

EXPERIMENTAL STUDY OF THE DIELECTRIC BEHAVIOR OF TINIDAZOLE IN ACETONE AT 25°C

ESTUDIO EXPERIMENTAL DEL COMPORTAMIENTO DIELECTRICO DEL TINIDAZOL EN ACETONA A 25°C

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ABSTRACT

Tinidazole is an anti-parasitic drug widely used nowadays in therapeutics. Nevertheless, it has not been well characterized from a physicochemical point of view. In this context, by means of optical experimental methods and dielectric spectroscopy applied to diluted solutions of Tinidazole in Acetone, molar polarizations of the solute, ${}_0P_2$, and the solvent, ${}_0P_1$, and the average dipolar moment $\bar{\mu}$ of tinidazole associated to acetone, were estimated, resulting in $\bar{\mu} = 9.18$ D. Since this value is higher than the theoretical μ of the two main conformers of isolated tinidazole, that is, 3.22 and 4.29 D, respectively, the formation of interactions between the solute and the solvent is assumed. An effect of intermolecular association by van der Waals and hydrogen bond interactions conducting to a modification of the partial molar volume of the solute, \bar{V}_2 , is thus expected. From the experimental and analysis by using the Halverstadt-Kumler's method, it can be seen that \bar{V}_2 had a 24% reduction as compared with the theoretical value, which would confirm the presence of such interactions.

Keywords: Tinidazole, polarization, permittivity, dipole moment.

RESUMEN

El tinidazol es un fármaco antiparasitario ampliamente utilizado en la actualidad. Sin embargo, este fármaco no ha sido bien caracterizado desde un punto de vista fisicoquímico. Por esta razón, mediante algunos métodos ópticos y espectroscopía dieléctrica, aplicados a soluciones diluidas de tinidazol en acetona, se estimaron las polarizabilidades molares del soluto, ${}_0P_2$, y del solvente, ${}_0P_1$, además del momento dipolar promedio, $\bar{\mu}$, del fármaco asociado a la acetona, obteniendo un valor $\bar{\mu} = 9,18$ D. Puesto que este valor es mayor que el obtenido teóricamente para los dos conformeros principales, osea 3,22 y 4,29

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D, respectivamente, se asume la presencia significativa de interacciones entre el soluto y el solvente. Por lo tanto, se espera que la asociación intermolecular soluto-solvente, establecida por fuerzas de van der Waals y por enlaces de hidrógeno, conduzca a la modificación del volumen molar parcial del soluto, \bar{V}_2 . A partir del análisis experimental mediante el método de Halverstadt-Kumler se observa una reducción del 24% en el valor de \bar{V}_2 , al compararlo con el valor teórico, lo cual podría confirmar la presencia de tales interacciones.

Palabras clave: Tinidazol, polarización, permitividad, momento dipolar.

INTRODUCTION

Tinidazole (TN, see figure 1), Fasigyn[®] or Simplotan[®], whose molecular formula is $C_8H_{13}N_3O_4S$, exhibits physical properties as white or pale yellow crystals when it is crystalized in benzene. TN has antibacterial activity and therapeutical efficacy on anaerobic microorganisms (1). It is practically insoluble in water, but soluble in acetone and highly soluble in methanol (2).

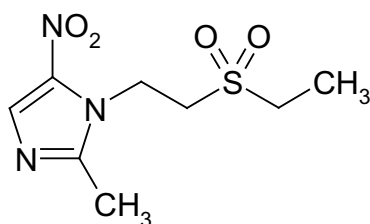


Figure 1. Molecular structure of tinidazole.

Tinidazole, on the same way as metronidazole (MN) and ornidazole (ON), integrates a group of structurally related drugs. They chemically derive from 5-nitroimidazole and they are therapeutically used against amebiasis and infections caused by anaerobic bacteria in humans. MN and TN have been used against *Helicobacter pylori* (3). The pharmacological applications of these drugs also extend to the veterinary medicine field.

TN has been reported to hydrolyze by following a first order kinetics within a wide pH range, although its highest stability could be seen between 4 and 5 (4). TN was also found to be a suitable drug to study substance transfer through different biological membranes, and thus, a bank of human organs has been used for that purpose (5).

