Improvement in Solubility and Dissolution Rate of 1,2-Dithiole-3-thiones upon Complexation with β-Cyclodextrin and Its Hydroxypropyl and Sulfobutyl Ether-7 Derivatives

GILLES DOLLO,*,† PASCAL LE CORRE,† MARYLÈNE CHOLLET,‡ FRANÇOIS CHEVANNE,† MARCEL BERTAULT,§ JEAN-LOUIS BURGOT,‡ AND ROGER LE VERGE†

Contribution from *Laboratoire de Pharmacie Galénique et Biopharmacie and Laboratoire de Chimie Analytique, Faculté des Sciences Pharmaceutiques et Biologiques, Université de Rennes I, 35043 Rennes Cedex, France, and Groupe Matière Condensée et Matériaux, UMR 6626 au CNRS, Université de Rennes I, Campus de Beaulieu, 35042 Rennes Cedex, France.*

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Abstract □ Inclusion complexes between β-cyclodextrin derivatives and 1,2-dithione-3-thiones were studied in aqueous solution and in the solid state. Phase solubility study was used to evaluate the complexation in solution, at 37 °C, of three cyclodextrins, i.e., β-cyclodextrin (βCD), hydroxypropyl-β-cyclodextrin (HPβCD), sulfobutyl ether-7-β-cyclodextrin (SBE7βCD), and four 1,2-dithiole-3-thiones, i.e., the parent compound dithiolethione (DTT), dimethyldithiolethione (DMDTT), 5-phenyldithiolethione (5PDTT), and anetholetrithione (ATT). Stability constants of the DTT complexes with HPβCD and SBE7βCD were also determined spectrophotometrically using a nonlinear least-squares methodology. Differential scanning calorimetry (DSC) and scanning electronic microscopy (SEM) were used to characterize spray-dried complexes formed between 5PDTT and SBE7βCD, ATT and SBE7βCD. Dissolution studies using the USP paddle method were carried out in water at 37 °C for both ATT and 5PDTT binary systems with HPβCD and SBE7βCD. Solubility enhancements were much greater with the more lipophilic ATT and 5PDTT compared to DTT and DMDTT, whatever the cyclodextrin used, in the rank order SBE7βCD > HPβCD ≫ βCD. Stability constants obtained (between 120 and 12800 mol^{-1}) were also the highest for the more lipophilic drugs and in the same rank order SBE7βCD > HPβCD ≫ βCD. Results obtained by UV spectrophotometry were in good agreement with those obtained by phase-solubility study. DSC thermograms of spray-dried complexes of ATT and 5PDTT with HPβCD and SBE7βCD lacked the endothermal peak of pure drug peak which was found for the physical mixtures (107 °C and 125 °C for ATT and 5PDTT, respectively). Finally, dissolution profiles of spray-dried inclusion complexes studied displayed a faster dissolution rate compared to physical mixtures and pure drugs. The present study showed that complexation of 1,2-dithiole-3-thiones with β-cyclodextrin derivatives resulted in an increase in solubility, allowing intravenous formulation for bioavailability and metabolism studies and an increase in the dissolution rate of the drugs, which shoud be of interest for oral absorption of these lipophilic compounds.

Introduction

1,2-Dithiole-3-thiones are sulfur heterocyclic compounds naturally found in cruciferous vegetables.[1] Among these compounds of pharmaceutical interest, anetholetrithione (ATT) has been marketed since 1947[2] and is prescribed for its choleretic and sialagogue properties. In the last 15 years, it has been shown that these molecules were able to inhibit carcinogenesis by increasing the activity of electrophile detoxification enzymes (phase II enzymes conjugating with carcinogens favoring their elimination)[3] and by increasing intracellular glutathione levels (increasing protection against free radicals, oxidants...). For that reason the pharmaceutical interest of 1,2-dithiole-3-thiones is growing since they may be useful for cancer chemoprevention in humans.[4−6] An oral formulation allowing a chronic administration of these compounds would be highly desirable.

Since 1,2-dithiole-3-thiones are highly lipophilic compounds,[7−8] and since too high a lipophilicity can result in low permeability,[9] there is a need from a biopharmaceutical standpoint to increase the poor aqueous solubility of these nonionizable molecules (around 0.001 mg/mL) in order to circumvent the low and highly variable absorption generally seen with such products.[10] To reach that goal, we used cyclodextrins, since preliminary solubilization studies with cosolvents such as DMSO or PEG resulted in drug precipitation upon dilution. The β-cyclodextrins (βCD) are α-1,4-linked cyclic oligosaccharides composed of seven D-glucopyranose units with a relatively hydrophobic central cavity and an hydrophilic outer surface.[11] These products are able to entrap poorly soluble drug molecules of appropriate size and polarity in their cavities to form reversible noncovalent inclusion complexes. This may improve physical and chemical properties of the incorporated guest molecule allowing, for example, the improvement of stability, solubility,[12] in vivo drug delivery, and bioavailability.[13−14] βCD has been studied extensively despite a very low aqueous solubility but some derivatives such as 2-hydroxypropyl-β-cyclodextrin (HPβCD) and more recently an anionically charged derivative with an average degree of substitution of 7, sulfobutyl ether-7-β-cyclodextrin (SBE7βCD) have attracted growing interest due to their greater intrinsic solubilities allowing improved complexing abilities.[12,15] SBE7βCD and HPβCD appear to be parenterally safer materials, compared to the parent β-cyclodextrin.[16]

The objectives of this work were (i) to examine the potential of βCD, HPβCD, and SBE7βCD as solubilizing agents for four 1,2-dithione-3-thiones, the parent compound dithiolethione (DTT), dimethyldithiolethione (DMDTT), 5-phenyldithiolethione (5PDTT), and anetholetrithione (ATT), using the phase-solubility technique and UV−visible spectrophotometry, (ii) to characterize the complexes formed by differential scanning calorimetry (DSC) and by scanning electronic microscopy (SEM), and (iii) to evaluate the rate of drug dissolution when complexed or not with cyclodextrins according to the USP paddle method.

