SPECTROPHOTOMETRIC STUDIES OF SANGUINARINE-B-CYCLODEXTRIN COMPLEX FORMATION

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Abstract. The main aim of this study was to investigate the influence of pH and the presence of hydrophilic polymer polyvinylpyrrolidone on the formation of sanguinarine-β-cyclodextrin (SANG-β-CD) inclusion complex. Spectrophotometric studies of the SANG-β-CD systems in the presence and without 0.1 % PVP at the pH 5.0 did not show any evidence of the complex formation. However, the same systems showed several obvious evidences at the pH 8.0: the hyperchromic and the hypochromic effects and the presence of the isosbestic point in the region of 200 – 210 nm. The association constants calculated by three linear methods: Benesi-Hildebrand, Scott and Scatchard, were two times higher for the systems with addition of 0.1% PVP than for the systems without it.

Keywords: sanguinarine, β-cyclodextrin, inclusion complex, UV-vis spectrophotometry, association constant

Introduction

Sanguinarine (SANG) is a quaternary benzo[c]phenanthridine alkaloid derived from the plants of *Papaveraceae* and *Fumaraceae* families (*Macleya cordata, Sanguinaria canadesis, Chelidonium majus*). It has been found to be a potent antimicrobial, anti-inflammatory, antioxidant agent [1, 2]. Moreover, recent studies have shown its *in vitro* and *in vivo* antiproliferative activity toward skin and prostate cancer cells [3-5].

Among the main obstacles of the pharmaceutical application of SANG are low solubility in water-based liquids at the physiological pH and possible mucosal and skin irritation. The low solubility is caused by the prevalence of SANG "pseudobase" form in the neutral and weak alkaline media (Figure 1). However, namely this form of SANG is supposed to penetrate membranes of the target cells.

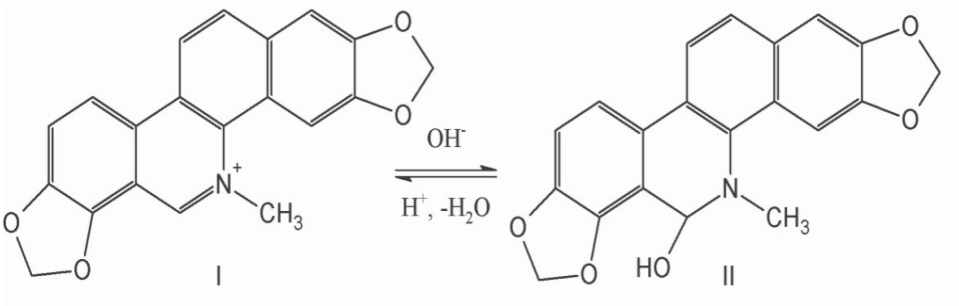


Figure 1. Sanguinarine iminium (I) and "pseudobase" forms (II)

One of the methods that can be used to enhance the water solubility and to reduce the irritating properties of different drugs, is formation of drug-cyclodextrin inclusion complexes.

Cyclodextrins (CDs) are cyclic oligosaccharides with a shape of truncated cone with a somewhat hydrophobic cavity and hydrophilic outside surface. α-, c and γ-CDs, with six, seven and eight, α-1,4-linked glucose residues respectively, are the most widely used. Inside of their cavity CDs can accommodate a wide range of lipophilic drug molecules. The process of drug-cyclodextrin inclusion complexes formation depends on several factors: type of cyclodextrin used (α-, β- or γ-CD), chemical and physical properties of the drug molecule, the complexation medium properties [6, 7].

In order to influence the efficiency of the complexation process different methods have been used: controlled change of pH of the complexation media [8, 9]; addition of different co-complexing agents to it: hydrophilic polymers (polyvinylpyrrolidone, sodium carboxymethylcellulose, hydroxypropyl methylcellulose, polyethylene glycol) [10, 11], hydroxyacids [12], amino acids [13]; treatment of the complexation media with microwaves [14].

The aim of this study is to evaluate the effect of the pH control and addition of hydrophilic polymer polyvinylpyrrolidone on the complexation efficiency of β-CD with SANG.