Despite of the various applications of TN, physicochemical studies referred to its dielectric behavior were not found in literature. In this work, the behaviour of TN in acetone (AC) solution under the effect of an electrical field at proper conditions to apply Buckingham and Halverstadt-Kumler's

method was studied (6-8). For that purpose, it was necessary experimentally to determine refraction indexes, densities, specific volumes, permittivities and solution dipolar moments. It is important to note that AC is not used as ingredient in pharmaceutical dosage forms because of its toxicity but it is widely used as model solvent for several physicochemical treatments with drugs and other pharmaceutical compounds. AC acts in solution mainly as a Lewis base, because of its carbonyl moiety, which can interact with drug Lewis acids groups, besides of its low polarity, which makes it a good solvent for semipolar compounds. Moreover, polarity of TN and AC are thus similar as will be indicated later.

Dielectric spectroscopy

Dielectric spectroscopy, also known as impedance spectroscopy, measures the dielectric properties of a medium as a function of frequency. In this context, a substance behavior under the action of an electric field could be understood and explained in function of the concept of dielectric constant or permittivity, ϵ . An electric field applied to a parallel plate capacitor causes the localization of opposed charges on its plates and a difference of potential, ΔV . Capacitance, C , is expressed in Farads (defined as Coulomb/Volt) (9, 10). For a capacitor full of a dielectric, the capacitance obtained, C_x , divided by the capacitance of reference, C_0 , is called dielectric constant or permittivity, ϵ :

$$\epsilon = \frac{C_x}{C_0} \quad \text{Equation 1.}$$

If the capacitor is occupied by a substance whose molecules exhibit a permanent dipolar moment, such molecules tend to be oriented by following the direction of the applied field; as a response, a maximum dipolar moment and a total molar polarization are obtained, being their magnitudes dependent on the applied field intensity.

When a polar substance is dissolved in a non-polar solvent and subjected to the action of an electric field, the dielectric response is due to the solute dipoles. When solute and solvent are polar, the events in the solution are more complex because different associations that influence the effective value of the molecular polarizations, ${}_0P_2$ and ${}_0P_1$, are thus generated (11).

Both properties are estimated by using equations 2 and 3 applying the Buckingham's theory (6-8) that relates optical, dielectric, and structural properties to the solutions composition.

When combining the Buckingham's method with that of Halverstadt-Kumler, applied to highly diluted solutions, linear correlations are obtained on plotting permittivity, ε , specific volume, v , and the square of the refraction index, n^2 , of the solutions, in function of the solute mass fraction, w_2 . Thus, the ε_1 , \bar{v}_1 , and n_1^2 values correspond to the pure solvent and they are obtained from extrapolation at infinite dilution (11). The specific volume, v , is estimated as the reciprocal of density, $1/d$.

$${}_0P_2 = \frac{1}{x_2} \left[\frac{\varepsilon + (n_2^2 - \varepsilon)A_2}{1 + (n_2^2 - 1)A_2} \right]^2$$

$$\left[\frac{\varepsilon - n^2}{\varepsilon(2\varepsilon + n^2)} \frac{x_1M_1 + x_2M_2}{d} - x_1x_0P_1 \left[\frac{1 + (n_1^2 - 1)A_1}{\varepsilon + (n_1^2 - \varepsilon)A_1} \right]^2 \right]^2$$

Equation 2.

$${}_0P_1 = \frac{(\varepsilon - n^2) \left[\varepsilon + (n^2 - \varepsilon)A_1 \right] M_2}{\varepsilon(2\varepsilon + n^2) \left[1 + (n^2 - 1)A_1 \right]^2 d}$$

Equation 3.

where, ${}_0P$ is the molar polarization, n is the refraction index, A is the form factor, ε is the permittivity, x is the molar fraction, M is the molar mass, and d is the density. In the expressions of ${}_0P_1$ and ${}_0P_2$, the subindexes 1 and 2 refer to the solvent and solute data, respectively, whereas those symbols with no subindex correspond to the solution.

Form factor

In order to estimate the form factors, A_1 and A_2 , different methodologies are used. Taking into account the Onsager's model, the solute molecule is considered to be a sphere containing a puntual dipole of dipolar moment in its center, whereas the solvent is regarded as a continuous, homogeneous

and polarizable medium of dielectric constant, ε . These conditions lead to a reaction field that relates different dielectric and geometric parameters (8, 11, 12).