* Corresponding author. Tel: (33) 2-99-33-62-65. Fax: (33) 2-99-33-68-91. e-mail: Gilles.Dollo@univ-rennes1.fr.
† Laboratoire de Pharmacie Galénique et Biopharmacie.
‡ Laboratoire de Chimie Analytique.
§ Groupe Matière Condensée et Matériaux.

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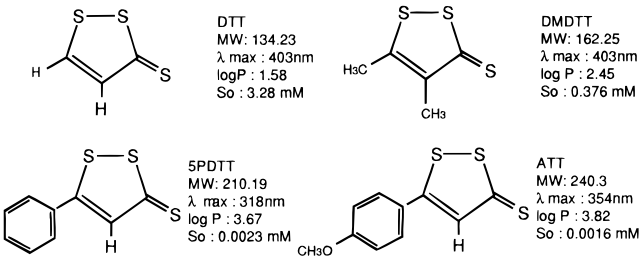


Figure 1—Chemical structures of 1,2-dithiole-3-thiones studied, their molecular weights (MW), λ_{max}, log *P*, and intrinsic water solubility (S_0) values.

Experimental Section

Materials—βCD (KLEPTOSE, MW = 1135 g/mol) and HPβCD (ref 615446, D. S. (degree of substitution) = 5.88; MW = 1476 g/mole) were kindly provided by Roquette (Lestrem, France) and SBE7βCD (CAPTISOL, cat. ref no. 7585−39−9; D. S. = 6.4; MW = 2163 g/mole) by CyDex (Overland Park, KS).

All 1,2-dithiole-3-thiones studied (Figure 1) were synthesized by our team, except that ATT was supplied by Solvay Pharma (Laboratoire de Thérapeutique Moderne, Suresnes, France).

All other reagents and solvents (E. Merck, Darmstadt, Germany) were of analytical grade, and freshly prepared distilled water was used throughout the study.

Phase-Solubility Study—Solubility measurements were determined according to the method of Higuchi and Connors.[17] Excess amounts of 1,2 dithiole-3-thione (10 mg) were weighted into 1 mL screw-cap polypropylene tubes to which were added aqueous solutions containing increasing concentrations of CDs, ranging from 0% to 10% (w/v) for HPβCD and SBE7βCD, 0% to 2% (w/v) for βCD. The suspensions formed were then rotated on a top to bottom shaker, thermostatically controlled at 37 ± 0.1 °C. After one week of agitation (equilibrium and absence of drug degradation were confirmed in preliminary studies), the suspensions were centrifugated (20000*g* for 10 min) and appropriately diluted with the mobile phase, and the total concentration of 1,2-dithiole-3-thione in the filtrate was analyzed by a reversed phase HPLC system according to Lefeuvre et al.[18] It consisted of a Waters Model 6000A pump (Waters Assoc., Milford, MA) equipped with a Waters Model WISP 710 B automatic injector, an LDC Milton Roy Model Spectromonitor 3100 variable-wavelength UV detector (LDC Milton Roy, Riviera Beach, FL), and a Delsi Model Enica 21 integrator (Delsi, Suresnes, France). The analytical chromatography column was a Lichrospher RP−SelectB (Interchim, Montluçon, France) (125 × 3 mm i.d.; particle size 5 μm). The chromatographic conditions were as follows: injection volume, 20 μL; column temperature, 30 °C; mobile phase, mixture of acetonitrile and water (50:50, v/v); flow rate, 0.5 mL/min; detector operated at 318 and 354 nm for 5PDTT and ATT, respectively.

The experiment was carried out in triplicate, and the stability constant of each drug-cyclodextrin system (K_s) was then calculated from the linear portion of the phase solubility diagrams (reporting drug concentration vs cyclodextrin concentration), assuming that a 1:1 stoichiometric ratio complex was formed at the initial step (slope smaller than 1) according to eq 1.[17]

$$K_s = \text{slope}/s_o(1 - \text{slope}) \quad (1)$$

where s_o is the drug solubility in water.

UV−Visible Spectrophotometry—The determination of the stability constants (K_s) of the parent compound DTT with the cyclodextrins HPβCD and SBE7βCD was also realized in solution by UV−visible spectrophotometry ultraviolet (UV) absorption changes of DTT (5×10^{-5} M) in the presence of aqueous solutions of HPβCD or SBE7βCD (various concentrations of 5×10^{-5} M, 10^{-4} M, 2.5×10^{-4} M, 5×10^{-4} M, 10^{-3} M, 2.5×10^{-3} M, 5×10^{-3} M, 10^{-2} M, 2.5×10^{-2} M and 5×10^{-2} M) were recorded from 200 to 500 nm with a Uvikon model 922 UV−visible spectrophotometer (Bio-Tek Kontron instruments, Saint Quentin en Yvelines, France). Absorbance data were treated by a nonlinear least-squares methodology. The stability constants were obtained together with the molar absorptivity coefficient of the DTT−CD complex studied which could not be experimentally determined since the complex could not stand alone in solution owing to the dilution effects. The mathematical model allowing the calculation of absorbances to be compared to the experimental ones according to the nonlinear least-squares methodology, issued from the basic equations:

