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Utilization of Ion-Associate Formation in Spectroscopic and Conductometric Determination of Mebeverine Hydrochloride in Pharmaceutical Formulations

Manal S. Elmasry, Marwa S. Elazazy[*]*, Wafaa S. Hassan*

Analytical Chemistry Department, Faculty of Pharmacy, Zagazig University Zagazig, Egypt
[*]E-mail: marwaelazazy78@yahoo.com

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Spectroscopic and conductometric determination of Mebeverine hydrochloride (MVH) with two ion-pairing reagents; Ammonium Reineckate (Amm.Rt) and Chromotrope 2R (C2R) has been described. The molar combining ratio reveals that (1:1) (drug: reagent) ion associates are formed by both reagents with MVH. The proposed conductometric procedure has been utilized for the determination of equilibrium constant of the reaction as well as other functions such as solubility product of the formed MVH ion associates. In addition, working upon the formerly obtained data operating differential conductivity methods (numerical first and second derivative) and Boltzmann nonlinear fitting was more adequate and systematic for data analysis compared to the conventional method. The described procedures allowed the determination of MVH within the range of 3-15 mg using both reagents. Moreover, the obtained precipitate has been spectroscopically characterized using IR and ^{1}H-NMR. The proposed conductometric method was applied successively to pharmaceutical formulations containing MVH and the results obtained were favorably compared with those obtained using the official method.

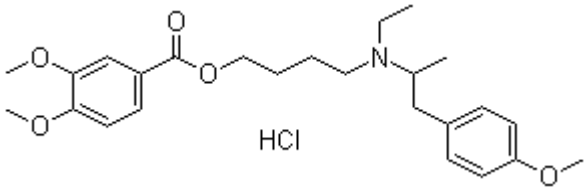
Keywords: Conductometry; Mebeverine; Pure form; Pharmaceuticals; Solubility product; Differential Conductivity, IR, NMR.

1. INTRODUCTION

Mebeverine hydrochloride (MVH) belongs to a class of anti-spasmodic agents known as musculotropic drugs. MVH is used mainly in treatment of irritable bowel syndrome and gastrointestinal spasm secondary to organic disorders [1, 2].

MVH is chemically designated as (*RS*)-4-(ethyl[1-(4-methoxyphenyl)propan-2-yl]amino)butyl-3,4-dimethoxybenzoate hydrochloride "Scheme 1". The drug and its pharmaceutical formulations are official in the B.P.2007 [2]. The official method utilizes non-aqueous titration with 0.1 M perchloric

acid for the determination of MVH in its pure form. Literature survey reveals that several approaches have been reported for the determination of this drug either pure or in formulations. In this concern, the following techniques have been used: spectrophotometric methods [3-9], electrochemical methods [10-12] and chromatographic methods [13-16].



Scheme 1. Mebeverine HCl

Reinecke salt which is ammonium tetrathiocyanato-diamminechromate (III) monohydrate and Chromotrope 2R, chemically known as 4,5-Dihydroxy-3-(phenylazo)-2,7-naphthalenesulfonic acid disodium salt, have been used for quantitative determination of many pharmaceutical compounds applying spectrophotometric, conductometric and AAS procedures [17-21].

In the current endeavor, we continue our previous efforts [22] and employ Amm.Rt and C2R as ion pairing reagents for conductometric and spectroscopic determination of MVH in its pure form and in pharmaceutical formulations. In addition, we elaborate on the data obtained from conductometric titration of MVH to calculate the solubility product of the formed ion associates and hence the equilibrium constant of the investigated reactions.

The availability of precise procedures for locating the equivalence point and hence the corresponding drug concentration is crucial. Typically, the endpoint is obtained via ordinary graphical procedures depending on the intersection of two straight lines. Usually, it is not easy to corroborate the exact break point and to a great extent this issue depends on the fine assessment of the investigator. This issue; in turn and with no doubt would be reflected on the quality and the outcomes of the validation procedures in terms of accuracy and precision [23].

Analysis of conductivity vs. concentration data operating the differential conductivity methods [24-29] and the integral of Boltzmann sigmoid fitting model [24, 29] have been extensively investigated with a purpose of locating an accurate *cmc* value of drugs and surfactants and to avoid the inadequacies of the conventional procedure.

In the present work, conductivity-volume data were analyzed operating the differential conductivity and Boltzmann models with a perspective of avoiding the uncertainty arising from locating the endpoint as the break in the conductance-volume curves. Spectroscopic characterization of MVH-Reineckate ion associate has been done using IR and ^{1}H-NMR.

2. EXPERIMENTAL

Apparatus:

HANNA Conductivity / TDS Meter (HI 8033), with a HANNA Conductivity Probe (HI 76301W) was used. FT-IR measurements were recorded as KBr disks using Mattson 1000

spectrophotometer, Micro analytical Center, Cairo University, Giza. ^{1}H-NMR spectra were measured in DMSO-d_6, using Avance II 600 MHz NMR spectrometers, National research Center, Cairo, Egypt. Chemical shifts (ppm) were reported relative to TMS.

