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Solubilization and preformulation of carbendazim

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Abstract

The solubilization of carbendazim by pH in combination with cosolvents, surfactants or complexants was investigated. At pH 7 the total drug solubility is 6.11 ± 0.45 μg/ml which increases by 1–7 fold with cosolvent, surfactant or complexant. However, at pH 2 the solubility increases by 250 times. Cosolvents have a negligible effect (50% increase) on the total drug solubility at pH 2 because of the high polarity of the cationic drug. Also pH combined with nonionic surfactants does not improve solubility, as relatively less polar micelles are not able to accommodate the cationic drug. Interestingly, the total drug solubility increases by combining pH 2 with complexants, as they can form a complex with the isolated aromatic ring of both the unionized and the ionized drug. The proposed oral formulation of 1 mg/ml carbendazim at pH 2 does not precipitate in the presence of Seven Up or water. But it does precipitate with pH 7 buffer when diluted 1:10 but not 1:100 or 1:250.

Keywords: Solubility; Carbendazim; Buffer; Surfactant and Complexant

1. Introduction

Carbendazim (methyl-2-benzimidazolecarbamate) is a well known anti-fungal agent that may have significant anti-cancer activity. Unfortunately its projected oral dose up to 100 mg per day is far greater than its water solubility of 6.11 μg/ml. Furthermore, because of its high melting point its dissolution rate is very low. Therefore, it must be solubilized before it can be adequately tested in the clinic.

According to Yalkowsky (1999), buffers, cosolvents, surfactants, and complexants are the most commonly used excipients to improve the solubility of a nonpolar drug in aqueous media. These can be used either alone or in combination (Martin, 1993; Li et al., 1998, 1999a,b). It is well known that ionized species are more soluble than their unionized counterparts in aqueous solution. Li and Yalkowsky (Li et al., 1998, 1999a,b) have reported that the ionization of a drug not only increases the solubility of the ionized species in water, but also can increase its solubilization by cosolvents, micelles, or complexants. Carbendazim has a calculated octanol-water partition coefficient of 1.71 by using CLOGP® 4.0 software (Bio Byte Corp., Claremont, CA). Its amphoteric

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structure (Gauthier et al., 2000) makes combining pH with other solubilization techniques a feasible method to increase its solubility.

This paper will address the solubilization of carbendazim by some pharmaceutically acceptable cosolvents, surfactants, and complexants, alone and in combination with pH. In addition, the precipitation of the solubilized drug upon dilution with various aqueous media is evaluated.

2. Materials and methods

2.1. Materials

Carbendazim was provided by the Procter & Gamble Company and used as received. Hydroxypropyl β-cyclodextrin (HPβCD) with an average molecular weight of 1390 and an average degree of substitution of 4.4 was obtained from Cyclodextrin Technologies Development Inc. (Gainesville, FL). Sulfobutyl ether β-cyclodextrin (SBEβCD) with an average molecular weight of 2160 and an average degree of substitution of 7 was a gift from Cydex, L. C. (Overland Park, KS). All other chemicals were of reagent grade, purchased from Sigma (St. Louis, MO) or Aldrich (St. Louis, MO) and used without further purification. Buffers were prepared according to Henderson–Hasselbalch equation.

2.2. pH-Solubilization profile

The buffer systems used for the pH-solubilization profile are 0.01 M sodium citrate/HCl with pH range 1.2–4.0, 0.01 M citrate-phosphate-borate/HCl with pH range 4.0–8.0, and 0.01M glycine/NaOH with pH range 8.0–12.0. In all the above mentioned buffers the ionic strength was maintained at 0.1M using sodium chloride. The effect of buffer species on the solubility of carbendazim at very low and high pH was not observed because the K_{sp} of each salt was not reached. Solubility at different pH was determined in the same manner as described below.