$$K_s = [\text{DTT−CD}]/[\text{DTT}][\text{CD}] \quad (2)$$

$$[\text{DTT}] + [\text{DTT−CD}] = c_2 \quad (3)$$

$$[\text{CD}] + [\text{DTT−CD}] = c_3 \quad (4)$$

where [DTT], [CD], [DTT−CD] are free equilibrium concentrations of DTT, CD, and complex, respectively; c_2 and c_3 are the analytical concentrations of DTT and cyclodextrin, respectively. The above system of three equations reduced to the second-order equation in [DTT-CD]:

$$K_s[\text{DTT−CD}]^2 - [K_s(c_2 + c_3) + 1][\text{DTT−CD}] + K_s c_2 c_3 = 0 \quad (5)$$

The absorbances were calculated for each of the relative concentrations used according to the Beer−Lambert Law:

$$A^{\text{calc}} = \epsilon_{\text{DTT}}[c_2 - (\text{DTT−CD})] + \epsilon_{\text{DTT−CD}}[\text{DTT−CD}] \quad (6)$$

where ϵ_{DTT} and $\epsilon_{\text{DTT−CD}}$ are the molar extinction coefficients of the pure drug and from the complex, respectively. The minimization of the least-squares function $U = \sum(A^{\text{calc}} - A^{\text{exp}})^2$, calculated over the 10 concentration ratios mentioned above, was achieved by an algorithm developed in our department.[19,20]

Preparation of Solid Complexes—Solid complexes of 1,2-dithiole-3-thiones and cyclodextrins were obtained by spray-drying of stoechiometric amounts of drug (ATT or 5PDTT) and cyclodextrin (HPβCD or SBE7βCD) (1:1 or 1:2 drug−cyclodextrin mole ratio). A solution containing the drug (500 mg in 500 mL 95% ethanol) to which was added 250 mL of an aqueous solution of the cyclodextrin was spray-dried in a Büchi Mini Spray-drier B-191 apparatus (Büchi, Switzerland) under the following conditions: flow rate of the solution, 10 mL/min; drying air flow rate, 600 L/h; air inlet temperature, 100 °C; air outlet temperature, 65 °C; aspirator capacity, 35 m^3/h. The yield of the spray-drying process was about 60% in the receiving vessel.

Physical mixtures of 1,2-dithiole-3-thione−cyclodextrin systems were prepared by gently mixing the drug and the cyclodextrin in a mortar, in the same 1:1 or 1:2 molar ratio.

Particle Size Measurements—Size distribution of pure materials, physical mixtures, and spray-dried products was determined by laser diffractometry using a Mastersizer S (Malvern Instruments, Orsay, France) particle size analyzer. All results were expressed as the median volumetric diameter *d*(v,0.5) which is the diameter that divides the volume distribution curve of the sample analyzed in two equal parts.

Differential Scanning Calorimetry—DSC was used to characterize the interactions between both lipophilic 1,2-dithiole-3-thiones, 5PDTT and ATT, with SBE7βCD. The calorimetric measurements of raw materials as well as 1:1 physical mixtures and 1:1 and 1:2 spray-dried complexes were performed with a Perkin-Elmer DSC-7 differential scanning microcalorimeter (Perkin-Elmer, Norwalk, CT) connected to a DEC 425 calculator. The measurement head was flushed with pure nitrogen gas, and its temperature was stabilized by circulating cold water−ethanol from a thermostatic bath controlled at 7 ± 0.1 °C. Temperatures were calibrated using the melting points of *p*-nitrotoluene (99.99%, mp = 51.5 °C, Carlo Erba, Milan, Italy) and indium (99.99%, mp = 156.6 °C, Perkin-Elmer, Norwalk, CT). Energy was calibrated from the melting enthalpy of indium (28.45 J/g). Samples weighting from 0.1 to 0.2 mg (for pure compounds 5PDTT and ATT allowing precise determination of the fusion point) to 10−20 mg (for physical mixtures and spray-dried complexes with SBE7βCD) were carefully encapsulated in aluminum pans with a crimped cap into which was made a needle hole, allowing water evaporation. Only heat flow measurements were made on all samples at a scanning heating rate of 10 °C/min in the 85−130 °C temperature range.

Scanning Electronic Microscopy—Morphological features of the raw materials were compared with a 1:1 physical mixture and 1:1 and 1:2 spray-dried complexes for the binary system (ATT, SBE7βCD), after examination by SEM (JEOL model JSM-6400, Tokyo, Japan). The samples were fixed on a brass stub using