Buckingham (11) developed a treatment to evaluate the polarization of molecules immersed in an ellipsoidal cavity. In such a case, A_2 can be calculated using equation 4 according to Farrell (13, 14), which requires to know not only the main axes of the ellipsoid, a , b and c , but also the direction of the dipolar moment vector with respect to its axes.

$$A_2 = -0.09 + 0.36 \frac{b}{a} \quad \text{Equation 4.}$$

An extension of Onsager's model was carried out by Diehl and Freeman (12, 15), which is applied to ellipsoidal molecules. These authors propose equation 5 in order to estimate the form factor A when the main axes of the ellipsoid are not available.

$$B = \frac{n^2 A}{1 - B} \quad \text{Equation 5.}$$

The term B acquires different values when spheric molecules, flattened ellipsoids (oblate) or elongated ellipsoids (prolate), are considered (15).

Molar refraction and refraction index relationship

Due to the fact that molar refraction is independent both of temperature and aggregation state and it is an approximate measure of the molecule total volume without considering free spaces, its value is useful to characterize such substances. Equation 6, developed by Halverstadt-Kumler allows to calculate the solute molar refraction R_{2D} (7, 16) from theoretical and experimental parameters.

$$R_{2D} = \left[\frac{3\mathcal{W}_1}{(n_1^2 + 2)^2} + \frac{(n_1^2 - 1)}{(n_1^2 + 2)} (\bar{v}_1 + \beta) \right] M_2$$

Equation 6.

When the solute refraction index is not known, it can be estimated from the RM_{Theor} value which is obtained by means of the sum of the bonding refractions corresponding to the sodium line D by using the Lorentz-Lorenz equation (Eq. 7) (17, 18).

$$RM_{\text{Exp}} = \frac{(n_2^2 - 1) M_2}{(n_2^2 + 2) d_2} \quad \text{Equation 7.}$$

Dipolar moments

On working with concentrated solutions of polar solutes, molecule dipolar moments are oriented in a colinear or parallel way, which, added to the established solute-solute interactions, produce effects in the medium that should be taken into account in order to interpret the obtained results. To avoid the interaction influence and simplify the respective effects, solutions with concentrations tending to infinite dilution should be studied. Under these conditions, the solute dipolar moment, μ , associated with the solvent can be estimated with Equation 8, based on Debye's theory (19, 20) from the polarization experimental data, ${}_0P_2$, at operating temperature.

$$\mu = 0.01281({}_0P_2T)^{1/2} \quad \text{Equation 8.}$$

Solubility parameters

The solubility parameter (δ) is a polarity index that can be estimated by means of the Hildebrand-Scott equation, from the root square of the quotient of the cohesion energy variation, ΔU , and the molar volume variation, ΔV (Eq. 9). If ΔU is expressed in cal mol⁻¹ and ΔV in cm³ mol⁻¹, δ is expressed in Hildebrand units, H. In his theory, Hildebrand proposes that a solute dissolves better in a given solvent if both have similar solubility parameters (21-23).

$$\delta = \left(\frac{\Delta U}{\Delta V} \right)^{1/2} \quad \text{Equation 9.}$$

On the basis of Hildebrand's theory and knowing the δ values for solute and solvent, the most suitable solvent can be predicted for the solute under study.

Dipolar moment, permittivity, polarizations and solubility parameters are properties that give idea of the polarity degree of the species in solution.

MATERIALS AND METHODS

Reagents

Samples of TN, Sigma, $\bar{v} = 214.9 \text{ cm}^3 \text{ mol}^{-1}$, $n = 1.599$ and $RM = 57.74 \text{ cm}^3$ (24) were used. Before the solubility assays were carried out, the samples had been dissecated by taking into account that the loss does not exceed 0.5% when they were treated in an oven at 100-105°C (2). Acetone, AC, was purchased from Merck, A.R. quality; $d = 0.7844 \text{ g cm}^{-3}$, $\varepsilon = 20.7$, $n = 1.35596$, $\mu = 2.78 \text{ D}$ and $v = 1.2673 \text{ cm}^3 \text{ g}^{-1}$ was used. These experimental values were determined at 25°C (25).

Solution preparation

A set of 10 diluted solutions of TN in AC, whose compositions range between $1.47 \times 10^{-3} \leq w_2 \leq 2.05 \times 10^{-2}$, was prepared, being w_2 the solute mass fraction. Each solution was prepared immediately before measurements were carried out in order to prevent atmosphere pollution by humidity and solvent evaporation. All systems were thermostated at 25°C in a Haake thermostatic bath previous to experimental determinations.

