J Incl Phenom Macrocycl Chem (2011) 71:149–159
DOI 10.1007/s10847-010-9919-9

ORIGINAL ARTICLE

Characterization and in vivo efficacy of inclusion complexes of sulphadoxine with β-cyclodextrin: calorimetric and spectroscopic studies

Renu Chadha · Sushma Gupta · Geeta Shukla · D. V. S. Jain · Surjit Singh

Received: 6 August 2010 / Accepted: 23 December 2010 / Published online: 26 January 2011

Abstract The study reports the characterization and pharmacological activity of sulphadoxine which is encaged by β-CD and its hydroxypropyl and methyl derivatives. Phase solubility studies depict A_L type of curves suggesting 1:1 complexation. Inclusion of drug in solid state was evidenced by Differential scanning calorimeter (DSC), Powder X-ray diffractometery (PXRD), Fourier transform infrared spectroscopy (FTIR) and in solution phase by NMR and solution calorimetry. In the proton NMR chemical shifts of aniline ring and methoxy group of pyrmidine ring moved downfield upon inclusion indicating the potentiality of both sides of the drug to interact with the cavity. Insertion of whole of the drug molecule inside the cavity is ruled out because of its three dimensional (3D) structure. Thus, co-existence of two 1:1 complexes is proposed in the present study. The calorimetric data are fitted into two class binding model to evaluate the values of stability constants (K_1 and K_2) along with enthalpy of binding (ΔH_1 and ΔH_2). Efficiency of the encaged drug was evaluated by in vitro drug release studies and provided useful information for the selection of appropriate form for further studies. The best performance in terms of dissolution rate enhancement was displayed by the Sulpha-M-β-CD lyophilized product. This was further evaluated on the basis of its pharmacological activity using Balb/C mice and showed significantly higher survival rate (83.3%) after 30 days as to the uncomplexed drug which showed (33%) survival.

Keywords Sulphadoxine · β-cyclodextrins and its derivatives · Inclusion complexes · Stoichiometry · Thermodynamic parameters · In vivo studies

R. Chadha (✉) · S. Gupta
University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh 160014, India
e-mail: renukchadha@rediffmail.com

G. Shukla
Department of Microbiology, Panjab University, Chandigarh 160014, India

D. V. S. Jain
Department of Chemistry, Panjab University, Chandigarh 160014, India

S. Singh
Guru Nanak Dev University, Amritsar, Panjab, India

Introduction

Sulphadoxine (Fig. 1) is an ultra-long-lasting blood schizonticidal sulfonamide, used in combination with pyrimethamine in the prophylaxis and treatment of malaria caused by chloroquine-resistant strains of *Plasmodium falciparum*. The combination offers two step synergetic blockade of plasmodial division because the parasite *Plasmodium falciparum* may be able to use the exogenous folic acid which is present in the parasite's environment [1–6]. Sulphadoxine competitively inhibits dihydrofolic acid synthesis by inhibiting dihydropteroate synthetase and dihydrofolate reductase, which is necessary for the conversion of para aminobenzoic acid (PABA) to folic acid and also exploits the difference between mammal cells and other kinds of cells [7–12]. Unfortunately, the poor aqueous solubility of sulphadoxine (2.96e-01 mg/mL) limits its extent of oral bioavailability [13, 14]. Encaging the drug by hydrophilic cyclodextrins (CDs) can be a useful approach to alleviate the solubility related problems [15–21]. The present work reports the preparation of the inclusion complexes of sulphadoxine (sulpha) with β-CD, M-β-CD

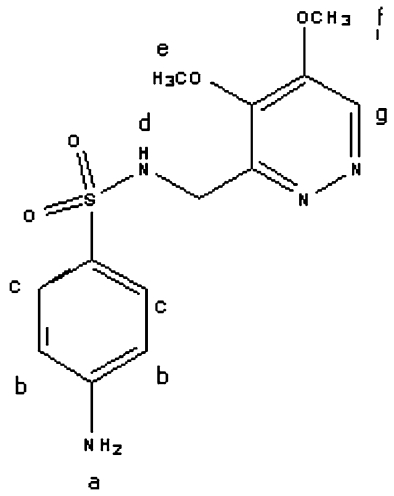


Fig. 1 Chemical structure of sulphadoxine

and HP-β-CD. The ability of different cyclodextrins to solublize the given drug is frequently evaluated by comparing their stability constants [22–25]. The focus of the study is to determine the stability constant, enthalpy of binding and entropy of binding along with their characterization. The thermodynamic parameters are necessary to draw a complete picture of the driving forces governing the CD-drug interaction. The pharmacological activity of complexed drug is performed and correlated with the complexing abilities of different CDs.

Experimental

Materials

Sulphadoxine was obtained as a gift sample from Biochem Pharmaceuticals Ltd. Mumbai. India.

β-CD, M-β-CD and HP-β-CD were obtained from Sigma-Aldrich. The other chemicals such as sodium hydroxide, potassium hydrogen phosphate, sodium dihydrogen phosphate were procured from SD fine Chemicals. All these chemicals were of analytical grade.

Phase solubility studies

Phase solubility curves of sulphadoxine with various CDs in phosphate buffer (pH 6.8) were obtained according to Higuchi and Connors [26]. An excess of drug was added to 10 mL of CD buffered solutions with different concentrations (0.002–0.010 M) in 20 mL glass vials. The suspensions were sealed and shaken in water-bath shaker MSW-275 (Macroscientific Works, Delhi) at 37 ± 0.5 °C for 24 h to ensure equilibrium. After equilibration, aliquots of the supernatant were withdrawn, filtered through 0.45 μm Millipore filter paper, and the sulphadoxine content, after suitable dilution, was determined spectrophotometerically at λ 270 nm (UV/VIS. Spectrophotometer, Perkin Elmer Lamda 15, USA). The presence of CDs did not interfere with the spectrophotometric assay of the drug.

Preparation of CD complexes

Various Sulpha-CD complexes were prepared in a 1:1 molar ratio on the basis of the results obtained from preliminary phase solubility studies by the following methods:

(a) Physical mixing: Physical mixture (PM) were prepared by simple mixing of drug with different CDs in mortar and to ensure uniform mixing, the vials filled with mixture were subjected to vortex mixing for 5 min.
(b) Kneading: The method involved the formation of paste of cyclodextrin with drug using small quantity of water to form the kneaded mass. This was dried in dark at 37 °C for 48 h, pulverized and sieved from 150 μm mesh and stored in glass vials in a vaccum dessicator.
(c) Freeze-drying method: The required 1:1 stoichiometric quantity of drug was added to aqueous solution of different CDs solution and agitated on magnetic stirrer for 24 h. The resulting solutions were frozen at (−80 °C) in deep freezer for 24 h. These were then lyophilized under 17.2 mTorr for 48 h. The samples were transferred immediately into a vacuum desiccator and dried over silica gel under vacuum for at least 24 h.

