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Conductometric and Spectroscopic Determination of Mebeverine Hydrochloride and the Solubility Products of its Ion Recognition Species

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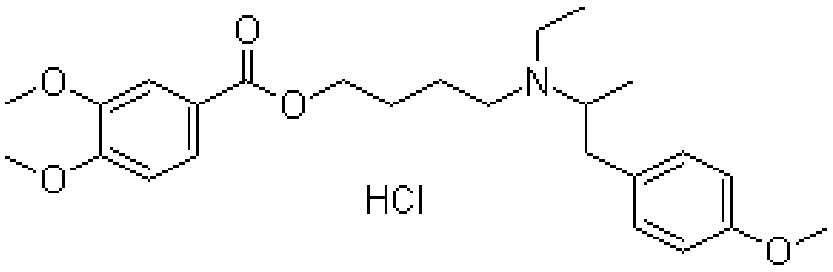
Conductometric and spectroscopic determination of Mebeverine hydrochloride (MVH), a widely used antispasmodic, with two precipitating reagents; phosphomolybdic acid (PMA) and silver nitrate ($AgNO_3$) has been investigated. Exploiting the conductometric procedure, the equilibrium constants and other functions associated with the process of precipitating MVH were determined. Moreover, two new maneuvers towards equivalence point detection were carried out. In this itinerary, data processing was performed applying numerical derivatization (first and second derivatives) and Boltzmann algorithm. The newly prescribed techniques were found to be more reasonable compared to the classical routine in terms of impartiality and rationality. The described procedures allowed the determination of MVH within the range of 2-15 mg using PMA and 1-20 mg using $AgNO_3$ respectively. The molar ratio and as confirmed by the molar conductance – mole ratio plots, reveal that (1:1) (drug: reagent) ion associates are formed for both reagents with MVH. Furthermore, the precipitate obtained by ion pairing MVH with PMA has been spectroscopically characterized using IR and ^{1}H-NMR. The method was further applied successively to pharmaceutical formulations containing MVH and the results obtained were favorably compared with those obtained using the official method.

Keywords: Conductometry; Mebeverine HCl; Solubility product; Differential conductivity, Boltzmann sigmoid plot, Spectroscopy.

1. INTRODUCTION

Mebeverine hydrochloride (MVH) is chemically known as (*RS*)-4-(ethyl[1-(4-methoxyphenyl)propan-2-yl]amino)butyl 3,4-dimethoxybenzoate hydrochloride "Scheme 1". Mebeverine belongs to a category of anti-spasmodics known as musculotropic drugs and is used largely in treatment of irritable bowel syndrome and gastrointestinal spasm secondary to organic disorder [1,2]. The official procedure described in B.P.2007 [2] depends on non-aqueous

potentiometric titration with 0.1 M perchloric acid for the determination of MVH in its parent form. Several methods have been reported in literature for the determination of this drug either per se or in formulations. In this concern, the following techniques have been described: Spectrophotometric methods [3-9], Electrochemical methods [10-12] and Chromatographic methods [13-16].



Scheme1. Mebeverine HCl

Considering the widespread usage of MVH as an OTC drug in Egypt and many countries in the area, there was a need to have a simple, cost-effective technique for the determination of MVH and its formulations. In this course; interactions between MVH and two precipitating agents, $AgNO_3$ and PMA have been investigated both by spectroscopic and conductometric techniques. Both titrants have been used for quantitative determination of many pharmaceutical compounds applying conductometric procedure [17-22].

Moreover and bearing in mind that the sharpness of the endpoint is greatly dependent on the solubility of the formed ion-pairs [22-25]; the conductance data were employed to calculate the solubility products and so the equilibrium constant of the considered precipitation reactions.

A major concern that will be thoroughly considered through this article is the availability of an accurate recipe for locating the endpoint and accordingly the corresponding drug concentration, an issue that affects the validation of the studied procedure in terms of accuracy and reproducibility [26]. By and large, the endpoint is established via an experiential graphical process that depends the intersection of two straight lines as the equivalence point. Generally, it is not easy to substantiate a precise break point using this procedure and to a great extent it is human contingent. Analysis of conductivity/concentration data employing the differential conductivity schemes [27-32] and the integral of Boltzmann type sigmoid [27, 32] has been expansively inspected with the purpose of locating an accurate cmc of drugs and surfactants.

The current paper implies the use of the differential conductivity methods and Boltzmann model "without integration" for locating the equivalence point. The first derivative data were fitted to a built-in non-linear regression model while approximations to Gaussians of the second derivative data were followed to locate the endpoint. The rationale behind such exploitation of data is to surmount the uncertainty coming up from establishing the endpoint as the break in the conductance-volume curves. Additionally, structural elucidation of MVH-PMA ion associate was performed using IR and ^{1}H-NMR.

