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Solubility and stability of taxol: effects of buffers and cyclodextrins

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Abstract

The degradation kinetics of taxol in aqueous solutions were investigated at 37°C over a pH range of 1–9. The hydrolysis rates followed pseudo first-order kinetics with respect to residual taxol concentration. The pH-rate profile at 37°C showed that a maximum stability of taxol occurred in the pH 3–5 region. The effect of γ-cyclodextrin (γCD), hydroxypropyl-γ-cyclodextrin (HPγCD) and hydroxypropyl-β-cyclodextrin (HPβCD) on the solubility and stability of taxol was also investigated. Taxol was more stable in cyclodextrin solution than in buffer solution of comparable pH. The solubility of taxol in water increased in the presence of cyclodextrins with HPβCD giving the greatest increase in taxol solubility. Taxol (as received) was anhydrous and on suspension in water, it dissolved to form a supersaturated solution which recrystallized as a hydrate of lower solubility. Taxol formed predominantly second order complexes with the cyclodextrins. Complexes of taxol with HPβCD were more stable than those of HPγCD or γCD. Further increase in the solubility of taxol was observed when ethanol was added as a co-solvent.

Keywords: Taxol; Solubility; pH-stability; Cyclodextrins complexation

1. Introduction

Taxol, an anticancer agent isolated from the bark of *Taxus brevifolia* (chemical structure shown in Fig. 1), has a broad range of antineoplastic activity with a unique mechanism of action (Wani et al., 1971; Schiff et al., 1979). It promotes polymerization of tubulin dimers to form microtubules and stabilises microtubules by preventing depolymerisation. Taxol has also been shown to

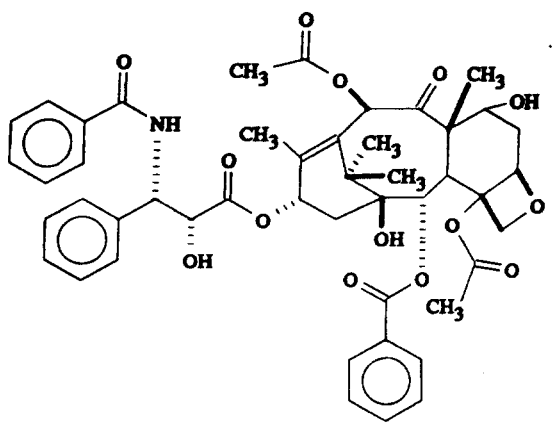

Fig. 1. The chemical structure of taxol.

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be a potent antiangiogenic agent (Burt et al., 1995; Dordunoo et al., 1995). Taxol is currently formulated in a vehicle composed of 50:50 blend of Cremophor EL and ethanol which is diluted with normal saline or dextrose solution (5%) before administration (Waugh et al., 1991). However, Cremophor EL has been implicated in some adverse reactions when administered intravenously and several attempts have been made to develop new drug delivery systems for taxol such as emulsions (Tarr et al., 1987), liposomes (Sharma and Straubinger, 1994), nanocapsules (Bartoli et al., 1990) and microspheres (Burt et al., 1995).

In addition to the problem of very low water solubility, taxol has been shown to be susceptible to solvolysis of its ester linkage with the removal of the ester side chain leading to a loss of cytotoxic activity (Parness et al., 1982). Mild methanolysis of taxol gave a methyl ester of the side chain and a tetraol, 10-deacetylbaccatin III (Wani et al., 1971). A more vigorous methanolysis at pH 9 gave baccatin III as the major product and minor compounds identified as 10-deacetyltaxol, 7-epitaxol, 10-deacetyl-7-epitaxol, baccatinV, 10-deacetylbaccatin V, 10-deacetylbaccatin III, some of which are biologically active (Ringel and Horwitz, 1987; Lataste et al., 1984). For example, Ringel and Horwitz (1987) have shown that taxol undergoes epimerization in culture media, forming 7-epitaxol with activity comparable to that of the parent molecule. Epimerization of taxol to 7-epitaxol was observed when taxol was heated with the free radical initiator azobis (isobutyronitrile) (Huang et al., 1986). Taxol may also undergo oxidation forming 7-oxotaxol or 2,7-dioxotaxol (Magri and Kingston, 1986). Despite several studies of the hydrolysis of taxol, to our knowledge, there have been no published reports of the effect of pH on the stability of aqueous taxol solutions.