Characterization

These complexes were characterized in solid state by DSC, PXRD and FTIR. In solution phase these were characterized by NMR and solution calorimetry.

Differential scanning (DSC) calorimetry

DSC thermograms of sulphadoxine, pure CDs and their inclusion complexes were obtained on DSC, Q20 TA instruments, (Waters LLC, USA). The samples were sealed in aluminium pans and the DSC thermograms were recorded at a heating rate of 10 °C per minute from 25 to 350 °C.

Powder X-ray (PXRD) diffraction analysis

Powder diffraction pattern of sulphadoxine and their inclusion complexes were recorded on an X-ray diffractometers (XPERT-PRO PANanalytical, Netherlands, Holand) using

Cu as tube anode. The diffractogram were recorded under the following conditions: voltage 40 kV, 35 MA, angular range 5, fixed divergence slit.

Fourier transform infrared (FTIR) spectroscopic studies

The FT-IR spectra of sulphadoxine and their inclusion complexes were recorded on FTIR spectrometer, (Mode spectrum RXI, Perkin Elmer, England) over the range 400–4000 cm^{-1}. Dry KBr (50 mg) was finely ground in mortar and samples of drug and their complexes (1–2 mg) were subsequently added and gently mixed. A manual press was used to form the pellets.

2D COESY, proton nuclear magnetic resonance (^{1}H-NMR) and ^{13}C NMR spectroscopy

^{1}H-NMR, ^{13}C NMR and 2D COESY spectra of sulphadoxine and inclusion complexes in d_6DMSO were recorded with a Brucker AC 300 °C NMR spectrometer operating at 300 MHz using tetramethylsilane as an internal standard. For 2D COESY experiments, samples were equilibrated for at least 24 h.

Solution calorimetry

Isoperibol solution calorimeter (ISC) model 4300 (Calorimetry Science Corporation, Utah, USA) was used for thermal measurements. It is a semi-adiabatic calorimeter with temperature resolution, after noise reduction, close to 1 μK, which corresponds to a heat resolution of 1–4 mJ in a 25 mL buffer (pH 7) reaction vessel. The details are given in our previous papers [27, 28]. The performance of the system was tested by measuring the enthalpy of solution of potassium chloride (17.301 kJ/mol) in triple distilled water, which is in good agreement with known enthalpy of solution of 17.322 kJ/mol. The precision of any individual measurement was better than ±0.03 kJ/mol for three consecutive experiments.

Dissolution studies

The dissolution studies of the complexes was performed in 500 mL of phosphate buffer (pH 7) using USP (12) apparatus at pre-equilibrated temperature 37 ± 0.5 °C and at a stirring rate of 50 rpm. The sink conditions were maintained throughout the period of dissolution study. Drug and its complexes, each containing 100 mg of sulphadoxine were filled in hard gelatin capsules and subjected to dissolution studies. Samples were withdrawn at different intervals for a period of 4 h and assayed spectrophotometrically at λ 270 nm. The dissolution efficiency was evaluated on the basis of the dissolution efficiency parameter at 30 min (DE_{30}) and at 180 min (DE_{180}). The dissolution parameters were calculated from the area under the dissolution curve and expressed as a percent of the area of the rectangle described by 100% dissolution in the same time period.

In vivo efficacy

Four to five weeks old BALB/c mice (25–30 g) were procured and maintained in the Central Animal house. They were provided with standard pellet diet and water ad libtum. Experiments were performed as per guidelines of Control and Supervision on Experiments on Animals (CPC-SEA) committee. The experimental protocol was approved by Institutional Animal Ethics Committee (A. I. E. C.). *Plasmodium berghei* (NK 65) strain was used for evaluation of antimalarial activity in vivo studies and was maintained in the mice. All the mice belonging to control group were challenged with 10^6 *P. berghei* infected RBCs intraperitonial (i/p). After challenge mean percent parasitaemia, percent activities of various complexes of sulphadoxine along with animal survivality were monitored. Mean percent parasitaemia was calculated for each group on every alternate day up to 30 days by tail blood smear, fixed in methanol and stained in Giemsa stain by counting at least 500 cells.

$$\text{Mean percent parasiteamia} = \text{infected RBCs/Total no. of RBCs} \times 100$$

Animals were divided into 3 groups and each group comprised of 6 animals ($n = 6$). These were treated with single dose therapy (6 mg/kg) two times a day on 1 day of PI for 7 days to monitor the efficacy and potency of prepared lyophilized complexes. Each animal was treated with 100 μl pyrimethamine and its various lyophilized complexes.

1. Control group—treated with 0.5% carboxymethyl cellulose (CMC) suspension;
2. Standard group—administered sulphadoxine in 0.5% CMC suspension;
3. Test group—treated with Sulpha-M-β-CD complex in 0.5% CMC suspension;

Statistical analysis

The differences between multiple groups of dissolution efficiency data (DE 30 min, DE 180 min) were assessed by analysis of variance followed by Turkey's posttest to determine the level of significance between different groups. Mean differences with $P < 0.05$ were considered to be significant.

Data from in vivo studies was expressed as mean ± SD and parasitaemia of the sulphadoxine and its inclusion

complexes were statistically assessed by one-way ANOVA followed by Turkey test using Jandel sigma stat 2.0 version. Differences were considered significant at $P < 0.05$.

Results and discussion

Phase solubility analysis

The phase solubility diagrams were found to be linear characterizing their A_L type nature. This suggests the formation of first order soluble complexes of sulphadoxine with all the three CD's (Fig. 2).

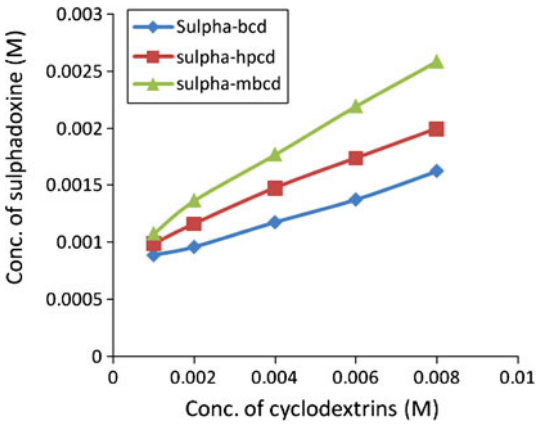


Fig. 2 Phase solubility curves of sulphadoxine with β-CD and its derivatives at 37 °C

Analysis of complexes in solid state

Differential scanning calorimetry (DSC) analysis

The characteristic endothermic peak of sulphadoxine at 197.87 °C is present in physical mixtures as well as kneaded complexes indicating incomplete inclusion (Fig. 3). Complete disappearance of the endothermic peak in the lyophilized systems suggests the formation of true inclusion complexes.