Experimental

Preparation of the SANG-β-CD systems for the spectrophotometric studies

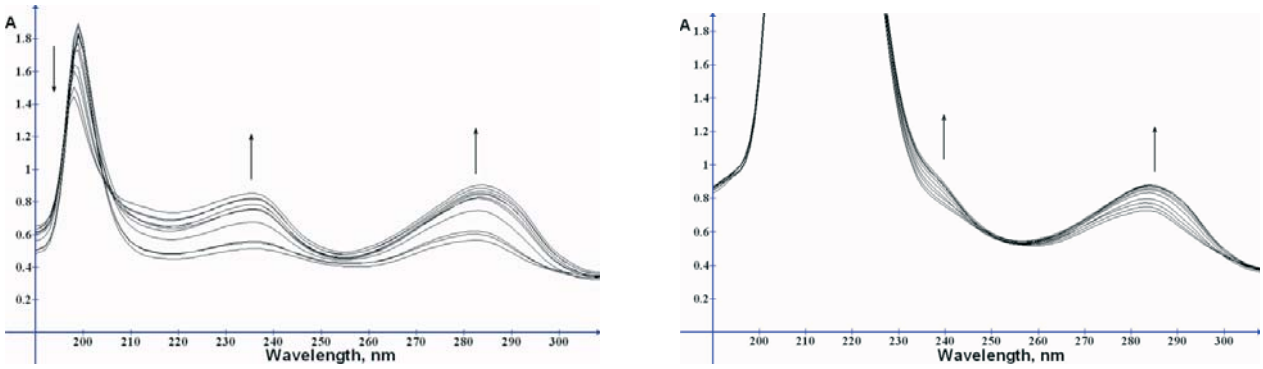
The studied systems of SANG at constant concentration 0.03 mM and βCD at increasing concentrations 0.03, 0.06, 0.10, 0.30, 0.50, 0.80, 1.00, 1.30, 1.50, 1.80, 2.00 mM have been prepared with and without addition of 0.1% (w/v) PVP in phosphate buffer-alcoholic solution (95:5 v/v) at different pH. The obtained solutions were mixed for 2 hours at 35 °C wrapped in the aluminium foil to protect them from the direct light and then left for 24-hour equilibration.

UV-vis spectrophotometry. UV-vis absorption spectra were recorded on a Jasco UV/VIS spectrophotometer, model V-550 equipped with a quartz cells with 1.0 cm optical path length.

Results and discussions

The spectrophotometric studies of the SANG-β-CD systems with and without addition of 0.1% (w/v) PVP at pH 5.0 did not show any evidence of stable complex formation. These results can be explained by the prevalence of iminium form (Figure 1) of SANG in the studied systems at this level of pH. The iminium form is charged and is more hydrophilic than 'pseudobase' form, therefore, it can not be included into the hydrophobic cavity of the β-CD.

At the pH 8.0, close to physiological levels, SANG-β-CD systems with and without addition of 0.1% (w/v) PVP showed several significant evidences of the inclusion complex formation: the hyperchromic and the hypochromic effects and the presence of the isosbestic point in the region of 200 – 210 nm (Figure 2. (a) and (b)).



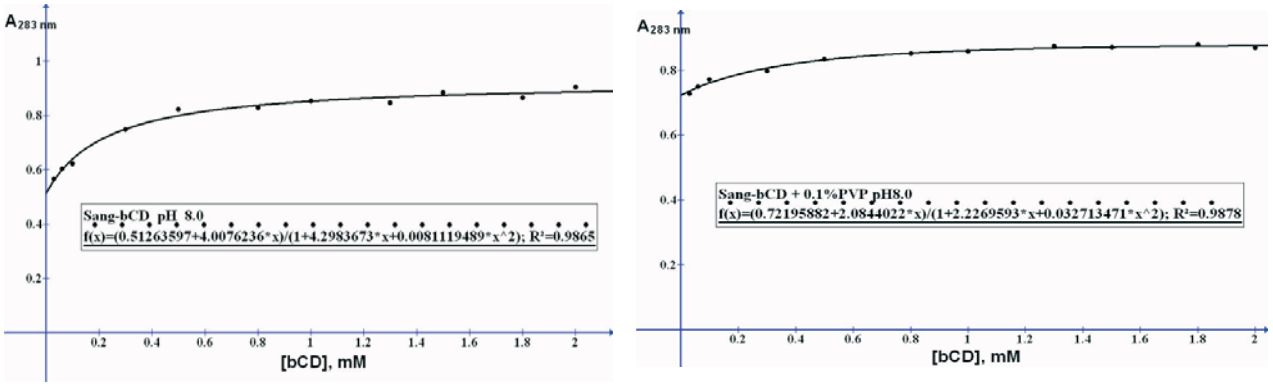
(a) **(b)**

Figure 2. (a) UV-VIS absorption spectra of 0.03 mM SANG at different concentrations of βCD: 0.03, 0.06, 0.10, 0.30, 0.50, 0.80, 1.00, 1.30, 1.50, 1.80, 2.00 mM, in phosphate buffer-alcoholic solution (95:5 v/v), pH 8.0; **(b)** UV-VIS absorption spectra of 0.03 mM SANG at different concentrations of βCD: 0.03, 0.06, 0.10, 0.30, 0.50, 0.80, 1.00, 1.30, 1.50, 1.80, 2.00 mM in the presence of 0.1% PVP, in phosphate buffer-alcoholic solution (95:5 v/v), pH 8.0.