2. EXPERIMENTAL

2.1. 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, using AvanceII 600 MHz NMR spectrometers.

2.2. Materials and reagents

All employed chemicals were of analytical grade and doubly distilled water was used throughout the study. 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. Silver Nitrate ($AgNO_3$), and Phosphomolybdic acid (PMA) 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.

2.3. Procedure for pure pharmaceuticals

Aliquots of the standard drug solution containing 1 – 20 mg of MVH 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 MVH was calculated using the following equation:

$$\text{Amount of the drug (mg)} = VMR / N$$

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. Mole Ratio

Mole ratio was established using a fixed concentration of the drug and varying concentrations of the titrants. The tested drug concentrations were (2 x 10^{-4} $mol.l^{-1}$ and 5 x 10^{-4} $mol.l^{-1}$), while the reagent concentrations were (0 – 6 x 10^{-4} $mol.l^{-1}$ and 0 – 1 x 10^{-3} $mol.l^{-1}$) using PMA and $AgNO_3$ respectively. The experimental data were fitted to a non-linear predefined fitting model (PSI Plot software).

2.5. Preparation of ion-associates

The ion associate with PMA was prepared by mixing solutions containing 10^{-2} M of PMA, and the requisite amount of 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 [33].

2.6. Procedure for Tablets

An amount of pulverized tablets equivalent to 100 mg of the active ingredient were weighted accurately and transferred into a 100 mL conical flask. The drug was extracted three times with 30 mL of distilled water. After extraction, the flask was washed with a few mL of water, then, combined washings, and extracts were filtered into a 100 mL volumetric flask. The volume was completed with distilled water. The nominal content of the active component in tablets was determined as described in the Procedure section.

2.7. Conductometric Determination of the Solubility Products for Ion-Exchangers:

The conductivities of solutions of different concentrations (C) were measured at 25˚C for MVH, PMA and $AgNO_3$. The specific conductivities (K_s), corrected for the effect of dilution, were calculated and used to get the equivalent conductivities (Λ) of these solutions.

Plots of Λ *vs.* $\sqrt{C}$ were constructed and Λ_{oMVH}, Λ_{oPMA} and Λ_{oAgNO3} were obtained from the intercept of the respective straight lines with the Λ axis. The activity coefficients of the ions employed were taken as unity because all the solutions were sufficiently dilute. The values of $\Lambda_{oMVH\text{-}PMA}$ and $\Lambda_{oMVH\text{-}AgNO3}$, were calculated using Kohlrausch's law of independent migration of ions [34]. 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 and Λ_o is the intercept of the Λ *vs.* $\sqrt{C}$ curve.

3. RESULTS AND DISCUSSION

3.1. Conductometric Procedure

In this paper, formation of ion- associates between two precipitating agents and MVH has been shown. Both titrants were found to react with MVH forming stable ion pairs with different aqueous

solubilities. The formed ion associate complexes, which can be perceived as a transition stage between two different systems, create the featured shape of the titration curve. Accordingly, and following the classical procedure, conductivity/volume plots show a linear behavior - with a smooth transition - prior to and after the inflection point. The drug concentration can be determined at this point by intersecting the two straight lines. A model titration curve is shown in Figure 1. Deeming that conductivity is a linear function of dilution, dilution factor was calculated using the equation: $\mathbf{X_{corr.} = X_{obs.}\,[(v_1 + v_2) / v_1]}$; where $X_{corr.}$ and $X_{obs.}$ are the corrected and observed conductance values, respectively; v_1 is the initial volume, and v_2 is the volume of the added reagent [35].