Powder X-ray diffraction (PXRD) analysis

The PXRD patterns of the physical mixtures as well as kneaded complexes (Fig. 4) reveal the characteristic peaks of drug with evident reduction in their intensity indicating the presence of drug as an isolated solid in the complex. The complete disappearance of drug peaks in the PXRD diffraction pattern of lyophilized complexes indicates the entrapment of drug inside the CD cavity.

Fourier-transform infrared (FTIR) spectroscopic studies

IR spectroscopy also provides an insight about the interactions through the detection of changes in bands that could be related to complexation process. The IR spectra of sulphadoxine shows the characteristic peaks at 3465.8 cm^{-1}, 3238.4 cm^{-1}, 1584.2 cm^{-1}, 1319.2 cm^{-1}, 1157.4 cm^{-1} due to –N–H asymmetric & symmetric stretch, N–H bend

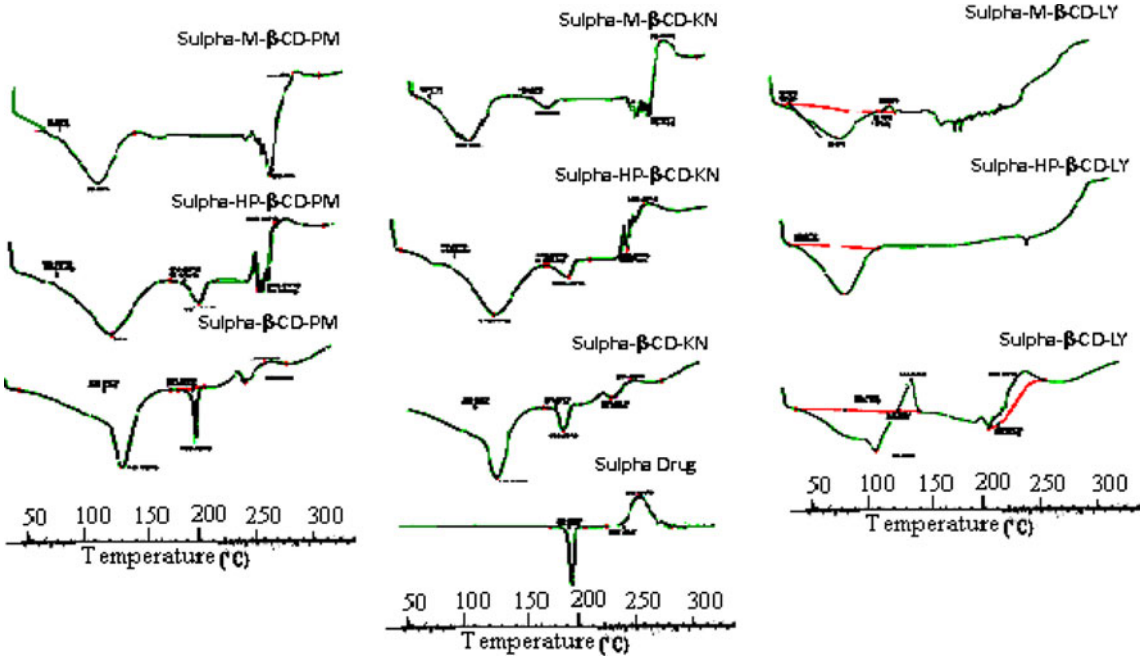


Fig. 3 DSC thermograms of sulphadoxine-CD solid systems: Sulphadoxine, (CDs) β-CD, M-β-CD and HP-β-CD, (PM) physical mixtures, (KN) kneaded complex and lyophilized complex (Ly)

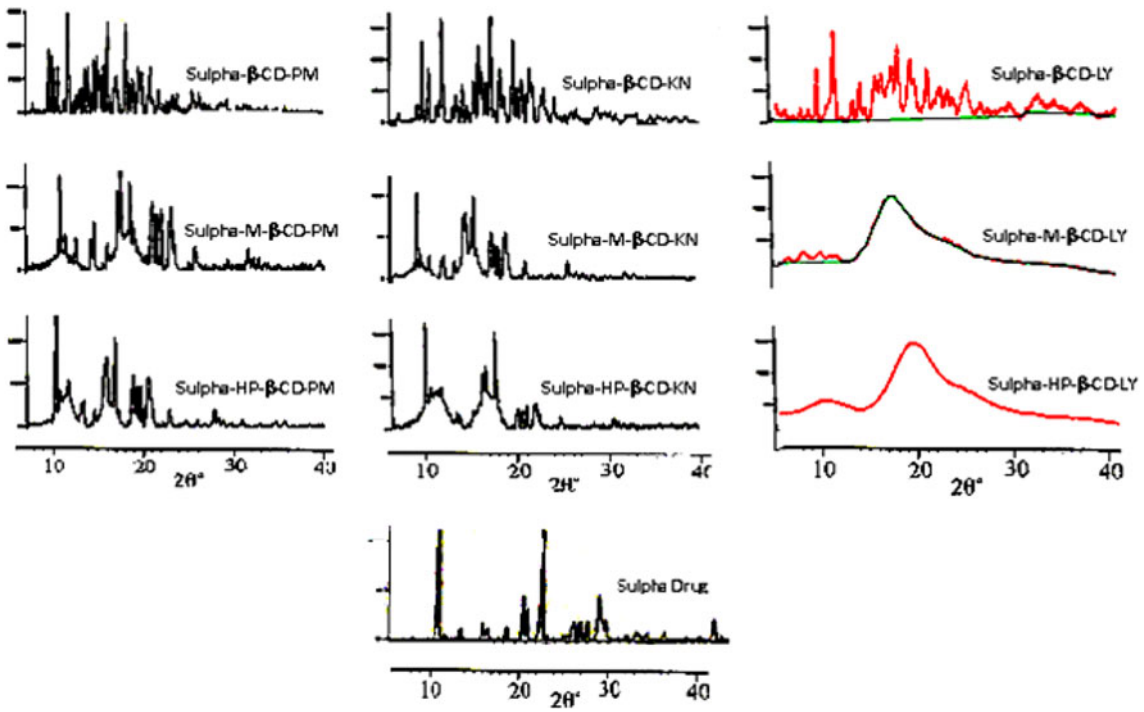


Fig. 4 PXRD diffraction patterns of sulphadoxine-CD solid systems: Sulphadoxine, (CD) β-CD, M-β-CD and HP-β-CD, (PM) physical mixture, (KN) kneaded complex and lyophilized complex (Ly)

and –S=O asymmetric & symmetric stretch, respectively. These characteristic peaks are dominated by the CD bands in kneaded and lyophilized complexes confirming interaction between the host and guest molecule (Fig. 5). However, the peaks are obvious in physical mixtures.

