*IOSR Journal of Applied Physics (IOSR-JAP)*
*e-ISSN: 2278-4861.Volume 8, Issue 6 Ver. III (Nov. - Dec. 2016), PP 67-75*
*www.iosrjournals.org*

# Self-Aggregation of Itopride HCl in Aqueous Solution: $^1$H NMR and Conductivity Study

Manal S. Elmasry[1], Heba M. El-Sayed[1]*
[1]*Analytical Chemistry Department, Faculty of Pharmacy, Zagazig University, Zagazig 44519, Egypt*

***Abstract*:** *The self-aggregation of itopride HCl in water at different temperatures has been studied. Critical aggregation concentrations (CAC) and the degree of ionization (α) have been determined by conductivity measurements over the temperature range from 293.15 – 318.15 K. The thermodynamics parameters of self-aggregation; the standard Gibbs energy change $\Delta G°_m$, the standard enthalpy change, $\Delta H°_m$, and the standard entropy change, $\Delta S°_m$, were calculated applying the pseudo-phase model. $^1$H NMR was also applied for the quantitative determination of itopride HCl in pure and in pharmaceutical tablets based on the change of the aromatic protons chemical shift as a function of concentration that was also used to calculate the critical aggregation concentration of itopride HCl The proposed quantitative $^1$H NMR method is fast, selective, accurate, and simple compared with other reported methods.*

***Keywords*:** *Itopride HCl, $^1$HNMR, Conductivity, Thermodynamics, Self-aggregation.*

## I. Introduction

Itopride hydrochloride, N-{p-[2-(Dimethylamino)ethoxy]benzyl}veratramide hydrochloride, is used for its prokinetic and antiemetic actions [1]. Itopride has antiacetylcholinesterase activity as well as dopamine $D_2$ receptor antagonist activity and it can be safely used for the treatment of functional bowel disorders such as functional constipation and constipation-dominant irritable bowel syndrome in addition to other gastrointestinal symptoms like nausea, vomiting, non-ulcer dyspepsia, emesis and chronic gastritis [2,3]. Different analytical methods were reported for the quantification of itopride HCl in pharmaceutical formulations or human plasma using spectrophotometry [4–10], Spectrofluorimetry [11,12], potentiometry [13], chemiluminescence [14], HPTLC [15–17] and liquid chromatography [11,17–23]. The aggregation characteristics and surface properties of a wide variety of amphiphilic drugs were investigated and found to be a result of the π-π interactions between aromatic units and the nature of this interaction is mainly dependent on the electrostatic effect and the type of the ring [24–26]. The investigation of aggregation due to the $\pi$-stacking interaction of aromatic compounds is most frequently achieved using NMR spectroscopy being a precise and easy technique that provides structural information for the aggregates by interpretation of the chemical shift data [27–33]. Conductivity measurement is another tool for studying the self-aggregation of amphiphilic drugs and determination of their critical aggregation concentration (CAC) [34–36]. This work was based on studying the concentration-dependent chemical shift variations in $^1$H NMR spectra of itopride HCl in $D_2O$ solutions as well as the inflection of conductance above CAC. The degree of dissociation ($\alpha$) and other thermodynamic parameters of aggregation were also calculated.

## II. Experimental

### *2.1. Materials and methods*

#### *2.1.1. Materials and reagents*

All chemicals were of analytical grade. Itopride HCl (purity >98%) was provided by Eva Pharma, Egypt. Deuterium oxide $D_2O$ (99.9%D) was from Cambridge Isotope Laboratories, Inc., USA. Doubly distilled water was used throughout the conductivity study. Garopride®, 50 mg itopride HCl per tablet, Eva Pharma for Pharmaceutical & Medical Appliances S.A.E., Kafr El Gabal - Haram - Giza – Egypt.

#### *2.1.2. Conductivity measurements*

The conductance was measured by using a HANNA Conductivity/TDS Meter (HI 8033), with a HANNA Conductivity Probe (HI76301W). Specific conductivity was 1413 µS/cm. The temperature was controlled by means of a thermstated water bath with a precision of ± 0.01$^o$C. All conductivity measurements were done using the direct current (DC) method and the uncertainty of the electrical conductivity measurements k was ±1.0%.

#### *2.1.3. $^1$H NMR measurements*

All NMR measurements were done on Bruker 400 MHz NMR spectrometer (Bruker Corporation, Rheinstetten, Germany). Samples were measured in $D_2O$.

### *2.1.4. General procedure*

Itopride HCl (0.06 mol.kg$^{-1}$±0.02) solution was prepared in 100 ml of doubly distilled water and then the solution was transferred into the conductivity measuring cell. The temperature of the measured solution was maintained constant at the desired measured temperature (ranging from 293.15, 298.15, 303.15, 308.15, and 313.15 and 318.15 K) by a thermostated water bath. The conductivity data of the solution was recorded after stabilization of the display of the instrument scale.

One - two ml of the solution was removed and an equal volume of pure doubly distilled water having the same temperature of the measured solution was added to the measuring cell and the conductivity of the diluted solution was recorded. The previous step was repeated till the solution was diluted to a concentration of 0.01 mol.kg$^{-1}$.

For $^1$H NMR measurements, different concentrations (2.53-151.9) mmol.kg$^{-1}$were prepared in $D_2O$ and aliquots of 0.7 ml from each concentration were transferred into 5mm tubes and each $^1$H NMR spectra was recorded at T = 298.15 K.

For commercial tablets, ten tablets of Garopride® were powdered and different amounts equivalent to (25.32-126.61) mmol.kg$^{-1}$were dissolved in $D_2O$ and filtered through 0.45 µm nylon membrane filters before recording $^1$H NMR spectra at T = 298.15 K.

## III. Results and discussion

### *3.1. Conductivity measurements*

The conductivity of different concentrations of itopride HCl was measured in water at different temperatures ranging from 293.15 to 318.15 K. The plots of specific conductivity versus the molal concentration of itopride HCl are presented in Fig. 1. The values of the critical aggregation concentrations (CAC) were determined from the intersection of the two straight lines of each plot. Different values of CAC at different temperatures are represented in Table1.