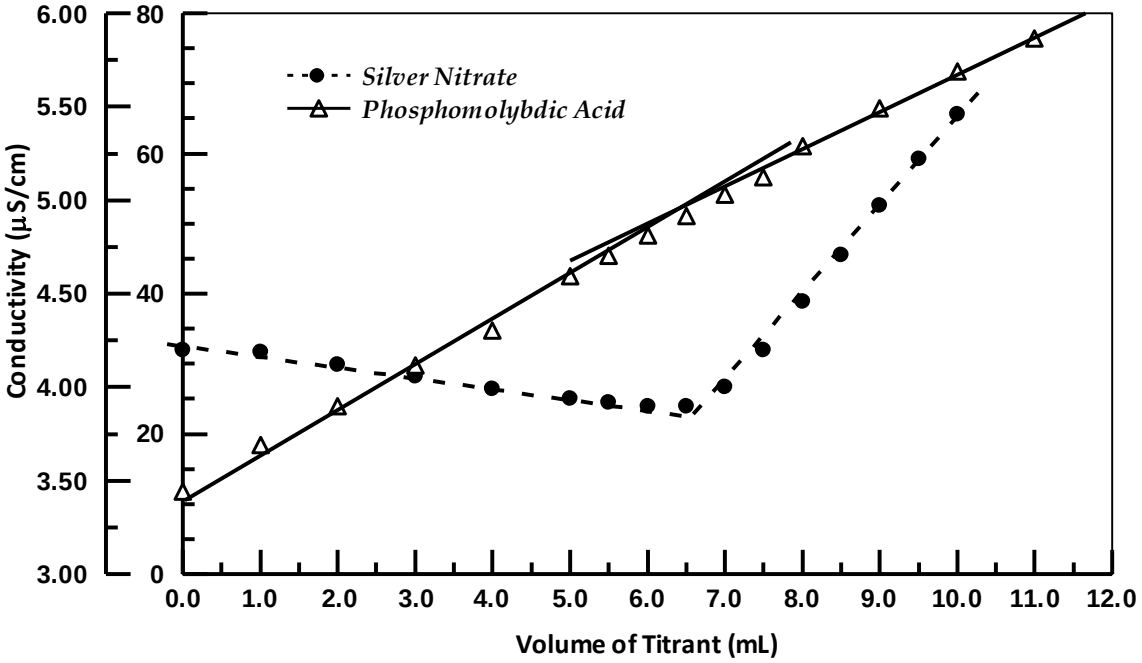


Figure 1. Conductometric titration curve of 15 mg MVH titrated with 0.005 mol/L of both reagents. The endpoint is located following the conventional procedure.

3.2. Reaction Mechanism and Molar Ratio

Using PMA as a titrant and as shown in Figure 1, the investigated system showed a steady increase in conductance values up to the equivalence point, where a gradual change in the slope transpires. Subsequent to this point, the measured conductance halted to increase linearly and became lower than anticipated. This divergence from linearity can be attributed to formation of an ion-associate, presumably, by replacing the drug cations (MVH^+) with the highly mobile H^+ ions, so the conductivity increases. After the endpoint, more acid reagent is added and the conductivity increases more rapidly [36]. A curve break is noted at a drug-reagent molar ratio of 1:1, for all curves constructed using the conventional scheme, Figure 1. Plots of molar conductance – mole ratio, Figure 2, were used to confirm this finding. As seen in this figure, the molar conductance showed a regular rise as the concentration of PMA increases. This increase starts to recede at the point where [D]/[R] is

around unity. The following equation describes the reaction mechanism and the expected structure of the ion pair is given in Scheme 2:

$$MVH^{+}.Cl^{-} + H_3(PMA) \longrightarrow \{[MVH]\,[H_2(PMA)]\}_{(s)} + H^{+}$$

When $AgNO_3$ was used as a titrant, silver chloride precipitated and the first segment of the titration curve was linear. The second part of the curve corresponded to the excess of $AgNO_3$ [17, 18]. Monitoring the molar ratio against the molar conductance, Figure 2, showed that addition of $AgNO_3$ resulted in almost no change in molar conductance. This comportment started to deviate at the point where [D]/[R] comes to unity, confirming the 1:1 molar ratio. Right after this point the molar conductance showed an increase demonstrating the presence of more mobile species compared to the first segment. The reaction can be represented by the following equation:

$$MVH^{+}.Cl^{-} + AgNO_3 \longrightarrow MVH^{+}.NO_3 + AgCl_{(s)}$$

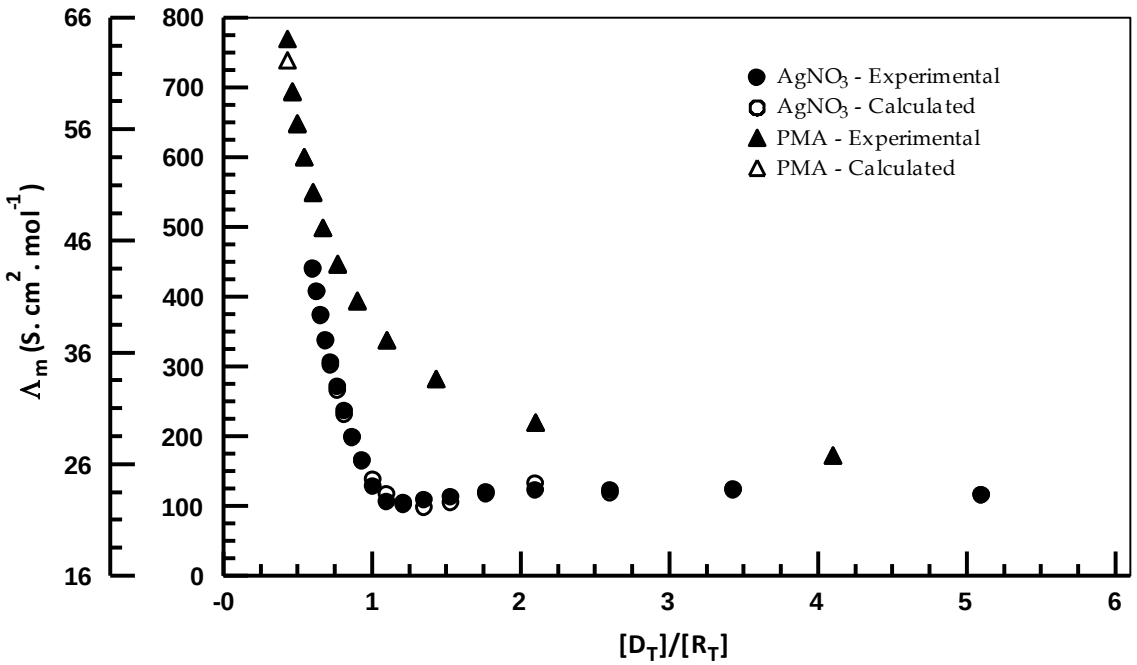


Figure 2. Molar Conductance – Mole ratio plots for the complexes of PMA and $AgNO_3$ with MVH in pure water. Experimental values are represented by Close geometries while the calculated values are represented by open ones. Calculated values are obtained by fitting the experimental values using non-linear least squares fitting algorithm. Indistinguishable geometries indicate that experimental and calculated points are the same within the resolution of the plot.

3.3. Conductivity – Volume Data Analysis:

The classical procedure for locating the endpoint has been described in the previous sections, Figure 1. The chief intricacy encountered trailing this technique came from the weak curvature observed around the intersection point of the two straight lines. Besides, a few number of data points

affects to a great extent the value of the endpoint [27, 32]. Consequently, there was a need for a more objective and a methodical prescription that prevails the negative features of the so-called conventional procedure. In the present endeavor, two proposals were considered. The first plan depended on numerical derivatization of the raw data, while in the second, experimental figures were implemented into a Boltzmann type model.

One of the suggested schemes was the mathematical differentiation of the obtained conductivity data against the corresponding reagent volume, Figure 3. Applying the first derivative mode, the endpoint was located as the halfway point-on the fitted line-between two shoulders. For a second derivative, data were fitted to Gaussian and the endpoint was located as the curve maximum and as defined by the fitting parameters. Such maneuver provides an equitable and a procedural protocol that prevails over the inadequacies of the classical procedure. Alternatively, the process of numerical handling of data creates unusual behavior represented in a noisy first derivative sigmoid (figure is not shown). Noticeably, this problem stems from consolidating the instinctive experimental errors. These errors, in turn, are amplified after the numerical processing.