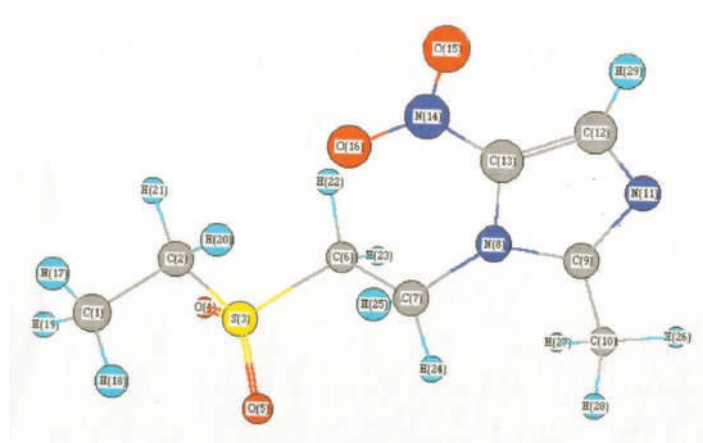
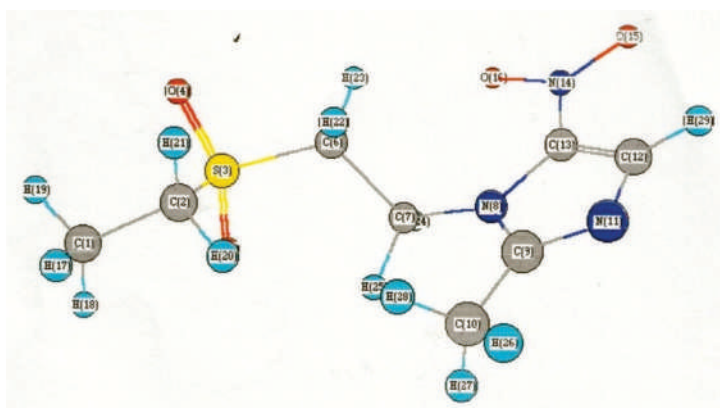
Methodology and equipment

Densities of the solutions and AC were experimentally determined with a digital densimeter (Anton Paar, model DM 58), calibrated at operating temperature (26). Refraction indexes were determined with an immersion refractometer (Aus Jena) with a sodium-discharge lamp and using prism E₁, being this equipment useful for the $1.3254 \leq n \leq 1.3664$ range. Permittivities of the pure solvent and of each solution were determined from capacitance values obtained by a dipolometer (WTW type DM 01) at fixed frequency (2 MHz). The equipment, which operates as an oscilloscope, is based on the heterodyne shaking method and is intended to give a linear correlation between the read capacitance and the permittivity. A cell MFL 3/S is coupled to the dipolometer, useful for liquid samples whose permittivities range between $20 \leq \varepsilon \leq 90$. The cell acts as a capacitor and during its filling the air bubble formation should be avoided. Solute and solvent polarization and TN dipolar moment were estimated from the experimental values of ε_1 , v , n_1 and d_1 whose respective values are 19.39, 1.2671 cm³ g⁻¹, 1.599, and 0.7891 g cm⁻³, all of them being obtained by our work team. The methodology applied in the experimental stage is based on previous works (16, 27).

RESULTS AND DISCUSSION

Tinidazole structure and dipolar moment relationship

The dipolar moment is a property associated with the molecule polarity. These molecules will have dipolar moment different from zero if the effective centers of positive and negative charges do not coincide. The μ values are in the order of 10^{-18} e.s.u., so Debye's unit is introduced and $1 \text{ D} = 10^{-18}$ e.s.u. (19). The two TN most stable conformers are shown in figure 2. The respective dipolar moments in the gas phase were estimated by using the Gaussian 98 computational method.

A (conformer I) $\mu_1 = 3.22$ D (gas phase)B (conformer II) $\mu_{II} = 4.29$ D (gas phase)**Figure 2:** TN structure, I and II conformers and dipole moments, μ , in gas phase, respectively.

The μ theoretical values were confronted with those that result from the application of Debye's equation (Eq. 8) after experimental polarization determinations were carried out by using Buckingham's equation (Eq. 2).

Refraction index estimation

Molar refraction, molar volume, and polarizability, are molecular descriptors appearing as a response to the combined effects of a number of intermolecular forces between the solute and its surroundings. RM is highly used in QSAR studies for drug design (18, 28).

Due to the fact that for a great number of compounds RM is practically additive from bonding refractions corresponding to the sodium D line (7), RM_{Theor} of TN can be estimated by taking into account its molecular structure, and this value can be used to estimate the solute refraction index when RM is not available.