The simulated curves of the spectrophotometric titration (at 283 nm) of 0.03 mM SANG with βCD in phosphate buffer-alcoholic solution (95:5 v/v) with and without addition of 0.1% PVP, at pH 8.0, are shown in the Figure 3. (a) and (b). The hyperbolic character of the obtained curves is one of the indicators of the 1:1 stoichiometric ratio in the inclusion complex formation between the host (βCD) and the guest molecule (SANG), according to the reaction shown in Scheme 1.

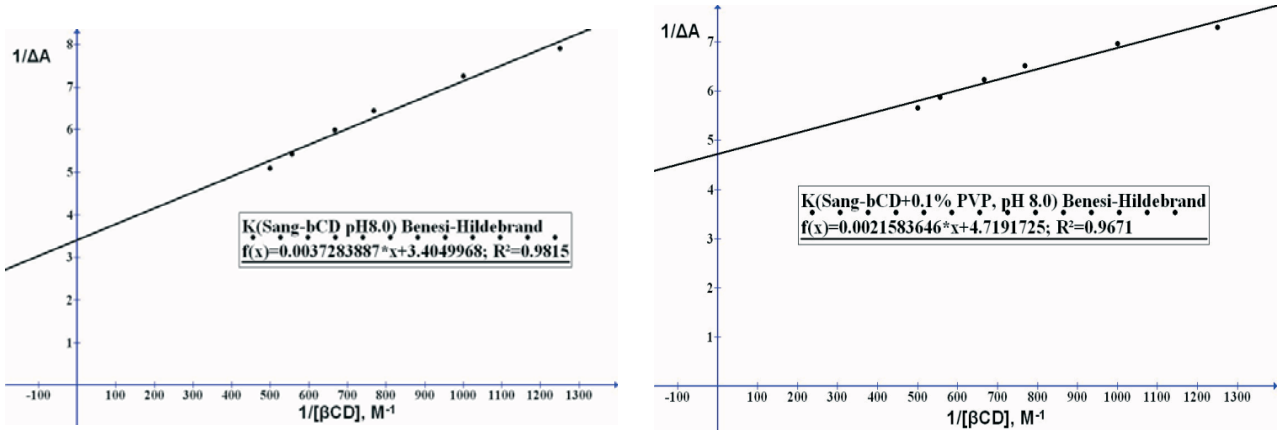
$$\text{SANG} + \beta\text{CD} \overset{KaKa}{\rightarrow\rightarrow} \text{SANG-}\beta\text{CD}$$

The curves resulted from the spectrophotometric titration of SANG with βCD (at 283 nm) were processed to obtain the values of the association constant K_a of SANG-βCD systems, in the presence and without of 0.1% PVP. The constants were calculated using different linear methods: Benesi-Hildebrand, Scott, Scatchard [15]. The plots of these three methods for the studied host-guest complexes at pH 8.0, are shown in the Figures 4, 5 and 6. Among them, the Scott method showed the highest levels of the correlation coefficients of the obtained results, ranging in the values 0.98 – 0.99. The correlation coefficients for the Benesi-Hildebrand and the Scatchard methods were in the limits of 0.96 – 0.98 and 0.93 – 0.94 respectively.