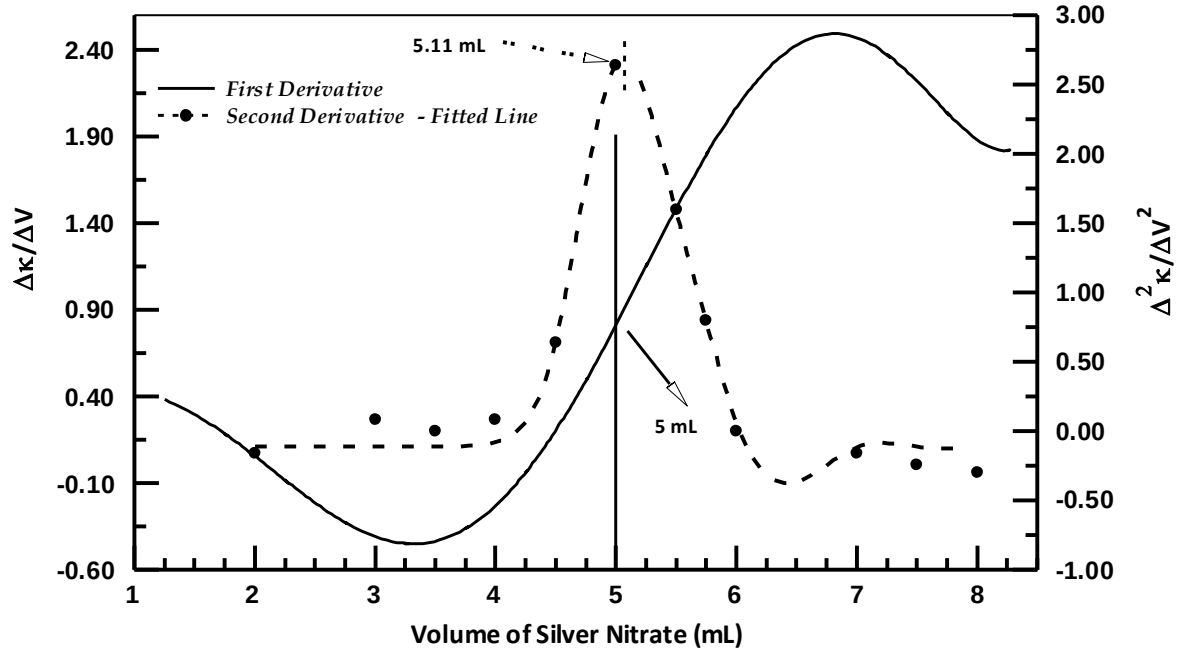


Figure 3. Conductometric titration of 11.65 mg MVH with $5x10^{-3}$ M $AgNO_3$ applying the numerical first derivative plot ($\Delta\kappa/\Delta V$), fitted to a PSI-Plot predefined non-linear regression model and numerical second derivative plot ($\Delta^2\kappa/\Delta V^2$), fitted to Gaussian. Arrows denote the equivalence points determined by each contrive.

The other proposal, Boltzmann paradigm, was suggested in an attempt to avoid the errors encountered by the arithmetical derivatization of conductivity data. Besides, being a predefined model in most of softwares, Boltzmann type sigmoid, provides a straightforward and simple correlation between the function parameters and the conductivity-volume curve characters. This model has been described by the following equation [27, 32]:

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

The parameters A_1 and A_2 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 Δx corresponds to the width of the function. Figure 4 shows 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 $f(x_o) = (A_1 + A_2)/2$.

An evaluation of the recovery percent and so the error encountered using the endpoint located by each method is given in Table 1. The shown results do not represent the best case scenario in which the same endpoint is obtained using the four techniques.