Characterisation of complexes in solution phase:

NMR spectroscopic studies

NMR spectroscopy assures the existence of complexes in solution and also predicts the geometry of the complexes by determining the changes in chemical shift of drug protons due to its insertion into the hydrophobic cavity of cyclodextrins. The induced changes ($\Delta\delta$) in chemical shifts for protons a, b, c, of aniline ring and protons e, f of methoxy group of pyrimidine ring indicate their deep penetration into the CD cavity (Table 1).

Sulphadoxine is a big molecule and is unable to fully fit into the cavity. So, there is a possibility of co-existing of two 1:1 complexes in the solution. To get an insight about the exact geometry of the inclusion mode, two-dimensional (2D) COESY spectra were used. COESY provides the information about the spatial proximity between host and guest atoms by observing intermolecular cross-relations. The off-diagonal peaks are displayed between the H-3 and H-5 protons of cyclodextrins and methoxy protons of pyrimidine ring as well as NH_2 protons of aromatic ring (Fig. 6). This supports our proposed inclusion mode which involves insertion of the drug molecule either from the pyrimidine ring side or from aniline ring side. Thus, two types of complexes coexist in the solution (Fig. 7). Similar, cross peaks were found in M-β-CD and HP-β-CD. However, peaks could not be clearly identified due to random substitution.

Solution calorimetry

Solution calorimetry has been employed to confirm the stoichiometry as well as to evaluate thermodynamic parameters associated with the binding process by determining the enthalpy of solution of sulphadoxine in presence and absence of cyclodextrin. The molar enthalpy of solution ($\Delta_{sol}H_{(M)}$) of sulphadoxine is slightly exothermic (−0.0896 kJ/mol) in phosphate buffer (pH 7) and is found to be higher in magnitude in presence of cyclodextrins ($\Delta_{sol}H_{(M)(CD)}$). This is due to interaction between drug and cyclodextrins. The enthalpy of interaction was calculated by the Eq. 1:

$$\Delta H_{\text{int L(exp)}} = \frac{\Delta_{sol}H_{(CD)} - \Delta_{sol}H}{v(l)} \quad (1)$$

$\Delta H_{\text{int,(exp)}}$ = enthalpy of interaction between drug and cyclodextrin per liter of solution

$\Delta_{sol}H$, $\Delta_{sol}H_{(CD)}$ are the enthalpy of solution of drug in buffer and in buffered aqueous solution of cyclodextrin, respectively, $v(l)$ = volume of sample cell in liters (0.025 L).

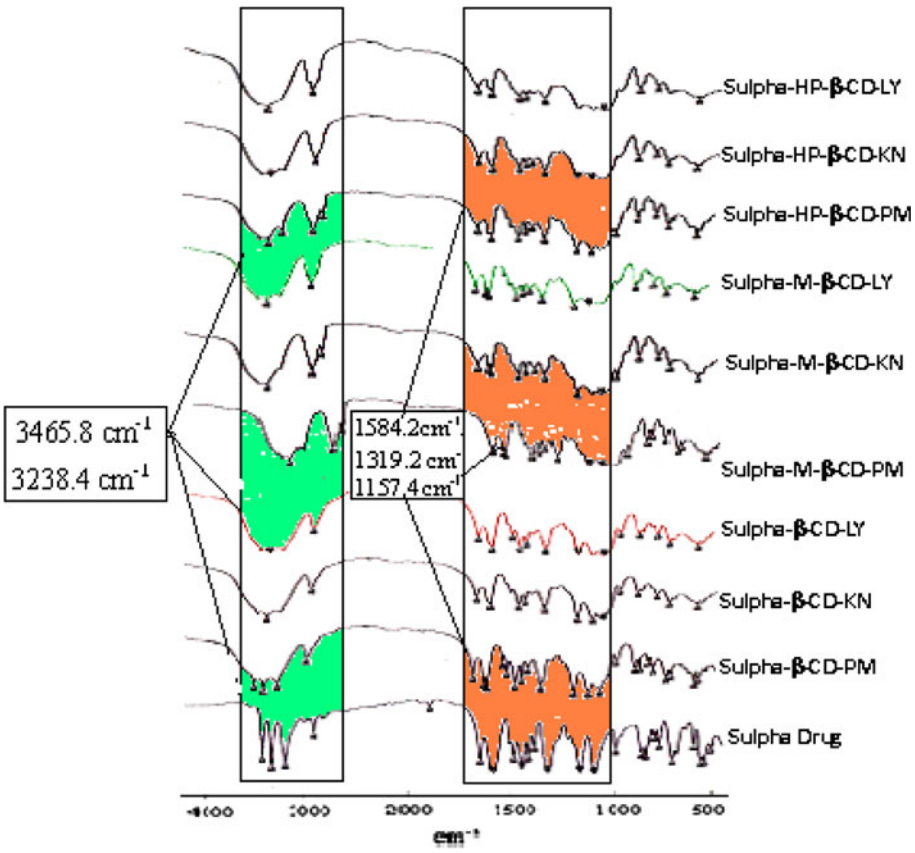


Fig. 5 FTIR spectra of sulphadoxine-CD solid systems: Sulphadoxine, (CD) β-CD, M-β-CD and HP-β-CD, (PM) physical mixture, (KN) kneaded complex and lyophilized complex (Ly)

Table 1 Values of ^{1}H chemical shifts before and after inclusion

Sulphadoxine	$\Delta\delta_{bCD}$ (ppm)	$\Delta\delta_{HP\text{-}B\text{-}CD}$ (ppm)	$\Delta\delta_{M\text{-}B\text{-}CD}$ (ppm)
H-a	−0.0006	−0.0004	−0.0009
H-b	−0.0007	−0.0003	−0.0012
H-c	−0.0023	−0.0022	−0.0037
H-d	−0.0004	−0.0017	−0.0127
H-e	−0.0492	−0.0124	−0.0427
H-f	−0.0124	−0.0163	−0.0176
H-g	−0.0015	−0.0035	−0.0047

$\Delta\delta = \delta$ (complex) − δ (Free)

Enthalpy of interaction per mole of drug and cyclodextrin calculated using Eq. 2.

$$\Delta H_{\text{int(M)}} = \frac{\Delta H_{\text{int L}_{(\text{exp})}}}{a+b} = \frac{\Delta_{\text{sol}}H_{(\text{M})(\text{CD})} - \Delta_{\text{sol}}H_{(\text{M})}}{1+\frac{x_2}{x_1}} \qquad (2)$$

Here a and b are the initial molar concentration of drug and cyclodextrin in the solution.

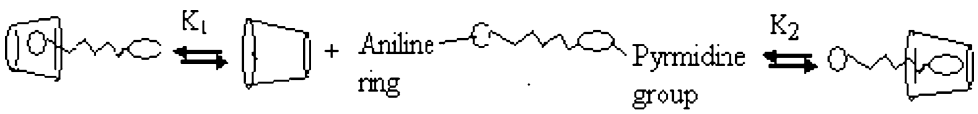
x_1 and x_2 are the apparent mole fractions of the drug and cyclodextrin ignoring the other ingredients.

