RESONANCE RAYLEIGH SCATTERING STUDY OF STREPTOMYCIN - CONGO RED IONIC ASSOCIATION IN VIEW OF ANALYTICAL APPLICATION

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Abstract

Streptomycin (STR) and Congo red (CR) react in weak acidic medium to give an ionic association complex which was studied by resonance Rayleigh scattering (RRS) and UV-VIS absorption. The combinative sites were defined by calculating the charge distribution on the STR and CR molecules in the ground state using Austin Model 1 quantum chemistry method. The maximum intensity of RRS signal (384 nm) was obtained at pH 5.5 created with Britton-Robinson buffer at a $2 \cdot 10^{-8}$M Congo red concentration; the reaction time was of 30 minutes. The ratio of the reagents (1:1) was determined using Job's method. The linear relationship between the RRS intensity and the antibiotic concentration was found within the concentration range 6.49 – 9.74 $\mu g \cdot mL^{-1}$. The method was successfully applied for the determination of streptomycin in pharmaceutical formulations.

Rezumat

În mediu slab acid, streptomicina (STR) şi roşul de Congo (CR) reacţionează cu formarea unui complex prin asociere ionică. Acesta a fost studiat prin spectrometrie Rayleigh de rezonanţă (RRS) şi spectrometrie de absorbţie UV-VIS. Grupările funcţionale implicate in formarea asocierii ionice au fost definite prin calcularea distribuţiei de sarcină pe moleculele de STR şi CR, în stare fundamentală, utilizând metoda de calcul a chimiei cuantice, Austin Model 1. Intensitatea maximă a semnalului RRS (384 nm) s-a obţinut la pH 5.5 creat de sistemul tampon Britton-Robinson şi o concentraţie a roşului de Congo de $2 \cdot 10^{-8}$M, timpul de reactie fiind de 30 minute. Raportul de combinare a celor doi reactivi (1:1) a fost determinat utilizând metoda Job. Între concentraţia de STR şi intensitatea semnalului RRS există o relaţie liniară în intervalul de concentraţii 6.49 – 9.74 $\mu g \cdot mL^{-1}$. Metoda a fost aplicată cu bune rezultate la dozarea streptomicinei din forme farmaceutice.

Keywords: resonance Rayleigh scattering, ionic association, streptomycin, Congo red, pharmaceuticals

Introduction

Streptomycin (STR) belongs to the group of aminoglycoside antibiotics. It is a water soluble organic triacidic base made up of three

components - streptidine, streptose, N-methyl-L-glucosamine - which are linked together by glycosidic bonds. STR is active against a wide range of bacteria, both Gram-positive and Gram-negative, being commonly used in clinical practice as a valuable therapeutic agent for all forms of tuberculosis [1].

Toxicity of the drug consists mostly in allergic reactions, renal irritation and neurotoxicity (permanent deafness). Because STR is frequently used in veterinary medicine, antibiotic residues can be found in meat, liver, kidney, milk, eggs and honey. Consequently, the development of fast, reliable, and sensitive methods for STR assay is of great interest.

The published methods reporting the assay of streptomycin are based on various techniques: microbiological [2], HPLC [3-6], capillary electrophoresis [7,8], voltammetry [9]. Among spectrophotometric methods, some fluorimetric [10,11] and UV - VIS [1, 12] methods are reported. Among spectrophotometric methods, fluorimetric [10,11] and UV – VIS absorption [1,12] methods are reported. Due to the absence of chromophores and/or fluorophores groups in the molecule, most of these methods are using derivatizing agents.

Resonance Rayleigh scattering (RRS) is a new sensitive, simple and fast analytical method proposed in 1993 by Pasternack et al. [13] RRS is a specific elastic light scattering effect produced when the wavelength of the Rayleigh scattering is located within or near the molecular absorption band of the compound. In this case, the frequency of the absorbed electromagnetic wave is equal to its scattering frequency. The process of scattering-absorption-rescattering that takes place results in enhanced scattered light due to the resonance effect. The scattering intensity is enhanced by several orders of magnitude compared to the common Rayleigh scattering and no longer obeys the Rayleigh law ($I \sim 1/\lambda^4$). In recent years, RRS method has been successfully applied in the pharmaceutical analysis [14]. Most of the published works present methods that are based on ionic association equilibrium. Thus RRS methods were developed as an alternative to UV-VIS or microbiological methods for the assay of aminoglycoside antibiotics [15-17].