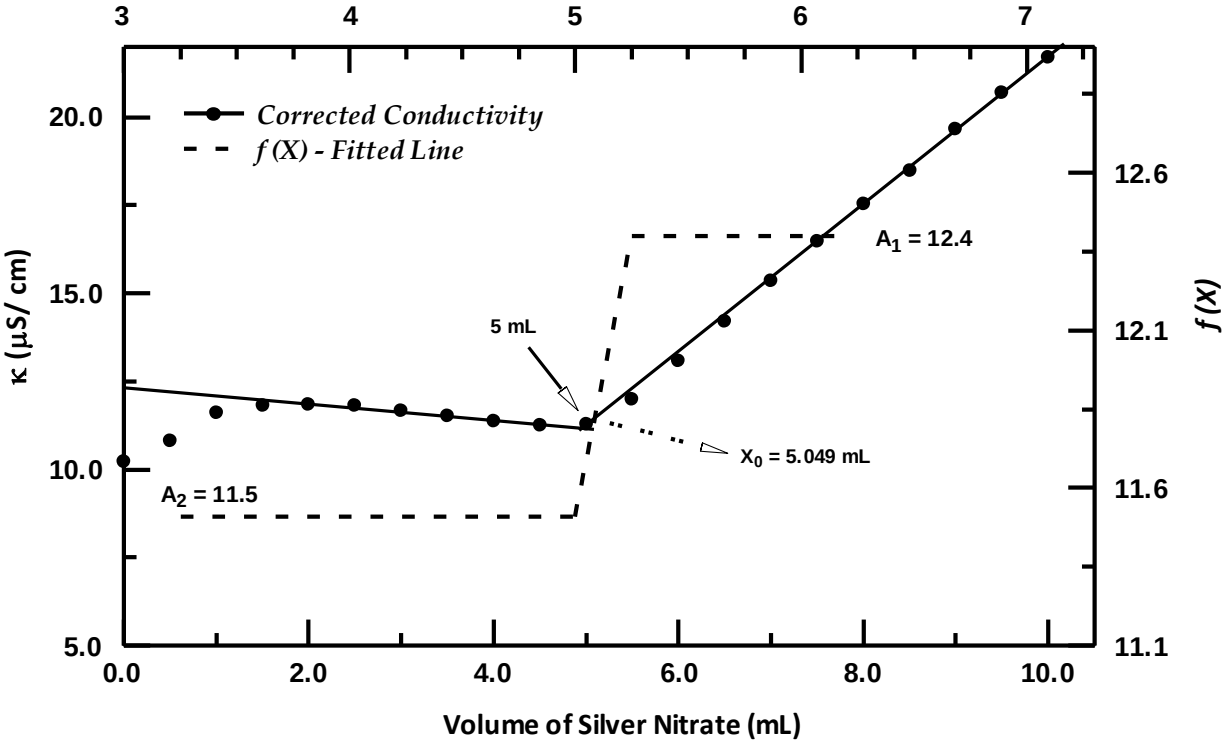


Figure 4. Conductometric titration of 11.65 mg MVH with $5x10^{-3}$ M $AgNO_3$ applying the Boltzmann sigmoid method *f(x)* compared to the conventional method. Value of x_0 stands for the equivalence point determined using Boltzmann model.

Table 1. A comparison between the four suggested procedures for conductivity – volume data analysis. $AgNO_3$ was used as a titrant for the determination of 11.65 mg of MVH.

Procedure	Found (mg)	Recovery %	% Error
Conventional	11.65	100.00	0
First Derivative	11.65	100.00	0
Second Derivative	11.90	102.15	2.15
Boltzmann Sigmoid	11.76	100.94	0.94

3.4. Determination of Solubility Product

Conductance of the investigated solutions has been employed to find out the solubility product of the formed precipitates. The solubility products together with the parameters related to ion pairing of MVH are listed in Table 2 and illustrated in Figure 5. The calculated equilibrium constant values (K) are high enough to show the high degree of completeness of the ion pairing reactions. At equilibrium, the solubility of the undissociated ion-associates in water (the intrinsic solubility) was omitted as it has an insignificant contribution to the total solubility because the ion-associates are sparingly soluble in water and their saturated solutions are consequently very dilute.

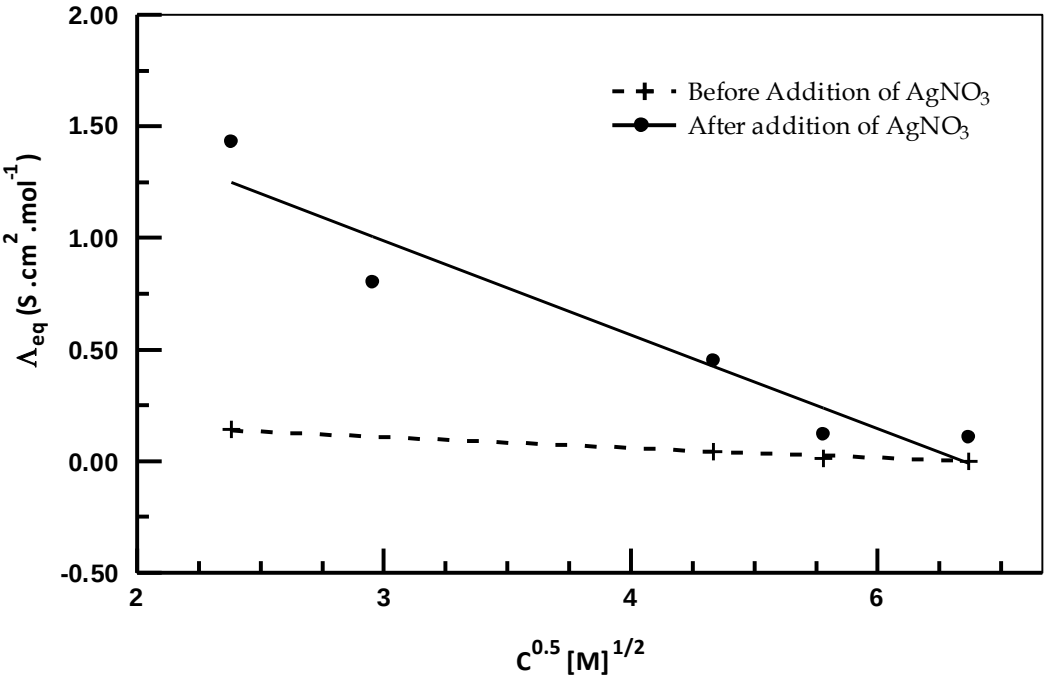


Figure 5. Equivalent conductance (**Λeq.**) *vs*. the square root of concentration $\mathbf{C^{0.5}}$ for MVH (Before and After addition of $AgNO_3$). Data points are fitted to straight line described by the equation y = ax + b.

