

WATER STRUCTURING EFFECT OF METHANOL AND ETHANOL ON ION PAIRING OF SOME GUANIDIUM CONTAINING PHARMACEUTICAL SUBSTANCES. ANALYTICAL APPLICATIONS

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Abstract

The influence of water structuring effect of methanol and ethanol on ion pairing of two guanidinium containing pharmaceutical compounds, namely streptomycin and arginine, was studied. Congo red was used as counterion. For this purpose, the molecular modelling, physico-chemical constants (dielectric constant, conductivity) and absorption and resonance light scattering spectrometry were used. Determinations were performed using water/alcohol binary solvent mixtures containing molar fractions of 0.05 methanol, respectively 0.03 ethanol. The results showed that, in low concentrations, methanol and ethanol favours ion pairing. For streptomycin, analytical applications were studied.

Rezumat

A fost studiată influența efectului structurant al apei determinat de metanol și etanol asupra echilibrului de asociere ionică a unor compuși farmaceutici care conțin în structură grupări guanidinice. Roșul de Congo a fost folosit drept contraion. Analiza a fost efectuată folosind modelarea moleculară, constante fizico-chimice (constantă dielectrică, conductivitate) și spectrometria de absorbție moleculară și de difuzie de rezonanță. Determinările au fost efectuate folosind amestecuri apă/alcool conținând o fracție molară de 0,05 metanol, respectiv 0,03 etanol. Rezultatele obținute au arătat că, în concentrații mici, metanolul și etanolul favorizează formarea perechilor de ioni. Pentru streptomicină au fost studiate aplicațiile analitice.

Keywords: ion pairing, guanidinium, streptomycin, arginine, resonance light scattering

Introduction

Ion pairing, one of the fundamental interaction in chemistry and biology, describes the association of oppositely charged ions in electrolyte solutions with the formation of distinct chemical species called ion pairs [23]. Since the introduction of the concept, ion pairs are intensively studied by physical chemistry and used in pharmaceutical field [3, 5, 7, 12]. Also known as ionic associations or ionic association complexes, ion pairs were defined by Bjerrum in 1926. He stated that ion pairs are formed when two oppositely charged ions approach each other to a distance smaller than the q value which equals

$$q = \frac{8.36 \cdot 10^6 z^+ z^-}{\epsilon_r T} \text{ pm}$$

(z^+ and z^- are the charge numbers of the ions, ϵ_r is the relative permittivity of the medium) [10]. Thus ion pairing, based on the electrostatic interactions, is dependent on the dielectric constant of the solvent,

on the temperature and on the charge of the ions. According to the Bjerrum model, ion pairs were considered only in solvents with relative permittivity between 10 and 40. At this point the ions were treated as hard spheres and only pairwise interactions between them were considered, assumptions nowadays known as the "restricted primitive model" (RPM) [23]. The subsequent development of organic synthesis and the physical-chemical study of association of more complex molecules, concomitant with the development of new analysis methods, indicated that, when forming an ion pair, the interacting ions cannot be considered as being rigid and spherical [11]. Diamond proposed, in 1963, hydrophobic interaction, typical for large un-hydrated (hydrophobic) univalent ions, as a mechanism of ion-pair formation in aqueous solutions [4]. Such ions break the water structure producing hydrophobic cages around them, the driving force for the ion pairing being the water molecule preference to interact with itself by hydrogen bonding. The equilibrium is named water-structure enforced ion