2.3. Solubility determination

An excess amount of carbendazim was added to vials containing 2 ml of an aqueous solution of pH 2.00 ± 0.15 and 7.00 ± 0.15 at different concentrations (0, 1, 2.5, 5, and 10%) of cosolvents (EtOH, PG, PEG 400, and Glycerol), surfactants (Tween 20, Tween 80, sodium lauryl sulfate (SLS), and myristoyl carnitine (MC)) and cyclodextrins (HPβCD and SBEβCD). In addition the cosolvents and cyclodextrins were studied at 20%. The sample vials were rotated at 20 rpm using an end-over-end mechanical rotator (GlasCol Laboratory rotator, Terre Haute, IN) at ambient temperature for 10 days. Ten days were selected to ensure equilibrium because the strong crystal structure and high melting point (305 °C) (Budavari, 1996) of carbendazim contribute to a very slow dissolution rate. For solutions having pH greater than 9, the solubility was measured after 3 days rotation instead of 10 days because of the unstable nature of carbendazim in alkaline condition (Budavari, 1996). The samples were filtered through a 0.45-μm filter and the pH at equilibrium was measured before performing HPLC analysis. The maximum concentrations of different solubilizing agents were selected because these concentrations have been used in the marketed formulations without any problem (Wade and Weller, 1994).

2.4. High performance liquid chromatography (HPLC) analysis

The HPLC assay used an Econosphere C8 column (150×4.6 mm, Alltech, Los Altos, CA) with a mobile phase composed of pure methanol. The flow rate was controlled at 1.0 ml/min (125 solvent Module, Beckman, Fullerton, CA) and the effluent was detected at 280 nm (168 detector, Beckman, Fullerton, CA). All experimental data are the average of duplicate values with an average error less than 3%.

2.5. Precipitation studies for oral formulations

0.1 ml potential oral formulation was added to a test tube containing 1, 10, or 25 ml each of soda

solution (Seven Up), 0.01M pH 7 phosphate buffer solution, or water. The mixed solution was shaken by hand for 5 s. The presence or absence of carbendazim crystal was determined visually and the final pH was recorded. The visual determination of the carbendazim crystals was also performed at one day later after dilution.

3. Results and discussions

Fig. 1 shows the unionized and the ionized forms of carbendazim. The formation of a resonance-stabilized cation and anion is responsible for the high solubilities at low and high pH that are shown in Fig. 2. The figure shows that carbendazim has an intrinsic solubility of 6.11 μg/ml and its solubility increases with decreasing pH below 4.5 (the pK_a of its basic guanadinium group), and with increasing pH above 10.6 (the pK_a of its carbamide group). Fig. 2 also shows that the

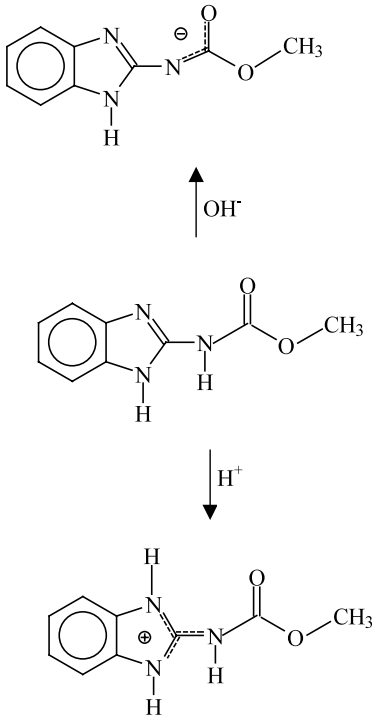

Fig. 1. Structure of carbendazim (methyl-2-benzimidazolecarbamate).

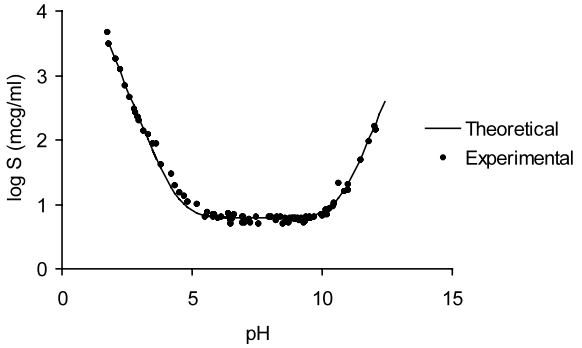

Fig. 2. pH-solubilization profile of carbendazim.

experimental pH-solubilization profile fits very well with the theoretical line calculated by the Henderson–Hasselbalch equation for an intrinsic solubility of 6.11 μg/ml, a basic pK_a of 4.5 and an acidic pK_a of 10.6.

