PREFORMULATION STUDIES USING COSOLVENT SYSTEMS TO INCREASE THE SOLUBILITY OF A NEW ENROFLOXACIN RUTHENIUM (III) COMPLEX WITH BIOLOGICAL ACTIVITY

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Abstract

Enhancement of solubility, dissolution rate and bioavailability of poorly water soluble new chemical entities is a very challenging task in pharmaceutical formulation development. Present study explores different technological approaches to enhance the solubility of a novel complex Ru(III)-enrofloxacin by using various co-solvent systems. Preliminary tests using water:dioxane co-solvent systems were used for optimizing the solubilization process and for determining the dielectric requirement value (DR). Dioxane was then substituted with the most frequently used low-toxicity co-solvents for pharmaceutical use: polypropylene glycol, polyethylene glycol 400, glycerol and ethanol, mixed in volume ratios so that the DR to be respected. The complex solubility was increased to roughly 1 mg/mL by using binary and ternary biocompatible co-solvent systems with apparent dielectic constant of 64.61, comparing to the water solubility of 0.034mg/mL. The release kinetics from saturated solutions prepared in biocompatible co-solvent systems was evaluated for 6 hours, by using a modified Franz diffusion cell fitted with a regenerated cellulose membrane. The obtained release kinetic data were best fitted by means of Higuchi model, suggesting a diffusion-based release mechanism.

Rezumat

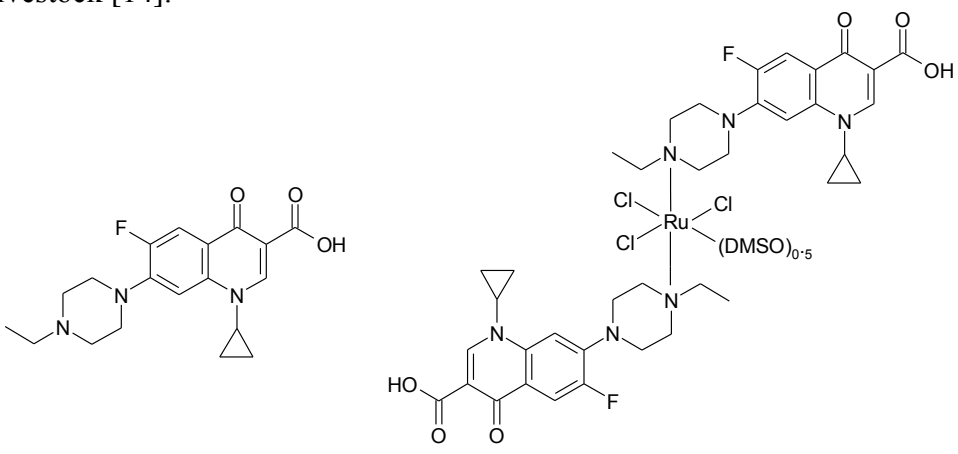
Principalele dificultăţi în dezvoltarea formulărilor farmaceutice pentru noi entităţi chimice greu solubile sunt legate de îmbunătăţirea solubilităţii, vitezei de cedare şi biodisponibilităţii lor. Prezentul studiu abordează diverse metode de îmbunătăţire a solubilităţii unui complex nou sintetizat al Ru(III) cu enrofloxacina prin tehnica cosolubilizării. În testele preliminare pentru stabilirea cerinţei dielectrice (DR) a complexului analizat au fost utilizate sisteme binare de cosolvenţi apă:dioxan. Din cauza incompatibilităţii dioxanului cu formulările farmaceutice, acesta a fost înlocuit cu solvenţi biocompatibili (propilenglicol, polietilenglicol 400, glicerol şi etanol) în proporţii care să asigure constante dielectrice aparente similare cu valoarea DR. Pentru sistemele biocompatibile binare sau ternare de cosolvenţi caracterizate de o valoare a constantei dielectrice aparente de 64,61, solubilitatea complexului analizat a crescut la aproximativ

1mg/mL, faţă de solubilitatea în apă de numai 0,034mg/mL. Studiile cinetice privind cedarea complexului din soluţiile saturate ale sistemelor biocompatibile de cosolvenţi s-au realizat pe o perioadă de 6 ore, utilizând o celulă de difuzie Franz modificată, prevăzută cu o membrană de celuloză regenerată. Modelul cinetic Higuchi a fitat cel mai bine datele cinetice experimentale, sugerând un mecanism difuzional al cedării.