The paper presents a new resonance Rayleigh scattering method for the assay of streptomycin. We studied the ability of the aminoglycoside antibiotic to form ion pairs with sulfonated azo dyes, and Congo red was found to be the appropriate reagent for this purpose. A quantum chemistry computation of the charge distribution of the proposed ion association and the experimental conditions that support the assay are also discussed. The method was tested for the assay of STR in a pharmaceutical formulation.

Materials and Methods

Apparatus

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

A Perkin Elmer LS50B spectrofluorimeter was used for recording RRS spectra using a 1 cm path length quartz cell and 1% emission attenuation filter.

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

Reagents

A $7 \cdot 10^{-4}$ M streptomycin stock solution was prepared by dissolving the appropriate amount of streptomycin sulphate standard substance (Sigma) in water. Working solution were prepared by diluting with water a 4 mL stock solution, using a 50 mL volumetric flask.

Congo red, solution A, was prepared by dissolving 0.1000 g substance (Scharlau) in water using a 250 mL volumetric flask. Solution B was prepared by diluting 8.8 mL solution A in water, using a 25 mL volumetric flask.

Britton-Robinson (BR) buffer was prepared by mixing 0.04 M phosphoric acid, 0.04 M acetic acid and 0.04 M boric acid in 1 L of water. The pH of the buffer was adjusted to the specified pH value with 0.1 $mol \cdot L^{-1}$ NaOH solution.

All reagents were of analytical grade and double-distilled water was used throughout.

General procedure

In a 10 mL volumetric flask 1 mL of Congo red, solution B, appropriate amounts of streptomycin working solution and 1 mL of Britton-Robinson buffer solution (pH 5.5) were successively added; the mixture was diluted to the mark with water and thoroughly shaken. The reagent blank was prepared using the same procedure, without streptomycin. The mixtures were allowed to stand still for 30 minutes at room temperature, then the UV-VIS and RRS spectra were recorded.

The UV-VIS absorption spectra were recorded against water; the RRS spectra were obtained by synchronous scanning in the range 200 – 600 nm, the fluorimeter monochromators being set at the same excitation and emission wavelength ($\Delta\lambda = \lambda_{excitation} - \lambda_{emission} = 0$).

The RRS intensity (I) for the ion pair and the reagent blank (I_o) were measured at the peak (384 nm), then the corrected scattered intensity, $\Delta I = I - I_o$, was computed and used for further evaluation.

Computation of the STR-CR ionic association

In order to define the groups involved in the STR-CR ionic association, we used AM1 (Austin Model 1) method of quantum chemistry to calculate the charge distribution on STR and CR molecules in the ground state. The calculations were performed with Hyperchem Release ver. 7.5 software.

The composition of STR-CR ion association was established using Job's method of continuous variation.

Validation parameters

Linearity range, detection and quantification limits, as well as precision parameters were investigated according to the validation guidelines for industry rules [18].

Results and Discussion

Spectral characteristics of the STR-CR ionic association

UV-VIS absorption spectra

STR has three absorption maxima, at 203, 271 and 327 nm, which can be registered only in concentrated solutions [19]. Absorption maxima of CR are found at 235, 343 and 497 nm. The 343 and 497 peaks undergo a slight hypsochromic shift ($\Delta\lambda$ = 5 nm and 9 nm), at 338 and 488 nm, respectively, upon complexation with STR (Figure 1), probably due to a charge distribution change on the associated molecules.

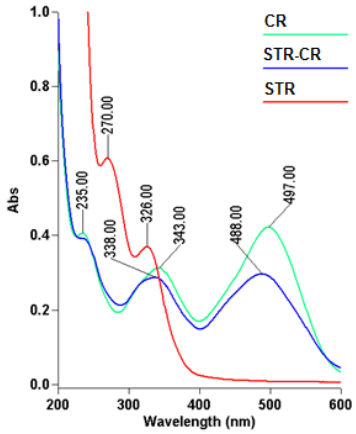

Figure 1.
UV-VIS absorption spectra of STR (16.20 mg·mL^{-1}), CR (14.12 µg·mL^{-1}) and corresponding STR-CR ionic association (6.49 µg·mL^{-1}) at pH=5.