Cyclodextrins (CDs) are cyclic oligosaccharides which have been used extensively to increase the solubility, dissolution rate and bioavailability of poorly water soluble drugs (Uekama and Otagiri, 1987; Szejtli, 1991). They have also been used to increase the stability of labile drugs (Uekama et al., 1983) and improve the performance of intravenous formulations (Estes et al., 1991). Uekama et al. (1994) have reviewed the safety profile of CDs. Since the nephrotoxicity of natural β-CD at higher doses is reported to be due to crystallization of the less soluble β-CD or its cholesterol complex in renal tissue, the more water soluble CDs show low toxicity and excellent tolerance. They suggested that hydroxy-β-cyclodextrin (HPβCD) may be safely used in parenteral formulations (Uekama et al., 1994). Cserhati and Hollo (1994) showed that taxol forms an inclusion complex with hydroxypropyl-β-cyclodextrin (HPβCD) and speculated that the solubility of taxol could be enhanced by forming an inclusion complex. Sharma et al. (1995) prepared β- and γ-CD complexes with taxol and determined solubilities, physical stability and maximum tolerated dose of the taxol-CD complexes in mice.

The objectives of this work were to determine the influence of temperature and pH on taxol stability and to investigate the formation of inclusion complexes of taxol with cyclodextrins in order to improve the solubility and chemical stability of taxol formulations for parenteral, ophthalmic or intra-vesicular drug delivery.

2. Materials and methods

2.1. Materials

Taxol was obtained from Hauser Chemicals Inc., Boulder, CO. Disodium phosphate (Fisher), citric acid (British Drug Houses), potassium chloride (British Drug Houses), monobasic potassium phosphate (Fisher), sodium hydroxide (British Drug Houses), hydrochloric acid (Fisher), dichloromethane (Fisher), acetonitrile (Fisher) and absolute ethanol (Fisher) were used as received. Distilled water was used throughout. Hydroxypropyl-β-cyclodextrin (HPβCD), γ-cyclodextrin (γ-CD) and hydroxypropyl-γ-cyclodextrin (HPγCD) were obtained from American Maize-Products Company (Hammond, Indiana) and were used as received.

2.2. pH-stability studies

The McIlvaine buffer (citric acid-disodium hydrogen phosphate) solutions (pH 2–8) and KCl-HCl (pH 1) and alkaline borate buffer (pH 9) were used to study the effect of pH on the stability of taxol. A stock solution of taxol was prepared to contain 10 μM of taxol in ethanol (100%) and an aliquot (0.1ml) of this solution was added to 20 ml of each buffer and incubated at 37°C. At various times, samples (in duplicate) of each solution were taken and analyzed for taxol by HPLC using a mobile phase of water:acetonitrile (50:50) at a flow rate of 1.5 ml min^{-1}. The analysis used a Beckman C18 Ultrasphere reverse phase column, Beckman isocratic pump (Model 110A), Shimadzu (SPD 6A) UV detector at 232 nm, Shimadzu integrator and Shimadzu Autosampler. The injection volume was 20 μl.

The experiments were repeated at 30°, 37°, 40°, 45° or 50°C using a solution of taxol in McIlvaine buffer at pH 8 in order to investigate the effect of temperature on the degradation of taxol. Buffer catalysis was investigated using citrate or phosphate buffer at pH 6.0 and the solutions were incubated at 45° or 55°C. The effects of buffer concentrations were studied using phosphate buffer concentrations of 0.05, 0.1 or 0.2M at 55°C.

The solutions containing 20% HPβCD or HPγCD had pH values of 3.9 and 5.2, respectively. The stability of taxol in cyclodextrin solutions was investigated by assaying taxol in solutions (20μg ml^{-1}) containing 10 or 20% HPγ-CD or HPβ-CD in either water or a 50:50 water-ethanol mixture at 37° or 55°C at various time intervals. In addition, stability of taxol in solutions (1μg/ml) containing 1, 2 or 5% HPβCD at 55°C were determined.

2.3. Mass spectroscopy

Preliminary degradation product profiling studies using liquid chromatography-mass spectroscopy (LCMS) were conducted on taxol degraded for 48 h in both acidic and alkaline solutions (pH 2 and pH 8, respectively). The equipment used was the Hewlett Packard Model 1090 Series II Liquid Chromatograph (Hewlett Packard, Avonsdale, PA) using Waters μBondapak ODS column (2.1 mm i.d. x 100 mm) and a mobile phase of 50% acetonitrile in water at a flow rate of 0.5 ml min^{-1}. The injection volume was 20μl.