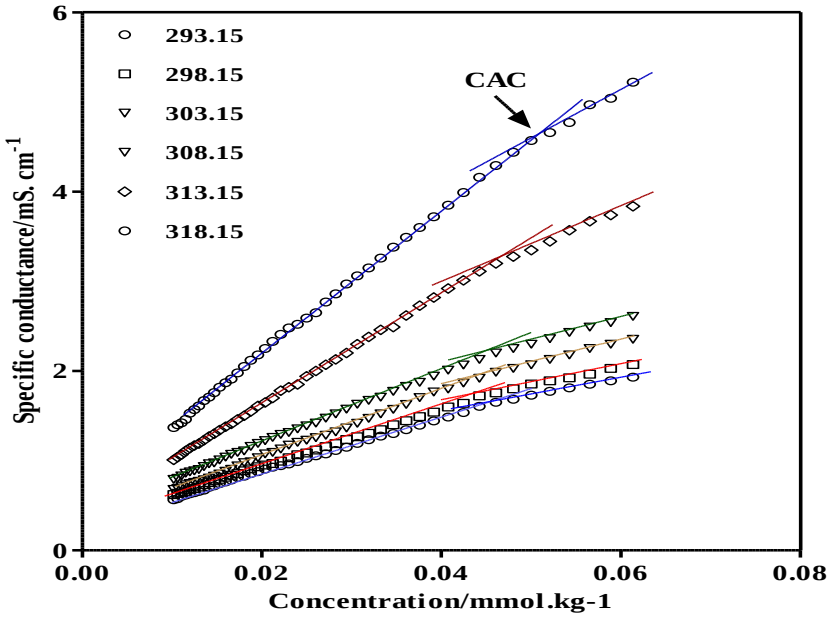



**Figure 1:** Specific conductance/mS.cm$^{-1}$ vs. concentration/mmol.kg$^{-1}$ plots of itopride HCl aqueous solutions at different temperatures.

**Table 1:** Critical aggregation concentration (CAC), degree of ionization ($\alpha$), standard Gibbs free energy change ($\Delta G°_m$), standard enthalpy change ($\Delta H°_m$) and the standard entropy change ($\Delta S°_m$)for itopride HCl various temperatures.

| T/K | CAC /mmol.kg$^{-1}$ | $\alpha$ | $\Delta G°_m$ kJ.mol$^{-1}$ | $\Delta H°_m$ kJ.mol$^{-1}$ | $\Delta S°_m$ J.Kmol$^{-1}$ |
|---|---|---|---|---|---|
| **293.15** | 44.4 | 0.623 | – 23.935 | – 11.009 | 44.090 |
| **298.15** | 43.8 | 0.649 | – 23.929 | – 14.168 | 32.738 |
| **303.15** | 44.5 | 0.669 | – 23.917 | – 17.482 | 21.227 |
| **308.15** | 45.2 | 0.688 | – 23.912 | – 20.913 | 9.732 |
| **313.15** | 47.1 | 0.702 | – 23.902 | – 24.542 | – 2.043 |
| **318.15** | 49.2 | 0.716 | – 23.874 | – 28.300 | – 13.911 |

### *3.1.1. Effect of temperature on CAC*

In general, CAC values decreases as the temperature decreases till a certain temperature ($T_m$) breakdown, then the values increase with further increase with temperature displaying a U-shaped behavior [37]. This can be explained as follow:

a) Increase the temperature decreases the hydration of the hydrophilic head groups of the amphiphilic structure and this behavior favors aggregation so aggregation takes place at lower concentration and CAC decrease.
b) Further increase in temperature leads to the breakdown of the structured water around the hydrophobic tail part so the thermal solubility of the amphiphile in water increases. In this case, the aggregation takes place at high concentration and the CAC value increase.

So the relative magnitude of these two opposing effects, therefore, determines whether the CAC increases or decreases over a particular temperature range. In case of itopride HCl, the CAC versus temperature plots show a typical U-shaped behavior [first decrease with increasing the temperature till a certain temperature ($T_m$= 298.15 K) and then increase with further increase in temperature] as illustrated in Fig. 2. The behavior of itopride HCl can be explicated as follows:

Below $T_m$, the dehydration effect predominates over the thermal solubility effect and so the CAC values start to decrease while above $T_m$, the thermal solubility effect predominates over the dehydration effect. The increase in the solubility of the amphiphile hinders its aggregation and a few number of head groups aggregate at high concentration so the CAC increases.

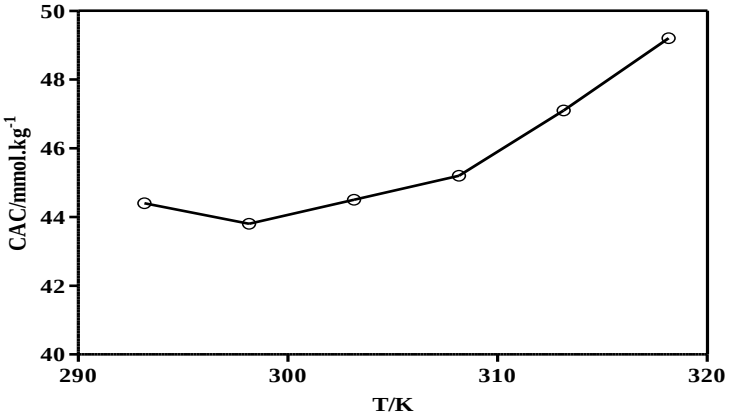



**Figure 2:** Temperature dependence of CAC/mmol.kg$^{-1}$ of itopride HCl aqueous solutions.

### *3.1.2. Degree of ionization*

The degree of ionization of the aggregate ($\alpha$) can be determined from conductance vs. molal concentration plots where $\alpha = S2/S1$

Where S2 = the slope of the conductivity–concentration plot above the CAC and $S1$ = the slope below the CAC [38]. Fig. 3 shows that $\alpha$ increased as a function of temperature due to the increase in thermal vibration [39]. A similar behavior was reported for decyldimethylbenzylammonim bromide and cationic surfactant solutions [40,41]. Results for the temperature-dependent values of $\alpha$ are provided in Table1.