Materials and reagents:

All reagents used were of chemically pure grade. Doubly distilled water was used throughout the experiments. Mebeverine Hydrochloride (MVH) was provided by EIPICO, Egypt; (M.wt = 466.01 g/mol and its purity was found to be 99.83 % according to B.P. method [2]). Pharmaceutical formulation (Spasmotaline® tablets; 100 mg of MVH/ tablet), was obtained from local pharmacy stores. Ammonium reineckate (Amm.Rt), and Chromotrope 2R (C2R) were obtained from Aldrich. The 5 x 10^{-3} mol.l^{-1} and 0.1% (w/v) (drug and reagent) solutions were prepared in doubly distilled water.

Procedure for pure pharmaceuticals:

A range of volumes containing 3 – 15 mg of the pure MVH solution 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 x 10^{-3} M of the titrant using a microburette. The conductance was measured 2 minutes subsequent to each addition of the reagent after thorough stirring. A conductivity (corrected for dilution) *vs.* volume plot for a particular titrant was constructed and the end point was determined. 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.

Determination of mole ratio was done using a fixed concentration of the drug (3 x 10^{-4} mol.l^{-1}) and varying concentrations of the titrants (0 - 7.5 x 10^{-4} mol.l^{-1}). Experimental data were fitted to a non-linear predefined fitting model implemented in PSI Plot software.

The same procedure was pursued for determination of the perceived content of the active ingredient in tablets.

Preparation of ion-associates for Spectroscopic Characterization:

Spectroscopic characterization of the ion associate formed between Amm.Rt and MVH was done by mixing solutions containing equimolar concentrations [1 x 10^{-2} M] of the reagent and MVH. The precipitate obtained was filtered, thoroughly washed with water, and dried at room temperature. The precipitate was subjected to IR and ^{1}H-NMR spectroscopy [30].

Conductometric Determination of the Solubility Products of Ion Associates:

Solutions of different concentrations (C) were prepared for MVH and Amm.Rt. The conductivities of these solutions were measured at 25˚C and the specific conductivities (K_s), corrected

for the effect of dilution, were calculated and used to obtain the equivalent/ molar conductivities (Λ) of these solutions.

Plots of Λ *vs.* √C were constructed and Λ_{oMVH} and $\Lambda_{oAmm.Rt}$ were determined from the intercept of the respective straight line with the Λ ordinate. All solutions were sufficiently dilute and hence the activity coefficients of the involved ions were taken as unity. The value of $\Lambda_{oMVH\text{-}Amm.Rt}$ was calculated using Kohlrausch's law of independent migration of ions [31]. The solubility (S) and solubility product (K_{sp}) values of a particular ion associate were calculated using the following equations;

$$S = K_s \times 1000/ \Lambda_{o\ \text{“ion-associate”}} \quad (1)$$

$$K_{sp} = S^2 \quad \text{(for 1:1 Ion Associates)} \quad (2)$$

$$K = 1/ K_{sp} \quad (3)$$

where, "K_s" are the specific conductivity of the saturated solution of the ion associate, Λ_o is the intercept of the Λ *vs.* √C curve and K is the equilibrium constant.

3. RESULTS AND DISCUSSION

Conductometric Procedure:

In this article, formation of ion- associates between two commonly used dyes and a quaternary ammonium compound, MVH, has been reported. Bearing in mind the widespread usage of MVH as an OTC drug in Egypt and the need for a cost-effective and simple procedure for its determination, we have undertaken this task. Two titrants were used for this purpose, C2R and Amm.Rt. Both titrants were found to react with MVH forming stable ion pairs with different aqueous solubilities. While the (MVH^+-$Amm.Rt^-$) ion pair was sparingly soluble; the (MVH^+-$C2R^-$) was soluble, under the mentioned experimental conditions.

Assuming that conductivity is a linear function of dilution, dilution factor was calculated using the equation: $X_{corr.} = X_{obs.} [(v_1 + v_2) / v_1]$, where $X_{corr.}$ and $X_{obs.}$ are the corrected and observed conductances, respectively; v_1 is the initial volume, and v_2 is the volume of the added reagent [32]. The corrected specific conductivities were plotted as a function of the titrants' volumes.

The obtained volume-conductivity empirical graphs showed a linear behavior - with an even transition - before and after the inflection point. This attitude is vindicated based on the fact that the formed ion associates can be recognized as a transition phase between two different systems. The point of intersection between these systems was believed to be the equivalence point at which the concentration of the drug can be calculated, Figure 1. Such a procedure is known as the "conventional procedure" for locating the endpoint.

