In order to establish whether the proposed method exhibits any fixed or proportional bias, a calibration graph was constructed by plotting the observed drug concentrations versus the corresponding theoretical values in mg and the regression

equation was computed [47].The regression plots were found to be linear over the range of 2-16 mg using both reagents. The yielded statistical results are summarized in Table 3.

Limits of detection and quantitation:

The limits of detection (LOD) and the limits of quantitation (LOQ) were calculated according to ICH guidelines from the following equations:

$$LOD = 3.3\,\sigma / S$$

$$LOQ = 10\,\sigma / S$$

Where σ is the residual standard deviation of a regression line and S is the slope of the calibration curve. LOD and LOQ values

Table 2 : Conductometrically measured solubility (S), solubility products (KSP), and formation constants (k) of various ion-associates:

Ion pair complex	λ_oIP	K_S	S	K_{SP}	$K=1/K_{SP}$
Lomefloxacin-reineckate	1.60×10^9	4.29	2.68×10^{-6}	7.19×10^{-12}	1.39×10^{11}
Lomefloxacin-tetraphenylborate	1.56×10^9	3.05	1.96×10^{-6}	3.82×10^{-12}	2.62×10^{11}

Table 3 : Regression and validation data for the determination of lomefloxacin hydrochloride by the proposed conductometric method:

Parameters	Proposed method	
	Ammonium reineckate	**Sodium tetraphenylborate**
Linearity range (mg)	2 - 16	2 - 16
LOD (mg)	0.401	0.363
LOQ (mg)	1.217	1.099
- Regression Equation - Slope (b) - Intercept (a)	$y^a = b x^b + a$ 0.9924 0.0127	$y^a = b x^b + a$ 1.0071 -0.0426
Correlation coefficient (r)	0.9995	0.9996
Accuracy (% R)	100.36	99.85
Precision (% RSD) Repeatability[c] Intermediate precision[d]	 0.857 0.697	 1.029 0.863

a The observed concentration in mg/ml of lomefloxacin hydrochloride.
b The theoretical concentration in mg/ml of lomefloxacin hydrochloride.
c Average of three concentrations of lomefloxacin hydrochloride (5, 10 and 15 mg) repeated three times within the day.
d Average of three concentrations of lomefloxacin hydrochloride (5, 10 and 15 mg) repeated three times in three days.

Table 4 : Recovery study of lomefloxacin hydrochloride by adopting standard addition technique using the proposed conductometric method in Lomex® tablet:

Pharmaceutical taken (mg)	Pharmaceutical found (mg)	Pure added (mg)	Pure found (mg)		Pure Recovery (%)	
			Ammonium reineckate	**Sodium tetraphenylborate**	**Ammonium reineckate**	**Sodium tetraphenylborate**
6	5.94[a] 6.06[b]	4	3.97	4.05	99.17	101.33
		6	5.92	5.91	98.74	98.48
		8	7.96	7.94	99.54	99.26
		10	10.07	10.19	100.68	101.94
Mean					99.53	100.25
% RSD					0.836	1.643

a Average of five determinations using ammonium reineckate reagent.
b Average of five determinations using sodium tetraphenylborate reagent.

were mentioned in Table 3and indicate good sensitivity of the methods.

Accuracy and precision:

Accuracy and precision of the method were determined by applying the proposed procedurefor determination of three different concentrations (5, 10 and 15 mg), each in triplicate, in pure formin the same day (intra-day) and in three successive days (inter-day), then the accuracy as percent recovery (%R) and precision as percent relative standard deviation (%RSD) were calculatedand results are listed in Table 3.

Accuracy of the method was also determined by applying the standard addition technique where the general procedure of the method was repeated using aliquots of standard lomefloxacin

hydrochloride solution (1 mg/ml) containing (4, 6, 8 and 10 mg) with aliquot of already analyzed Lomex® tablet solution (1 mg/ml) containing (6 mg), then the percent recovery (%R) of pure added concentrations were calculatedand results are listed in Table 4. From these data the method shows high accuracy and precision.

PHARMACEUTICAL APPLICATIONS

The proposed method was applied to the determination of lomefloxacin hydrochloride in Lomex® tablet. Satisfactory results were obtained in good agreement with the label claim, indicating no interference from excipients and additives. The obtained results were statistically compared to those obtained by the reported method [17]. No significant differences were found by

Table 5 : Determination of lomefloxacin hydrochloride in Lomex® tablet by the proposed conductometric andreported methods:

Parameters	Proposed conductometric method		Reported method*[17]
	Ammonium reineckate	Sodium tetraphenylborate	
Number of measurements	5	5	5
Mean % recovery of lomefloxacin	98.95	101.07	100.48
SD	0.796	0.736	1.320
% RSD	0.804	0.728	1.314
Student's *t*-test**	2.221 (2.306)	0.878 (2.306)	——
F-value**	2.752 (6.388)	3.217 (6.388)	——

* Reported method is using first derivative spectrophotometric method with zero crossing point at 303.4 nm.
** The values in parenthesis are tabulated values of "*t* " and "*F* " at (P = 0.05).

applying student's *t*-test and *F*-test at 95% confidence level, indicating good accuracy and precision of the proposed method for the analysis of the studied drug in its pharmaceutical dosage form, as shown in Table 5.

CONCLUSION:

This study described conductometric determination of lomefloxacin hydrochloride with two precipitating reagents; ammonium reineckate and sodium tetraphenylborate. The conductometric methods are characterized by low cost and simplicity, conductometric titrations are especially useful for very dilute solutions as the percentage changein conductance is independent of concentration and measurements need not be made close to theequivalence point. The proposed methods are simple, rapid and inexpensive. So, it is a good alternative to the other reported methods and to the high-cost HPLC methods.

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None.

CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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