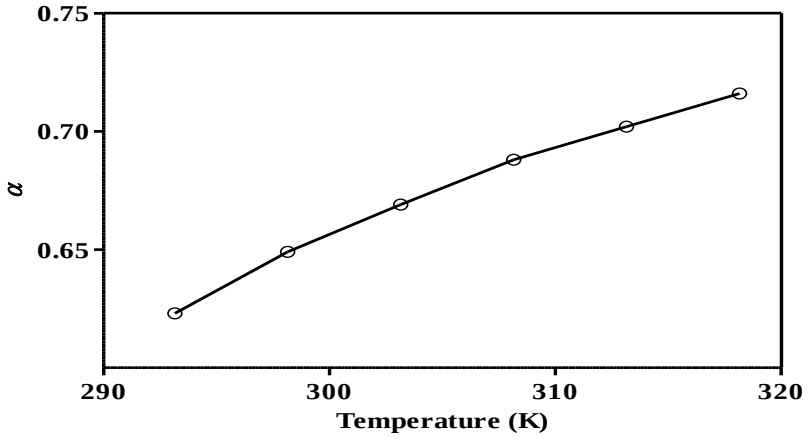
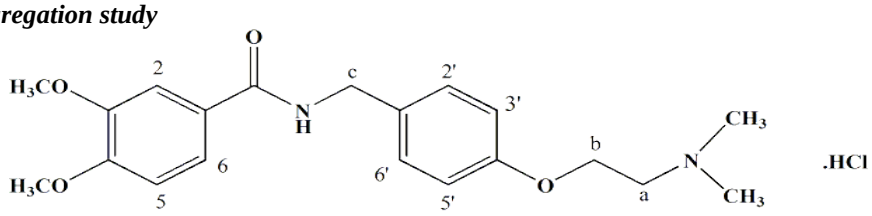



**Figure 3:** Temperature dependence of degree of ionization ($\alpha$) of itopride HCl aqueous solutions.

### *3.2. [1]HNMR measurements*

#### *3.2.1. Self-aggregation study*




The self-aggregation behavior of itopride HCl has been investigated by $^1$H NMR spectroscopy. Different concentrations of itopride HCl (2.53-151.9) mmol.kg$^{-1}$were measured in $D_2O$. The NMR spectrum was assigned as follow (2.53 mmol.kg$^{-1}$in $D_2O$, δ chemical shift in ppm):

7.36 (1H, d, H6), 7.29 (2H, d, H2',6'), 7.26 (1H, s, H2), 7.00 (1H, d, H5), 6.94 (2H, d, H3',5'), 4.43 (2H, s, Hc), 4.28 (2H, t, Hb), 3.82 (3H, s,4-$OCH_3$), 3.80 (3H, s, 3-$OCH_3$), 3.50 (2H, t, Ha), 2.88 (6H, s, N-$(CH_3)_2$).

The $^1$H NMR spectra of itopride HCl solutions at the studied range of concentrations showed a decrease in the chemical shift values of the aromatic ring protons (upfield shift) on increasing the concentration of itopride HCl, Fig. 4. The decrease in chemical shift is expected to occur as a result of self-aggregation that brings the protons very close to the phenyl ring, which reduces the effective field causing an upfield shift in the observed chemical shift. The phenyl groups of itopride HCl would stack, and thus the aromatic protons would be most greatly affected. However, these protons show different degrees of chemical shift variations. The values of the upfield shift were – 0.221, – 0.174, – 0.225, – 0.353, – 0.143 ppm for H6, H2',6', H2, H5 and H3',5' respectively when increasing the concentration from (2.53-151.9) mmol.kg$^{-1}$.