2.4. Solubility studies

Solubility studies were conducted according to the method of Higuchi and Connors (Higuchi and Connors, 1965). Excess amounts of taxol (5 mg) were added to aqueous solutions containing various concentrations of γ-CD, HPγ-CD, or HPβ-CD and tumbled gently for about 24 h at 37°C. After equilibration, aliquots of the suspension were filtered through a 0.45-μm membrane filter (Millipore), suitably diluted and analyzed using HPLC as described above. The mobile phase was composed of a mixture of acetonitrile, methanol and water (58:5:37) at a flow rate of 1.0 ml min^{-1}. Calibration plots of the peak area versus concentration showed a straight-line relationship over the concentration range of 1–250 μg/ml. The solubility of taxol in a solvent composed of 50:50 water and ethanol (95%) containing various concentrations, up to 10%, of HPβ-CD was also investigated. In addition, dissolution rate profiles of taxol were investigated by adding 2 mg of taxol (as received) to 0, 5, 10 or 20% HPγ-CD solutions or 2 mg of previously hydrated taxol (by suspending in water for 7 days) to pure water and tumbling gently at 37°C. Aliquots were taken at various time intervals and assayed for taxol.

2.5. Thermal analyses

Taxol was suspended in water for 24 h, filtered and dried overnight under vacuum (20 mmHg) at room temperature to give the hydrate. Differential scanning calorimetry (DSC) was conducted on taxol (as received) or taxol hydrate. About 5 mg samples (accurately weighed), in open aluminium pans, were heated from ambient temperature to 250°C at a heating rate of 10°C min^{-1} using Differential Scanning Calorimeter 910 S (TA Instruments). Thermogravimetric analysis (TGA)

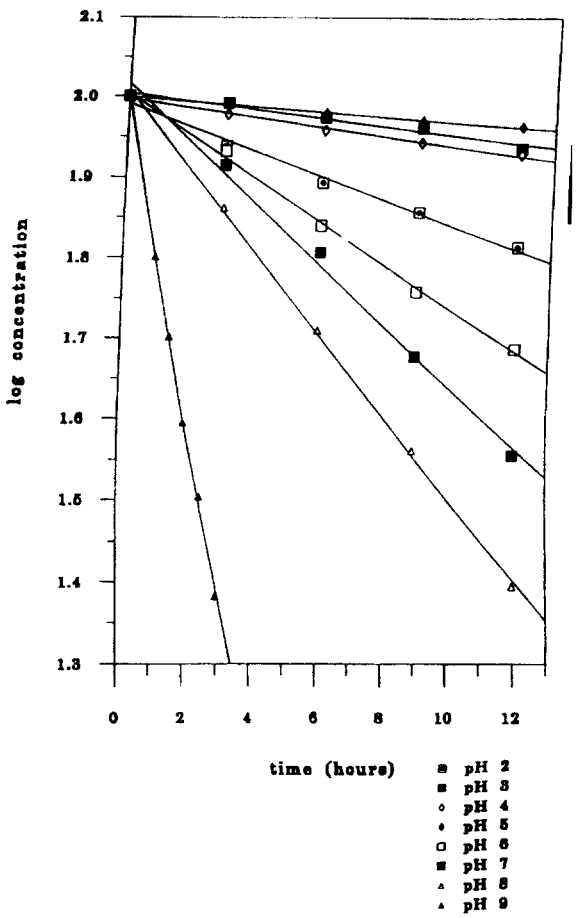

Fig. 2. Plots of the observed pseudo first order kinetic degradation of taxol in solution at 37°C and different pHs.

(TA Instruments) was also conducted on about 20 mg of the sample at a heating rate of 10°C min^{-1} to determine weight loss.

3. Results and discussion

3.1. Stability profiles

The HPLC chromatograms showed excellent separation of taxol and its decomposition products with a retention time of 8.4 min for taxol. At a constant pH and temperature, the degradation of taxol was found to undergo pseudo first-order kinetics with respect to the substrate. Fig. 2 shows the plots of log concentration of residual taxol versus time at different pH's at 37°C. The pH-rate profile showed the pH of maximum stability to be in the range pH 3–5 (Fig. 3). The U-shaped pH-rate profile suggests that the hydrolytic reactions would involve neutral or ionic species of taxol catalyzed by H^+, H_2O or OH^-. Between pH 3–5, the data showed a slope of nearly zero suggesting spontaneous but slow reactions catalyzed by water molecules.

Degradation product profiling studies using LCMS showed that in both acidic and alkaline solutions (e.g. pH 2 and pH 8, respectively) baccatin III (mol. wt. 586), deacetylbaccatin III (mol. wt. 544), baccatin V and their 7-epimers were the major decomposition products. Similar findings for the hydrolytic products of taxol were previously reported (Kerns et al., 1994).