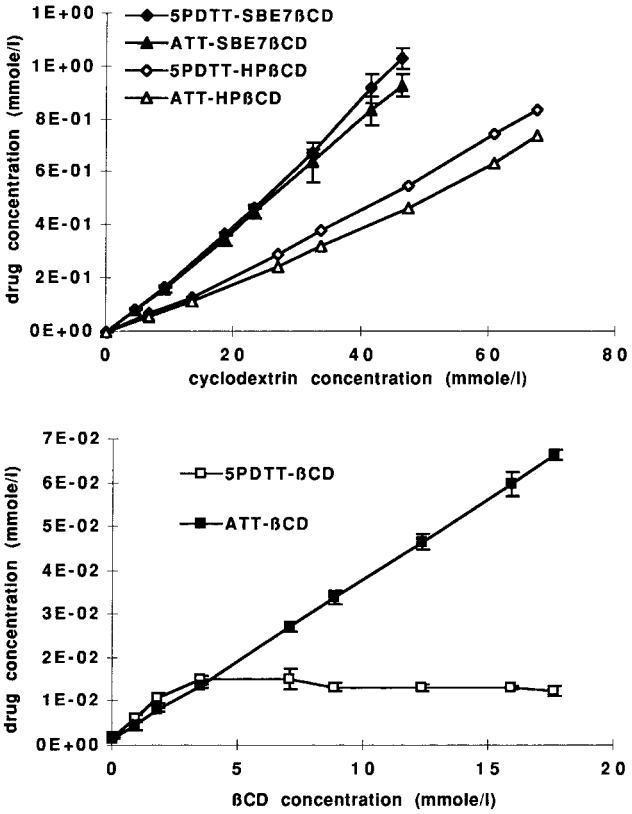


Figure 2—Phase-solubility diagrams of ATT– and 5PDTT–cyclodextrin systems. Top: SBE7βCD and HPβCD; bottom: βCD. Each point is the mean (±SD) of three determinations.

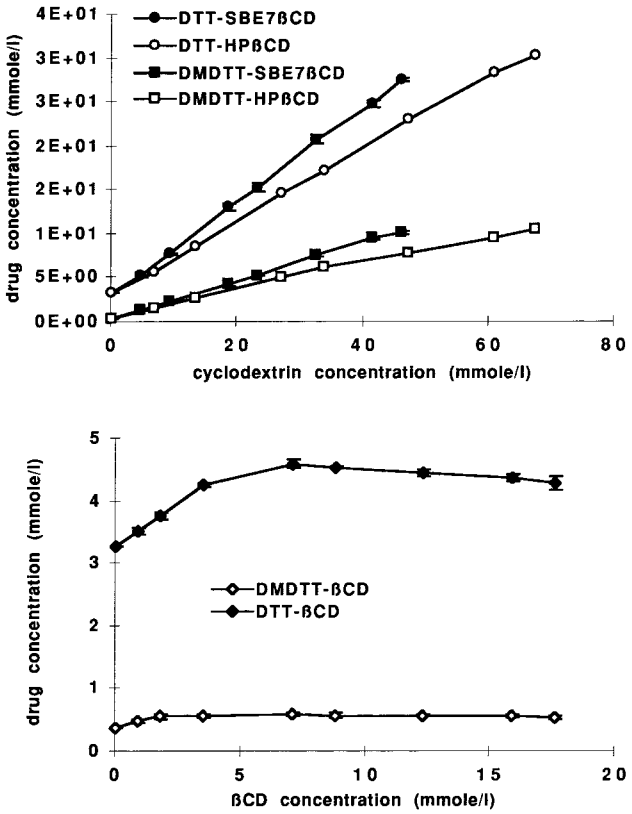


Figure 3—Phase-solubility diagrams of DTT– and DMDTT–cyclodextrin systems. Top: SBE7βCD and HPβCD; bottom: βCD. Each point is the mean (±SD) of three determinations.

double-sided tape and then made electrically conductive by coating in a vacuum with a thin layer of gold/palladium.

Dissolution Study—USP XX Type 2 apparatus (rotating paddle method) was employed to obtain all the dissolution profiles of pure drugs, 1:1 and 1:2 physical mixtures, and spray-dried complexes of ATT and 5PDTT with HPβCD and SBE7βCD. Due to the poor aqueous solubility of these nonionizable compounds, sink conditions (less than 20% of saturation concentration) could not be maintained during the tests. Each powdered sample contained 5 mg of 1,2-dithiole-3-thione. The dissolution medium consisted of 1000 mL of distilled water thermostated at 37 ± 0.1 °C, and the paddle speed was 100 rpm. Concentration of dissolved drug was measured continuously (every 15 min for drugs and physical mixtures, every 30 s for complexes at a wavelength of 318 and 354 nm for 5PDTT and ATT, respectively) using a UV spectrophotometer (Spectronic 1201 Milton, LDC Milton Roy, Riviera Beach, FL). Each experiment was carried out in triplicate.

Results and Discussion

Phase-Solubility Analysis—Phase-solubility diagrams for ATT and 5PDTT, DTT and DMDTT are shown in Figure 2 and Figure 3, respectively. Data obtained from these diagrams are presented in Table 1. Phase solubility diagrams obtained with HPβCD and SBE7βCD showed a linear relationship between the amount of 1,2-dithiole-3-thione solubilized and the concentration of cyclodextrin in solution (*Al* type diagram) for less lipophilic drugs DTT and DMDTT (log P = 1.59 and 2.45, respectively) (Figure 3). According to Higuchi and Connors theory,[17] this may be ascribed to the formation of soluble 1:1 (1,2-dithiole-3-thione–cyclodextrin) inclusion complexes. For more lipophlilic ATT and 5PDTT (log P = 3.82 and 3.67, respectively), the diagrams were of the *Ap* type (Figure 2), the positive curvatures indicating the existence of soluble complexes with an order greater than 1 in cyclodextrin. βCD, however, exhibited *Al* type diagrams according to Higuchi and Connors classification only with the more lipophilic ATT, while *Bs* type diagrams were observed with DTT, DMDTT, and 5PDTT, with an initial rising portion followed by a plateau and/or a very slight decrease in total drug concentration in the filtrate due to solid complex precipitation.