Keywords: ruthenium(III)-enrofloxacin complex, solubility, co-solvent systems, release kinetics

Introduction

Enrofloxacin [1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-ethyl-1-piperazin-1-yl)-quinoline-3-carboxylic acid] (enro, Figure 1a), is a second-generation fluoroquinolone antimicrobial agent with a broad-spectrum bactericidal activity against gram-positive and gram-negative bacteria [3]. Enrofloxacin has veterinary applications, which include the treatment of urinary tract, respiratory tract and skin infections in pets and livestock [14].



(a) (b)

Figure 1
Structure of enrofloxacin (a) and its complex with Ru (III) (b)

As other quinolone molecules, enrofloxacin forms metal chelates acting as bidentate ligand coordinated through the pyridone oxygen and one carboxylate oxygen. Metal complexes with divalent cations as $VO(erx)_2(H_2O)$ [5], $Mn(erx)_2(H_2O)_2$, $Co(erx)_2(H_2O)_2$, $Cd(erx)_2(H_2O)_2$,

$UO_2(erx)_2$ [7], $MoO_2(erx)_2$ [6], $Ni(erx)_2(H_2O)_2$ [7, 26-27], $Zn(erx)_2(H_2O)_2$, [7, 29], $Cu(erx)_2(H_2O)$ [4], $[Cu(erx)_2(H_2O)_2]$ [12], $[Cu(erx)_2]Cl$ [23] or trivalent cations as $Fe(erx)_3$ [7] have been synthesized and characterized and their antibacterial activity was tested comparing to the free ligand. A recent strategy in the field of metal complexes of quinolone antibiotics consists in the introduction of different quinolone molecules into the octamolybdates POMs. The MTT investigations of these complexes, including the corresponding enrofloxacin complex $[Cu^{II}(L_1)_2(H_2O)_2]H_2[\beta\text{-}Mo_8O_{26}]\cdot 4H_2O$ (L_1 = erx) showed that the antitumor activity of parent β-Mo_8 can be modulated by the introduction of metal complex onto the polyoxoanion surface [25].

Apart the bidentate manner of coordination found in chelates such as those described above, quinolones can be bound to the metal ion as monodentate ligand through the terminal piperazinyl nitrogen atom. The chelation of enrofloxacin through piperazine ring was not yet reported, but it is known for the complexes of Ag (I) with norfloxacin [13, 20] and pefloxacin [2], as well as in a complex of Au (III) with norfloxacin [14, 20]. In this coordination mode, the quinolone molecules keep free the carbonyl pyridone and the carboxyl groups with consequences for solubility and further interactions with metal ions. On the basis of the above considerations, the aim of this work was to initiate some preformulation studies for a novel complex of ruthenium(III) with enrofloxacin and dimethylsulfoxide (DMSO) with general formula $Ru(enro)_2(DMSO)_{0.5}Cl_3\cdot 4H_2O$ (Fig. 1b). In this complex, the quinolone agent acts as monodentate ligand coordinated through the piperazine nitrogen atom. The need for preformulation studies is justified by the biological potential of the complex, which proved a good antimicrobial activity on *Staphylococcus aureus* methicillin resistant (MRSA), *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Bacillus subtilis* strains [30], and which is currently in study for the antitumor activity.