Resonance Rayleigh scattering spectra

At pH 5.5, STR and CR alone produce very weak RRS signals. When the two agents react to form STR-CR ionic association a significant enhancement of the RRS intensity in the studied wavelength range is obtained (Figure 2) accompanied by a few changes in the shape of the spectrum (Table I). The maximum scattering peak was located at 384 nm.

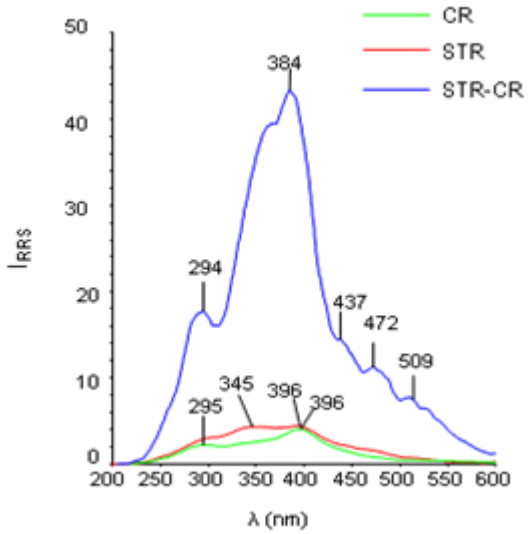

Figure 2.
RRS spectra of CR (14.12 μg·mL^{-1}), STR (6.49 μg·mL^{-1}) and STR-CR ionic association (6.49 μg·mL^{-1})

Table I
Characteristics of RRS spectra of CR, STR and STR-CR ionic association

Substance	λ_{max}/I_{RRS} (nm/a.u.)	Other RRS peaks (nm)				
CR	396/3.85	295	-	-	-	-
STR	396/4.25	-	345	-	-	-
STR-CR	384/43.48	294	-	437	472	509

Because the RRS spectra are the result of a scattering-absorption-rescattering process, they are strongly related to the absorption spectra. As can be seen from Figure 1, the formation of STR-CR ionic association is followed by hypochromic and hypsochromic shifts of two CR absorption maxima. A hypsochromic shift (of 12 nm) can be also observed for the RRS maximum, therefore the hypochromic shifts can be attributed to the resonance between Rayleigh scattering and absorption of the radiation with

the same frequency; consequently, the RRS signal increases. Figure 3 presents the superposed absorption and RRS spectra of STR-CR ionic association. It can be seen that the RRS maximum at 384 nm is located within the absorption band.

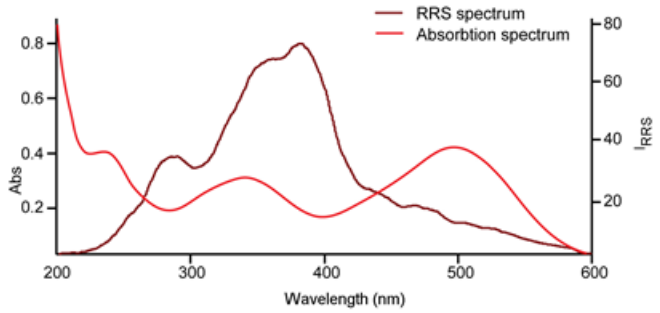

Figure 3.
Absorption spectrum and RRS spectrum of STR-CR ionic association

Composition of the STR-CR ionic association

In order to define the groups involved in the STR-CR ionic association, we used AM1 (Austin Model 1) method of quantum chemistry to calculate the charge distribution on STR and CR molecules in the ground state.

STR contains three groups with basic function (two guanidinic groups and one secondary amino group) and the charge distribution in ground state is not uniform: on the guanidinic groups there is a high negative charge density (- 0.168, - 0.157), while on the secondary amino group the negative charge density is lower (- 0.085) (Figure 4). Based on these data we can assume that, in weak acid conditions, the guanidinic groups are easily protonated to take positive charges. In the case of CR molecule, negative charge distribution is even in both sulfonic groups (- 0.328) (Figure 5). The charge density at weak acid pH for the two structures suggest the formation of the STR-CR ion pair with charge transfer, which was also suggested by the hypsochromic shift of the UV maximum described before.