A primary salt effect was observed in this study with an increased pseudo-first order rate constant for taxol at higher salt concentrations. Buffer

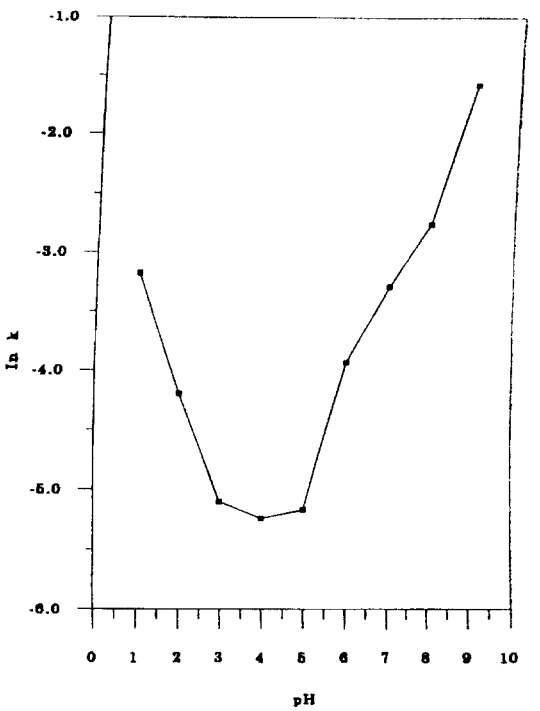

Fig. 3. pH-rate profile for taxol degradation in aqueous buffers at 37°C.

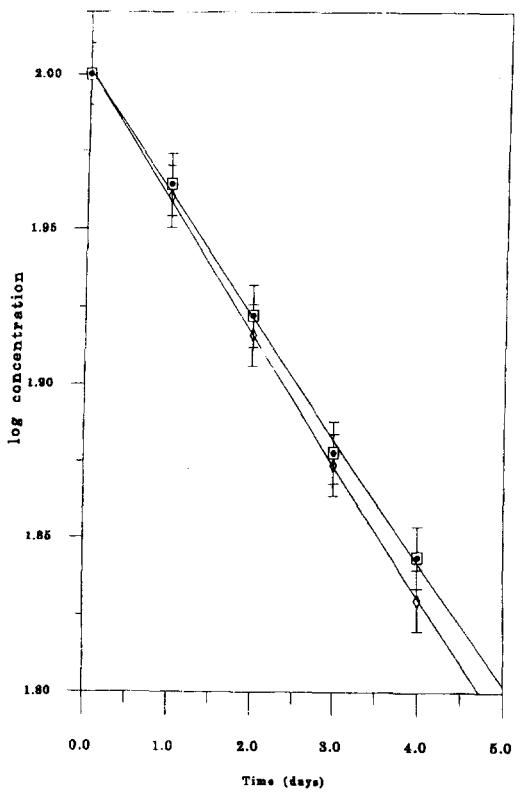

Fig. 4. Plots of the observed pseudo first order kinetic degradation of taxol (20μg ml^{-1} in 10% HPβCD and 10% HPγCD solutions at 37°C and pH of 3.7 and 4.9, respectively.

catalysis due to the type of buffer species was also observed. At 37°C and pH 6, there was a faster rate of taxol decomposition in phosphate buffer (observed k was 1.25 x 10^{-2} h^{-1}) compared with citrate buffer (observed k was 2.97 x 10^{-3} h^{-1}). In addition, the rate of degradation increased at higher buffer concentrations, necessitating the use of buffers with as low a concentration as possible for aqueous formulations of taxol.

Taxol showed excellent stability in solutions containing 20 μg ml^{-1} and 10 or 20% of each of the cyclodextrins in water or a 50:50 ethanol:water mixture at 37°C (pH 3.7–5.2); there was less than 1% decomposition of taxol in the cyclodextrin solutions stored for one month at 37°C. Degradation of taxol in a 10% (pH 3.7) or 20% HPβCD (pH 3.9) and 10% (pH 4.9) or 20% HPγCD (pH 5.2) solution at 55°C followed pseudo-first order degradation kinetics (e.g. Fig. 4) with observed degradation rate constants of 1.78 x 10^{-3} h^{-1} and 0.96 x 10^{-3} h^{-1} for taxol in 10% HPβCD and HPγCD, respectively. There was no appreciable difference between the rate of degradation of taxol in 10 or 20% cyclodextrin solutions. The rate of degradation of taxol at 55°C was significantly faster in solutions (1μg/ml taxol) containing 1% HPβCD (k = 3.38 x 10^{-3} h^{-1}). Taxol solutions (1μg/ml) containing 2, 4, 6 or 8% HPβCD did not show any significant difference in the rate of degradation from that obtained with the 10 or 20% HPβCD solutions (20μg/ml). Hence, very low concentrations of HPβCD (1% or lower) did not protect taxol against hydrolysis as effectively as higher CD concentrations. This may be due to the preferential formation of a more physically unstable 1:1 taxol-CD complex at low concentrations of CDs.