Table 1: Data Obtained from the Phase-Solubility Analysis for Each (1,2-dithiole-3-thione–cyclodextrin) Binary System: Type and Slope of the Diagrams, Apparent Stability Constant (K_s), and Solubility Increase with 2% βCD, 10% HPβCD, and 10% SBE7βCD

1,2-dithiole-3-thione	cyclodextrin	type of curve	slope of curve	stability constant[a] (M^{-1})	solubility increase[a]
ATT	βCD	Al	0.004	2841	41.3
	HPβCD	Ap	0.009	6227	458
	SBE7βCD	Ap	0.020	12834	576
5PDTT	βCD	Bs	0.049	2322	7.05
	HPβCD	Ap	0.009	2863	391
	SBE7βCD	Ap	0.022	10705	482
DMDTT	βCD	Bs	0.109	408	1.57
	HPβCD	Al	0.175	654	28.3
	SBE7βCD	Al	0.214	764	27.1
DTT	βCD	Bs	0.280	119	1.4
	HPβCD	Al	0.424	225	9.2
	SBE7βCD	Al	0.544	364	8.4

[a] Mean of three determinations.

Solubility enhancements obtained with the three cyclodextrin solutions, i.e. βCD (2% w/v), HPβCD (10% w/v), and SBE7βCD (10% w/v), were much greater with the more lipophilic 1,2-dithiole-3-thiones than with less lipophilic drugs, in the rank order SBE7βCD > HPβCD ≫ βCD (Table 1). The values obtained were near 480 times the intrinsic solubility for 5PDTT (which is 0.0023 mmol/L or 0.48 mg/L) and near 580 times the intrinsic solubility for ATT (0.0016 mmol/L or 0.39 mg/L) while they only reached 9 times the intrinsic solubility for DTT (3.28 mmol/L or 441 mg/L) and 28 times the intrinsic solubility for DMDTT

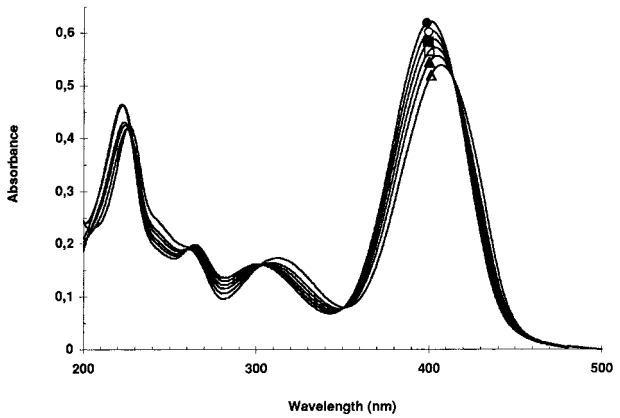


Figure 4—Effect of different concentrations of HPβCD (●: 0 mM; ○: 2.5×10^{-4} mM; ■: 10^{-3} mM; □: 5×10^{-3} mM; ▲: 10^{-2} mM; Δ: 5×10^{-2} mM) on the UV spectrum of DTT (5×10^{-2} mM).

Table 2: Data Obtained from UV Spectroscopy Study of the Complexation between DTT and the Cyclodextrins HPβCD and SBE7βCD: Stability Constants (K_s), Calculated (calcd) and Experimental (exp) Molar Absorptivities of Free (ϵ_{DTT}) and complexed (ϵ_{DTT-CD}) DTT

	K_s (M^{-1})	$(\epsilon_{DTT})^{calc}$ ($cm^{-1}\,M^{-1}$)	$(\epsilon_{DTT})^{exp}$ ($cm^{-1}\,M^{-1}$)	$(\epsilon_{DTT-CD})^{calc}$ ($cm^{-1}\,M^{-1}$)
DTT-HPβCD	178	12441	12458	9982
DTT-SBE7βCD	184	12376	12350	9831

(0.376 mmol/L or 61 mg/L). Apparent 1:1 stability constants (K_s) were obtained from the slopes of the linear portion of each diagram (slopes always lower than 1); the values, ranging from 119 to 12834 mol^{-1}, were also the highest for the more lipophilic drugs (exhibiting *Ap* type diagrams), in the same rank order SBE7βCD > HPβCD ≫ βCD (Table 1). Such results, showing the highest complexing ability of the SBE7βCD compared to HPβCD were also reported by Okimoto et al.[21] for binding constants of several neutral (uncharged) drugs with SBE7βCD and HPβCD, when studing the effect of charge on complexation.

UV−Visible Spectrophotometry—The stability constants (K_s) between the parent compound (DTT) and the cyclodextrins HPβCD and SBE7βCD could also be determined by UV−visible spectrophotometry taking into account the fact that the complex formation gave a bathochromic shift of the maximum located near 403 nm. Figure 4 shows the effect of different concentrations of HPβCD on the UV spectrum of DTT, for example; only 6 of 11 spectra are shown for clarity. The absorption maximum at 403 nm was shifted to higher wavelength with a concomitant decrease in the molar extinction coefficient (Table 2). The complexation of DTT with both cyclodextrins may be related to the high electron density inside the CD cavity on one hand,[22] and to the positive charge of the DTT