The main impediment in the biological testing was the low water solubility of the complex, and it could be corrected by an appropriate preformulation strategy. Having in consideration the previous studies which used the various solvent systems for solubility enhancement of enrofloxacin free [24], in the present study has been made an attempt to increase the solubility of metal complex using a series of co-solvents. The influence of co-solvent type, the influence of pH on solubility, the stability of test solutions obtained with appropriate co-solvent mixtures were determined.

The influence of the solubilization system on the release kinetics of complex from the aqueous systems was studied.

Materials and Methods

Reagents and chemicals

The complex $Ru(enro)_2(DMSO)_{0.5}Cl_3 \cdot 4H_2O$ was prepared according to previously reported procedure [1].

Dioxane, Polyethylene Glycol 400 (PEG 400), all of analytical grade, were purchased from Merck. The HPLC grade ethanol, glycerin (GLY) and propylene glycol (PG) were obtained from Sigma Aldrich.

All chemicals were of analytical grade and used without further purification.

Methods

The solubility of poorly soluble chemical entities, defined as concentration of solute in a saturated solution at a certain temperature, is often causing unsatisfactory bioavailability.

Water solubility of the newly synthesized Ru-enro complex is very low ($S=0.034*10^{-2}$ mg/mL), for which reason a solubilization enhancement technique is required in order to provide a sufficient amount of active substance to be available at the absorption site.

One of the most popular approaches for improving the solubility of drugs in pharmaceutical liquid formulations is the use of cosolvent systems, mixtures of water and one or more water miscible solvents used to create a solution with enhanced solubility for poorly soluble compounds [11]. In practice, to approximate the solubility of compounds in aqueous co-solvent systems, the dielectric requirement (DR) value is necessary [10].

The dielectric requirement (DR) for a chemical entity represents the apparent dielectric constant (ε_{app}) of the system corresponding to components ratio that provides the maximum solubility for the analyte. Estimation of the dielectric requirement for the Ru-enro system was performed by using water:dioxane mixtures. The choice was based on the extreme value of dielectric constant for the two solvents in pure state ($\varepsilon_{water}=78$ and $\varepsilon_{dioxane}=2$), providing a versatile model for studying drug solubility [18, 19].

The value of ε_{app} for a binary system was calculated by using:

$$x_1\varepsilon_1 + x_2\varepsilon_2 = \varepsilon_{app} \quad (1)$$

where:

x_1, x_2 are the molar ratios of the solvents (water, and dioxane, respectively);

$\varepsilon_1, \varepsilon_2$ are the dielectric constants of the solvents.

Although dioxane is a very useful solvent in solubility screening tests, its toxicity prevents the use in real pharmaceutical formulations. Selection of a biocompatible co-solvent system was performed by substituting dioxane with the most frequently used low-toxicity co-solvents for pharmaceutical use: propylene glycol (ε=25), ethanol (ε=32), glycerin (ε=43) or polyethylene glycol 400 (ε=19) [16]. The water : co-solvent ratio in the resulting systems was calculated according to equation (1), in order to obtain a system with apparent dielectric constant equal to DR, for which the solubility of Ru-enro complex is maximum.

Calculation of DR value for the Ru-enro complex

For evaluation of Ru-enro complex DR value, five different water:dioxane systems, with ε_{app} in the range 28.12-76.36 were prepared. The solubility of Ru-enro in each system was evaluated by using the shake-flask method: 10 mL of each co-solvent system was added to an excess of drug substance, using 10 mL volumetric flasks (all determinations were performed in triplicate). The vials were vigorously mixed for 30 seconds, and maintained under sonication for 30 minutes, at 50°C, by means of a SonoSwiss thermostated ultrasonic bath. The samples were placed for 24 hours on a Heidolph Vibramax 100 stirrer, at 200 rpm.

The samples were furthemore centrifuged and the supernatant was filtered through a cellulose-esther membrane with the average pore size 0.45 μm. For quantitative evaluation of the dissolved Ru-enro complex a spectrophotometrical method with UV detection at λ = 282 nm was developed, using a Jasco V-630 Spectrophotometer.