The presence of ethanol did not adversely affect the stability of taxol in the cyclodextrin solutions. The degradation rate constants for taxol in cyclodextrin solutions at 37°C were 8.3 x 10^{-5} h^{-1} and 9.5 x 10^{-5} h^{-1} for taxol in 10% HPβCD and HPγCD, respectively. The rate of degradation of taxol was, therefore, lower in the cyclodextrin solutions than the rate observed for taxol in citrate or phosphate buffer solutions of comparable pH's. Improved stabilities of drugs in cyclodextrin solutions have been previously reported (Uekama and Otagiri, 1987); the drug molecules are enclosed in the comparatively hydrophobic cavity of the cyclodextrins thereby protecting the drug from the hydrolytic effects of the medium.

3.2. Solubility studies

Fig. 5 shows the phase solubility diagrams for γ-CD, HPβCD and HPγCD and taxol at 37°C. The solubility of taxol increased over the entire CD concentration range studied; HPβCD producing the greatest increase in the solubility of taxol. This might be unexpected if a first order complex is solely responsible for the increase in solubility. Taxol, being a very large molecule (mol. wt. 853), would be expected to form more stable inclusion complexes with the γ-cyclodextrins than with the β-cyclodextrin derivative due

the larger cavity size of the former. However, the shape of the solubility curves (Fig. 5) suggests that the stoichiometries were of higher order than a 1:1 complex. Taxol formed Type A_P curves with both HPβCD and HPγCD and Type A_N curves with γCD. A Type A diagram indicates the formation of soluble complexes between CD and taxol, thereby increasing the total amount of the drug in solution (Higuchi and Connors, 1965). The Type A_N diagram obtained for taxol-γCD complex is expected since the water solubility of the γ-CD is lower (about 23% w/v; 0.19 M) than those of the hydroxypropyl derivatives (> 50% w/v; 0.34 M for HPβCD). The solubility of taxol in a 50% solution of HPβCD in water was 3.2 mg ml^{-1} at 37 °C which was about a 2000-fold increase over the solubility of taxol in water.

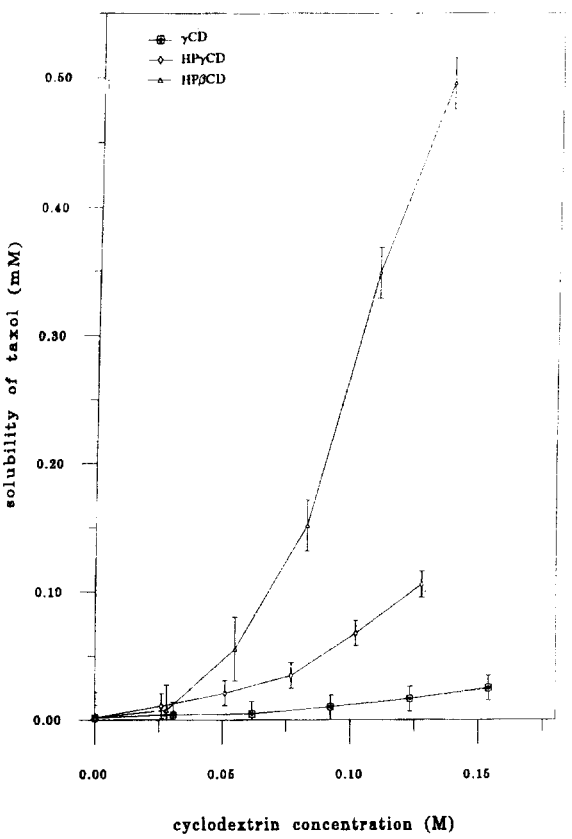

Fig. 5. The phase solubility diagrams for cyclodextrins and taxol in water at 37°C.