pairing and the complexes formed accordingly - water-structure enforced ion pairs. Ion pairing of the hydrophilic organic molecules of pharmaceutical interest, largely hydrated in water started to be studied using resonance light scattering (RLS) spectrometry. RLS, also known as resonance Rayleigh scattering, or enhanced Rayleigh scattering, is a simple, rapid and sensitive method for the study of aggregation of molecules. Starting with the 2000s, a series of studies underlined the utility of the method in the assay of pharmaceuticals as ion pairs with organic dyes [5]. In this case, the ion pairs are formed mostly by experimentally conducted hydrophobic ion pairing. An increased ionic strength determines the chemical species involved in the ion pairing to become more hydrophobic because the solvent molecules in their hydration shell are attracted in competitive solvation equilibria of the inorganic ions [5]. A favourable effect on ion pairing of organic molecules in water solutions may be obtained by adding small quantities of methanol (CH₃OH) or ethanol (C₂H₅OH) [5]. Solvent mixtures of water and alcohol have special importance because of their unique physical and chemical properties correlated with the concentration of alcohol [14]. At low molar fractions, CH₃OH and C₂H₅OH have a strong water-structuring effect [13, 15], so the hydrophobic interactions driving ion pairing can be enforced by engaging water and alcohol molecules in hydrogen bonds, thus dehydrating the substance of interest [5]. The aim of our research was to study the influence of water structuring effect of the alcohols (CH₃OH, C₂H₅OH) on ion pairing of organic molecules of pharmaceutical interest containing guanidine groups, namely streptomycin (STR) and arginine (AG). Congo red (CR), an azo dye, was used as counterion. The reason of using this dye in the study is the presence in its structure of two sulfonate groups. In biochemical researches, sulfonate moieties are given special attention, being considered in the molecular recognition schemes for purposes of drug development and for biological mimetic. In addition, in ion pairing, the advantage of using CR is that sulfonate groups are ionized in the aqueous media regardless of the surrounding medium pH [25].

AG is the most basic of all natural amino acids (pK_a = 12.5). In its free form or built into peptide, protein or receptor systems, can participate to electrostatic and directed hydrogen bond interactions with polar and anionic molecules by its planar, forklike guanidinium (Gdm⁺) functionality [25]. Gdm⁺ acts as hydrogen bond donor interacting strongly with water only in molecular plane, whereas its faces remain hydrophobic. Computational methods indicated like-charge ion pairing for Gdm⁺ ions in water, characteristic with significant biochemical implications [24, 25]. That property correlates with the geometry of the ion belonging to the class of planar Y-conjugated

quasi-aromatic structures [27]. The cerebrospinal fluid analysis of Alzheimer's patients has elevated levels of AG though it remains uncertain whether this is a cause or a result of the disorder [16]. Recent evidence suggest that alcohol use may play an important role in the development of early-onset dementia [21]. STR is the first aminoglycoside antibiotic discovered, being isolated in 1943 from *Streptomyces griseus*. It was the first antibiotic used for the successfully treatment of tuberculosis. Currently is less used for this purpose due to the emergence of other broad-spectrum antibiotics with fewer side effect. However, with the dramatically increasing rate of infections caused by multidrug-resistant bacteria, focus has returned to aminoglycoside antibiotics as one of the few remaining treatment options [1, 28]. Chemically STR is made up of a disaccharide unit linked to the 4 - position of a guanidinylated streptomine. The hydroxyl groups contribute to its high hydrophilicity [1]. It has basic function due to the two guanidino groups and the secondary amino group. Reported pK values are pK₁ = 11.81 ± 0.05, pK₂ = 11.51 ± 0.05, pK₃ = 7.7 ± 0.05 [18], STR being mostly protonated in physiological conditions. In a previous work, the interaction between STR and CR in aqueous solutions was studied by means of UV-VIS absorption and RLS spectrometry. A RLS method for the assay of STR was proposed [6].

In the present work, the interaction of STR and AG with CR in H₂O/CH₃OH and H₂O/C₂H₅OH binary solvent mixture was studied by means of absorption and RLS spectrometry, physico-chemical constants and molecular modelling. Analytical applications are also considered.

Materials and Methods

Reagents

Streptomycin sulphate (Sigma) as 3 · 10⁻⁵ M, arginine (Janssen Chimica) as 1.5 · 10⁻⁴ M and Congo red (Scharlau) as 2 · 10⁻⁴ M aqueous solutions were used. Britton-Robinson (BR) buffer solutions were prepared using an equimolar mixture of H₃PO₄, CH₃COOH and H₃BO₃ (4 · 10⁻² M) in water, the pH of the buffer being potentiometrically adjusted at values 5.5, 6.5 and 7.5 using NaOH 10⁻¹ M solution. CH₃OH (99.8%, ρ = 0.792 g/mL) and C₂H₅OH (96%, ρ = 0.810 g/mL) analytical grade solvents from Chemical Company and double-distilled water were used throughout.