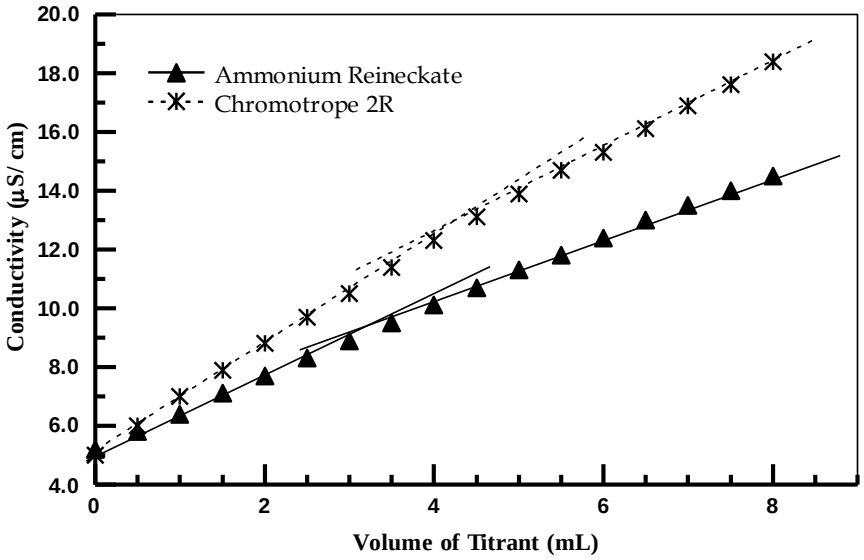


Figure 1. Conductometric titration curve of 8 mg MVH using 0.005 mol/L Amm.Rt and 10 mg MVH titrated with 0.005 mol/L C2R. Conventional procedure is used for locating the endpoint.

Reaction Mechanism and Molar Ratio:

Figure 1, shows that on using Amm.Rt as titrant, two straight lines were obtained; the first line is ascending, indicating a rapid increase in conductance. Above certain volume, the measured conductance ceased to increase linearly and became lower than estimated, constituting the second segment of the curve. This behavior can be attributed to formation of species of lower mobilities as a result of further ionic condensation and formation of slightly dissociated, less conducting and insoluble ion pair. All plots drawn following the conventional scheme showed a curve break around a point where the mole ratio (Drug: Reagent) is 1:1, [17-19].

Further investigation of molar ratio was done by monitoring the molar conductance (Λ_m) of the drug solution as a function of mole ratio ([D]/[R]) and the experimental figures were fitted using a built-in non-linear least squares fitting algorithm defined by PSI Plot software, Figure 2. As can be perceived, addition of Amm.Rt to the MVH solution causes a regular increase in molar conductance of the solutions. This might be an indication of formation of complexes that are more mobile compared to the solvated drug molecules. This continuous increase in molar conductance starts to smooth out at the point where the [D]/[R] ratio is equal to unity, such a behavior further corroborates the formation of stable (1:1) ion pairs, [22, 33]. The reactions may be denoted by the following equation:

$$MVH^{+}.Cl^{-} + NH_4[Cr(NH_3)_2(CSN)_4] \longrightarrow \{MVH.H^{+}[Cr(NH_3)_2(CSN)_4]^{-}\}_{(s)} + NH_4Cl$$

In case of C2R, an analogous behavior was detected (Figures 1 and 2), nevertheless; a soluble 1:1 ion pair was formed. The molar ratio was also 1:1 and the reaction can be represented by the following equation:

$$MVH^{+}.Cl^{-} + C2R \longrightarrow \{[MEH.H]^{+}[C2R]^{-}\}_{(aq.)} + NaCl$$

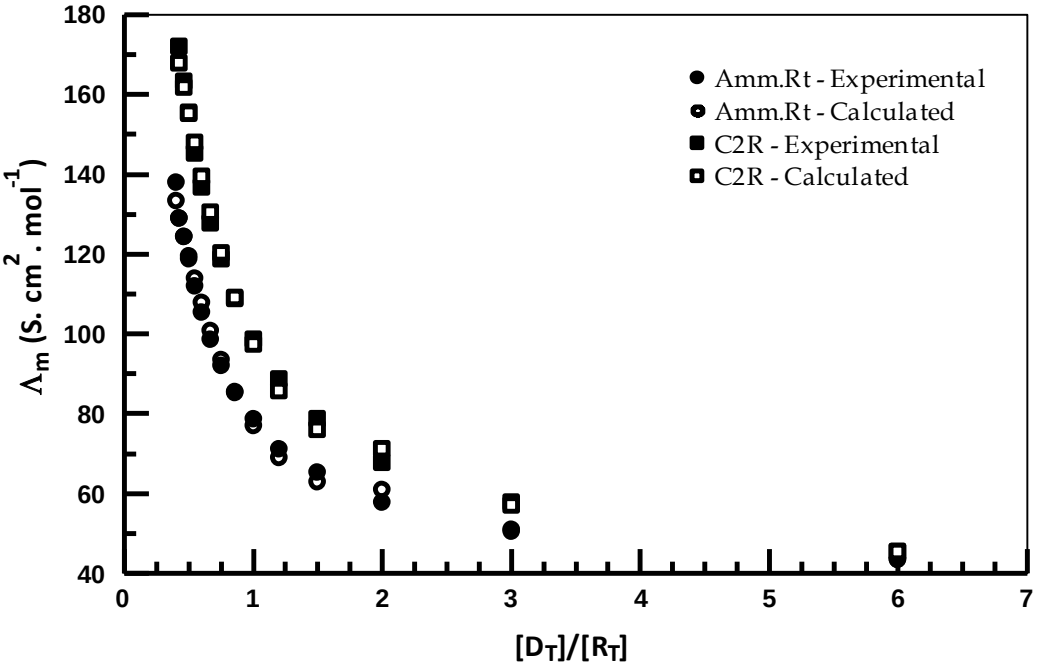


Figure 2. Molar Conductance – Mole ratio plots for the complexes of C2R and Amm.Rt with MVH in pure water. Experimental values are represented by Close geometries while the calculated values are represented by open ones. Calculated values are obtained by fitting the experimental values using non-linear least squares fitting algorithm. Indistinguishable geometries indicate that experimental and calculated points are the same within the resolution of the plot.

Volume-Conductivity Data Analysis:

A conventional method for obtaining the equivalence point has been illustrated in the preceding sections (Figure 1). Nonetheless, this method, though being commonly used by researchers; it divulges undeniable intricacies particularly when the plot exhibits a weak curvature. Add to that, the endpoint located by this method hinges largely on the number of data points [24, 29]. This behavior necessitates the need for a systematic approach that surmounts such negative aspects. In the present venture, two schemes were proposed; numerical derivatization (first and second derivatives) and fitting the experimental data to Boltzmann sigmoid paradigm.

Numerical differentiation of conductivity data against the volume of titrant was one of the suggested proposals, (Figure 3). In case of first derivative, the equivalence point was located as the intersection with the abscissa, while for second derivative, it was determined as the minimum. Though locating the intersection with the X-coordinate was easier than finding a minimum, fitting the second derivative data to Gaussian resolves this concern and the endpoint is determined as central point. Following such a scheme for locating the equivalence point provides a methodological and an impartial recipe that equivocates the shortcomings of the supposed classical procedure. Yet, getting a first derivative sigmoid with a noisy behavior was an inevitable problem. This problem usually originates from the numerical handling of data, an issue that would consolidate the intrinsic

experimental errors of the original conductivity-volume data. These errors, in turn, are augmented in the numerical differentiation procedure.

In a tryout to avoid the errors encountered by the arithmetical derivatization of conductivity data, many non-linear fitting patterns were probed. Among the tested models, Boltzmann type sigmoid, a built-in model in many types of software, provided a simple and direct association between the function parameters and the conductivity-volume curve features. This paradigm has been depicted by the following equation [24, 29]:

$$f(X) = \frac{A_1 - A_2}{1 + e^{(X - x_o)/\Delta x}} + A_2 \qquad (4)$$

The parameters A_1 and A_2 correspond to the asymptotic value for small and large values of x respectively, x_o represents the endpoint and Δx is related to the width of the function. Figure 4 shows the determination of MVH applying Boltzmann model. The simplicity of this model emanates from the value of x_o which represents the central point of transition and it is simply obtained as $f(x_o) = (A_1 + A_2)/2$.