The experimental parameters (sonication time and temperature, time of stirring) were optimized in order to increase Ru-enro solubility. All the preliminary tests were performed using a 1:1 (v/v) water:dioxane mixture.

Solubility of Ru-enro complex in biocompatible co-solvent systems

The solubility of Ru-enro in each biocompatible system was established by using the same experimental protocol used for evaluation of DR value.

Kinetic parameters analysis

The Ru-enro complex release from saturated solutions prepared in biocompatible co-solvent systems was evaluated by using a modified Franz diffusion cell fitted with a regenerated cellulose membrane as previously reported [9]. Briefly, 1 mL from each solution was introduced in the donor compartment. The receptor medium consisted in 1:1 (v/v) water: PG mixture, pre-warmed at 37ºC. Samples of 5 mL were collected at 15, 30, 45, 60, 75, 90, 120, 180, 240, 300 and 360 min and replaced with the same volume of pre-warmed fresh receptor medium.

The amount of ruthenium(III)-enrofloxacin complex released through the membrane was determined by means of a UV spectrophotometric method with detection at the maximum absorption wavelenght of the complex (282 nm) (Figure 2). A six-point calibration standard curve in the range 2-20 µg/mL was used for the quantitative determination of the amount of complex released.

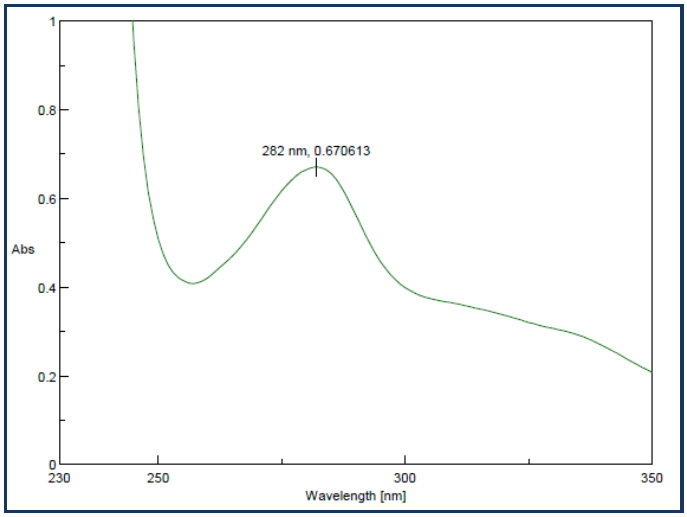


Figure 2
UV absorption spectrum of a 10 µg/mL standard solution of Ru-enro complex in 1:1 (v/v) water:PG mixture

Results and Discussion

Optimizing the variable parameters of solubilization process in binary solutions of water:dioxane

In order to optimize the experimental protocol for enhancing Ru-enro solubilization, influence of some experimental parameters, such as sonication time and temperature, time of stirring, pH was analyzed. All the preliminary testing was performed using a 1:1 (v/v) water:dioxane mixture.

A. Effect of pH

Because in some cases the solubilization capacity of the co-solvents may not be sufficient to ensure the required concentration in the pharmaceutical formulations, the possibility to combine their addition with other solubilization techniques, such as pH adjustment was investigated.

In order to evaluate the pH influence on Ru-enro solubility, six aqueous solutions with pH within the range 1.46-11.92 were used.
The results are presented in table I and in Figure 3.