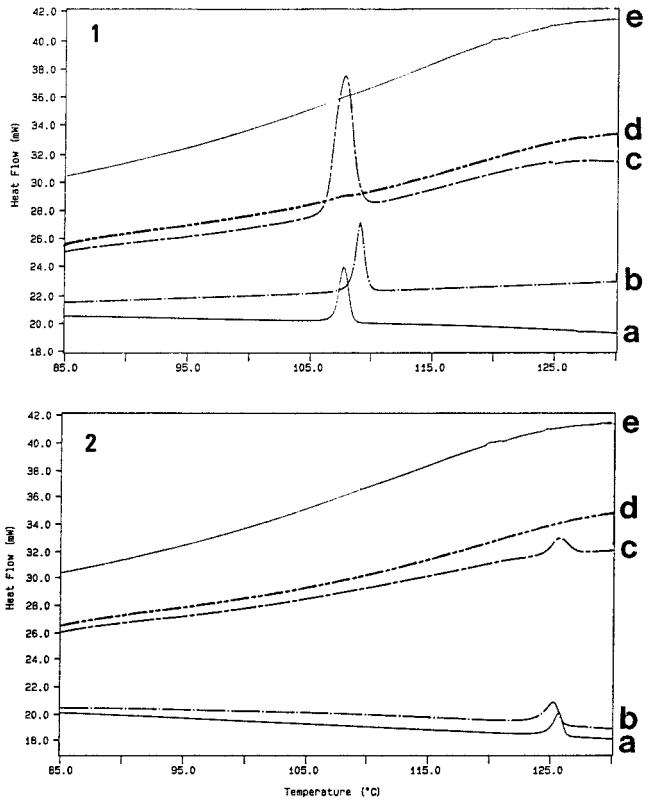
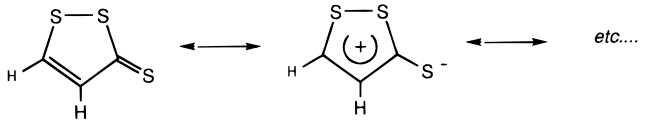


Figure 5—DSC thermograms of (1,2-dithiole-3-thiones, SBE7βCD) binary systems: Figure 5.1 (ATT,SBE7βCD) and Figure 5.2 (5PDTT,SBE7βCD). (a) 1:1 physical mixture (drug + SBE7βCD); (b) drug alone; (c) spray-dried complex obtained with a 1:1 initial mole ratio (drug–SBE7βCD); (d) spray-dried complex obtained with a 1:2 initial molar ratio (drug–SBE7βCD); (e) SBE7βCD alone.

nucleus on the other hand. Indeed, a lot of chemical and physical data indicate that the structure of 1,2-dithiole-3-thiones must be considered to be an hybrid resonance according to the following scheme:[23]



where the two mentioned structures have approximatively the same weight. The partially positive charge brought by the DTT nucleus may explain also on an electrostatic ground, the systematically enhanced stability for the complexes formed with the anionic SBE7βCD compared to those formed with neutral HPβCD, in agreement with Okimoto et al.,[21] i.e., a driving force for complexation being a combination of Coulombic and hydrophobic forces with regard to the binding of charged drugs to

Table 3: Data Obtained from DCS (sample weight, endothermic peak onset, ΔH, complexation efficiency) and Drug Content Determined by HPLC for 1:1 Physical Mixtures (1:1 PM), 1:1 Mole Ratio Spray-Dried Products (1:1 SD), and 1:2 Mole Ratio Spray-Dried Products (1:2 SD) for Each (1,2-dithiole-3-thione, cyclodextrin) Binary System Studied

binary system	product	sample (mg)	drug content (%)	onset (°C)	ΔH (J/g)	drug complexed (%)
ATT–SBE7βCD	ATT alone	0.19		109.0	101.50	
	1:1 PM	1.91	9.99	107.0	8.10	20.0
	1:1 SD	15.24	9.36	106.3	3.45	63.7
	1:2 SD	17.73	4.41	106.1	0.056	98.7
5PDTT–SBE7βCD	5PDTT alone	0.096		124.8	125.00	
	1:1 PM	1.70	8.86	123.8	10.25	7.4
	1:1 SD	18.41	5.44	124.3	0.70	89.7
	1:2 SD	20.13	4.55	none	none	100.0

