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A new insight about pharmaceutical dosage forms for benzathine penicillin G.

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ABSTRACT

In this work, a micellar system of benzathine penicillin G (BPG) in sodium deoxycholate (NaDC) was developed and evaluated physicochemically. The solubility profile of the drug in water and buffer solutions at various pH was determined, as well as its n-octanol/water partition coefficient. The Critical Micellar Concentration of NaDC and its ability to incorporate BPG were also assessed. The study was carried out at low and high ionic strength which was adjusted by the addition of sodium chloride. The results demonstrated the ability of the micellar system to incorporate BPG, as well as to increase its apparent solubility in water. The enhancement of the solubility of BPG by the presence of NaDC micelles could be analyzed quantitatively within the framework of the pseudo-phase model. Concentration analysis showed that the micellar system could attain up to 90% incorporation of BPG. The incorporated drug is expected to exhibit improved stability, since the antibiotic enclosed in the hydrophobic core of micelles is rather shielded from the aqueous external environment.

Keywords: Benzathine Penicillin G; micellar solubilization; micelles; pre-formulation; sodium deoxycholate.

INTRODUCTION

Benzathine penicillin G (BPG) is natural penicillin whose molecular structure is $C_{48}H_{58}N_6O_8S.4H_2O$. BPG is chemically designated as N, N-dibenzyl ethylenediamine dipenicillin and its molecular mass is 981.19 daltons. It occurs as a white, crystalline, odorless powder, and is very slightly soluble in water and sparingly soluble in alcohol. BPG concentrations are described in international units (IU), in which 1mg corresponds to 1,211 IU of penicillin (Shulman & Gerber, 2004). The antibacterial activity of BPG is mainly against Gram-positive bacteria (including actinomycetes) and some Gram-negative Cocci, as well as some spirochetes (Parfitt, 1999). BPG is widely used in the treatment of numerous infectious diseases, especially those related to obstetric and gynecologic conditions. In general, penicillin is effective in the treatment of localized skin and soft-tissue infections of the nose, throat, lower respiratory tract and genitourinary tract. It is also commonly used in prevention of bacterial endocarditis prevention, prophylaxis during gastrointestinal and/or genitourinary procedures, and treatment of rheumatic fever (Miller, 2002).

According to the American Academy of Pediatrics, the American Heart Association, the Infectious Diseases Society of America and the World Health Organization the first-line treatment for streptococcal infections consists of monthly injections of 1,200,000 UI of BPG (Currie, 1996; Kassem, et al., 1996). However, lack of patient compliance has meant in treatment failure (Currie, 1996; Carapetis et al., 2000; Peloso et al., 2003).

Sustained-release dosage forms could be a promising means of overcoming this drawback in relation to rheumatic fever treatment. When they are used, several come into play (Peloso et al., 2003), for example: less frequent dosing, improved bioavailability, reduced concentration, increased absorption and minimization of adverse drug reactions.

Pre-formulation studies are a preliminary stage in the development of new drug dosage forms. Such studies mainly focus on developing physically and chemically stable formulations, whose biopharmaceutical profile is suitable for their purpose. Partition coefficient analysis and solubility tests in liquid media are some of the most important tests at this stage (Blasco et al., 2001; Granero et al., 1999).

The drug dosage form for BPG which is currently available on the pharmaceutical market is an intramuscular injection suspension. Due to its variable physical properties, such as solubility degree, viscosity and crystal size, deviations on its bioavailability and release profile have been reported (Currie, 1996; Carapetis et al., 2000).