Table I
Solubility at different pH values of aqueous solution

pH	S (mg/mL)
1.46	0.017
5.00	0.016
7.08	0.019
8.12	0.400
10.40	0.700
11.92	1.210

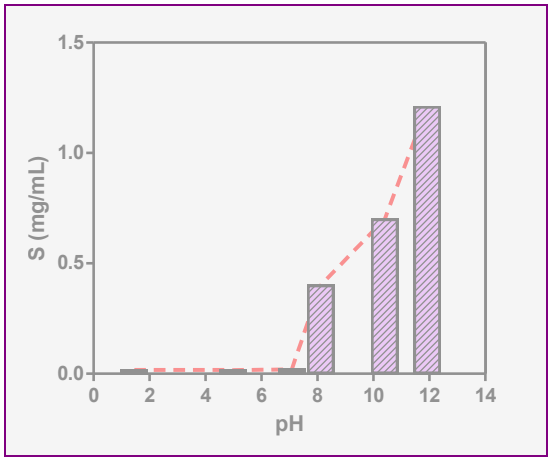


Figure 3
Solubility of Ru-enro complex as function of pH

A significant enhancement of solubility toward alkaline pH was observed, as consequence of salt formation based on the weak acid function of carboxyl group [17, 21]. Although the value of solubility in basic medium is significantly higher than in neutral solutions, additional studies are required in order to establish the compatibility with the pharmaceutical formulations and to verify the maintaining of a therapeutic effect comparable to that of neutral complex (often forming a salt reduces / cancels therapeutic effect).

B. Effect of sonication time and temperature

The influence of overall sonication process was studied through the quantitative determination of Ru-enro complex dissolved in the 1:1 (v/v) water:dioxane system unsonicated or sonicated.

The sonication process was performed at 30ºC and at 50ºC, in order to study the influence of temperature on Ru-enro solubilization (Figure 4). Based on the obtained data, the optimum sonication parameters: 30 minutes at 50°C were chosen for the experimental protocol.

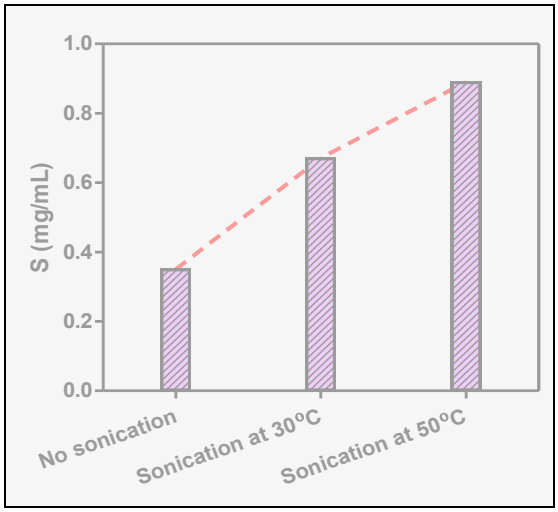


Figure 4
Influence of sonication process on Ru-enro solubility

C. Stirring time

The stirring time for the tested Ru-enro samples ranged between 1 and 48 hours. Signifficant differences were obtained for the first 24 hours (Figure 5). Further increase in stirring time didn't improve Ru-enro solubilization. In the final protocol a 24 hours stirring process of the complex was included.

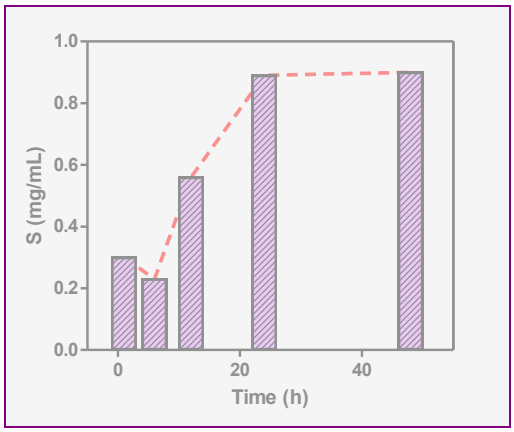


Figure 5
Influence of stirring time on Ru-enro solubility

Calculation of DR value for the Ru-enro complex

For evaluation of Ru-enro complex DR value, five different water:dioxane systems, with ε_{app} in the range 28.12-76.36 were prepared (Table II).

The ε_{app} value was calculated according to equation (1), and ε_{app} corresponding to the maximum solubility of Ru-enro was considered as DR value (Figure 6).