By using Lorentz-Lorenz equation (Eq. 7) and the data of $RM_{exp} = 60.805$ cm³ obtained by Halverstadt-Kumler's equation (Eq. 6), the refraction index, $n_2 = 1.42168$, was calculated in the present work. In the literature (24) an estimated value of 1.599 is reported, but it was not indicated the obtention method. Thus, the value calculated to quantify polarizations by our work team was used.

Solvent selection

To apply Buckingham's method two essential conditions are required: 1) that the solute will be soluble in the solvent; and 2) that the solutions in diluted concentrations will give a response in the oscilloscope in order to determine the permittivity of the solutions. In the operational stage, certain limitations were found, such as the low solubility of TN in water and the lack of dielectric response for solutions diluted in ethanol, which required other assays with different solvents.

Table 1. Permittivity (ϵ), dipolar moment (μ) and Hildebrand solubility parameter (d) of different solvents at 25°C.

Property	Water	Ethanol	Propylene glycol	Ethylene glycol	Acetone	Methanol
ϵ	78.5	24.5	29	37.7	20.7	32.6
μ (D)	1.82	1.66 ^(a)	2.25 ^(a)	2.31	2.69	2.87 ^(a)
δ (H)	23.4	13.0	14.8	16.1	9.8	14.5

^(a)Data corresponding at 20 °C.

By using Fedors' tables (23) for the different group contributions on the basis of TN structure (see figure 1), energies and molar volumes were obtained, resulting in 24090 cal mol⁻¹ and 214.9 cm³ mol⁻¹, respectively. With these data and Hildebrand-Scott's equation (Eq. 9), TN solubility parameter was estimated, resulting $\delta_2 = 10.59$ H. According to Hildebrand's theory, a suitable TN solvent is the one whose δ value is very similar to δ_2 . This premise was the criterion taken into account for solvent selection (21, 29).

Although Spanish Pharmacopeia (2) indicates that TN can be dissolved in AC or in methanol, other solvents were tested, according to their respective solubility parameters, dipolar moment and permittivity. These properties that account for its polarities are shown in table 1 (17, 25, 29).

AC was selected as a solvent since its δ value is very similar to δ_2 . By taking into account Table 1, AC is considered to be a semipolar solvent.

Form Factor

In this work the Diehl and Freeman's model was used to obtain the value of the solute and solvent form factor. By taking into account equation 5, the value adopted by A depends on the B term, that takes values 1, 2.86, or 0.5, for sphere-shaped, ellipsoid-flattened, or ellipsoid-elongated molecules, respectively (12).

By assuming that AC has an elongated ellipsoidal shape, value $B = 0.5$ is considered. According to this value and equation 5, a value $A_1 = 0.214$ is obtained.

According to Allan *et al.*, 1999 (30), AC cell constants a , b and c are 6.1219, 5.2029 and 10.244 Å, respectively. These values allowed to apply both the Farrell's traditional method and equation 4, resulting $A_1 = 0.216$. The agreement between the values obtained by means of both methods allows us to assert that Diehl and Freeman's equation is a valid alternative to estimate A when the cell constant values are not available.

By taking into account the TN conformers, it can be seen that they occupy a rather elongated than flattened ellipsoidal cavity. With these considerations, value $B = 0.5$ was used, which applied in turn to equation 5, leads to a form factor $A_2 = 0.163$.

Halverstadt-Kumler's method

Experimental results obtained for all the solutions are shown in table 2.

Table 2. Mass fractions (w_2), densities (d), permittivities (ϵ), specific volumes (v) and refraction indexes (n^2) of TN – AC solutions at 25°C.

Solution	w_2	d (g cm ⁻³)	ϵ	v (cm ³ g ⁻¹)	n^2
1	1.47 x 10 ⁻³	0.7897	20.86	1.2663	1.83958
2	3.34 x 10 ⁻³	0.7905	20.88	1.2650	1.84007
3	5.41 x 10 ⁻³	0.7911	20.94	1.2641	1.84107
4	7.07 x 10 ⁻³	0.7921	20.97	1.2625	1.84148
5	1.02 x 10 ⁻²	0.7933	21.00	1.2606	1.84327
6	1.29 x 10 ⁻²	0.7942	21.08	1.2591	1.84406
7	1.54 x 10 ⁻²	0.7952	21.13	1.2575	1.84479
8	1.74 x 10 ⁻²	0.7959	21.14	1.2564	1.84522
9	1.89 x 10 ⁻²	0.7963	21.20	1.2558	1.84577
10	2.05 x 10 ⁻²	0.7971	21.20	1.2545	1.84721