Micelles and other related systems have had considerable success in changing some of the physical and chemical properties of drugs, e.g. solubility and stability (Oliveira et al., 1990; Oliveira et al., 1991; Oliveira & Chaimovich, 1993). Micellar and directly-related systems are dynamic colloidal supramolecular aggregates containing surfactant molecules that can efficiently dissolve water-insoluble drug compounds (Oliveira et al., 1997; Oliveira & Scarpa, 1999). The micelization process takes place when the surfactant concentration exceeds the Critical Micellar Concentration CMC (Tascioglu, 1996). This is the results

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from an induced interaction of intermolecular forces, including electrostatic and steric repulsions, hydrogen bonds, and Van der Waals interactions. This approach is of particular interest in the pharmaceutical field, given the ability of these systems to increase the solubility of hydrophobic drugs (Rangel-Yagui et al., 2005). Besides, this technique involves easily-controlled parameters, as the factors that change the properties of micelles are well established. In this study the physicochemical properties of BPG were evaluated. The CMC was determined for sodium deoxycholate (NaDC) in aqueous solution, as well as its effect of sodium chloride on its CMC value. It was aimed to develop a micellar system containing BPG, using data obtained on ideal surfactant concentration need to dissolve the drug.

MATERIAL AND METHODS

Determining solubility profile of drug in liquid media is a critical stage in the development of new drug delivery systems. In fact, the profile plays a major role in drug dissolution, which is a rate-limiting step for drug absorption (Blasco et al., 2001). Another major point to consider is the analysis of the n-octanoll/water partition coefficient. This parameter is largely used to determine the degree of hydrophobicity in pharmacological models, as well as to study toxic properties (Roberts, 2002). In addition to that, it provides a theoretical approach to the partition of molecules in biological membranes during absorption processes.

In the study of micellar systems, the determination of both the CMC of the surfactant and the parameters that can modify its value are mandatory steps.

Drug Solubility Determination

In the experiments, a supersaturated solution of BPG was used, as described in the equilibrium method proposed by Granero et al. (1999).

Solubility in water and n-octanol

Excess of drug (250mg) was added to 5mL of solvent (distilled water or n-octanol), and was stirred magnetically for 24 hours at 25 °C. Afterwards, the mixture was allowed to rest for 12 hours. As equilibrium was reached, the samples were filtered through by using a 0.8μm membrane and the drug concentration in the filtrate was analyzed by UV-Vis spectrophotometry.

Solubility in different pH

The pH values of the buffer solutions varied from 5.0 to 9.0 to obtain solubilities in acid, neutral and alkaline conditions. Samples were stirred in an ultrasound bath for 3 hours to obtain saturated solution and allowed to rest for 1.5 hour. After that, the concentration of the dissolved BPG was determined by UV-Vis spectrophotometry.

Partition coefficient determination (log P)

This parameter was determined in n-octanol/water mixture (Roberts, 2002; Sangster, 1997). The two phases of solvents were stirred until equilibrium was reached, then an accurately weighed amount of BPG dissolved in a minimal amount of methanol was added. The mixture was stirred for 1 hour, and allowed to rest for 36 hours for phase separation. An aliquot of each phase was withdrawn by pipette, and analyzed by ultraviolet spectrophotometry. The partition coefficient was taken as the ratio of the drug concentration (w/v) in the n-octanol and water phases, respectively.

Determination of NaDC Critical Micellar Concentration

The CMC of sodium deoxyxcholate (NaDC) was evaluated by determining the electric conductivity of solutions, in which the concentration of the surfactant varied from 0.8 to 18.1mM. The results were plotted as a function of NaDC concentration and the CMC was taken as the point of intersection of the curves.

To evaluate the effect of ionic strength on the CMC, the electric conductivity of NaDC solutions was determined in saline solutions using the same method as for the solutions in water.

Preparation of micellar solutions of BPG

In order to prepare BPG micellar solutions a solution of NaDC containing NaCl was used. The drug incorporation took place under stirring in an ultrasound bath. Macroscopic analysis was performed against black background. Drug concentration was assessed by UV-Vis spectrophotometry after calibration of the apparatus. The concentration of BPG in micellar solution was established by subtracting its concentration in the supernatant liquid from the bulk (nominal) value.

RESULTS

The results for the solubility of the BPG in some n-octanol, water and buffers at several pH values are displayed in table 1, together with the n-octanol/water partition coefficient.

Table 1. Physicochemical properties of BPG and NaDC micelles.

BPG Solubility [mM]					log P
Water	Octanol	pH 5.4	pH 7.4	pH 8.9	
0.16	0.026	0.057	0.485	1.13	1.12

Note that the solubility of benzathine penicillin G in water was very low, decreasing to a value 6.2 times lower in n-octanol (Table 1). In addition, the solubility depended on pH increasing 8.5 fold as the pH enhanced from 5.4 to 7.4, and a further 2.3 fold from pH 7.4 to 8.9. The n-octanol/water partition coefficient was also low.