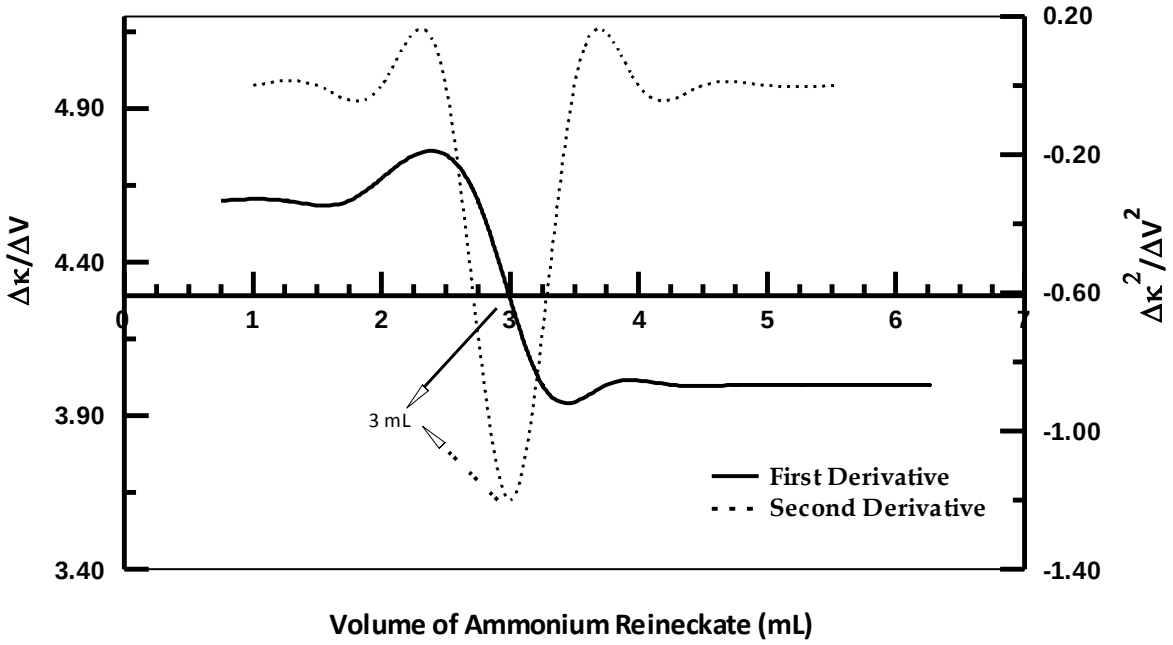


Figure 3. Conductometric titration of 6.99 mg MVH with $5x10^{-3}$ M Amm.Rt applying the numerical first derivative plot ($\Delta\kappa/\Delta V$), fitted to a predefined non-linear regression model [A] and numerical second derivative plot ($\Delta^2\kappa/\Delta V^2$), fitted to Gaussian [B]. Arrows denote the endpoint determined by each procedure.

Figures 3 and 4 show the best case scenario where nearly the same endpoint was localized by all techniques; yet, this is not constantly the picture. An assessment of % error encountered on calculation of recovery % obtained using the equivalence point determined by each procedure is shown

in Table 1. From this table, small discrepancies were observed, which most likely originate from data processing. Therefore, it seems more suitable to get such figures from the original data without additional treatment which eventually brings in defective results.

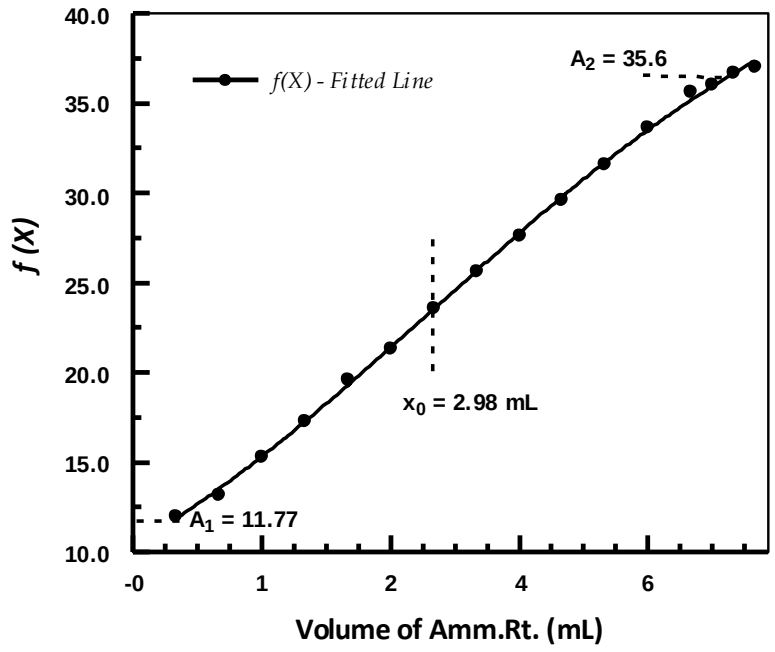


Figure 4. Conductometric titration of 6.99 mg MVH with $5x10^{-3}$ M Amm.Rt applying the Boltzmann sigmoid method *f(x)*. The same set of data was drawn following the conventional procedure and the endpoint was determined as 3.00 ml.

Table 1. A comparison between the proposed procedures for conductivity *vs.* volume data analysis. Amm.Rt was used as a titrant for the determination of 10 mg of MVH.

Procedure	Found (mg)	Recovery %	% Error
Conventional	9.95	99.50	- 0.5
First Derivative	9.90	99.00	- 1
Second Derivative	10.02	100.2	0.2
Boltzmann Sigmoid	9.97	99.70	- 0.3

Determination of Solubility Product:

Formation of ion-associates via conductometric titrations and the usage of titration data to calculate the solubility product of the formed sparingly soluble species have been described in many articles [34-37].

In this article, conductance of the tested solutions has been used to find out the solubility product of the formed precipitate. The solubility product value by conductometric measurements of the investigated ion-associate was found to be 4.52×10^{-17} using ammonium reineckate. The solubility

product constant values were gathered in Table 2 and illustrated in Figure 5. The equilibrium constant value (K) shown in Table 2 is high enough to indicate the high degree of completeness of the ion-associate formation reactions.