Table II

Evaluation of DR value for Ru-enro by using different water:dioxane systems

H_2O:dioxane (v:v)		ε_{app} **DR**	Solubility (mg/mL)
3	1	73.27	0.59
1	1	**64.61**	**0.97**
1	3	48.43	0.96
1	9	28.12	0.71
9	1	76.36	0.08

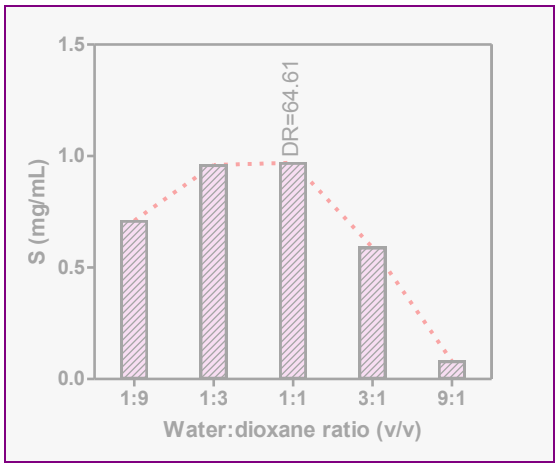


Figure 6

Influence of the water:dioxane ratio on Ru-enro solubility

Literature data emphasized that a number of active substances can present several solubility maxima [11]. Consistent with this observation, it was observed that the studied complex shows two maxima of solubility, at values of apparent dielectic constant significantly different. Thus, the solubility in the mixtures with ε_{app} 48.43 (mixture 1:3) and 64.61 (mixture 1:1) respectively, is almost identical. However, considering that for the

biocompatible systems is preferable that the addition of co-solvent to be minimal, the ratio 1:1 water:dioxane was chosen.

Solubility of Ru-enro complex in biocompatible co-solvent systems

Solubility of the complex was studied in binary or ternary systems using PG, PEG 400, GLY and ETOH as co-solvents, mixed in volume ratios so that the DR to be respected (Table III).

Table III

Composition of biocompatible co-solvent systems used for the solubilization of Ru-enro complex

System code	H_2O (mL)	PG (mL)	PEG400 (mL)	GLY(mL)	ETOH (mL)
SPG	4.3	5.7	-	-	-
SPEG	3.8	-	6.2	-	-
SGLY	7.2	-	-	1.8	-
SETOH	5	10	-	-	10

The solubility of Ru-enro in each biocompatible system was established by using the same experimental protocol used for evaluation of DR value, for avoiding the introduction of supplementary variables. The obtained solubility values are presented in Figure 7.

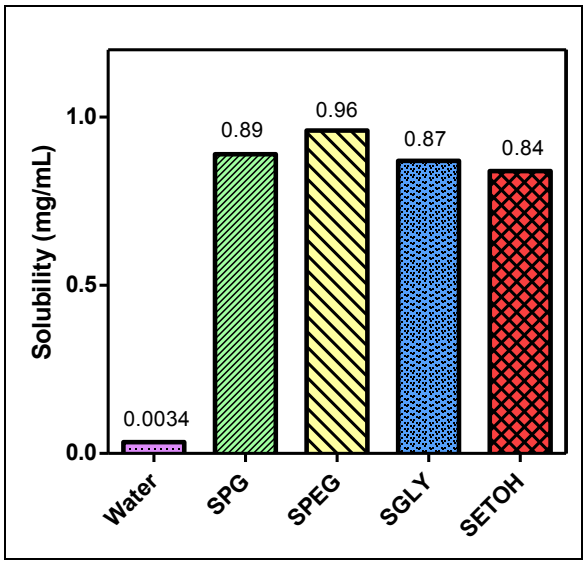


Figure 7

Solubility of Ru-enro complex in various biocompatible systems

Experimental data confirmed that the solubility values vary within narrow limits, *i.e.*, at the same value of DR, solubility remains practically constant. It was also revealed that for all systems analyzed the co-solvents used do not interfere with the active substance.