Each solution specific volume was estimated as the reciprocal of their respective density. Plots of ϵ , v and n^2 in function of w_2 gave a linear response with adjusted determination coefficients, r^2 , 0.987, 0.9974 and 0.9858, respectively (see Figs. 3 to 5). The good r^2 values obtained would confirm the Halverstadt-Kumler's method validity throughout the studied drug concentration range. Besides, it allows both to obtain the ϵ_1 , \bar{v}_1 and n_1^2 values for the solvent by an extrapolation at infinite dilution and to know the $d\epsilon/dw_2$, dv/dw_2 , and dn^2/dw_2 slopes, defined as, α , β and γ , respectively (11). The values obtained from this methodology are shown in table 3.

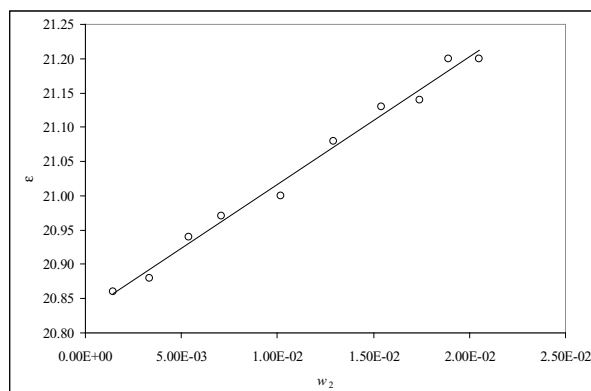


Figure 3: Permittivity (ϵ) vs. mass fraction (w_2) of TN in AC solutions. Linear equation obtained, $\epsilon = 18.7 (0.7) \times w_2 + 20.830 (0.009)$, with r^2 adjusted: 0.987 and typical error: 0.014.

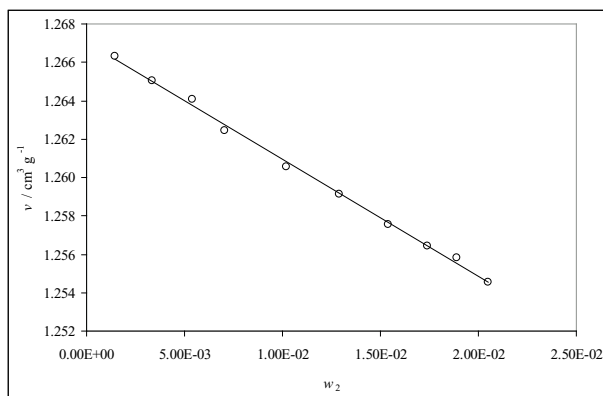


Figure 4: Specific volume (v) vs. mass fraction (w_2) of TN in AC solutions. Linear equation obtained, $v = -0.610 (0.010) \times w_2 + 1.26706 (0.00013)$, with r^2 adjusted: 0.9974 and typical error: 0.0002.

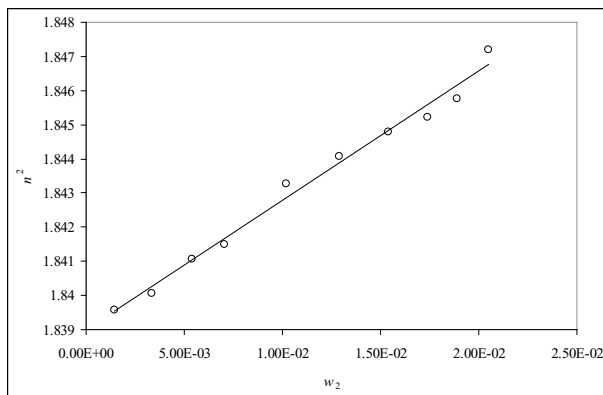


Figure 5: Refraction index (n^2) vs. mass fraction (w_2) of TN in AC solutions. Linear equation obtained, $n^2 = 0.380 (0.015) \times w_2 + 1.83897 (0.00020)$, with r^2 adjusted: 0.9858 and typical error: 0.0003.

Table 3. AC parameters values obtained by means of Halverstadt-Kumler's method.

Solvent	ϵ_1	\bar{V}_1 ($\text{cm}^3 \text{g}^{-1}$)	n_1^2	α	β	γ
AC (theor.)	20.70	1.2673	1.83863	--	--	--
AC (exp.)	20.83	1.2671	1.83897	18.7	-0.610	0.380

The agreement obtained between the experimental and theoretical values of ϵ_1 , \bar{v}_1 , and n_1^2 reinforces the method validity. This allows us to obtain all the parameters useful to estimate TN and AC polarizations.