General procedure

Into a 10 mL volumetric flask 1 mL of CR, appropriate amounts of STR or AG and 1 mL of BR buffer solution were mixed. The amount needed of water to 9 mL and 1 mL CH₃OH, C₂H₅OH respectively were added and thoroughly mixed. The reagent blank was prepared using the same procedure, without STR or AG.

Apparatus

Absorption spectra were recorded using a UV-VIS Cary 100 Bio (Varian Inc.) spectrophotometer.

RLS spectra were recorded on a Perkin Elmer LS50B spectrofluorimeter using a 1 cm path length quartz cell and 1% emission attenuation filter. Synchronous scanning at the same excitation and emission wavelength ($\lambda_{\text{excitation}} = \lambda_{\text{emission}}$), after 5 min, for H₂O/C₂H₅OH and 15 for H₂O/CH₃OH, at room temperature, was performed. The RLS intensity (I) for the ion pair and the reagent blank (I₀) at the maximum RLS wavelength were measured ($\Delta I = I - I_0$).

The pH measurements were made using a Metrohm 716 DMS Titrino pH-meter.

Conductivity measurements were performed at a Corning Conductivity Meter 441, and dielectric constant was determined on a Radelkis Dielectric Constant Meter.

Molecular modelling

In order to obtain the most stable conformations (with minimum internal energy), conformational studies were conducted by using the Conformational Search program from HyperChem Professional package 7.5

[29]. The following steps were run over: (1) for the structures in a random conformation, but with defined bond length and angles, all flexible bonds and rings were set up and used in the conformational analysis; (2) to minimize the energy, in order to obtain a more stable conformation of the molecules AMBER (Assisted Model Building and Energy Refinement) force field with distance dependent dielectric constant was used, electrostatic and van der Waals 1 - 4 scale factors were set to 0.5 and cut-offs set to none; (3) the minimizing of the conformation energy was conducted until the total root-mean-square (RMS). The geometry optimization parameters were: Polak-Ribiere (conjugate gradient) algorithm, RMS gradient of 0.1 kcal/mol * Å, *in vacuum*, without periodic boundary conditions [19].

Results and Discussion

Molecular modelling

Ion pairing of AG and CR can take place in two different ways: a) electrostatic interaction between Gdm⁺ and -SO₃⁻ or b) cation ... π interaction between Gdm⁺ and aromatic nucleus of CR (Figure 1).

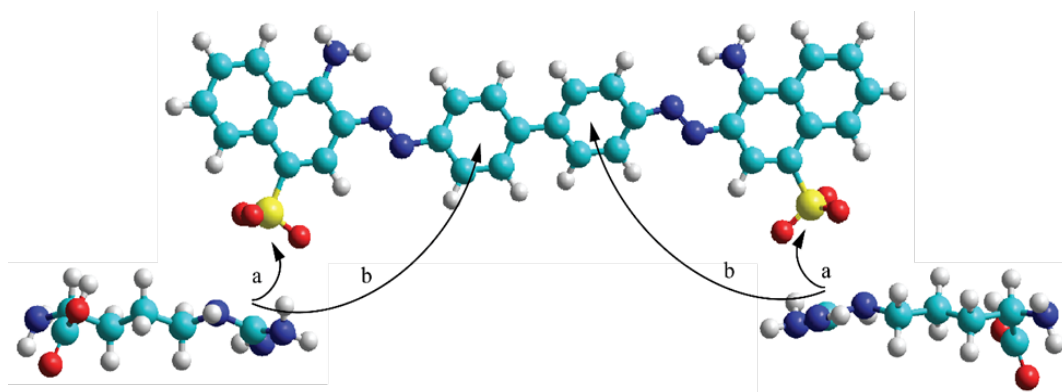
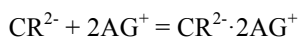


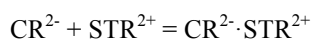
Figure 1.