The results of the CMC determination for sodium deoxycholate are described in Table 2 and show clearly that at high ionic strength due to sodium chloride concentration of the saline solution, the value of the CMC was much lower than in water favoring micelle formation at lower surfactant concentrations.

Table 2. Effect of NaCl on the Critical Micellar Concentration of NaDC. [NaCl] = 0.09g/mL (163.63mM)

CMC of NaDC [mM]	
Aqueous solution	**Saline solution**
3.6	0.8

The CMC was easily determined from the conductivity measurements at various NaDC concentrations, since that at CMC the curve has a marked discontinuity and a significant change in the inclination between the straight lines (Figures 1 and 2).

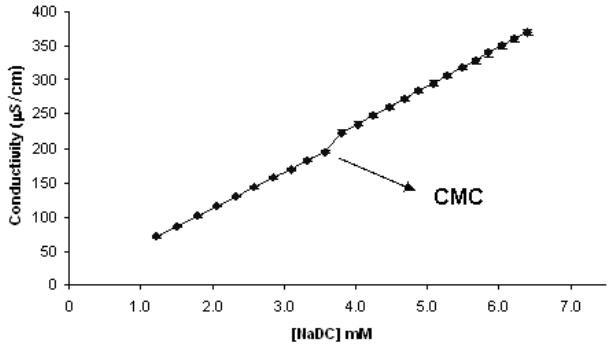

Figure 1. Determination of the Critical Micellar Concentration of NaDC in aqueous solution. (25ºC)

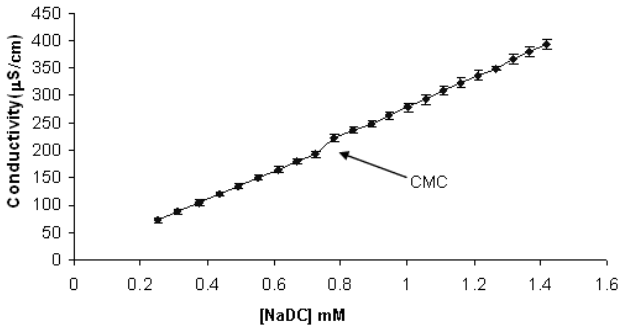

Figure 2. Effect of 0.9% (w/v) sodium chloride on the Critical Micellar Concentration of NaDC. (25ºC).

Considering that in the presence of saline solution the CMC of the NaDC was about 0.8 mM, the experimental determination of micellar incorporation of BPG was conducted at NADC concentrations below and above CMC (Table 3).

Table 3. Effect of the NaDC concentration on the solubilization of BPG into micellar solutions in saline.

Sample	NaDC (mM)	BPG		Abs	Incorporation % w/v
		Bulk	Supernatant		
1	0.49	5.81	2.83	0.14	48.76
2	0.98	6.01	3.51	0.15	58.33
3	1.96	6.15	4.03	0.20	65.60
4	2.45	5.98	5.00	0.25	83.67
5	2.94	5.84	5.28	0.26	90.28
6	3.92	6.32	5.63	0.28	89.10

The concentration of dissolved BPG increased with increasing surfactant concentration up to a plateau (Figure 3). This incorporation profile is typical of micelle-modified drug solubility where the drug solubility in the micellar pseudo-phase is higher than that in the aqueous phase.