Table 2. Solubility product constant of MVH - Amm.Rt ion-associate:

Ion Associate	Solubility (S) mol/L	K_{SP}	K = 1 / Ksp
MVH-Amm.Rt	6.72×10^{-9}	4.52×10^{-17}	2.22×10^{16}

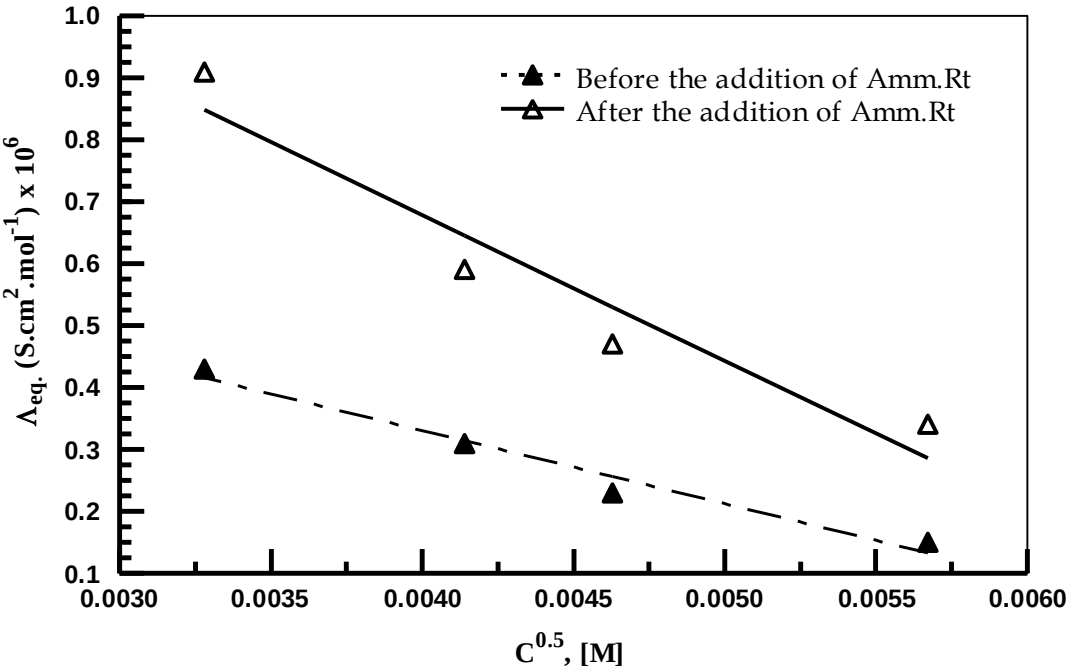


Figure 5. Equivalent conductance (Λeq.) vs. the square root of concentration $C^{0.5}$ for MVH (Before and After addition of Amm.Rt). Data points are fitted to straight line described by the equation: y = ax + b.

IR and ^{1}H-NMR Spectra:

Ion pairing of MVH with Amm.Rt was investigated by comparing IR, and ^{1}H-NMR spectra of the formed ion associate with those of the free ligand.

IR Spectra: The IR spectrum of MVH displays characteristic bands at 2945, 1717, 1265 and 1221 cm^{-1} assigned to ν_{CH} (aliphatic), $\nu_{C=O}$ (ester) and ν_{C-O} (ether) for the last two peaks, respectively.

On the other hand, the IR spectrum of Amm.Rt has a characteristic band at 2119 cm^{-1} due to $\nu_{(CN)}$ "in the Cr-NCS link" stretching vibration, a band at 766 cm^{-1} due to $\nu_{sym\,(C-S)}$ and at 499 cm^{-1} due to $\delta_{(NCS)}$ deformation vibration [38].