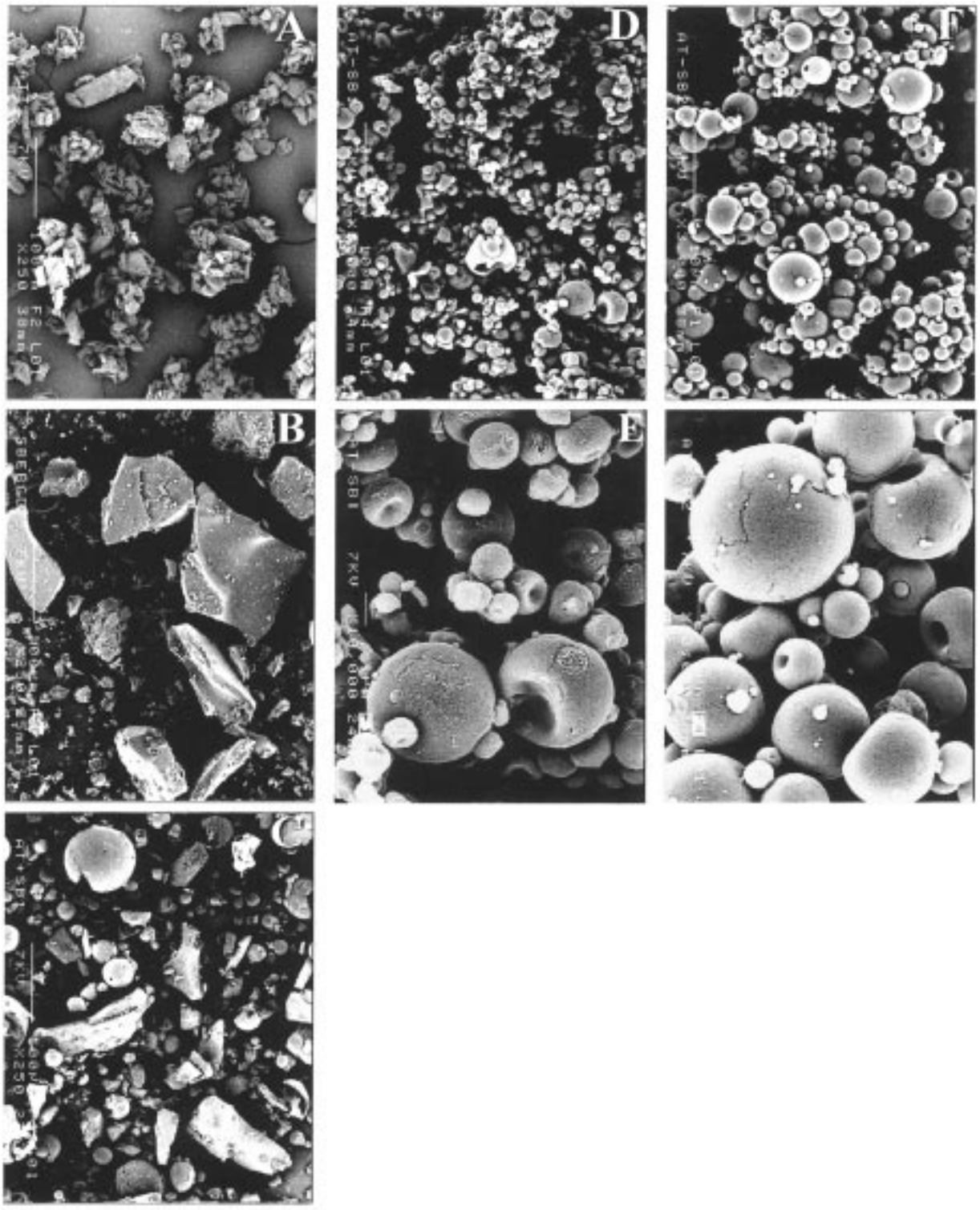


Figure 6—SEM micrographs of of binary system (ATT–SBE7βCD): (A) ATT alone; (B) SBE7βCD alone; (C): 1:1 physical mixture (ATT + SBE7βCD); (D and E) ATT–SBE7βCD spray-dried complex complex obtained with a 1:1 initial molar ratio (ATT–SBE7βCD1), × 3000 and × 10000, respectively; (F and G) ATT–SBE7βCD spray-dried complex complex obtained with a 1:2 initial molar ratio (ATT–SBE7βCD2), × 3000 and × 10000, respectively.

charged cyclodextrins. The occurrence of five isosbestic points at 227, 260, 303, 352, and 414 nm warranted the existence of only one equilibrium between the three species, i.e., the free DTT, the complexed DTT, and the cyclodextrin. This suggests that the model chosen (1:1 complexation) is appropriate and agrees well with the *Al*-type phase-solubility diagrams obtained with both cyclodextrins. Stability constants were extracted from the absorbance data obtained with the different relative concentrations of cyclodextrins and DTT, by a nonlinear least-squares methodology (see Experimental Section). Care was taken to ensure that equilibria were reached before determination of the absorbances. Results, which are given in Table 2, are in satisfactory agreement with those obtained by phase-solubility measurements (Table 1). It is interesting to note that another treatment of the experimental data performed by considering also the molar absorptivity coefficient of the pure DTT as unknown gave the same value for that

grandeur than that determined experimentally (Table 2). This is a strong argument to validate the accuracy of both the chemical model, i.e., the model with one equilibrium, and the experimental data.

Differential Scanning Calorimetry—DSC revealed some information on solid-state interactions between both 1,2-dithiole-3-thiones ATT and 5PDTT and cyclodextrin SBE7βCD. DSC thermograms are presented in Figure 5.1 and 5.2. Table 3 sums up all the data obtained from DSC. The DSC scan of SBE7βCD showed a typical broad endothermic peak between 85 and 150 °C (only the range 85−130 °C is shown) corresponding to its dehydration. DSC thermograms of pure drugs exhibited a sharp endothermic peak corresponding to their melting point, at 109 °C (ΔH = 101.5 J/g) and 124.8 °C (ΔH = 125 J/g) for ATT and 5PDTT, respectively. Both characteristic peaks (due to cyclodextrin water loss and drug melting) were also clearly visible in the physical mixtures of ATT and 5PDTT with SBE7βCD, even if the melting endotherms were slightly shifted to 107 and 123.8 °C for ATT and 5PDTT, respectively. This may be due to an interaction between the two species occurring during their mixing. From the ΔH values obtained (8.1 and 10.25 J/g for ATT and 5PDTT, respectively) it was easy to obtain the percentage of free drug, from the following equation:

$$\% \text{ free drug} = [\Delta H_{DTT,CD} \times 100]/[\Delta H_{DTT} \times (DC)/100)] \quad (7)$$

where ΔH_{DTT} and $\Delta H_{DTT,CD}$ are the enthalpy values of pure drug and of drug from each system with cyclodextrins (physical mixture or spray-dried complex), respectively, and DC is the drug content (%) of each system. From eq 7, we could estimate that the percentage of complexed drug (100 − % free drug) concerned 20 and 7.45% of the drug present in the mixture, for ATT and 5PDTT, respectively. Concerning the spray-dried products obtained with both drugs, increasing the initial concentration of the complexing agent led to a more complete or a total dissappearance of the drug characteristic melting endotherm and also to a higher complexation efficiency (Table 3 and Figure 5). The endothermic peak of pure 1,2-dithiole-3-thione was found for each 1:1 mole ratio spray-dried product with ΔH values of 3.45 and 0.70 J/g, for ATT and 5PDTT, respectively, indicating the presence of 36.3 and 10.3% free drug, respectively. In fact, only DSC thermograms of 1:2 drug−cyclodextrin mole ratio products lacked the melting peak (corresponding to 98.7 and 100% of complexed drug, for ATT and 5PDTT, respectively). As the disappearance of an endothermic peak may be attributed to an amorphous state and/or to an inclusion complexation,[24] these results suggest that only 1:2 spray-dried products can be considered as true and complete inclusion complexes, differing from simple physical mixtures.