Studies have shown a significant improvement in solubility of the Ru-enro complex in the selected biocompatible co-solvent systems in comparison with water ($S=3.4\times10^{-2}$ mg/mL). Should also be noted that the highest solubility value was obtained for the binary SPEG system, whereas for SPG, SGLY and SETOH systems the solubility values were comparable.

Kinetic parameters analysis

Ru-enro release kinetics through regenerated cellulose membranes was studied by using saturated solutions of the complex solubilized in the biocompatible co-solvent systems presented in Table III. The results obtained, represented by the arithmetic mean of three kinetic experiments conducted in identical experimental conditions, are presented in Table IV.

The release kinetic data were fitted by using different diffusion-based kinetic models [8, 15], in order to evaluate the release mechanism of the tested compound (Figures 8-10).

Table IV

Kinetic data regarding Ru-enro release from biocompatible co-solvent systems through a regenerated cellulose membrane

No.	Time (min)	Abs (a.u.)			$q*10^3$		
		SPG	SPEG	SETH	SPG	SPEG	SETH
1	15	-	0.023	0.028	-	0.201	0.245
2	30	0.014	0.029	0.041	0.122	0.264	0.371
3	45	-	0.034	0.059	-	0.31	0.534
4	60	0.029	0.041	0.071	0.26	0.374	0.647
5	75	-	0.046	0.086	-	0.421	0.784
6	90	0.043	0.052	0.101	0.389	0.476	0.922
7	120	0.054	0.062	0.123	0.491	0.566	1.121
8	180	0.064	0.079	0.146	0.584	0.719	1.332
9	240	0.069	0.092	0.167	0.632	0.84	1.526
10	300	-	0.103	0.182	-	0.942	1.667
11	360	0.087	0.112	0.204	0.792	1.026	1.866

where:

q (unitary mass released) = amount of drug released per surface unit of artificial membrane into the donor medium (g/cm^2).

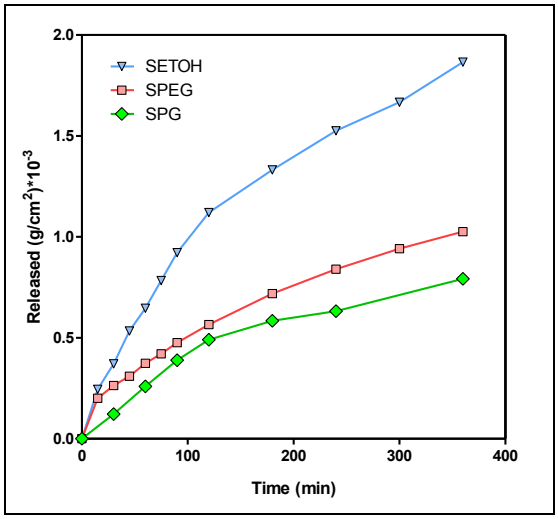


Figure 8

Release profiles of Ru-enro complex from various co-solvent systems through a regenerated cellulose membrane (n=3)

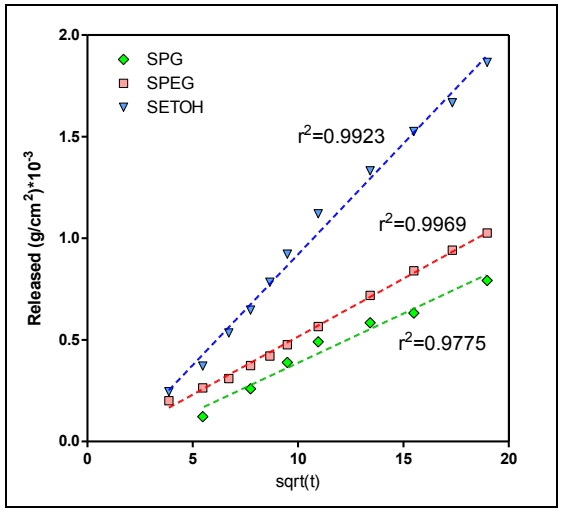


Figure 9

Modeling of the release kinetics of Ru-enro complex from various cosolvent systems by using Higuchi model (n=3)

For all co-solvent systems, Ru-enro release kinetic data were best fitted by means of Higuchi model. The analysis of experimental data using Peppas power-law model suggested a change in the Ru-enro release mechanism after two hours.