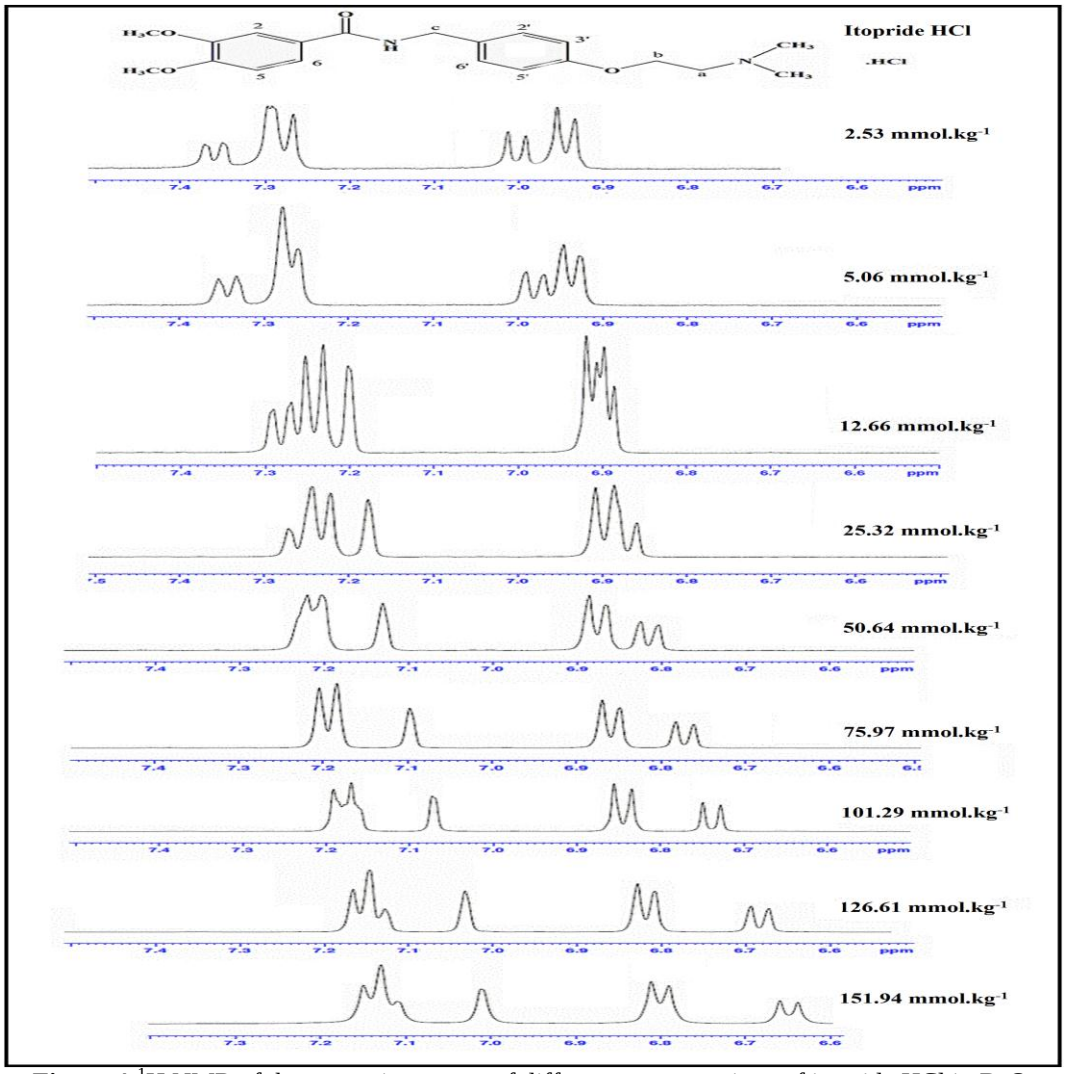



**Figure 4:**$^1$H NMR of the aromatic protons of different concentrations of itopride HCl in $D_2O$.

The chemical shifts of the aromatic ring protons were plotted versus the reciprocal molal concentration (Fig. 5) and the CAC values were determined from the intersection of the two linear regions of the plots above and below the inflection region. Values obtained for CAC from different aromatic protons plots at T = 298.15 K are 40.00, 47.61, 42.55, 40.81 and 43.4 mmol.kg$^{-1}$ for H6, H2',6', H2, H5 and H3',5', respectively, which are close to those values obtained from conductivity measurements.

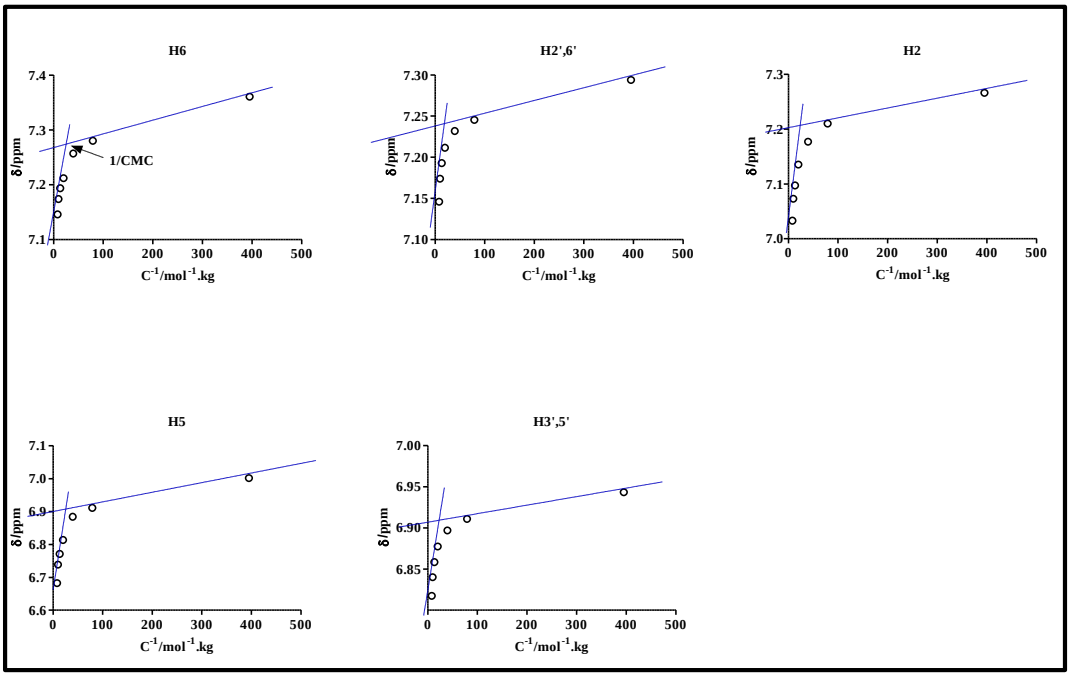



**Figure 5:** Plots of the chemical shift δ/ppm values against reciprocal molal concentration for different aromatic protons of itopride HCl in $D_2O$.

### *3.2.2. Quantitative determination of itopride*

The $^1$H NMR results of itopride HCl in $D_2O$ over a concentration range (2.53-151.9) mmol.kg$^{-1}$ were recorded and the chemical shift values for each aromatic proton were plotted against concentration. The plots showed a strong dependence of chemical shift on concentration and this dependence was found to be linear over a concentration range (25.32-126.61) mmol.kg$^{-1}$ as shown in Fig. 6.

The regression equation of the linear region for each proton was calculated (Table2). The regression equation obtained from H 3',5' showed the best regression coefficient so the regression equation of this proton was selected for the quantitative determination of itopride HCl in the authentic drug and in Garopride® tablets, Table3. The results of the quantitative method showed an excellent recovery verified by statistical comparison with the results of the reported method [6] using the Student's t-test and the Variance ratio test (F-test) which showed no significant difference between them (Table3).