Table 2. Solubility product constants and other functions related to precipitation of MVH using PMA and $AgNO_3$:

Ion Associate	Solubility (S) mol/L	K_{SP}	K = 1 / Ksp
MVH-PMA	2.73×10^{-8}	7.45×10^{-16}	1.34×10^{15}
MVH-$AgNO_3$	3.62×10^{-10}	1.31×10^{-19}	7.64×10^{18}

3.5. IR and ^{1}H-NMR Spectra

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

3.6. 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 PMA as shown in Figure 6 has two characteristic bands at 1624 and 1065cm^{-1} due to $\nu_{sym\ (P=O)}$ and $\nu_{as\ (P=O)}$ and a strong, broad peak at 3406 cm^{-1} due to $\nu_{(OH)}$ vibration; respectively. The IR spectra of the formed ion associate shows a band corresponding to ν_{CH} (aliphatic) at nearly the same frequency (2942 cm^{-1}) as that of MVH. The band corresponding to the stretching vibrations of C=O shifted to a lower frequency by ~ 18 cm^{-1}. In addition, the peak due to $\nu_{sym\ (P=O)}$ is shifted to a lower frequency by 24 cm^{-1}. The above arguments indicate that an ion associate has been formed between MVH and PMA.

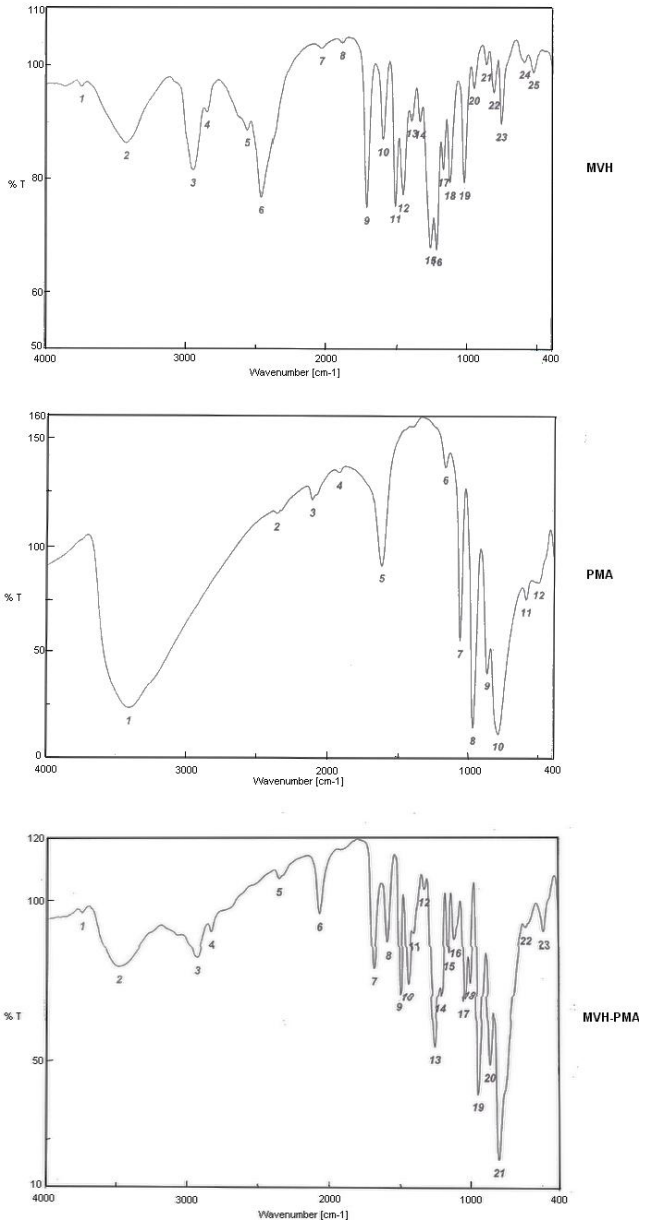
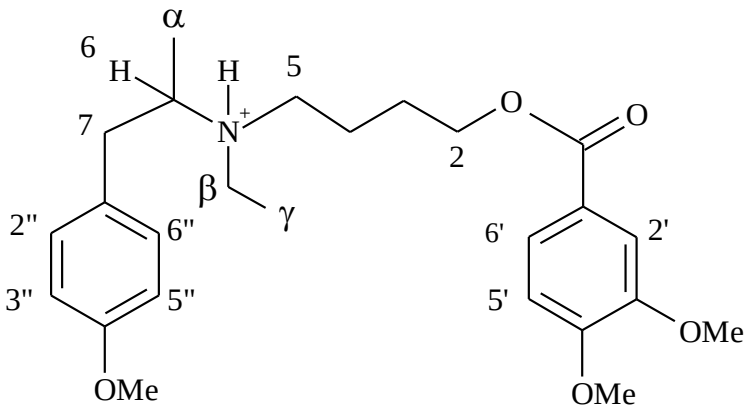


Figure 6. IR spectra of MVH, PMA and their ion associate.