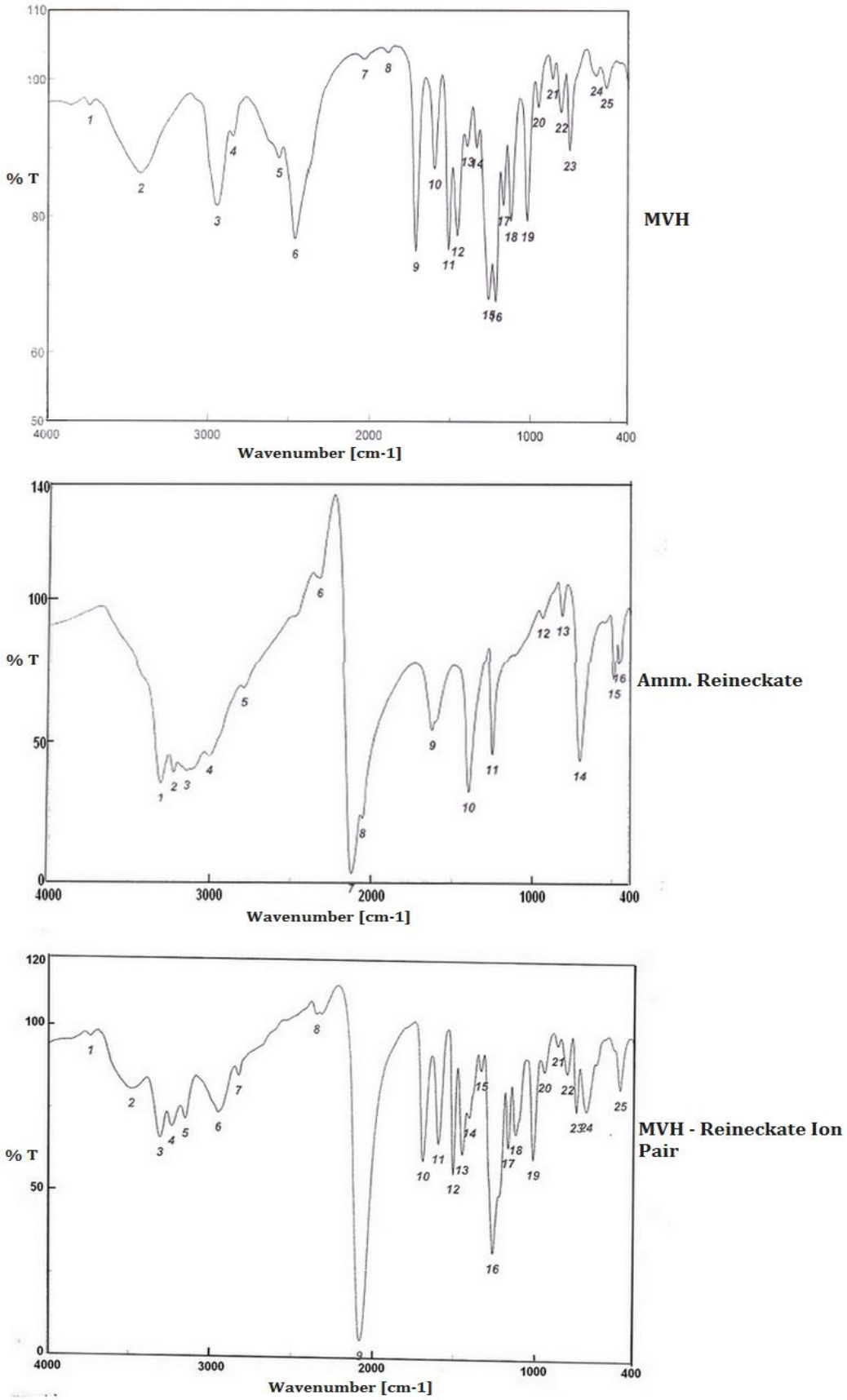
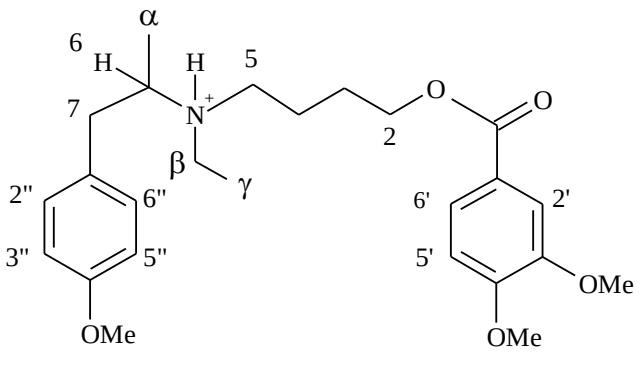


Figure 6. IR spectra of MVH, Amm.Rt and their ion associate

The IR spectrum of the formed ion associate shows a band corresponding to ν_{CH} (aliphatic) at 2960 cm^{-1}. The band corresponding to the stretching vibrations of C=O shifted to a lower frequency by ~ 20 cm^{-1}. In addition, the peak due to ν_{NCS} is shifted to a lower frequency by 44 cm^{-1}. Peaks due to $\nu_{sym\ (C\text{-}S)}$ and $\delta_{(NCS)}$ appear at 699 and 489 cm^{-1} respectively. The above arguments indicate that an ion associate has been formed between MVH and Amm.Rt. Figure 6 shows the IR spectra of the free ligands as well as the ion associate.

^{1}H-NMR Spectra



$Cr(SCN)_4(NH_3)_2$

Scheme 2. Mebeverine Reineckate (MVH-Amm.Rt) Ion Associate with NMR Numbering.

The NMR spectrum of MVH - Reineckate was analyzed. As shown in Table 3, some aliphatic protons signals assigned to MVH were shifted up-field while negligible changes were observed in the chemical shift for other protons. Also some NMR peaks of equivalent protons have been observed as singlet peak due to the rapid exchange of MVH between the ion pair sites and the bulk solution [39 - 40]. The suggested structure of the formed ion pair is shown in Scheme 2.

Table 3. Significant chemical shifts (ppm) of the formed MVH-Amm.Rt ion-associate compared to free MVH:

MVH	MVH-Reineckate
δ 7.15 (2H, dd, H2",6")	δ 7.08 (3H, s, H2",6",5')
δ 7.03 (1H, d, H5')	
δ 3.19 (1H, dd, H7)	δ 2.87(2H, s, H7)
δ 2.54 (1H, dd, H7)	
δ 1.88 (2H, m, H3)	δ 1.68 (4H, s, H3 and H4)
δ 1.76 (2H, m, H4)	
δ 1.29 (3H, t, Hγ)	δ 1.10 (3H, s, Hγ)
δ 1.06 (3H, d, Hα)	δ 0.95 (3H, s, Hα)

Applications:

The proposed method was applied to the determination of the studied drugs both in pure forms and in pharmaceuticals (Figure 7). Results given in Tables 4 and 5 show that the proposed method is satisfactorily accurate and precise. The accuracy and reproducibility with respect to the official [2] method were assessed by performing student's t and F tests, respectively. Mean values in Tables 4 and 5 do not show any systematic error and indicates no significant difference between the methods compared.