The possibilities of interaction between AG and CR

The assumption is based on the literature data [2] correlated with the geometry of the molecules. In both cases a 1:2 CR:AG ion pair is obtained as presented in the reaction:



In the pH range 5.5 - 7.5, according to the charge densities reported [6], STR is protonated at the guanidinium groups being STR²⁺. With CR²⁻ can form a 1:1 ion pair:



The interaction of STR with CR, due to the hydroxyl groups is more than electrostatic, H-bonds being formed.

Dielectric properties of H₂O/CH₃OH and H₂O/C₂H₅OH mixtures

For CH₃OH and C₂H₅OH, at low molar fractions in water, it was described a phenomenon of hydrophobic

hydration, interpreted structurally in terms of the “iceberg” picture [8, 17]. In the CH₃OH case, studies indicated that, at molar fractions of 0.048, the alcohol molecules are substituting a part of water molecules in the hydrogen - bonding network of water [22]. This property could have a favourable effect on ion pairing of organic hydrophilic molecules.

For the H₂O/CH₃OH and H₂O/C₂H₅OH mixtures used in the present study, dielectric constants were determined. In Figure 2 is presented the variation of the determined parameter as function of the molar fraction of CH₃OH.

For CH₃OH, a linear correlation of dielectric constant with the concentration of alcohol exist for molar fractions between 0.1 and 0.9 ($r = 0.9998$), and respectively for C₂H₅OH for molar fractions between 0.07 and 0.9 ($r = 0.9999$). The results confirm that, for the studied water/alcohol mixtures with molar fraction of methanol lower than 0.1 and for ethanol

lower than 0.07, respectively, characteristic structural properties exist. The following determinations were performed on solutions prepared in a water/alcohol mixture with a molar fraction of 0.05 and 0.03, CH₃OH respectively, of C₂H₅OH, obtained by mixing 9 mL of water and 1 mL of alcohol.

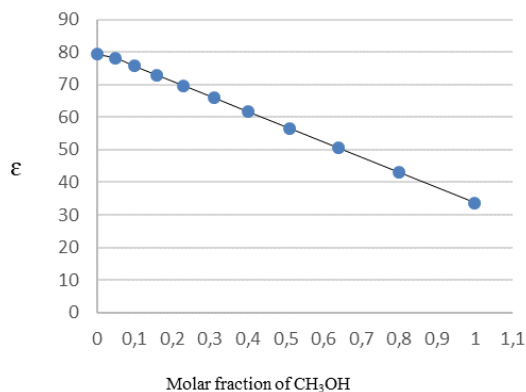


Figure 2.

Variation of dielectric constant of H₂O/CH₃OH mixture

Conductometric determinations

For asymmetric electrolytes (e.g., 2:1 electrolytes), ion pairing reduces the number of charge carriers in solution and the associated ions possess a lower mobility due to the increased hydrodynamic volume. Thus, ion pairing leads to a marked reduction of the conductance compared to the limiting case of a completely dissociated electrolyte solution. This fact renders conductance measurements an excellent tool to study the association behaviour of salt solutions [9]. Conductivity measurements were performed for solutions of 2 · 10⁻⁵ M CR, STR-CR (6.2 · 10⁻⁶ M STR) and AG-CR (1.5 · 10⁻⁵ M AG) in H₂O, H₂O/CH₃OH (9/1 v/v) and H₂O/C₂H₅OH (9/1 v/v) and BR buffer with pH 5.5, 6.5 and 7.5. During the determinations the temperature increased from 298.15 K to 230.15 K and the time interval during which they were performed was 2 hours. The results obtained are presented in Table I.

The results indicate electrostatic interactions in solutions of STR-CR in the entire pH range used, for H₂O/CH₃OH and H₂O/C₂H₅OH solutions and for AG-CR in H₂O/CH₃OH solution.

For AG-CR, in H₂O/C₂H₅OH solutions these interactions appear at pH 5.5 and 6.5. At pH 7.5 an increase of the

conductivity is observed for the solution of AG-CR in H₂O/C₂H₅OH. This may be due to the increase of the concentration of free Gdm⁺ ions by detachment from Gdm⁺:Gdm⁺ like ion pairs.