The detailed calorimetric results for Sulpha-M-β-CD complexation are given in Table 2. Similar calculations were made for β-CD and HP-β-CD.

The stoichiometry of the complexes were ascertained utilizing continuous variation method (Job's plot) [31] by plotting ($\Delta H_{\text{int(M)}}$) versus (x_2) (Fig. 8). It is clear that the minimum occurs at $x_2 = 0.5$ confirming the 1:1 stoichiometry as indicated by phase solubility.

As discussed above NMR data indicate the possibility of two 1:1 complexes co-existing in the solution. Therefore, a two class binding model was utilized to determine the concentration of drug: CD complex.

Now the binding constants are calculated assuming the following two equilibria.



(3)

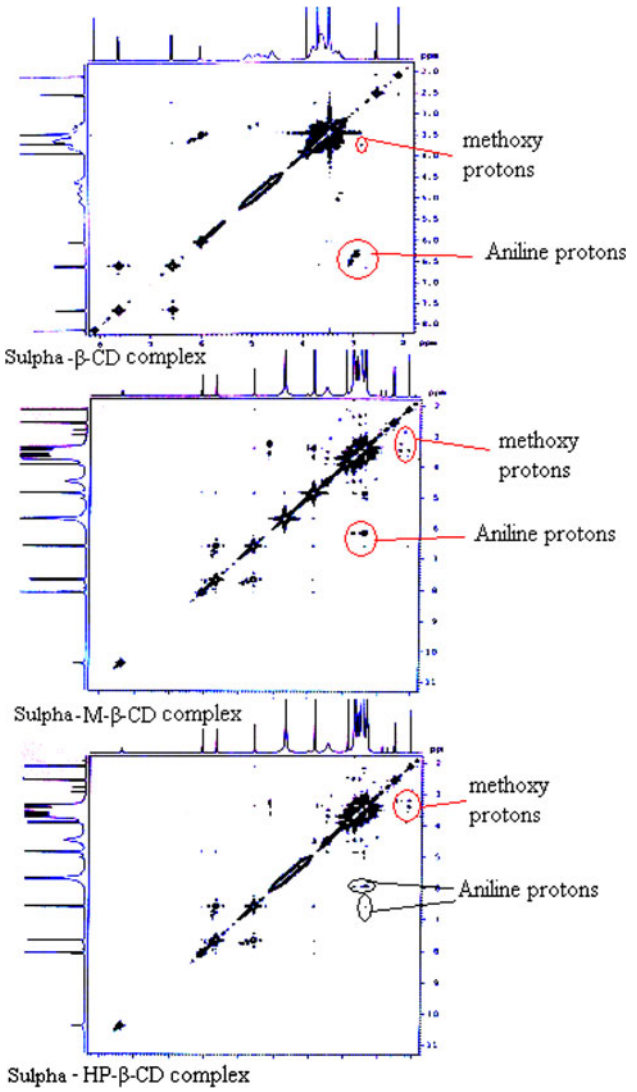


Fig. 6 COESY spectra of inclusion complexes of sulphadoxine with β-CD, M-β-CD and HP-β-CD

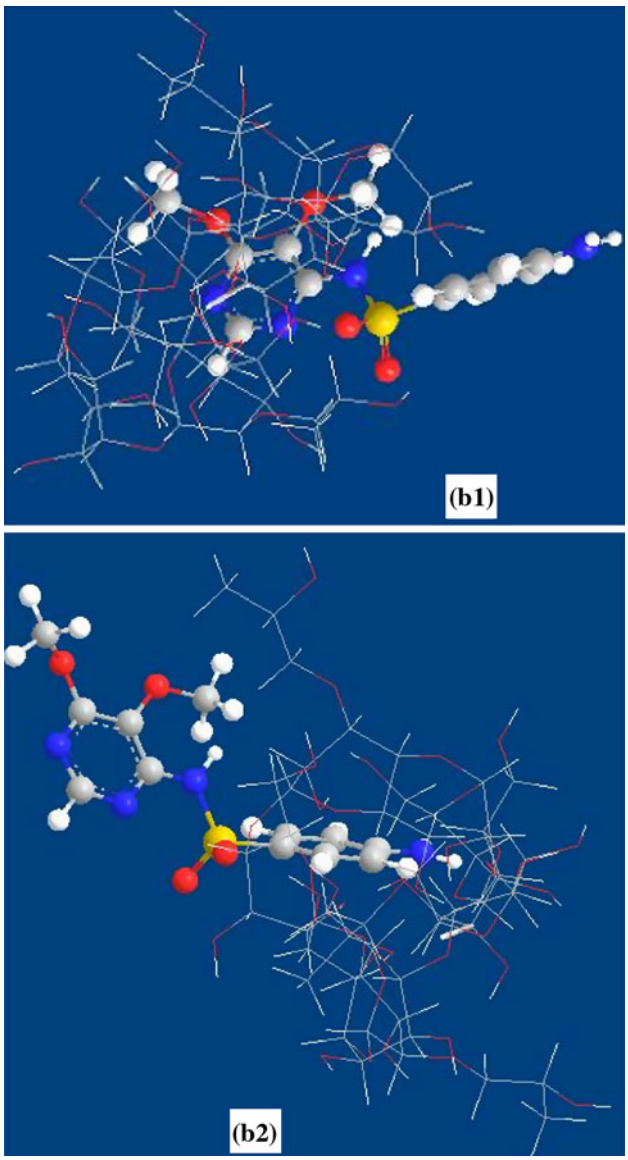


Fig. 7 **b1** molecular representation of inclusion mode of sulphadoxine with M-β-CD where ' pyrimidine ring enters the cyclodextrin cavity (**b2**) where the aniline ring enters the cavity

K_1 is the equilibrium constant for one type of complex where pyrimidine ring enters the cyclodextrin cavity;

K_2 is the equilibrium constant for second type of complex where the aniline ring enters the cavity.

The experimentally calculated enthalpy of interaction ($\Delta H_{\text{int L(exp)}}$) is proportional to the sum of the product of molar concentration of each type of CD: sulphadoxine complexes in the solution at equilibrium and associated molar enthalpy of binding.

$$\Delta H_{\text{int.L(exp)}} = \Delta H_1 \times c_1 + \Delta H_2 \times c_2 \tag{4}$$

ΔH_1 and c_1 are molar enthalpy of binding and the concentration of complex I and ΔH_2 and c_2 are molar enthalpy of binding and the concentration of complex II.

where

$$c_1 = K_1(C)\,(D) \tag{5}$$

$$c_2 = K_2(C)\,(D) \tag{6}$$

Here c_1 is the concentration of one type of the complex where pyrimidine ring enters the cyclodextrin cavity & c_2 is the concentration of the second type of the complex where the aniline ring enters the cavity.

(C) = free concentration of cyclodextrins, (D) = free concentration of drug.