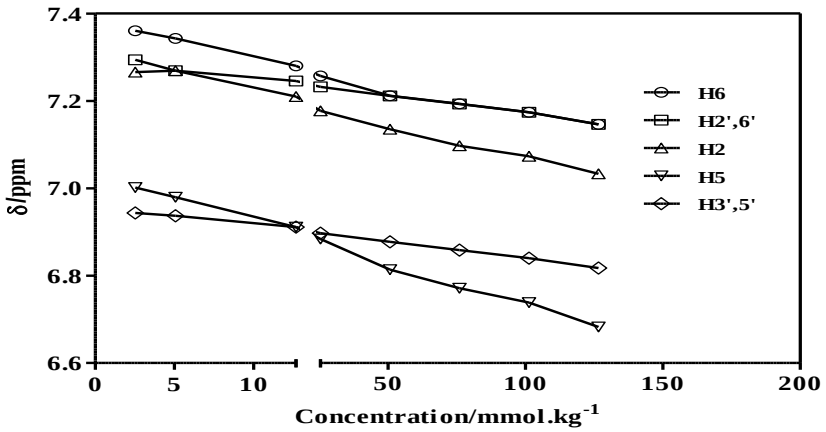



**Figure 6:** Concentration/ mmol.kg$^{-1}$ vs. chemical shift/ppm of different aromatic protons of itopride HCl in $D_2O$.

**Table 2:** Regression equations for different aromatic protons of itopride HCl.

| Proton | Regression equation | Regression coefficient/r |
|---|---|---|
| **H6** | δ = -0.0029C + 7.2841 | 0.9844 |
| **H2',6'** | δ = -0.0021C + 7.2551 | 0.9976 |
| **H2** | δ = -0.0038C + 7.2182 | 0.9932 |
| **H5** | δ = -0.005C + 7.9284 | 0.9936 |
| **H3',5'** | δ = -0.002C + 7.9171 | 0.9993 |

**Table 3:** Statistical comparison between the proposed $^1$H NMR method and a reported method for itopride HCl in its pure form and the results obtained from the pharmaceutical tablets.

| | **Proposed method** | **Reported method** [6] | **Garopride tablets** |
|---|---|---|---|
| **Mean** | 98.627 | 99.870 | 99.477 |
| **SD** | 1.627 | 0.851 | |
| **RSD%** | 1.649 | 0.852 | 1.931 |
| **Variance** | 2.647 | 0.724 | |
| **SE** | 0.727 | 0.283 | 1.941 |
| **n** | 5 | 9 | 3.728 |
| **F test** | 3.656 (3.84) | | 0.863 |
| **Student's t test** | 1.907 (2.179) | | 5 |

### *3.3. Thermodynamics of aggregation*

Aggregation is sensitive to temperature and the dependence of the CAC values on temperature can be used to evaluate the thermodynamic parameters of aggregation. The standard Gibbs free energy change $\Delta G^{o}_{m}$, the standard enthalpy change $\Delta H^{o}_{m}$ and the standard entropy change $\Delta S^{o}_{m}$ were derived according to the pseudo-phase model [38,42,43].

$\Delta G^{o}_{m}$ values were calculated from the following equation:

$$\Delta G^{o}_{m} = (2-\alpha)\, RT \ln X_{CAC} \quad (1)$$

where $\alpha$ is the degree of ionization, R is the universal gas constant, T is the absolute temperature and $X_{CAC}$ is the mole fraction of itopride HCl at the CAC.

The aggregation of itopride HCl was associated with large negative values of $\Delta G^{o}_{m}$ that increases on increasing the temperature. This indicates that the self-aggregation becomes more spontaneous and thermodynamically favored at lower temperature. Small differences in the $\Delta G^{o}_{m}$ values were observed over the studied temperature range. The negative values of $\Delta G^{o}_{m,}$ resulting from the large, positive $\Delta S^{o}_{m,}$ support that aggregation is an entropy-driven process particularly at law temperature, Fig. 7.

$\Delta H^{o}_{m}$ was obtained from the Gibbs-Helmholtz relation [44]:

$$\Delta H^{o}_{m} = -(2-\alpha)\, RT^2 \left(d \ln X_{CAC}/dT\right) \quad (2)$$

To obtain $(d \ln X_{CAC} / dT)$ value, the $\ln X_{CAC}$ versus temperature plot was fitted to the second order polynomial followed by differentiation, Fig. 8.

The temperature-dependent values of $\Delta H^{o}_{m}$ were found to be negative, confirming that aggregate formation is a spontaneous and exothermic process and prove the importance of London dispersion force in the aggregation process (Fig. 9) [38].

The standard entropy change $\Delta S^{o}_{m}$ was evaluated from the relation:

$$\Delta S^{o}_{m} = (\Delta H^{o}_{m} - \Delta G^{o}_{m})/T \quad (3)$$

The entropy of aggregation was positive at lower temperature and becomes negative at higher temperature, Fig. 10.

The decrease in $\Delta H^{o}_{m}$ and $\Delta S^{o}_{m}$ values with temperature suggests that the aggregate formation is an energy-driven process at higher temperature.

Linear relationship was found between $\Delta H^{o}_{m}$ and $\Delta S^{o}_{m}$ and effectively led to a negative and temperature-independent $\Delta G^{o}_{m}$. This phenomenon is called the enthalpy–entropy compensation [38].

The results of the temperature dependence of $\Delta H^{o}_{m}$ and $\Delta S^{o}_{m}$ were used to construct the enthalpy–entropy compensation plot (Fig. 11) that can be described in the form of a straight line equation [38]:

$$\Delta H^{o}_{m} = \Delta H^{*}_{m} + T_c \Delta S^{o}_{m} \quad (4)$$

The slope is the compensation temperature ($T_c$) that was found to be 298.25 and $\Delta H^{*}_{m}$ is the intercept at the origin and was –23.96 kJ mol$^{-1}$.