Polarizations

The physicochemical parameters obtained were introduced in equations 2 and 3 in order to estimate ${}_0P_2$ and ${}_0P_1$ values according to Buckingham's model (6, 8); and thus, through Debye's equation (Eq. 8) the dipolar moments of the respective solutions were estimated. Data are shown in table 4.

Table 4. Solvent and solute polarizations and dipolar moments of TN associated with AC at 25°C.

Solution	${}_0P_1$ ($\text{cm}^3 \text{mol}^{-1}$)	${}_0P_2$ ($\text{cm}^3 \text{mol}^{-1}$)	μ (D)
1	309.34	1728.34	9.22
2	309.29	1690.05	9.12
3	309.90	1725.83	9.22
4	309.94	1709.81	9.17
5	309.72	1744.24	9.26
6	310.56	1735.15	9.24
7	310.87	1728.76	9.22
8	310.70	1717.79	9.19
9	311.44	1722.37	9.21
10	310.94	1741.22	9.26

From values showed in table 4, the average dipolar moment of TN associated with AC resulted in $\mu_{\text{Exp}} = 9.18$ D, which is a value higher than the isolated μ data of any of the conformers, $\mu_1 = 3.22$ and $\mu_{\text{II}} = 4.29$ D. The hypothetical existence of TN-AC interactions is highly probable, since both of them have marked sites with a high charge density, which would justify that the solution μ increase can be determined.

To validate this assumption the partial molar volume of TN, \bar{v}_2 , was estimated from experimental density data in order to be compared to the theoretical value. Since \bar{v} is a function of state, an experimental value lower than the theoretical one would confirm the existence of solute-solvent interactions, at least in the whole studied concentration range (16, 27, 31). \bar{v} has the meaning

of the variation of the total volume, V , with respect to the variation in concentration at constant pressure and temperature. Each solution total volume was estimated in function of molality, m , and specific volume, v , in agreement with:

$$V = (mM_2 + 1000)v \quad \text{Equation 10.}$$

The values resulting from these estimations are shown in table 5.

Table 5. Molality (m), specific volume (v) and total volume (V) of TN-AC solutions at 25°C.

Solution	m (mol Kg ⁻¹)	v (cm ³ g ⁻¹)	V (cm ³ mol ⁻¹)
1	5.97×10^{-3}	1.2663	1268.17
2	1.36×10^{-2}	1.2650	1269.27
3	2.20×10^{-2}	1.2641	1270.94
4	2.88×10^{-2}	1.2625	1271.45
5	4.16×10^{-2}	1.2606	1273.52
6	5.28×10^{-2}	1.2591	1275.56
7	6.31×10^{-2}	1.2575	1277.17
8	7.16×10^{-2}	1.2564	1278.68
9	7.78×10^{-2}	1.2558	1279.98
10	8.48×10^{-2}	1.2545	1280.85

Total volume representation in function of m (figure 6) gives a linear response according to equation 11, whose slope, \bar{v}_2 , resulted in 162.4 cm³ mol⁻¹ (Eq. 11).

$$V = 1267.06 + 162.4m \quad \text{Equation 11.}$$

For AC, the value of \bar{v}_1 was estimated by dividing the ordinate to the straight line start (Eq. 11) by the number of AC moles, resulting in $\bar{v}_1 = 73.50$ cm³ mol⁻¹. AC moles resulted from dividing 1000 by AC molar mass by considering that the whole solution mass is AC, since those solutions tending to an infinite dilution are worked on.

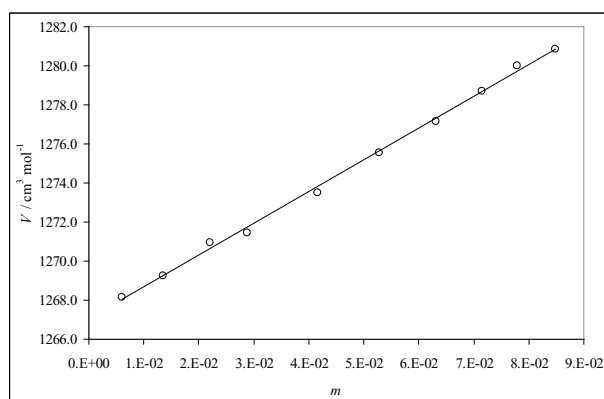


Figure 6: Total volume (V) vs. molality (m) of TN in AC solutions. Linear equation, $V = 162.4(2.6) \times m + 1267.06(0.14)$, with r^2 adjusted: 1.00 and typical error: 0.22.