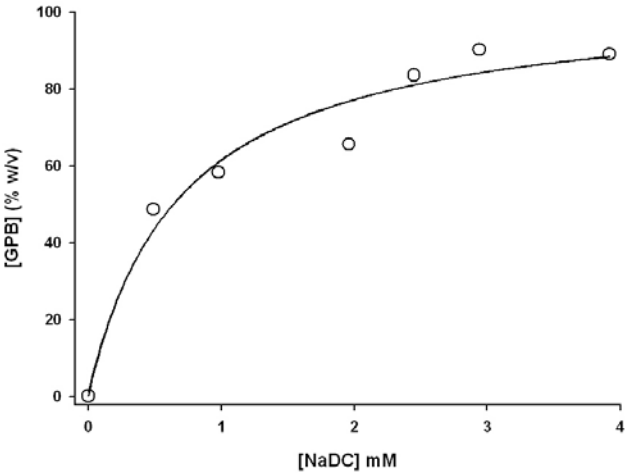

Figure 3. Effect of NaDC on the micellar incorporation of BPG in aqueous solution containing sodium chloride 0.9 % w/v (163.63mM). See table 3 for details. Solid line calculated from equation 1.

This increase in the solubility suggests that BPG may be distributed between the aqueous and micellar pseudo-phases. Then, the data in the Figure 3 can be analyzed quantitatively by the expression of the pseudo-phase model (Oliveira et al., 1991).

$$BPG_d = \frac{BPG_w + BPG_m \cdot Ks \cdot [NaDSC]}{(1 + Ks) \cdot [NaDSC]} \qquad (1)$$

Where the subscript d, w, and m refer to the concentrations of total dissolved BPG, BPG dissolved in the water phase and BPG dissolved in the micellar phase, respectively. Ks is the constant that describes the incorporation of the BPG into micellar system. The value of BPG_w was obtained from the solubility of BPG in the absence of NaDC.

The calculated value of Ks was about 106, demonstrating clearly the incorporation of the BPG into the micellar system.

This feature can be seen in the photographs in Figure 4, which shows that at low surfactant concentrations, BPG is partially dissolved, generating opaque and semi-transparent dispersions which are completely dissolved at high surfactant concentrations.

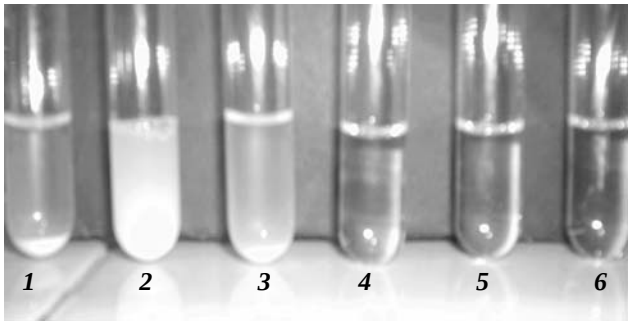

Figure 4. Macroscopic aspect of the system during BPG incorporation into micellar aqueous NaDC. (**1**) pre-micellar dispersion of BPG; (**2-6**) Stages in incorporation of BPG into NaDC micelles at various concentrations of NADC (see Table 1).

DISCUSSION

In the experiments of BPG solubility in different solvents a high degree of insolubility was observed in both lipophilic and hydrophilic media. In fact, these data agree with those in the literature (Kreuzig, 1982), and suggest the amphiphilic character of the drug molecule. However, it was observed that the solubility increased with the pH (Table 1), possibly due to the fact that BPG is an acid drug, with the pKa of the carboxyl group around 2.65, depending on the solvent. Thus, in the range of pH studied the equilibrium is displaced towards ionized species, which are fast hydrated, becoming more soluble (Atwood & Florence, 1985).

The determination of partition coefficient provided log P for the n-octanol/water system. The low value of log P indicates low partition capacity and medium-low hydrophobicity. Thus, the value described in Table 1 reveals a medium ability of the drug to diffuse to the hydrophobic core of the micelles.

Surfactant molecules, constituted by a polar head-group and a hydrocarbon-chain longer than eight methylene groups, associate spontaneously in water to form dynamic aggregates denominated micelles (Oliveira & Chaimovich, 1993). In dilute solutions below the CMC these molecules are dispersed individually in the medium as monomers and such solutions exhibit ideal physical and chemical properties. As the surfactant concentration increases the properties deviate from ideal indicating the aggregation of monomers above the CMC forming micelles, provoking the differentiated properties of the micellar pseudo-phase (Tascioglu, 1996).