Fig. 3a shows the aqueous solubility of carbendazim versus the concentration of EtOH, PG, PEG 400, and Glycerol at pH 7. In all cases there

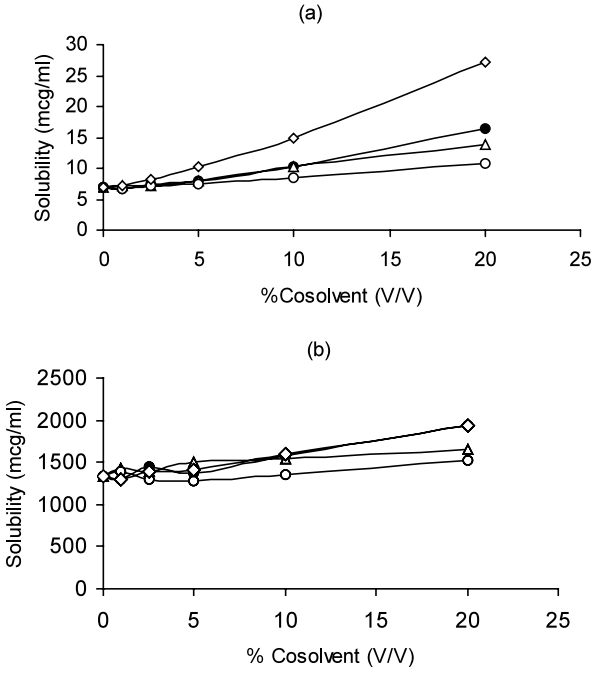

Fig. 3. Total solubility of carbendazim in cosolvent solutions at (a) pH 7.00, (b), pH 2.10. ◇: PEG 400; ●: EtOH; △: PG; ○: Glycerol.

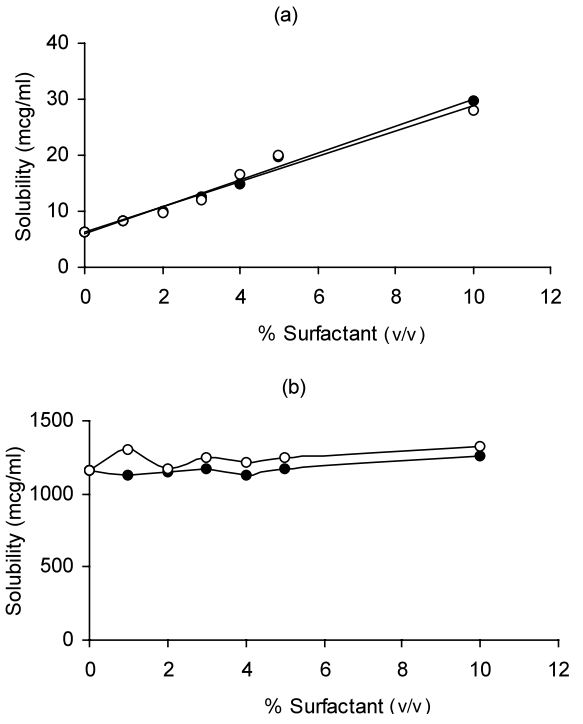

Fig. 4. Total solubility of carbendazim in surfactant solutions at (a) pH 7.00, (b), pH 2.15. ●: Tween 20; ○: Tween 80.

is an exponential increase in the solubility with increasing cosolvent concentration. The extent of solubilization depends both on the concentration and the polarity of cosolvents. The order of solubilization by four cosolvents is PEG 400 > EtOH > PG > Glycerol, which is in agreement with the polarities of cosolvents as reflected by their reported dielectric constants or solubility parameters (Etman and Nada, 1999).

Fig. 3b shows the aqueous solubility of carbendazim versus the concentration of the same cosolvents at pH 2. From Fig. 3b, we can conclude that there is only a slight effect of cosolvent on the total drug solubility at pH 2, with the cosolvents following the same order as at pH 7. While the number of milligrams solubilized is larger, the percentage increase is much smaller at pH 2.