Successive iteration were used to compute the interaction parameters ($K(i)$ & $\Delta H(i)$) for the drug-CD systems. For any choice of K_1 and K_2, initial concentration and free concentration of drug as well as of cyclodextrin at equilibria can be expressed by the following equation

$$C_t = a = (C) + K_1(C)\,(D) + K_2(C)\,(D) \tag{7}$$

Table 2 Interaction enthalpy of inclusion complexes of sulphadoxine with M-β-CD at pH 7

X_2	MD (a) (mM)	$M_{m\text{-}b\text{-}CD\text{-}}$ (b) (mM)	$\Delta_{sol}H_{(CD)}$ (J) × 10	$\Delta_{sol}H_{int(exp)}$ (J/l)	$\Delta H_{int.(M)}$ (kJ/mol) × 10
0.893	0.365	3.042	−1.59	−3.096	−9.09
0.787	0.412	1.524	−1.71	−3.156	−16.29
0.697	0.325	0.747	−1.21	−1.936	−18.07
0.583	0.549	0.767	−1.99	−3.024	−22.98
0.514	0.311	0.328	−1.08	−1.532	−23.95
0.397	0.468	0.308	−1.49	−1.776	−22.87
0.291	0.741	0.304	−2.12	−1.836	−17.56
0.188	0.688	0.159	−1.81	−1.084	−12.78
0.099	1.377	0.151	−3.36	−1.112	−7.27
0.844	0.284	1.534	−1.21	−2.316	−12.76
0.636	0.425	0.744	−1.57	−2.476	−21.2
0.458	0.761	0.642	−2.54	−3.348	−23.87
0.344	1.164	0.611	−3.54	−3.72	−20.96
0.239	0.606	0.191	−1.68	−1.312	−16.9

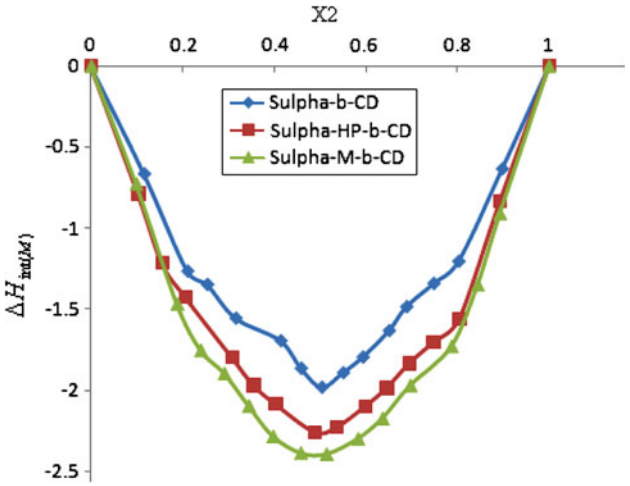


Fig. 8 Plot between $\Delta H_{int(M)}$ vs. X_2 of β-CD, M-β-CD and HP-β-CD of ulphadoxine (Sulpha) at pH7 in phosphate buffer

$$D_t = b = (D) + K_1(C)(D) + K_2(C)(D) \quad (8)$$

Hence,

$$C = C_t/(1 + K_1(D) + K_2(D)) \quad (9)$$

$$D = D_t/(1 + K_1(C) + K_2(C)) \quad (10)$$

where C_t = Total initial concentration of cyclodextrin, D_t = Total initial concentration of drug.

The values of C and D were calculated by iteration until their values within selected limits were obtained by putting any reasonable initial values of K_1 and K_2. Once C and D are determined, then

$$\Delta H_{intL}(calc) = [\Delta H_1 \times K_1(C)(D)] + [\Delta H_2 \times K_2(C)(D)] \quad (11)$$

The values of K_1 & K_2, ΔH_1 and ΔH_2 were evaluated by using a self consistent iterative non-linear least square regression programme after successive iteration to minimize the values of $\Sigma\{(\Delta H_{int\ L(exp)} - \Delta H_{int\ L(calc)})^2\}$.

The values of the binding constants thus determined for all the three β-CDs are given in Table 3. The binding constants K_1 and K_2 increased in the order of M-β-CD > HP-β-CD > β-CD which means better interaction with M-β-CD. Higher complexing efficiency of M-β-CD compared to HP-βCD is revealed by its more hydrophobic environment due to methylation of hydroxyl groups which increases adaptability and flexibility of CD towards the guest but in HP-β-CD, the hydroxyl groups make the CD cavity partially hydrophilic.

Table 3 Stability constant and thermodynamic parameters of sulphadoxine-β-CD's interaction at pH 7 in phosphate buffer for each type of complex

System	K_1 (M^{-1})	ΔH_1° (kJ mol^{-1})	ΔG_1° (kJ mol^{-1})	ΔS_1° ($Jmol^{-1}$ K^{-1})	K_2 (M^{-1})	ΔH_2° (kJ mol^{-1})	ΔG_2° (kJ mol^{-1})	ΔS_2° ($Jmol^{-1}$ K^{-1})
Sulpha + β-CD	1380	−6.90	1516	−5.9	−18.6	37.78	−18.87	41.79
Sulpha + HP-β-CD	1580	−8.88	1614	−7.92	−18.98	32.59	−19.04	35.86
Sulpha + M-β-CD	1817	−9.79	1886	−8.67	−19.32	30.81	−19.44	34.73

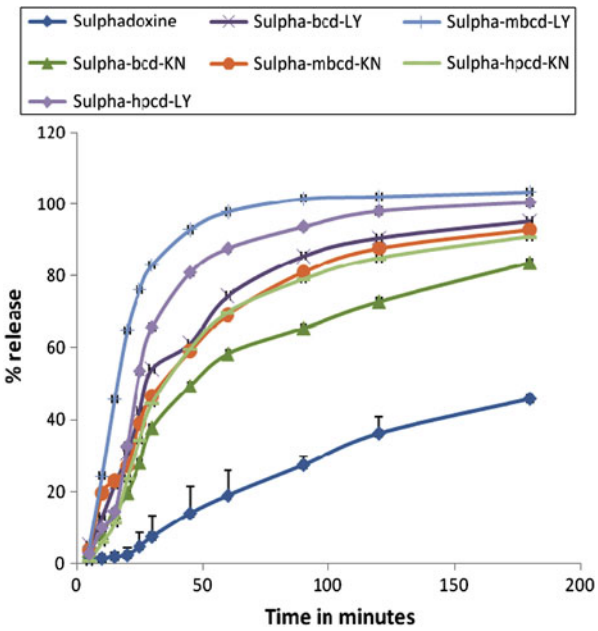


Fig. 9 Dissolution profile of sulphadoxine and its complexes

Table 4 Mean ± SD values of dissolution efficiency at 30 min and 90 min (DE_{30min}, DE_{90min}) of sulphadoxine and its complexes with β-CD, M-β-CD and HP-β-CD at 37 °C in phosphate buffer at pH 7