3.7. ^{1}H-NMR Spectra

When the ion pair is formed during titration of MVH with PMA, the rapid exchange of MVH between the ion pair sites and the bulk solution will cause the NMR peak of a set of equivalent protons to be a collapsed singlet [37]. This is mostly obvious in the two multiples at 1.8 and 1.75 (H3 and H4) in MVH spectra changed to one singlet at 1.78 in the ion pair spectra. Also the triplet at 1.29 (Hγ) changed to singlet at 1.25, the doublet at 1.06 (Hα) changed to singlet at 1.05, Table 5. Based on the above discussion, the following structure for the ion associate "Scheme 2" was suggested:



. $H_2(Mo\ O_3)_{12}\ PO_4$

Scheme 2. Mebeverine Phosphomolybdate (MVH-PMA) Ion Associate with NMR Numbering.

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

MVH	MVH-PMA
δ 1.88 (2H, m, H3) δ 1.76 (2H, m, H4)	δ 1.78 (4H, s, H3 and H4)
δ 1.29 (3H, t, Hγ)	δ 1.25 (3H, s, Hγ)
δ 1.06 (3H, d, Hα)	δ 1.05 (3H, s, Hα)

3.8. Analytical Applications

The proposed methods were applied effectively for determination of MVH in its pharmaceutical dosage forms. The results obtained were validated by comparison with the official method [2]. No significant difference was found by applying *t*- and *F*-tests at 95% confidence level indicating good accuracy and precision (Table 4). Recovery studies were also carried out. The results in Tables 4 and 5 indicate good recoveries (99.75 to 100.28% for pure form and 99.68 to 100.03% for formulations) and confirm that there is no interference from frequently encountered excipients or additives.

Table 4. Quantitative determination of MVH using the proposed PMA and $AgNO_3$ methods compared to the official method [2]:

Proposed Method						Official Method [2]
PMA			$AgNO_3$			
Taken, mg	Found, mg	Recovery %*	Taken, mg	Found, mg	Recovery %*	
2	1.99	99.50	1	0.99	99.00	
3	3.02	100.66	4	4.03	100.77	
5	5.009	100.18	8	8.03	100.48	
10	9.902	99.02	15	15.14	100.97	
15	14.91	99.40	20	20.03	100.19	
Mean ± SD = 99.75 ± 0.65 n = 5 RSD = 0.65 V = 0.42 SE = 0.29 *t* = 0.23 (1.83)[a] *F* = 1.61 (5.19)[b]			Mean ± SD = 100.28 ± 0.77 n = 5 RSD = 0.77 V = 0.59 SE = 0.34 *t* = 1.16 (1.83)[a] *F* = 2.25 (5.19)[b]			Mean ± SD = 99.83 ± 0.51 n = 6 RSD = 0.51 V = 0.26 SE = 0.21

[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).

PMA			$AgNO_3$		
Taken, mg	Found, mg	Recovery %*	Taken, mg	Found, mg	Recovery %*
1	1.001	100.19	2	2.01	100.50
4	4.007	100.19	3	2.98	99.33
8	7.92	99.02	5	4.96	99.20
15	14.91	99.41	10	10.019	100.19
20	19.92	99.60	15	15.14	100.93
Mean ± SD = 99.68 ± 0.51 n = 5 RSD = 0.51 V = 0.26 SE = 0.22			Mean ± SD = 100.03 ± 0.74 n = 5 RSD = 0.74 V = 0.55 SE = 0.33		

4. CONCLUSION

In this work, we have shown by means of conductivity and spectral data that MVH forms 1:1 ion pair complexes with PMA and $AgNO_3$. The proposed procedure is very simple, accurate and can be readily adopted for routine analysis in quality control laboratories. Additionally, the proposed methods can be easily applied for determination of MVH in pharmaceutical formulations.

A comparison between the proposed models for data processing showed that fitting the experimental data directly into the proposed Boltzmann model is more acceptable for data manipulation compared to both the classical and the mathematical derivatization approaches. Provided that the endpoint is directly defined in this model, Boltzmann type sigmoid can be viewed as a technique of choice, particularly when transition occurs in a steady way. The errors encountered due to proposing the fitting parameters were incomparable to those obtained by data processing.

With the small divergences observed between the conventional and numerical schemes, which most probably come from data processing, it might seem more appropriate to have such figures directly from the raw data without further processing, which finally brings in flawed results. However, the availability of a systematic procedure that does not depend on the researcher's decisive factors in addition to having many processing schemes to select from outweighs these defects.

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