Another interesting observation is that for the solutions prepared in H₂O/CH₃OH and H₂O/C₂H₅OH, the measured conductivity remains constant as the temperature increase, in the entire pH range studied, unlike solutions prepared in H₂O where an increase of the measured conductivity appears. The observation is an evidence for the stabilization effect determined by the presence of the alcohols in the reaction media.

Table I

Conductivity values measured for solutions of CR, STR-CR and AG-CR

Solvent	Conductivity (μS/cm)		
	RC	STR-CR	AG-CR
pH = 5.5			
H ₂ O	429 → 459	450 → 473	428 → 458
H ₂ O/CH ₃ OH	393	393	380
H ₂ O/C ₂ H ₅ OH	373	373	372
pH = 6.5			
H ₂ O	536 → 542	489 → 519	519 → 527
H ₂ O/CH ₃ OH	443	432	440
H ₂ O/C ₂ H ₅ OH	418	412	413
pH = 7.5			
H ₂ O	627 → 636	615 → 630	606 → 628
H ₂ O/CH ₃ OH	532	519	521
H ₂ O/C ₂ H ₅ OH	489	490	497

Spectral characteristics

Ion pair formation is revealed spectrophotometrically by a shift of the absorption peak of the chromophore. Spectral changes are determined by the additional interactions (aromatic staking, charge transfer, hydrogen bonds) that occur with the electrostatic ones when an ion pair is formed. When the interaction between ions is strictly electrostatic in the absorption spectrum, no changes occur [5].

UV-Vis absorption spectra

Changes in the UV-Vis absorption spectra correlated with the formation of STR - CR ion pair are a hypochromic effect and a hypsochromic shift of the two maxima of the dye. In Table II, changes are summarized and presented comparative with the changes reported for the STR-CR ion pairing in water.

Table II

Spectral changes in the absorption spectra indicating STR-CR ion pairing

Solvent	CR		STR - CR ion pair		Blue shift Δλ	Hypsochromic effect (ΔA)
	λ (nm)	Absorbance	λ (nm)	Absorbance		
H ₂ O/CH ₃ OH (9/1 v/v)	499	0.407	493	0.318	6 nm	-0.089
	341	0.322	338	0.311	3 nm	-0.011
H ₂ O/C ₂ H ₅ OH (9/1 v/v)	500	0.436	497	0.363	3 nm	-0.073
	341	0.305	380	0.277	1 nm	-0.028
H ₂ O [6]	497	0.430	488	0.310	9 nm	-
	343	0.308	338	0.290	5 nm	-

Blue shift in the absorption spectra is dependent on the medium polarity. Compared with water, 9/1 v/v H₂O/CH₃OH and H₂O/C₂H₅OH mixtures have lower relative permittivity: 78.20 and 78.56 respectively. Accordingly, blue shifts of the absorption maxima are lower. The intense hypochromic effect indicate STR - CR ion pairing by hydrophobic interaction, thus confirming the favourable effect assumed for the CH₃OH and C₂H₅OH, at low molar fractions in water.

For the solutions containing AG-CR ion pairs, no changes in the absorption spectra were observed.

Resonance light scattering spectra

In the RLS spectra, for H₂O/CH₃OH and H₂O/C₂H₅OH solutions of STR and CR alone very weak RLS signals were obtained. When the two agents react with each other, a significant enhancement of the RLS intensity appeared in the range 300 - 450 nm, with the maximum scattering peak located at 380 nm (Figure 3).

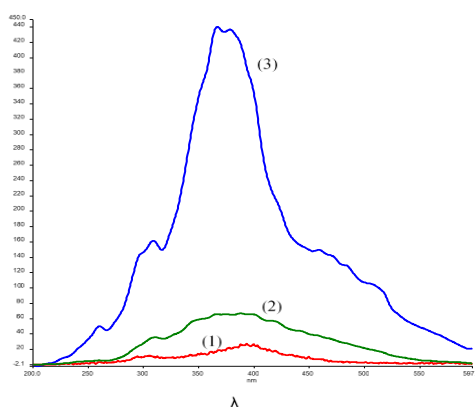


Figure 3.