Sample	DE^{a}_{30}	DE^{a}_{90}
Sulpha	2.73 ± 0.68	29.94 ± 0.92
Sulpha-β-CD PM	7.82 ± 1.2	36.83 ± 1.34
Sulpha-β-CD KN	6.47 ± 0.87	40.19 ± 0.8
Sulpha-β-CD LY	9.69 ± 1.03	44.89 ± 3.67
Sulpha-HP-β-CD PM	8.54 ± 0.59	38.93 ± 4.89
Sulpha-HP-β-CD KN	9.18 ± 0.98	43.45 ± 4.27
Sulpha-HP-β-CD LY	11.90 ± 1.12	48.62 ± 3.27
Sulpha-M-β-CD PM	10.66 ± 0.79	42.33 ± 5.23
Sulpha-M-β-CD KN	12.09 ± 1.23	47.02 ± 3.60
Sulpha-M-β-CD LY	14.38 ± 1.01$^{\#}$	51.11 ± 5.28

$^{\#}$ $P < 0.05$ as compared to all groups

^{a} DE_{30min} and DE_{90min} was calculated as described in the text from area under the dissolution curve at 30 min and 90 min expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time

Dissolution studies

The dissolution behavior of sulphadoxine and its inclusion complexes with β-CD, M-β-CD and HP-β-CD was studied to select the most appropriate system for the in vivo studies (Fig. 9). Significant improvement ($P < 0.05$) in dissolution rate and dissolution efficiency was observed for all the complexes (Table 4). However, lyophilized complexes of Sulpha-M-β-CD exhibit the highest dissolution profile and is selected for in vivo studies.

In vivo efficacy of sulphadoxine in mice

Lyophilized complex of sulphadoxine with M-β-CD was used to monitor the in vivo antimalarial activity and efficacy against *P. berghei* infection. CMC (control group), sulphadoxine (standard group) and M-β-CD complexed (test group) suspensions were administered for 5 days on day 1 of PI to check their protective efficacy to prevent the mortality rate. It was found that all the preparations are ineffective in preventing the mortality but survival time was increased for test group (25–30 days) compared to standard group (12–16 days). Percent mortality rate was decreased from 100% (control group), to 83.3% and 16.7% for standard group and test group, respectively. Test group showed significantly less ($P < 0.001$) mean percent parasitaemia (4.73 ± 1.28) as compared to control group (53.23 ± 9.49) on day 8 (Table 5). Mean differences of M-β-CD drug complex for antimalarial activity was found significant ($P < 0.05$) by analysis of variance (ANOVA) followed by Turkey's posttest (Figs. 10, 11)).

Conclusions

The study shows that M-β-CD is the best of all the three CDs (β-CD, M-β-CD and HP-β-CD) to solubilise sulphadoxine. Co-existence of two 1:1 complexes was indicated by proton–NMR studies and was confirmed by solution calorimetry by determining the two stability constant K_1 (aniline ring) and K_2 (pyrmidine ring). The numerical value

Table 5 Antimalarial activity of M-β-CD lyophilized complexes of sulphadoxine in *P. berghi* infected mice

S. No.	Groups	Treatment	⅄ Mean % Parasitaemia on day 8th PI	% Mortality ($n = 6$, $t = 30$ days)
1	Control group	0.5% CMC solution	53.23 ± 9.49	100
2	Standard group	Sulphadoxine ~ (5 mg/kg)	12.44 ± 2.21*	83.3
3	Test group	Sulpha-M-β-CD ~ (5 mg/kg of sulpha)	4.73 ± 1.28*$^{\#}$	16.7

⅄ Values were expressed as mean ± SD (standard deviation)

t = no. of days; n = no. of animals per group

PI post inoculation, *Sulpha-M-β-CD* Sulphadoxine-methyl-β-cyclodextrin complex

* $P < 0.001$ as compared to the control group, $^{\#}$ $P < 0.05$ as compared to standard group

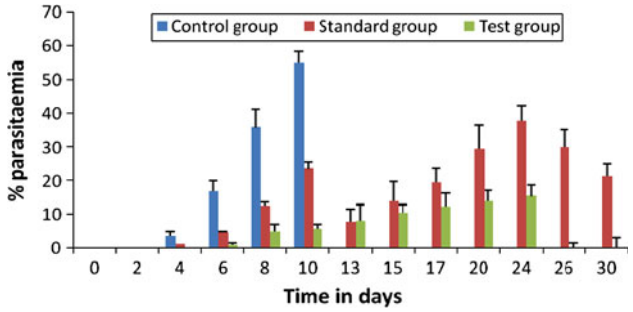


Fig. 10 Percent parasitaemia observed in *P. berghei* infected mice "($n = 6$)"

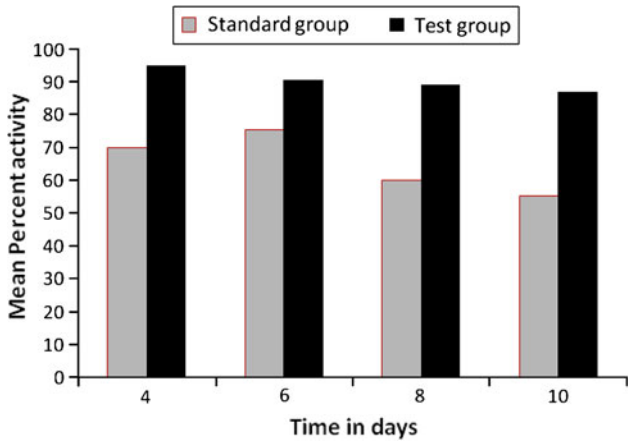


Fig. 11 Antimalarial activity of M-β-CD lyophilized complexes of sulphadoxine in *P. berghei* infected mice "($n = 6$)" as compared to drug

of stability constants increases in the order β-CD < HP-β-CD < M-β-CD and is supported by the in vitro dissolution rate and dissolution efficiency. Significantly less mean percentage parasitaemia and better antimalarial efficacy of M-β-CD complex as to drug is observed in in vivo studies. It concludes that the encapsulation of sulphadoxine by cyclodextrin is a successful approach for improving its pharmacological activity.

Acknowledgments The financial assistance provided by Indian Council of Medical research, New Delhi, India and Instrumentation assistance by Department of Science Technology (DST), New Delhi is gratefully acknowledged.