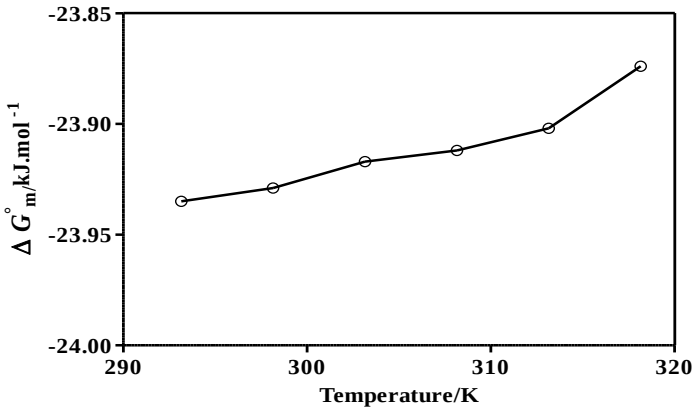



**Figure 7:** Plots of $\Delta G^{\circ}_{m}$/kJ.mol$^{-1}$ vs. temperature/K for itopride HCl aqueous solutions.

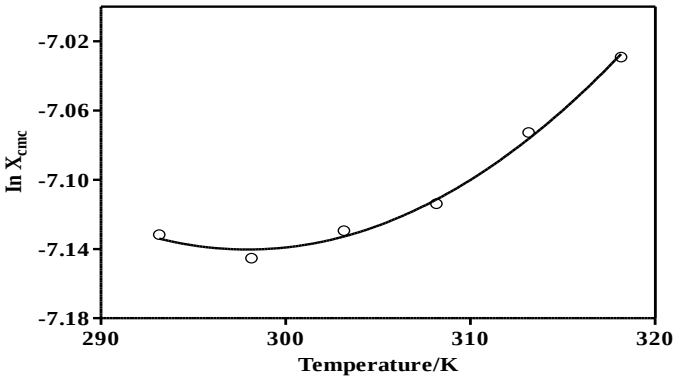



**Figure 8:** The second polynomial fit of ln $X_{CAC}$ of itopride HCl aqueous solutions vs. temperature/K.

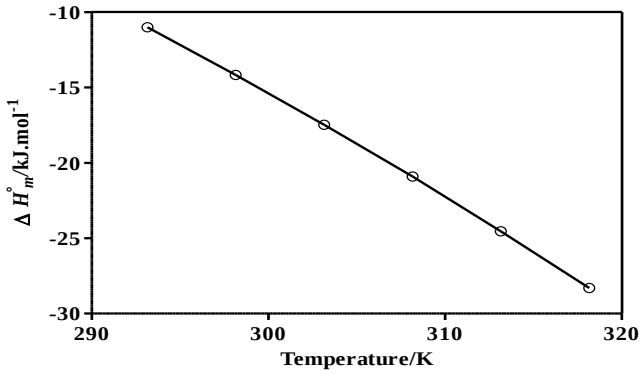



**Figure 9:** Plots of $\Delta H^{\circ}_{m}$/kJ.mol$^{-1}$ vs. temperature/K for itopride HCl aqueous solutions.

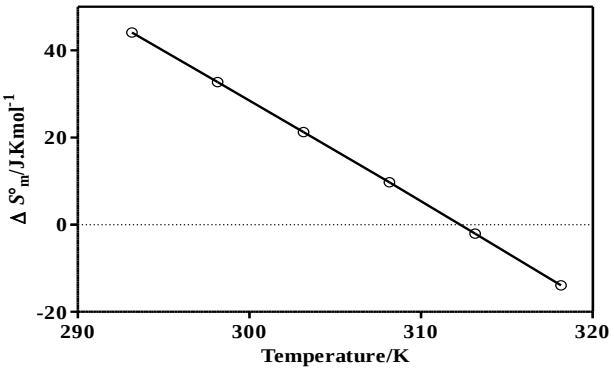



**Figure 10:** Plots of $\Delta S^{\circ}_{m}$/kJ.mol$^{-1}$ vs. temperature/K for itopride HCl aqueous solutions.

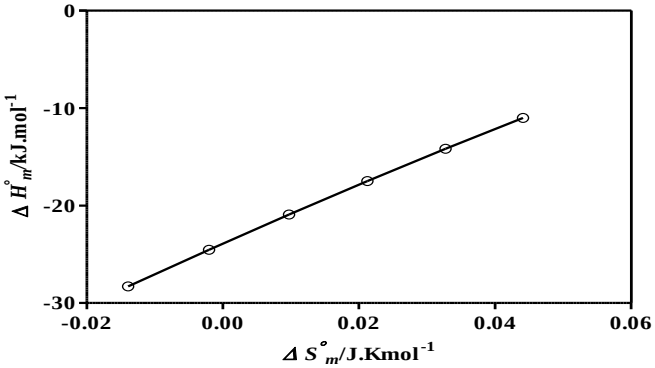



**Figure 11:** Typical enthalpy–entropy compensation plot for itopride HCl aqueous solution.

## IV. Conclusion

Aggregation of itopride HCl was investigated in water as a function of temperature. The CAC and $\alpha$ value were calculated from the concentration-dependent conductivity data. The values of CAC were confirmed by $^1$H NMR based on the concentration-dependent chemical shift variation. Gibbs free energy, enthalpy and entropy of aggregation were evaluated as a function of temperature by pseudo phase separation method. The calculated enthalpy and entropy of aggregation was correlated by enthalpy - entropy compensation phenomenon.

$^1$H NMR was also used for quantitative determination of itopride HCl in pure form and in pharmaceutical tablets without interference from excipients with the aromatic protons signals. The new quantitative $^1$H NMR method is selective, accurate, precise and does not require the use of internal or external standard so many disadvantages like solute-solute interaction and broadening of signals can be avoided.