When the conductivity is plotted against surfactant concentration, the data points fit on two different lines (Figures 1 and 2). The first corresponds to concentrations below the CMC, in which only surfactant monomers exist in the solution (Atwood & Florence, 1985; Oliveira & Chaimovich, 1993). At higher concentrations, micelles appear, and conductivity curve suffers discontinuity at the CMC. The second parte of the curve has a different slop. The determination of the CMC was a first step in the BPG incorporation study, used to indicate exactly the surfactant concentration at which the micelles were formed in solution.

Addition of inorganic salts is able to change micelle size and shape. Therefore, ionic strength influences the solubilization rate of drugs. In fact, salt tends to reduce the mutual electrostatic repulsion of the head groups of surfactants, which become more hydrophobic. This effect induces surfactant aggregation at lower concentrations. As a result the CMC is lower and the final cost of these systems is reduced (Tascioglu, 1996). This phenomenon was observed here for NaDC (Figure 2), and also found to function in BPG micellar system. The incorporation of BPG into the micelles was feasible by a simple and fast method that was able to increase drug incorporation markedly in solutions above the CMC (Table 3 and Figure 3).

The value of Ks calculated from the plot of the data by equation 1 (Ks=106) demonstrated that the incorporation of BPG into the micellar system is due mainly to the hydrophobic property of the drug molecule. It is known that in several cases the effect of micelles on drug solubility can be attributed to the free energy of activation resulting from transfer of the substrate to a medium of lower dielectric constant (Oliveira et al., 1991). Indeed, our results show clearly that the solubility of BPG was increased when the antibiotic was transferred to the low dielectric constant medium of the hydrophobic core of the NaDC micelles. In addition, this value for Ks is in agreement with the partition coefficient (log P = 1.12) indicating that antibiotic molecule is moderately lipophilic.

The determination of the analytical concentration of the drug while [NaDC} was raised showed that the percentage of incorporated BPG could reach about 90% (Table 3). Hence, micellar solutions appear to be a promising approach improving the physical-chemical properties of BPG for therapeutic use. The dibenzyl ethylenediamine moiety, added to penicillin G to produce BPG, delays its release by the formation of a water-insoluble deposit at the site of injection, due to molecular aggregation (Martinez-Ladeira, et al., 2004).

The incorporation of BPG into micellar systems is intended to reduce the incompatibility of this molecule with water (Blaha et al., 1976). Its resistance against the hydrolytic and enzymatic degradation may be also be improved. As a consequence, its pharmacological effect may be achieved at lower doses. Besides, assuming that drug molecules do not undergo degradation, they will remain pharmacologically active for longer. The incorporation of BPG in micellar solutions was feasible by a simple and fast method that was also able to increase markedly the amount of incorporated drug in solutions above the CMC. Hence, micelle solutions seem to be a promising approach to improving the physical-chemical properties of BPG for therapeutic use.

RESUMO

Novas perspectivas para sistemas de liberação para a penicilina G benzatina

O objetivo desse trabalho foi o de desenvolver e avaliar do ponto de vista físico-químico um sistema micelar de penicilina G benzatina (BPG) em desoxicolato de sódio (NaDC). Foram estudadas as características físico-químicas da BPG quanto à solubilidade em água e em soluções tampões com diferentes pHs, além do coeficiente de partição octanol-água. Foram avaliadas as propriedades da concentração micelar crítica (CMC) das soluções micelares de Desoxicolato de sódio (NaDC) em baixa e alta força iônica provocada pela presença de cloreto de sódio. O estudo da incorporação da BPG em soluções micelares de NaDC usando várias concentrações de NaDC também foi realizado. O aumento da solubilidade da BPG provocada pela presença de micelas de NaDC foi analisada quantitativamente pelo formalismo do modelo de pseudo-fase. Houve indicação da aplicabilidade do sistema micelar estudado quanto à incorporação de penicilina e aumento se sua solubilidade aparente, com taxa de incorporação de até 90%. Espera-se que a formulação micellar de BPG apresente melhor estabilidade, considerando-se que o antibiótico incorporado na região hidrofóbica das micelas está protegido do meio aquoso externo.

Palavras-chave: penicilina G benzatina; solubilização micelar; micelas; pré-formulação; desoxicolato de sódio.

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