Fig. 4 shows the total solubility of carbendazim at pH 7 and 2, respectively, for different concentrations of Tween 20 and 80 solutions. The critical micelle concentrations of Tween 20 and 80 are 0.006 and 0.0014%, respectively (Florence and Attwood, 1988) which is well below the minimum concentration of surfactant used for solubilization. At pH 7, the total drug solubility increases equally with increasing the concentration of either surfactant. On the other hand, there is no significant change in the total solubility with increasing the surfactant concentration at pH 2, because the polar cationic species does not partition into the nonpolar region of the micelle. Also at high concentrations of micelles (which do not form homogeneous aqueous solutions), the volume of free water is reduced. This reduces the amount of the ionized species in the free water. Therefore, the net effect of micellization on the total drug solubility is the result of the increase in the solubility of the unionized drug by the micelle, the decrease in the amount of the ionized drug in the free water, (i.e. the volume of the solution not occupied by the micelles), and the increase (if any) in the solubility of the ionized drug in the micelle.

Fig. 4 also shows that there is no significant difference on a weight basis between Tween 20 and 80 in solubilizing carbendazim at either pH 7 or 2. However, the more nonpolar Tween 80 is a more efficient solubilizer on a molar basis.

Due to the high polarity of ionized carbendazim at pH 2, the ionic surfactants, sodium lauryl sulfate (SLS) and myristoyl carnitine (MC), were also studied at pH 2. Fig. 5 shows the total drug solubility of carbendazim at pH 2 for different concentrations of SLS and MC. The figure shows that SLS increases the solubility of carbendazim more than MC. Since the drug is positively

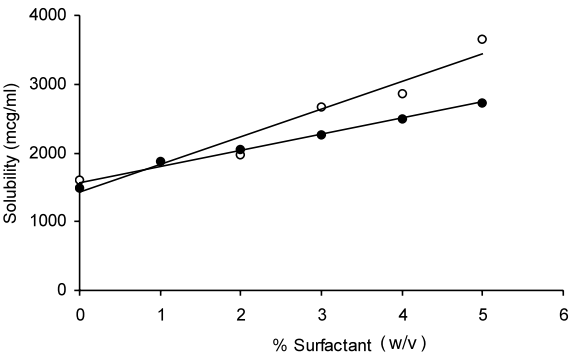

Fig. 5. Total solubility of carbendazim in surfactant solutions at pH 2.05. ●: MC, ○: SLS.

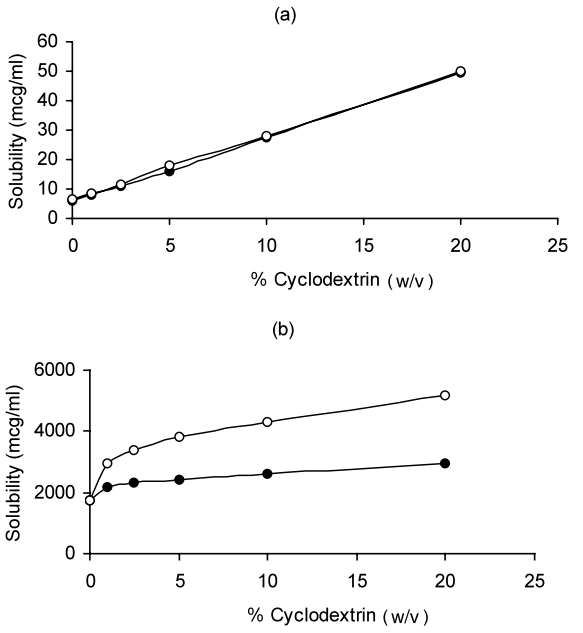

Fig. 6. Total solubility of carbendazim in complexation solutions at (a) pH 7.00, (b), pH 1.95. ●: HPβCD; ○: SBEβCD.

charged at pH 2.0, the non-ionic Tween 80 and 20 and cationic MC are less effective than the anionic SLS as solubilizing agents.

Fig. 6 shows that the total drug solubility increases both at pH 7 and 2 for HPβCD and SBEβCD. This suggests the formation of an inclusion complex between β-cyclodextrin and the benzene ring of drug molecule even though a large polar group is attached to benzene ring when the drug is ionized at pH 2. Fig. 6a shows that both HPβCD and SBEβCD have the same capacity to solubilize unionized carbendazim at pH 7. On the other hand, Fig. 6b shows that SBEβCD increases the solubility more than HPβCD at pH 2, because the resultant complex is stabilized by the interaction between the anionic cyclodextrin and the cationic drug.