## References

[1]. S.C. Sweetman, *Martindale, the complete drug reference* (London: The Pharmaceutical Press, 2009).
[2]. T. Tsubouchi, T. Saito, F. Mizutani, T. Yamauchi and Y. Iwanaga, Stimulatory action of itopride hydrochloride on colonic motor activity in vitro and in vivo, *The Journal of Pharmacology and Experimental Therapeutics. 306(2)*, 2003, 787–793.
[3]. K.D. Tripathi, *Essentials of medical pharmacology* (New Delhi,: Jaypee Brothers medical publishers, 2013).
[4]. N.K. Ramadan, N.A. El-Ragehy, M.T. Ragab and B.A. El-Zeany, Simultaneous determination of a binary mixture of pantoprazole sodium and itopride hydrochloride by four spectrophotometric methods, *Spectrochimica Acta Part A, Molecular and Biomolecular Spectroscopy*, *137*, 2015, 463–470.
[5]. H.M. Mohamed, A study of selective spectrophotometric methods for simultaneous determination of itopride hydrochloride and rabeprazole sodium binary mixture: Resolving sever overlapping spectra, *Spectrochimica Acta Part A, Molecular and Biomolecular Spectroscopy,136*, 2014, 1308–1315.
[6]. K.R. Gupta, R.R. Joshi, R.B. Chawlaand S.G. Wadodkar, UV spectrophotometric method for the estimation of itopride hydrochloride in pharmaceutical formulation, *E-Journal of Chemistry*, *7(S1)*, 2010, S49–S54.
[7]. A.M. Yehia, Development and validation of new spectrophotometric ratio H-point standard addition method and application to gastrointestinal acting drugs mixtures, *Spectrochimica Acta Part A, Molecular and Biomolecular Spectroscopy*, *109*, 2013, 193–200.
[8]. B. Choudhary, A. Goyaland S.L. Khokra, New visible spectrophotometric method for estimation of itopride hydrochloride from tablets formulations using methyl orange reagent, *International Journal of Pharmacy and Pharmaceutical Sciences, 1(1)*, 2009, 159–162.
[9]. S.S. Sabnis, N.D. Dhavale, V.Y. Jadhavand S.V. Gandhi, Spectrophotometric simultaneous determination of rabeprazole sodium and itopride hydrochloride in capsule dosage form, *Spectrochimica Acta Part A Molecular and Biomolecular Spectroscopy, 69(3)*, 2008, 849–852.
[10]. P. Pattanayak, R. Sharma and S.C. Chaturvedi, Simultaneous spectrophotometric estimation of rabeprazole sodium and itopride HCl, *Analytical Letters, 40(12)*, 2007, 2288–2294.
[11]. F. Ibrahim and J.J. Nasr, Fourth-derivative synchronous spectrofluorimetry and HPLC with fluorescence detection as two analytical techniques for the simultaneous determination of itopride and domperidone, *Luminescence : The Journal of Biological and Chemical Luminescence*, *31(1)*, 2016, 255–263.
[12]. M.I. Walash, F. Ibrahim, M.I. Eid and S.A. El Abass, Stability-indicating spectrofluorimetric method for determination of itopride hydrochloride in raw material and pharmaceutical formulations, *Journal of Fluorescence, 23(6)*, 2013, 1293–1300.
[13]. M.T. Ragab, M.K. Abd El-Rahman, N.K. Ramadan and N.A. El-Ragehy, B.A. El-Zeany, Novel potentiometric application for the determination of pantoprazole sodium and itopride hydrochloride in their pure and combined dosage form, *Talanta, 138*, 2015, 28–35.
[14]. X. Hun and Z. Zhang, Electrogenerated chemiluminescence sensor for itopride with Ru(bpy)3(2+)-doped silica nanoparticles/chitosan composite films modified electrode, *Sensors and Actuators B: Chemical, 131(2)*, 2008, 403–410.
[15]. D. Bageshwar, V. Khanvilkar and V. Kadam, Stability indicating high performance thin-layer chromatographic method for simultaneous estimation of pantoprazole sodium and itopride hydrochloride in combined dosage form, *Journal of Pharmaceutical Analysis*, *1(4)*, 2011, 275–283.
[16]. A. Suganthi, S. John and T.K. Ravi, Simultaneous HPTLC determination of rabeprazole and itopride hydrochloride from their combined dosage form, *Indian Journal of Pharmaceutical Sciences, 70(3)*, 2008, 366–368.
[17]. N. Kaul, H. Agrawal, P. Maske, J.R. Rao, K.R. Mahadik and S.S. Kadam, Chromatographic determination of itopride hydrochloride