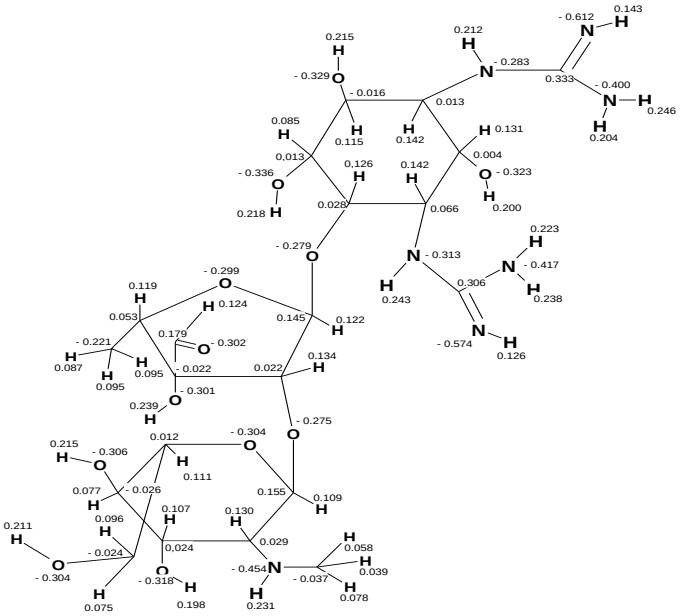

Figure 4.
STR charge distribution in the ground state

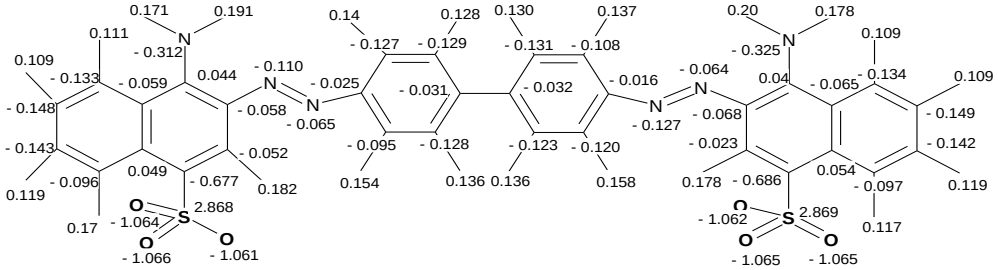

Figure 5.
STR charge distribution in the ground state

When STR and CR are mixed, the dye in anionic form (CR^{2-}) reacts with diprotonated cation of STR ($STRH_2^{2+}$) and the neutral $STRH_2^{2+}\cdot CR^{2-}$ ionic association is formed: $STRH_2^{2+} + RC^{2-} \rightarrow [STRH_2^{2+}\cdot RC^{2-}]$. Consequently, the hydrophilicity of both reagents decreases and a hydrophobic liquid-solid interface appears. The hydrophobic interface is an important reason for the scattering enhancement. Also, an increase of molecular volume is a significant factor in the enhancement of RRS intensity. When the ionic association is formed the molecular weight increased from 581.58 to 1278.38. The increase of the molecular weight corresponds to an increase of its molecular volume.

The composition of STR-CR ionic association was established using Job's method of continuous variation. The results obtained showed that STR and CR form a 1:1 ionic association, thus confirming the supposition made before.

Optimization of the experimental conditions for the STR-CR ionic association equilibrium

Stability of scattering intensity

At room temperature, the reaction completed in 30 minutes and the RRS intensity remained constant for 2 hours.

Effect of the pH

The influence of the pH on ionic association equilibrium was studied in the pH range 4.0 – 9.0, using various buffer systems (Britton-Robinson, acetate, citrate, phosphate). The best results were obtained using the BR buffer with pH 5.5. The optimum volume of the buffer solution was found to be 1 mL.

At pH values below 5.5, CR exists in its molecular form [20]. At pH values higher than 6.0 deprotonation of STR takes place [21]. Thus pH values lower than 5.0 and higher than 6.0 are not convenient to the STR-CR ionic association equilibrium.

Effect of Congo red concentration

The influence of CR concentration on ionic association equilibrium was studied by registering the RRS spectra for the aqueous solutions (pH 5.5) with constant concentration of STR and various concentrations of CR (7.06 – 28.24 $\mu g \cdot mL^{-1}$). We found that the optimum CR concentration range was 13.80 - 14.20 $\mu g \cdot mL^{-1}$. If the reagent concentration is lower than 13.80 $\mu g \cdot mL^{-1}$ the ionic association equilibrium would be incomplete. If the concentrations of CR are higher than 14.20 $\mu g \cdot mL^{-1}$ the intensity of I_{RRS} decreases, probably due to CR self-association.