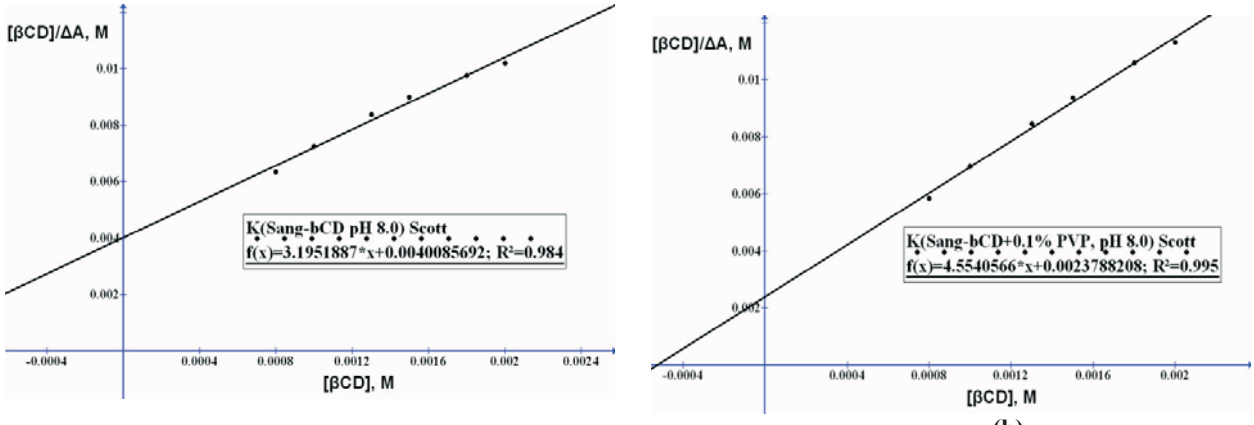
(a) **(b)**

Figure 3. **(a)** Simulated curve of the spectrophotometric titration (at 283 nm) of 0.03 mM SANG with βCD in phosphate buffer-alcoholic solution (95:5 v/v) pH 8.0; **(b)** simulated curve of the spectrophotometric titration (at 283 nm) of 0.03 mM SANG with βCD in presence of 0.1% PVP, in phosphate buffer-alcoholic solution (95:5 v/v), pH 8.0



(a) **(b)**

Figure 4**.** Benesi-Hildebrand plots for the SANG-βCD systems in the presence of 0.1 % PVP (b) and without it (a), pH 8.0



(a) **(b)**

Figure 5. Scott plots for the SANG-βCD systems in the presence of 0.1% PVP (b) and without it (a), pH 8.0

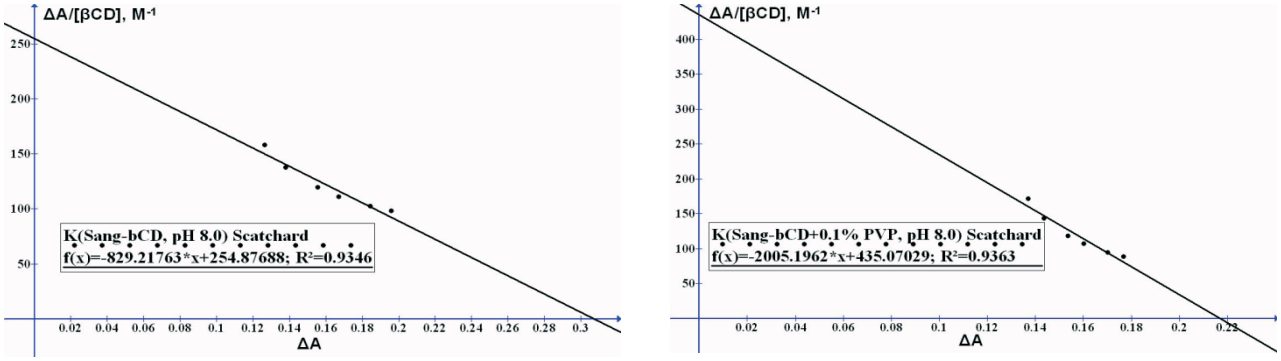


Figure 6. Scatchard plots for the SANG-βCD systems in the presence of 0.1 % PVP (b) and without it (a), pH 8.0

The values of the association constants calculated by the three methods (Table 1) differ from each other not more than by their errors. This fact suggests a satisfactory level of correlation between the obtained results.

The main conclusion based on the analysis of the obtained values is a two fold increase of the association constant of SANG-βCD complex in the presence of 0.1% PVP at the pH 8.0. This fact can be explained by the prevalence of the pseudobasic form of SANG (Figure 1) at this level of pH. This form is more lipophilic and, thus, it can be easier included inside of the hydrophobic cavity of the βCD.

Table 1

Association constants of complexation of SANG with βCD - without and in the presence of 0.1% PVP - in phosphate buffer-alcoholic solution (95:5 v/v), pH 8.0

Method of binding constant calculation	K_a(SANG-βCD), M^{-1} [PVP] = 0 %	K_a(SANG-βCD), M^{-1} [PVP] = 0.1 %
Benesi-Hildebrand	903±58	2164±132
Scott	798±43	1919±100
Scatchard	829±47	2005±111

Conclusions

The obtained results suggest that pharmaceutical formulations containing sanguinarine and β-cyclodextrin should be prepared in the weak alkaline media. Moreover, in order to improve the complexation efficiency of the cyclodextrin a small amount (~0.1%) of hydrophilic polymer (polyvinylpyrrolidone) should be added.

Acknowledgements

The research described in this publication was made possible in part by Award No. MTFP-012/05 Follow-On Award of the Moldovan Research and Development Association (MRDA) and the U.S. Civilian Research & Development Foundation (CRDF).

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