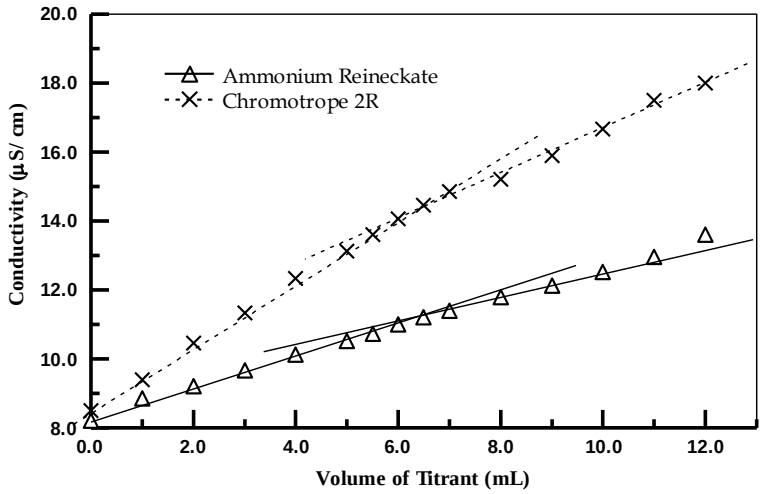


Figure 7. Conductometric titration of 15 mg MVH in Spasmotaline® tablets with $5x10^{-3}$ M Amm.Rt and C2R.

Table 4. Quantitative determination of MVH using the proposed Amm.Rt and C2R methods compared to the official method [2]:

Proposed Method						Official Method [2]
Amm.Rt			C2R			
Taken, mg	Found, mg	Recovery %*	Taken, mg	Found, mg	Recovery %*	
3	3.005	100.19	3	3.01	100.58	
5	5.03	100.66	5	4.96	99.26	
8	7.99	99.90	8	8.06	100.77	
10	9.949	99.49	10	9.949	99.49	
15	15.12	100.81	15	15.07	100.50	
Mean ± SD = 100.21 ± 0.54			Mean ± SD = 100.12 ± 0.69			Mean ± SD = 99.83 ± 0.51
n = 5			n = 5			n = 6
RSD = 0.53			RSD = 0.68			RSD = 0.51
V = 0.29			V = 0.47			V = 0.26
SE = 0.24			SE = 0.308			SE = 0.21
t= 1.20 (1.83)[a]			0.84 (1.83)[a]			
F = 1.11 (5.19)[b]			1.8 (5.19)[b]			

[a] and [b] are the Theoretical *t*-values and *F*-ratios at $p = 0.05$.

Table 5. Statistical analysis of results obtained by the proposed methods for the analysis of Spasmotaline® tablets (Amriya Co., Egypt) (100 mg of MVH/ tablet).

Amm.Rt			C2R		
Taken, mg	Found, mg	Recovery %*	Taken, mg	Found, mg	Recovery %*
3	2.99	99.66	3	2.98	99.57
5	4.98	99.60	5	5.009	100.19
8	8.03	100.48	8	8.01	100.19
10	10.06	100.60	10	10.04	100.42
15	14.98	99.88	15	14.95	99.72
Mean ± SD = 100.04 ± 0.46 n = 5 RSD = 0.45 V = 0.21 SE = 0.205			Mean ± SD = 100.01 ± 0.35 n = 5 RSD = 0.34 V = 0.12 SE = 0.15		

4. CONCLUSION

The proposed conductometric procedures are simple, accurate, rapid and reproducible (RSD = 0.53 – 0.68%). Application of the proposed procedure for the determination of MVH in its dosage forms was successful without interference from excipients.

Moreover, we have shown, using conductivity data, that the suggested fitting model seems more satisfactory in terms of simplicity and objectiveness. Numerical derivatization of data, as well, provided a methodological maneuver for data analysis, however, processing of data usually results in unavoidable numerical errors. It is noteworthy to mention that errors resulting from estimating the fitting parameters are much less than these obtained from mathematical processing of data. The classical procedure, though being commonly used, it depends to a great extent on the human's judgment.

Reaction of Reinecke salt with MVH resulted in formation of insoluble ion associate with the formula $[C_{25}H_{36}NO_5]^+[Cr(NCS)_4(NH_3)_2]^-$. The solubility product of this precipitate and hence the equilibrium constant of the reaction were calculated utilizing the conductance data. The attained values were very high suggesting the high degree of completion of the ion association reaction.

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