Effect of ionic strength

The effect of the ionic strength was studied by registering RRS spectra for the aqueous solutions (pH 5.5) with constant concentration of STR and CR, and various amounts of 1M NaCl solution. The results (Figure 6) showed that the intensity of RRS decreased with the increase of ionic strength. So, constant and low ionic strength conditions are favorable for the ionic association reaction.

Effect of the reagents addition order

The effect of the addition order of the reagents on the RRS intensity was also investigated. The results showed that RRS is higher for the following sequence of reagents addition: (1) CR solution, (2) STR solution, (3) BR buffer solution and (4) water.

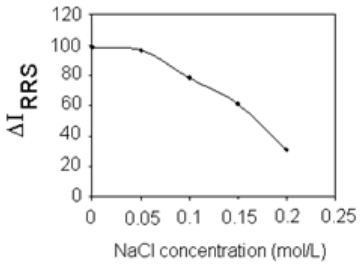

Figure 6.
Effect of the ionic strength on the ionic association equilibrium

Calibration and sensitivity of method

Validation studies were performed [22-23]. According to the procedure described above, a calibration curve was constructed. The linearity was tested within the range 6.49 – 9.74 $\mu g \cdot mL^{-1}$ and the linear regression equation calculated resulted in a correlation coefficient of 0.9981.

We also performed studies regarding the determination of detection (LOD) and quantification (LOQ) limits. Experimental results showed that, at concentrations of STR lower than 6 $\mu g \cdot mL^{-1}$, under established experimental conditions, the RRS spectrum has no changes compared with RRS spectrum of CR, suggesting that STR-CR ionic association is not formed. Consequently, it can be considered that LOD is the minimum concentration of STR required for the ionic association formation, 6 $\mu g \cdot mL^{-1}$, and LOQ is the lower limit of linear range, 6.49 $\mu g \cdot mL^{-1}$.

Based on the linear range limits, one can be considered that the sensitivity of the proposed method is comparable with other instrumental methods used for the STR assay (Table II).

Table II

Comparison of the sensitivity of RRS method with other methods for the determination of STR

Method	Samples	Linear range ($\mu g \cdot mL^{-1}$)	Reference
HPLC (DAD, 200 nm)	human serum	10 – 80	[3]
IP-RP-HPLC (evaporative light scattering detection)	raw materials, pharmaceutical formulations, culture media, plasma	2 – 120	[6]
CZE (DAD, 195 nm)	bactericidal products	20 – 200	[8]
Voltammetry	powders for injections	5 - 50	[9]
RRS	powders for injections	6.49 – 9.74	proposed method

DAD = Diode Array Detector
IP-RP-HPLC = Ion Pair – Reversed Phase – High Performance Liquid Cromatography
CZE = Capillary Zone Electrophoresis

Accuracy studies

The accuracy of the RRS method was determined using standard samples at three levels of streptomycin concentration (7.25, 8.26 and 9.22 $\mu g \cdot mL^{-1}$). Every sample was analyzed in triplicate using the procedure mentioned previously. The percent recovery ranged between 98.90 and 101.20%, proving a good accuracy of the method.

Analytical application

The applicability of the proposed method was studied via assay of STR in pharmaceuticals. Commercial powder for injections (Strevital® 1g streptomycin sulphate, Antibiotice SA Iasi) was analyzed. Under the established experimental conditions, the evaluation of the steptomycin resulted in a recovery coefficient between 99.01 and 101.02%.

Conclusions

The interaction between streptomycin and Congo red in aqueous solution was studied by means of UV-VIS absorption and resonance Rayleigh scattering. Based on the characteristics of the resonance Rayleigh scattering spectrum of the STR-CR ionic association, a fast and simple method for the assay of streptomycin was developed. The sensitivity of the method is comparable with the modern instrumental methods (HPLC, capillary electrophoresis, voltammetry) being more simple, more rapid and less expensive. The proposed method was successfully applied to the assay of streptomycin in pharmaceutical formulations.

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