Although several complex species can theoretically exist in solution, the solubility curves in Fig. 5 suggested the presence of higher order complexes and the data were treated assuming that the majority of the complexes were second-order. The stability constants for the complexes were calculated from equation 1 (Higuchi and Connors, 1965):

$$\frac{[D]_t - [D]_o}{[L]} = K_{1:1}[D]_o + [D]_o K_{1:1} K_{1:2}[L] \quad (1)$$

where $[D]_o$ is the solubility of taxol in the absence of cyclodextrin, $[D]_t$ is the total amount of taxol in solution in the presence of free complexing agent (cyclodextrin) concentration [L] and $K_{1:1}$ and $K_{1:2}$ are the apparent stability constants of first order and second order complexes, respectively. Assuming the extent of complexation is fairly small, the concentration of the free complexing agent, [L], may be equated to the total concentration of the complexing agent $[L]_t$. Plots of $[D]_t$ - $[D]_o$/ $[L]_t$ against $[L]_t$, gave straight lines (Fig. 6) from which the apparent stability constants for the first ($K_{1:1}$) and second order ($K_{1:2}$) complexes were estimated from intercept/$[D]_o$ and slope/intercept, respectively (Higuchi and Connors, 1965). The estimated stability constants for first order complexes of taxol-cyclodextrins were 3.1, 5.8 and 7.2 M^{-1} for γ-CD, HPγCD and HPβCD and those for second order complexes were 0.785 x 10^3, 1.886 x 10^3 and 7.965 x 10^3 M^{-1} for γ-CD, HPγCD and HPβCD, respectively. Comparing the values of the apparent stability constants showed that the second order stability constants were about 500, 1000 and 2000 times greater than the stability constants for the first order complexes for taxol and γCD, HPγCD and HPβCD, respectively. The values of the observed stability constants support the assumption that the inclusion complexes formed by taxol with cyclodextrins were predominantly second order complexes.

The solubility of taxol in 50:50 water:ethanol mixture increased with an increase in the cyclodextrin concentration (Fig. 7) as observed for complexation in pure water. The apparent stability constant for the complexation of taxol and HPβCD in the presence of 50% ethanol (26.57

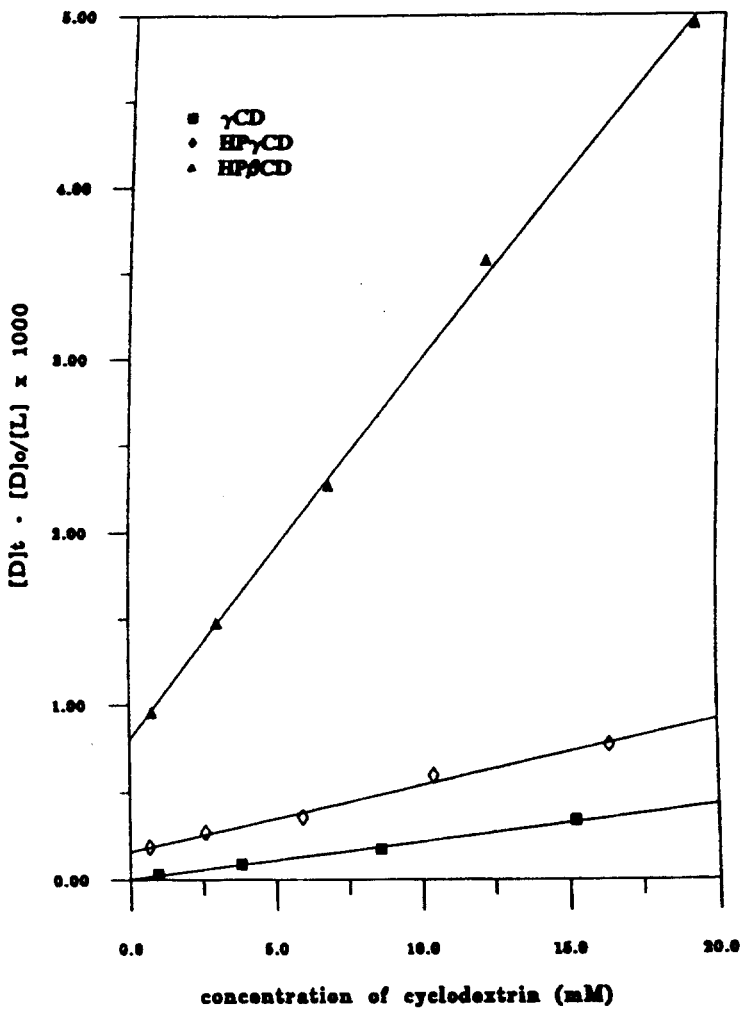

Fig. 6. Second order plots of the complexation of taxol and γCD, HPβCD or HPγCD at 37°C.