in the presence of its degradation products, *Journal of Separation Science, 28(13),* 2005, 1566–1576.
[18]. S.S. Perumal, S.P. Ekambaram and S. Raja, Analytical method development and validation of simultaneous estimation of rabeprazole, pantoprazole, and itopride by reverse-phase high-performance liquid chromatography, *Journal of Food and Drug Analysis, 22(4),* 2014, 520–526.
[19]. Y. Sun, Z. Zhang, Z. Xi, Z. Shi and W. Tian, Determination of itopride hydrochloride by high-performance liquid chromatography with Ru(bpy)3(2+) electrogenerated chemiluminescence detection, *Analytica Chimica Acta, 648(2),* 2009, 174–177.
[20]. J. Ma, L.-H. Yuan, M.-J. Ding, J. Zhang, Q. Zhang, Q.-W. Xu and X.-M. Zhou, Determination of itopride hydrochloride in human plasma by RP-HPLC with fluorescence detection and its use in bioequivalence study, *Pharmacological Research, 59(3),* 2009, 189–193.
[21]. P. Ptácek, J. Klíma and J. Macek, Optimized method for the determination of itopride in human plasma by high-performance liquid chromatography with fluorimetric detection, *Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences, 877(8-9),* 2009, 842–846.
[22]. H.-W. Lee, J.-H. Seo, S.-K. Choi and K.-T. Lee, Determination of itopride in human plasma by liquid chromatography coupled to tandem mass spectrometric detection: application to a bioequivalence study, *Analytica Chimica Acta, 583(1),*2007, 118–123.
[23]. S.S. Singh, M. Jain, K. Sharma, B. Shah, M. Vyas, P. Thakkar, R. Shah, S. Singh and B. Lohray, Quantitation of itopride in human serum by high-performance liquid chromatography with fluorescence detection and its application to a bioequivalence study, *Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences, 818(2),*2005, 213–220.
[24]. M.L. Waters, Aromatic interactions in peptides: impact on structure and function, *Biopolymers, 76(5),*2004, 435–445.
[25]. E.C. Lee, B.H. Hong, J.Y. Lee, J.C. Kim, D. Kim, Y. Kim, P. Tarakeshwar and K.S. Kim, Substituent effects on the edge-to-face aromatic interactions, *Journal of the American Chemical Society, 127(12),* 2005, 4530–4537.
[26]. E.C. Lee, D. Kim, P. Jurecka, P. Tarakeshwar, P. Hobza and K.S. Kim, Understanding of assembly phenomena by aromatic-aromatic interactions: benzene dimer and the substituted systems, *The Journal of Physical Chemistry A, 111(18),* 2007, 3446–3457.
[27]. X. Ding, T.C. Stringfellow and J.R. Robinson, Self-association of cromolyn sodium in aqueous solution characterized by nuclear magnetic resonance spectroscopy, *Journal of Pharmaceutical Sciences, 93(5),* 2004, 1351–1358.
[28]. S. Michaleas and E. Antoniadou-Vyza, A new approach to quantitative NMR : Fluoroquinolones analysis by evaluating the chemical shift displacements, *Journal of Pharmaceutical and Biomedical Analysis, 42(4),* 2006, 405–410.
[29]. H. Sun, Y. Zhao, Z. Huang, Y. Wang and F. Li, 1H NMR study on the self-association of quinacridone derivatives in solution, *The Journal of Physical Chemistry A, 112(45),* 2008, 11382–11390.
[30]. I.S. Blagbrough, M.S. Elmasry, T.J. Woodman, H.M. Salehand A. Aboul Kheir, Quantitative determination of mebeverine HCl by NMR chemical shift migration, *Tetrahedron, 65(25),* 2009, 4930–4936.
[31]. P. Agrawal, S.K. Barthwal and R. Barthwal, Studies on self-aggregation of anthracycline drugs by restrained molecular dynamics approach using nuclear magnetic resonance spectroscopy supported by absorption, fluorescence, diffusion ordered spectroscopy and mass spectrometry, *European Journal of Medicinal Chemistry, 44(4),* 2009, 1437–1451.
[32]. P. Zeng, G. Zhang, A. Rao, W. Bowles and T.S. Wiedmanna, Concentration dependent aggregation properties of chlorhexidine salts, *International Journal of Pharmaceutics, 367(1-2),* 2009, 73–78.
[33]. M.S. Elmasry, W.S. Hassan and M.S. Elazazy, Self-association and thermodynamic behavior of etilefrine hydrochloride in aqueous electrolyte solution, *Journal of Chemical Thermodynamics, 79,* 2014, 76–83.
[34]. P. Taboada, D. Attwood, J.M. Ruso, M. García, F. Sarmiento and V. Mosquera, Self-association of the penicillin sodium nafcillin in aqueous solution, *Langmuir, 16(7),* 2000, 3175–3181.
[35]. Kabir-ud-Din, Z. Yaseen and M.S. Sheikh, Modulation of aggregation behavior of amphiphilic drug AMT under the influence of polymer molecular weight and composition, *Colloids and Surfaces. B, Biointerfaces, 87(2),* 2011, 340–345.
[36]. M.S. Alam, D. Samanta and A.B. Mandal, Micellization and clouding phenomenon of amphiphilic antidepressant drug amitriptyline hydrochloride: Effect of KCl, *Colloids and Surfaces B: Biointerfaces, 92,*2012, 203–208.
[37]. M.J. Rosen, Surfactants and interfacial phenomena (New York: John Wiley & Sons, 2004).
[38]. S. Chauhan and K. Sharma, Effect of temperature and additives on the critical micelle concentration and thermodynamics of micelle formation of sodium dodecyl benzene sulfonate and dodecyltrimethylammonium bromide in aqueous solution: A conductometric study, *Journal of Chemical Thermodynamics, 71,*2014, 205–211.
[39]. Kabir-ud-Din, M.A. Rub and A.Z. Naqvi, Self-association behavior of amitriptyline hydrochloride as a function of temperature and additive (inorganic salts and ureas) concentration, *Colloids and Surfaces B: Biointerfaces, 82,* 2011, 87–94.
[40]. A. González-Pérez, J.L. Del Castillo, J. Czapkiewicz and J.R. Rodríguez, Thermodynamics of micellization of decyldimethylbenzylammonium bromide in aqueous solution, *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 232(2-3),* 2004, 183–189.
[41]. J.J. Galán, A. González-Pérez, J.A. Seijas, E. Uriarte and J.R. Rodríguez, Effect of counterion on thermodynamic micellar properties of tetradecylpyridinium in aqueous solutions, *Colloid and Polymer Science, 283(4),* 2005, 456–460.
[42]. Kabir-ud-Din, M.A. Rub and A.Z. Naqvi, Mixed micelle formation between amphiphilic drug amitriptyline hydrochloride and surfactants (Conventional and Gemini) at 293.15-308.15 K, *Journal of Physical Chemistry B, 114 (19),*2010, 6354–6364.
[43]. D. Tikariha, B. Kumar, N. Singh, K.K. Ghosh and P. Quagliotto, Micellization behavior of cationic gemini surfactants in aqueous-ethylene glycol solution, J*ournal of Surfactants and Detergents, 14(4),* 2011, 555–562.
[44]. S. Kumar and K. Parikh, Influence of temperature and salt on association and thermodynamic parameters of micellization of a cationic gemini surfactant, *Journal of Applied Solution Chemistry and Modeling, 1(1),*2012, 65–73.