Due to the high melting point (305 °C) and low clogP (1.71) cosolvency, micellization, and complexation are not very efficient methods to improve the solubility of carbendazim. The high crystallinity of carbendazim more significantly contributes to its insolubility in water than its non-polarity.

Fortunately, carbendazim is relatively soluble at either high or low pH. While it is not practical to give oral preparations at very high pH, low pH is acceptable for oral administration. Emetrol® (anti nausea liquid) is available over the counter. It is a solution of phosphoric acid and sugars with a pH between 1 and 2 that is an excellent vehicle for carbendazim. Anti nausea liquid can be given in 15–30 ml doses, five times in an hour, four times a day. This amounts to a maximum daily dose of 600 ml. Since the solubility of carbendazim at pH 1 and 2 are 16 and 1.6 mg/ml, up to 100 mg carbendazim can be given orally in 100 ml anti nausea liquid. Provided, of course, that the drug does not precipitate mixing with the contents of the stomach.

Since the oral formulation is administered along with a fluid like water or juice it is essential to study the effect of dilution of the formulation with these fluids. This can be investigated by a simple serial dilution precipitation study. The formulation is serially diluted using equal volumes of Seven Up, water, and pH 7 buffer.

Table 1 shows the result of a precipitation study for two oral formulations, viz. 1 mg/ml carbendazim buffered solution at pH 2 and 2 mg/ml carbendazim in 5% buffered MC solution at pH 2. It is evident from Table 1 that both above formulations did not precipitate in Seven Up and water at dilutions varying from 10 to 250 times. The final pHs of the diluted solutions were also measured and listed in Table 1. When the formulation is diluted to the point at which the concentration of hydrogen ion or hydroxide ion is not sufficient to maintain the solubility of the drug above the concentration present, precipitation will occur. Therefore, soda and water can be used efficiently to administer above formulation orally.

The dramatic shift in the pH of the formulations from 2 to 6.9 is responsible for the precipitation when diluted with pH 7 buffer solution by 1:10 ratio. However, when diluted 100 and 250 times with pH 7 buffer solution no precipitation is observed visually. Although on observation under the microscope, the formulations diluted 100 times did show some small crystals of the drug.

Table 1
Precipitation results for oral formulations

Formulation	Dose (mg/ml)	Dilution with								
		Vehicle (pH)	Seven Up (3.26)			Water (5.50)			pH 7 Buffer	
		Ratio	1:10	1:100	1:250	1:10	1:100	1:250	1:10	1:100	1:250
		pH	3.01	3.18	3.22	3.00	3.75	4.15	6.50	6.96	6.97
pH 2 Buffer	1	Initial	−	−	−	−	−	−	+	−	−
		1 day	−	−	−	−	−	−	+	−	−
5% MC pH 2	2	Initial	−	−	−	−	−	−	+	−	−
		1 day	−	−	−	-	−	−	+	−	−

(+): precipitation; (−): no precipitation.

Table 2
Summary of solubility of carbendazim in different vehicles

Vehicle	Solubility of carbendazim (mg/ml)	
	Unionized (pH 7.00 ± 0.15)	Ionized (pH 2.00 ± 0.15)
Buffered solution	0.006	1.64
20% Ethanol	0.016	1.94
20% PG	0.014	1.66
20% Glycerine	0.010	1.52
20% PEG 400	0.027	1.93
10% Tween 20	0.030	1.26
10% Tween 80	0.028	1.33
5% SLS	N/A	3.66
5% MC	N/A	2.73
20% HPβCD	0.049	2.96
20% SBEβCD	0.050	5.20

4. Conclusion

The solubilization of carbendazim by combining pH with cosolvents, surfactants, and complexants was investigated. Table 2 summarizes the solubility of carbendazim in different vehicles. Although various cosolvents, surfactants, and complexants increase the solubility of carbendzaim at pH 2, their use would reduce the maximum acceptable dosage of the vehicle and increase cost. Thus, they would reduce the maximum dose of the drug that could be given. Formulation containing 1 mg/ml carbendazim does not precipitate when mixed with water or Seven Up. It does precipitate with pH 7 buffer when diluted 1:10 but not 1:100 or 1:250.

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