The theoretical partial molar volume of AC was estimated as the quotient between molar mass and the respective density, whereas for TN it was estimated as the sum of group contributions from data obtained from Fedors' tables (23). Theoretical and experimental values are shown in table 6.

Table 6. TN and AC partial molar volumes (\bar{v}).

\bar{v}_2 theor. (cm ³ mol ⁻¹)	\bar{v}_2 exptl. (cm ³ mol ⁻¹)	\bar{v}_2 theor. (cm ³ mol ⁻¹)	\bar{v}_1 exptl. (cm ³ mol ⁻¹)
214.9	162.4	73.94	73.50

When confronting values of partial molar volumes, \bar{v}_{2-Exp} was observed to have a 24% decrease with respect to the theoretical value.

In highly diluted solutions of polar components, an intermolecular association effect by permanent dipole–permanent dipole, permanent dipole–induced dipole, dispersion forces, and hydrogen bonding, having direct influence on the solute apparent molar volume, and consequently, on \bar{v}_2 , is expected. The associations established between the solute and the solvent allow us to assume the formation of a solvation layer that would increase the μ value of the solute in solution.

An understanding of intermolecular forces is the key that allows the comprehension of the solubility properties, interfacial phenomena, and stabilization of emulsions. Considering the particular structure of TN and AC, different types of attraction are possible between them, such as permanent dipole–permanent dipole (Keesom), permanent dipole–induced dipole (Debye), and induced dipole–induced dipole (London) (9).

AC molecules, on being smaller than those of the solute, would contribute to weak and break the solute–solute interactions to generate TN-AC interactions. This leads us to assert that the obtained volumetric behavior is the result of the existence of such interactions.

In order to account for associations, both structures should be taken into account. TN offers several sites for possible associations with AC, they being located in $-\text{NO}_2$ and $-\text{SO}_2-$ groups, nevertheless the pair of free electrons on N of each imidazole ring should not be discarded, although the highest electron densities would be located in N_{14} , O_{15} , O_{16} , S_3 , O_4 and O_5 (see Fig. 2).

With respect to AC, although it is considered to be a semipolar solvent, it can induce certain

polarization in dissolved molecules. Jedlovsky and Palinkas, 1995 (32) and Ferrario *et al*, 1990 (33) have proposed AC polarizable and non-polarizable models by simulation, and reported that AC structure is rather influenced by steric effects than by electrostatic forces.

In the liquid phase, some other authors found that the vectors of carbonyl bondings of nearby molecules tend to orient them in an antiparallel way, whereas for molecules located in the first coordination sphere they do not have a preferential orientation (34). In liquid AC, the fact that molecules can be associated via Hydrogen bonding when they are in enolic form was also reported. More recent studies demonstrated that AC acts as a selective solvent to form micelles (35), and on the other hand, the diffusion coefficient variation of AC in copolymers has also been reported (36). In both cases, the authors attributed these observations to AC capacity to form Hydrogen bonding associations (32, 33).

This evidence as a whole would lead to reinforce the idea of the existence of TN-AC interactions that correlate with the dielectric behavior observed in the solutions. Furthermore, TN has recently been resurrected and FDA approved it because of its important role in different pharmaceutical formulations and other authors carried out some investigations in order to elucidate the kinetics oxidation and voltammetric responses (37, 38).

CONCLUSIONS

If the solubility of a substance in polar or semipolar solvent is taken into account to depend on chemical, electric and structural effects leading to reciprocal interactions between them, this study would contribute to elucidate the behavior of TN in AC. Buckingham and Halverstadt-Kumler's methods were well applied to the studied concentration range of TN-AC systems and allowed us to estimate polarizations from theoretical and experimental data.

The different volumetric parameters studied allowed to justify the dielectric behavior of TN in AC, and to explain why the dipolar moment of TN in solution underwent an important increase and account for the TN-AC associations that determine certain deviations concerning theoretical considerations.

The systems analyzed should be taken as a model to carry out similar studies for other solvents of pharmaceutical application in order to obtain results that justify TN behaviour in such a field. Further dielectric relaxation time, cosolvency phenomena and RMN spectroscopic studies are also suggested in order to confirm the nature of interactions, and therefore, to extend our conclusions.

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