M^{-1}) was significantly lower (about 300 times) than the stability constant in the absence of ethanol. The stability constant is known to be affected by such factors as the pH, temperature, ionic strength and competing agents (Tokumura et al., 1986; Uekama and Otagiri, 1987). In the present study, the lower stability constant may be attributed to a change in the dielectric constant or the polarity of the solvent in the presence of ethanol which will influence the equilibrium interactions between water, cyclodextrin and taxol.

The solubility of taxol in a 10% HPβ-CD solution in 50:50 water:ethanol was about 3.7 mg ml^{-1}. This notwithstanding, physically stable solutions were prepared containing 6.25 mg ml^{-1} taxol when taxol was first dissolved in required amount of ethanol and the cyclodextrin (HPγ-CD or HPβ-CD) solution (equivalent to 10%) was gradually added with agitation. This solution, albeit moderately supersaturated, was still clear after one month storage at 37°C suggesting that the order of mixing may be important in preparing stable solutions. The absence of solid taxol which could act as a seed to initiate the precipitation of

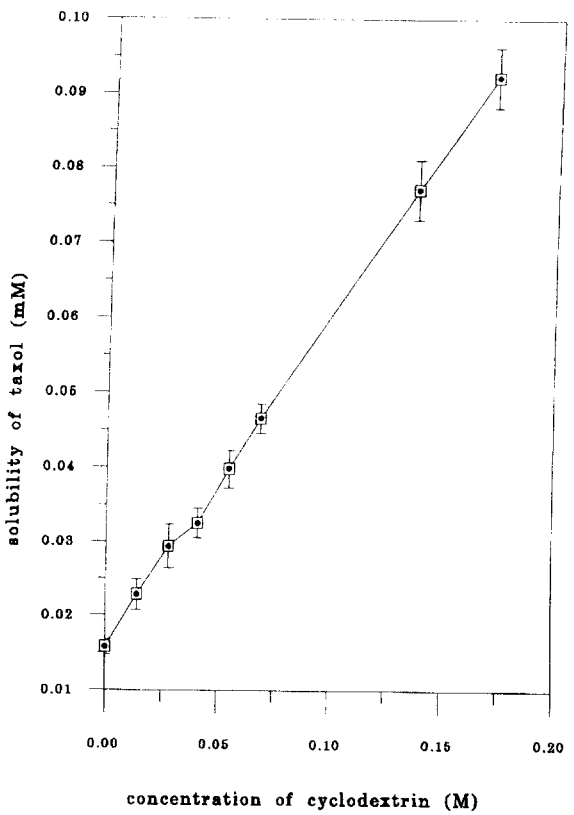

Fig. 7. The phase solubility diagram for taxol at 37°C and hydroxypropyl-β-cyclodextrin in 50:50 water:ethanol solutions.

the complex may, partly, explain the physical stability of the supersaturated solutions in the present study. However, temperature fluctuation or a dilution with water, normal saline or 5% dextrose solution was found to induce precipitation of taxol from aqueous solutions containing cyclodextrins and/or ethanol as a co-solvent. For example, a 1:1 dilution of a solution of taxol (5mg/ml) containing 25% HPβCD and 50% ethanol with normal saline or 5% dextrose solution caused precipitation of the drug. Sharma et al. (1995) prepared taxol-CD complexes by freeze drying water-alcohol mixtures of CDs and taxol followed by reconstitution of the solid matrix in water. They showed weak interactions of taxol with CDs in solution, with precipitation upon dilution with some CDs.

The dissolution profiles of taxol in 0, 5, 10 and 20% γCD solutions (Fig. 8) illustrates the formation of a metastable solution of taxol in pure water or the cyclodextrin solutions; the amount of taxol in solution gradually increased, reached a maximum and subsequently decreased. Other studies have reported this phenomenon. Torres-Labandeira et al. (1991) prepared solutions of pancrastatin in hydroxypropylcyclodextrins containing 9 mg ml^{-1} pancrastatin which was more than a five-fold increase over the equilibrium solubility of the drug. However, these solutions were prone to precipitation on storage (Uekama et al., 1983; Torres-Labandeira et al., 1991) but the precipitation could be delayed by storing the supersaturated solutions in siliconized glass or polyethylene containers The stability of the metastable solution may also be increased by incorporating viscosity increasing agents such as hydroxypropylmethyl cellulose (Uekama et al., 1983).