The RLS spectra of (1) CR (14.10 μg/mL), (2) STR (9.81 μg/mL) and STR-CR ion-pair (9.81 μg/mL) in H₂O/C₂H₅OH 9/1 (v/v) binary solvent mixture

In the AG case, in the RLS spectra, changes were observed in H₂O/C₂H₅OH solutions when BR buffer with pH 7.5 was used. For the solution containing AG and CR, a decrease of the intensity of the maximum at 396 nm with the increase of the concentration of AG was observed. This can be due to the detachment from Gdm⁺-Gdm⁺ like ion pairs, result that is in accordance with the observations on the conductivity measurements.

Analytical applications of the STR-CR ion pairing

For STR-CR ion pair formed in H₂O/CH₃OH and H₂O/C₂H₅OH 9/1 (v/v) optimum experimental conditions (buffer pH, counterion concentration, ion pair stability, reagents addition order) were determined for a quantitative ion pairing that can be used for the determination of STR.

Thus, spectral changes indicated that optimum pH value of the buffer is 5.5. 1 mL of pH = 5.5 BR buffer was used. CR optimum concentration was established to be $2 \cdot 10^{-5}$ M.

The reaction time is 5 minutes in H₂O/CH₃OH (9/1 v/v) and 15 minutes for H₂O/C₂H₅OH (9/1 v/v) reaction media. The RLS intensity remains constant for at least 2 hours indicating a good stability of STR-CR ion pair. The optimum sequence of reagents addition: (1) CR solution, (2) STR solution, (3) BR buffer solution, (4) water and (5) alcohol.

In the established experimental conditions for H₂O/CH₃OH solvent mixture, we obtained a linear correlation between the decrease of the absorbance and concentration of STR at the wavelength 493 nm. When H₂O/C₂H₅OH solvent mixture was used, a linear correlation between the intensity of the scattered light and concentration of STR at the wavelength 380 nm was observed. In Table III, a comparison of the sensitivity of the proposed methods with the previously published is presented.

Table III

The comparative presentation of the performance parameters of the STR assay methods based on ion pairing with CR

Solvent	Linearity range (μg/mL)	LOQ (μg/mL)	LOD (μg/mL)	Reference
H ₂ O/CH ₃ OH	2.29 - 6.87	0.09*	0.32*	Present work
H ₂ O/C ₂ H ₅ OH	9.73 - 16.22	9.73**	2.05**	Present work
H ₂ O	6.49 - 9.47	6.49	6	[6]

*based on the slope of calibration curve and standard deviation of the blank, **according to the procedure described in the reference 6

Accuracy studies for the RLS method were performed, using standard samples at three levels of STR concentration, every sample being analysed in triplicate, using the *General procedure* previously described. The percent recovery ranged between 99.2% and 101.4% for the absorption based method and 99.2 - 101.9% for the RLS method, providing a good accuracy.

Although widely used in the treatment of tuberculosis [20], and formalized over time in various pharmacopoeias [26], at the time of making the determinations, on

the local pharmaceutical market no pharmaceutical formulations were available containing STR. Consequently, the methods proposed could not be applied to the assay of STR in pharmaceuticals, but they can be considered as alternatives to the analysis of other types of probes.

Conclusions

The influence of water structuring effect of CH₃OH and C₂H₅OH on the ion pairing of STR respectively AG, with CR was studied. The optimum solvent

mixture - 9/1 v/v water/alcohol, corresponding to 0.05 molar fraction of CH₃OH and 0.03 molar fraction of C₂H₅OH - was selected based on dielectric constant determinations. Conductometric measurements indicated the existence of the electrostatic interactions in the case of the STR - CR ion pair in the pH range 5.5 - 7.5 in both reaction media. The same observations were made for the interaction of AG with CR, with the exception of H₂O/C₂H₅OH mixture when BR with pH 7.5 was used. In this case, the conductivity value of the solution increased, change that can be attributed to the dissociation of the Gdm⁺:Gdm⁺ like ion pair, and suggested also by the RLS spectra.

Spectral characteristics of the STR-CR ion pair, in both solvent mixtures, using BR with pH 5.5 were considered for analytical applications. Linearity range obtained for the maximum in the RLS spectra, 9.73 - 16.22 µg/mL, is larger than that obtained in the previously RLS published method (6.49 - 9.74 µg/mL) for the assay of STR.

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