Scanning Electronic Microscopy—From SEM (Figure 6) we can see the particle morphology of pure compounds ATT and SBE7βCD (micrographs A and B, respectively), both irregular three-dimensional particles with parallelogram shape. The physical mixture (micrograph C) showed particles of SBE7βCD embedded with ATT particles and a comparable morphology with pure compounds taken separately, revealing no apparent interaction between both species. In contrast, a drastic change in the morphology and shape of particles was observed in 1:1 (micrographs D and E) and 1:2 (micrographs F and G) spray-dried products, revealing an apparent interaction in the solid state. Spherical microparticles obtained also showed a reduction in size due to the spray-drying process, confirmed by laser diffractometry where the median volume diameters d(v,0.5) obtained, i.e., 39.87 μm, 11.54 μm, and 63.53 μm for SBE7βCD, ATT, and their physical mixture, respectively, decreased to 4.13 μm and 3.85 μm for a 1:1 and 1:2 mole ratio of spray-dried products, respectively.

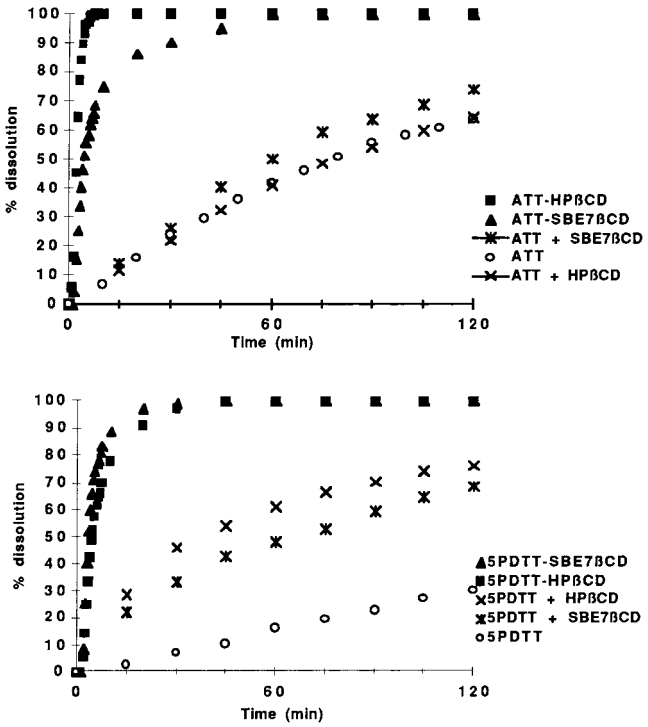


Figure 7—Dissolution rate profiles for ATT (top) and 5PDTT (bottom) systems with SBE7βCD or HPβCD. (○) 1,2-dithiole-3-thione alone; (*) physical mixture with SBE7βCD; (×) physical mixture with HPβCD; (▲) spray-dried complex with SBE7βCD; (■) spray-dried complex with HPβCD.

Dissolution Study—Dissolution profiles of pure ATT and 5PDTT, their 1:1 mole ratio physical mixtures, and spray-dried inclusion complexes with HPβCD and SBE7βCD are presented in Figure 7. From these curves we can see that all binary systems displayed an increase in drug dissolution rate with respect to pure drug. However, the dissolution enhancements of the drugs from the physical mixtures were not as marked, as they were from the spray-dried products (especially for ATT, the more lipophilic drug) and may be explained by mean of greater solubility of the drug in aqueous solution of cyclodextrins because of the hydrophilic environment surrounding the drug in the early stages of dissolution process, resulting in a better wettability of the drug. Concerning the significant enhancement of the dissolution rate that occurred with all spray-dried products, this may be attributed to an increase of solubility upon complexation, to the amorphous state generally occurring during spray-drying (i.e. a decrease in cristallinity), and to the reduction in the particle size resulting in an increase of the surface area of the drug, according to the Noyes−Whitney equation:[25]

$$dC/dt = D/h \times S\,(C_s - C_t) \quad (8)$$

where dC/dt is the dissolution rate, D is the diffusion coefficient of the drug, h is the thickness of the diffusion layer, S is the surface area of the dissolving solid, C_s and C_t are the aqueous solubility and the concentration of the drug in the aqueous medium at time t, respectively.

Conclusion

The present study showed that 1,2-dithiole-3-thiones could form inclusion complexes with β-cyclodextrin deriva-

tives, in solution as well as in the solid state, allowing an increase in solubility, a possible intravenous administration without using cosolvents, and also an increase in the dissolution rate of the drugs. These data shoud be of interest for improving oral absorption of these lipophilic compounds, allowing bioavailability and metabolism studies. An oral formulation allowing a chronic administration of these compounds for cancer chemoprevention is under investigation.

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