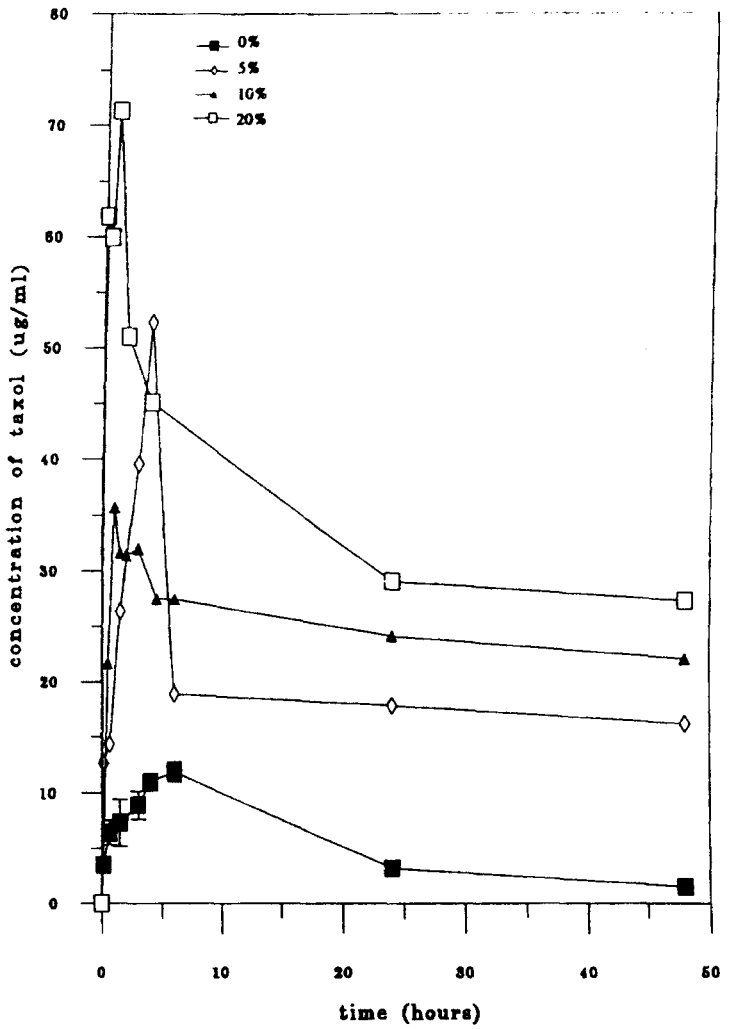

Fig. 8. Dissolution rate profiles of taxol in 0, 5, 10 or 20% HPγCD solutions at 37°C.

The formation of a metastable solution of a drug may be due to the presence of different solid states (Shefter and Higuchi, 1963). The drug in an anhydrous or a solvate (not a hydrate) form may have a higher apparent solubility in water giving rise to a supersaturated solution which may recrystallize as the stable hydrate with lower solubility (Shefter and Higuchi, 1963). In the present study, dissolution studies using taxol samples which were previously hydrated by suspending in water for 48 h did not show the formation of the metastable solution. In addition, DSC analysis of the hydrated taxol (dried in a vacuum oven at room temperature) showed two broad endothermic peaks between 60 and 110°C. These peaks were accompanied by about 4.5% weight loss (determined by thermogravimetric analysis) indicating the presence of hydrate(s). A loss in weight of about 2.1% would suggest the formation of a taxol monohydrate. Therefore, the occurrence of the DSC peaks between 60 and 110°C and the loss in weight of about 4.5% suggests the presence of a dihydrate. The addition of water to taxol forming a monohydrate with a m/z peak of 871 in LCMS studies was previously reported (Kingston et al., 1990) and was also observed in the present LCMS

study. There was no evidence of endothermic peak(s) between 60 and 110°C (DSC results) or a weight loss (TGA results) for taxol samples as received. Therefore, (as received) taxol was anhydrous and on suspension in water it dissolved to form a supersaturated solution which recrystallized as a hydrate of lower solubility (Fig. 8). Further studies of taxol phase changes are in progress. A time-dependent decrease in taxol solubility was observed for dried amorphous taxol films equilibrated in water but no evidence of a phase change was reported when taxol was directly suspended in water (Sharma et al., 1995).

This study showed that the solubility of taxol could be increased by complexation with γ-CD, HPγCD and HPβCD. The greatest increase in solubility was obtained for HPβCD. The stability of taxol was found to be pH dependent and taxol was most stable in the pH 3–5 region. The stability of taxol was also improved using cyclodextrins. Dilution of water/ethanol solutions of taxol-CD complexes with water, normal saline or 5% dextrose solution caused precipitation of taxol which may preclude the use of CD formulations for IV infusions of taxol. Nevertheless, these aqueous based formulations may have potential for ophthalmic application in the treatment of pathological corneal neovascularization and for intra-peritoneal or intravesicular administration of taxol for the treatment of various cancers.

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