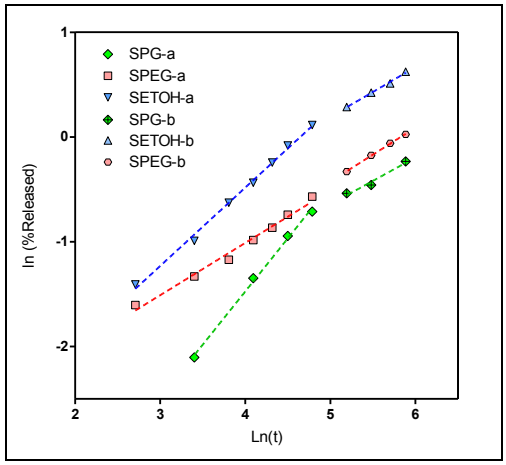


Figure 10
Peppas modeling of Ru-enro release kinetics

Influence of co-solvent system on the complex release was quantified by means of Ru-enro % released after 6 hours. The results are presented in figure 11.

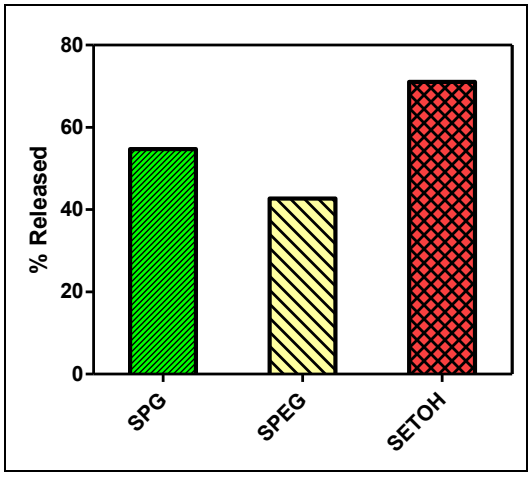


Figure 11
Comparative analysis of % Ru-enro released from different biocompatible co-solvent systems (t=6 hours)

Comparative analysis for the two binary systems (SPG and SPEG) suggests a more rapid release of Ru-enro from the water:PG system. The fastest transfer was obtained for ternary system water: PG: ETOH, which might suggest that ethanol could act as permeation enhancer.

Conclusions

The present study evaluated and compared aqueous solubility enhancement of Ru-enro complex by using cosolvency. The preliminary tests using water:dioxane co-solvent systems were used for optimizing the solubilization experimental protocol and for evaluating the DR value for the tested Ru-enro complex.

Selection of a biocompatible co-solvent system was performed by substituting dioxane with the most frequently used low-toxicity co-solvents for pharmaceutical use: PG, PEG 400, GLY and ETOH, mixed in volume ratios so that the DR to be respected.

Ru-enro aqueous solubility (0.034mg/mL) was increased to roughly 1 mg/mL by using binary and ternary biocompatible co-solvent systems with ε_{app} of 64.61 (corresponding to the DR value for the Ru-enro complex).

The Ru-enro complex release kinetics was evaluated from saturated solutions prepared in biocompatible co-solvent systems was evaluated for 6 hours, by using a modified Franz diffusion cell fitted with a regenerated cellulose membrane. The obtained Ru-enro release kinetic data were best fitted by means of Higuchi model, suggesting a diffusion-based release mechanism.

The complex proved to be stable in the biocompatible systems proposed, providing real benefits in future formulation processes (regardless the route of administration chosen).

Acknowledgements

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