References

1. Hawser, S., Lociuro, S., Islam, K.: Dihydrofolate reductase inhibitors as antibacterial agents. Biochem. Pharmacol. **71**(7), 941–948 (2006). doi:10.1016/j.bcp.2005.10.052
2. Hopkins Sibley, C., Hyde, J.E., Sims, P.F., Plowe, C.V., Kublin, J.G., Mberu, E.K., Cowman, A.F., Winstanley, P.A., Watkins, W.M., Nzila, A.M.: Pyrimethamine-sulfadoxine resistance in Plasmodium falciparum: what next? Trends Parasitol. **17**, 582–588 (2001)
3. Sirawaraporn, W., Yuthavong, Y.: Potentiating effect of pyrimethamine and sulfadoxine against DHFR from pyrimethamine-sensitive and pyrimethamine-resistant *P. chabaudi*. Antimicrob. Agents. Chemother. **29**, 899–905 (1986)
4. Sims, P.F.G., Wang, P., Hyde, J.E.: Comments on 'The efficacy of antifolate antimalarial combination in Africa'. Parasitol. Today **14**, 136–137 (1998)
5. Chulay, J.D., Watkins, W.M., Sixsmith, D.G.: Synergistic antimalarial activity of pyrimethamine and sulfadoxine against Plasmodium falciparum in vitro. Am. J. Trop. Med. Hyg. **33**(3), 325–330 (1984)
6. Hurly, M.G.: Potentiation of pyrimethamine by sulphadiazine in human malaria. Trans. R. Soc. Trop. Med. Hyg. **53**, 412–413 (1959)
7. Brown, G.M.: The biosynthesis of folic acid. II. Inhibition by sulfonamides. J. Biol. Chem. **237**, 536–540 (1962)
8. Ferone, R.: Folate metabolism in malaria. Bull. W. H. O. **55**, 291–298 (1977)
9. Wang, P., Read, M., Sims, P.F., Hyde, J.E.: Sulfadoxine resistance in the human malaria parasite *Plasmodium falciparum* is determined by mutations in dihydropteroate synthetase and an additional factor associated with folate utilization. Mol. Microbiol. **23**, 979–986 (1997)
10. Laing, A.B.: Studies on the chemotherapy of malaria. I. The treatment of overt falciparum malaria with potentiating combinations of pyrimethamine and sulphormethoxine or dapsone in The Gambia. Trans. R. Soc. Trop. Med. Hyg. **64**, 562–568 (1970)
11. Christian, F., Swedberg, G.: Adaptation to sulfonamide resistance in *Neisseria meningitidis* may have required compensatory changes to retain enzyme function: kinetic analysis of dihydropteroate synthases from *N. meningitides* expressed in a knockout mutant of *Escherichia coli*. Am. Soc. Microbiol. **179**, 831–837 (1997)
12. Huovinen, P., Sundström, L., Swedberg, G., Sköld, O.: Trimethoprim and sulfonamide resistance. Antimicrob. Agents Chemother. **39**, 279–289 (1995)
13. The International Pharmacopoeia; 4th edition—2006, Monographs: Sulfadoxine Thursday, 21 June 2007. http://www.artepal.org
14. DrugBank: Showing Sulfadoxine (DB01299) www.drugbank.ca/cgi-bin/getCard.cgi?CARD=DB01299
15. Yang, B., Lin, J., Chen, Y., Liu, Y.: Artemether/hydroxypropyl-β-cyclodextrin host–guest system: characterisation, phase solubility and inclusion mode. J. Bioorg. Med. Chem. **17**, 311–317 (2009)
16. Mura, P., Corti, G., Maestrelli, F., Cirri, M.: The influence of chitosan on cyclodextrin complexing and solubilizing abilities towards drugs. J. Incl. Phenom. Macrocycl. Chem. **59**, 307–313 (2007). doi:10.1007/s10847-007-9329-9
17. Loftsson, T., Brewster, M.E.: Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. J. Pharm. Sci. **85**, 1017–1025 (1996)
19. Onyeji, C.O., Omoruyi, S.I., Oladimeji, F.A., Soyinka, J.O.: Physicochemical characterization and dissolution properties of binary systems of pyrimethamine and 2-hydroxypropyl-β-cyclodextrin. Afr. J. Biotechnol. **8**(8), 1651–1659 (2009)
20. de Araujo, M.V.G., Vieira, E.K.B., Lazaro, G.S., Conegero, Ld.S., Ferreira, O.P., Almedia, L.E., Barreto, L.S., da Costa, N.B., Gimenez, I.F.: Inclusion complexes of pyrimethamine in 2-hydroxypropyl-β-cyclodextrin: characterisation, phase solubility and molecular modeling. J. Biol. Med. Chem. **15**, 5752–5759 (2007)
21. Rekharsky, M.V., Inoue, Y.: Complexation thermodynamics of cyclodextrins. Chem. Rev. **98**, 1875–1917 (1998)
22. Royall, P.G., Gaisford, S.: Application of solution calorimetry in pharmaceutical and biopharmaceutical research. Curr. Pharm. Biotech. **6**, 215–222 (2005)

23. Figueiras, A., Ribeiro, L., Vieira, M.T., Veiga, F.: Preparation and physicochemical characterization of omeprazole: methyl-beta-cyclodextrin inclusion complex in solid state. J. Incl. Phenom. Macrocycl. Chem. **57**, 173–177 (2007)
24. Sinha, V.R., Anitha, R., Ghosh, S., Nanda, A., Kumria, R.: Complexation of celecoxib with β-cyclodextrin: characterization of the interaction in solution and in solid state. J. Pharm. Sci. **94**, 676–687 (2005)
25. Brun, H., Paul, M., Razzouq, N., Binhas, M., Gibaud, S., Astier, A.: Cyclodextrin inclusion complexes of the central analgesic drug nefopam. Drug Dev. Ind. Pharm. **32**, 1123–1134 (2006)
26. Higuchi, T., Connors, K.A.: Phase solubility techniques. Adv. Anal. Chem. Instr. **4**, 117–212 (1965)
27. Chadha, R., Kashid, N., Jain, D.V.S.: Characterization and quantification of amorphous content in some selected parenteral cephalosporins by calorimetric method. J. Therm. Anal. Calorim. **81**, 277–284 (2005)
28. Chadha, R., Kashid, N., Jain, D.V.S.: Microcalorimetric evaluation of the in vitro compatibility of amoxicillin/clavulanic acid and ampicillin/sulbatam with ciprofloxacin. J. Pharm. Biomed. Anal. **36**, 295–307 (2004)
29. Dotsikas, Y., Loukas, Y.L.: Efficient determination and evaluation of model cyclodextrin complex binding constants by electrospray mass spectrometry. J. Am. Soc. Mass Spectrom. **14**, 1123–1129 (2003)
30. Guernelli, S., Lagana, M.F., Mezzina, E., Ferroni, F., Siani, G., Spinelli, D.: Supramolecular complex formation: a study of the interactions between β-cyclodextrin and some different classes of organic compounds by ESI-MS, surface tension measurements, and UV/Vis and ^{1}H NMR spectroscopy. Eur. J. Org. Chem. **24**, 4765–4776 (2003)
31. Job, P.: Recherches sur la formation de complexes mineraux en solution et sur leur stabilite. Ann. Chem. **9**, 114–1132 (1928)