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Research paper

Cyclodextrin complexes of valdecoxib: properties and anti-inflammatory activity in rat

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Abstract

The influence of natural β-cyclodextrin and its hydrophilic derivatives (HPβCd and SBE7βCd) on the in vitro dissolution rate, in vivo absorption and oral bioavailability of a poorly water soluble anti-inflammatory agent, valdecoxib (VALD) was studied. Equimolar drug–cyclodextrin solid complexes were prepared by kneading and coevaporation methods and characterized by infrared spectroscopy, differential scanning calorimetry, X-ray diffraction. In the liquid state, the cyclodextrin complexes were studied using phase solubility analysis, ^{1}H nuclear magnetic resonance and circular dichroism spectroscopy. Drug solubility and dissolution rate in distilled water were notably improved by employing the βCds. The DP_{15} (i.e. percent of dissolved VALD at 15 min) was 10.5% for the pure drug and 50, 91 and 93% for VALD-βCd, VALD-HPβCd and VALD-SBE7βCd complexes, respectively. Moreover, it was found that in the, the cyclodextrin complexes of drug showed significant improvement in the anti-inflammatory activity.

Keywords: Valdecoxib; Cyclodextrin complexation; Modified β-cyclodextrins; Anti-inflammatory activity

1. Introduction

Valdecoxib 4-(5-methyl-3-phenyl-4-isoxazolyl) benzenesulfonamide, a novel highly selective COX-2 inhibitor is used for a variety of acute and chronic inflammatory diseases. However, its very low aqueous solubility and poor dissolution could cause formulation problems and limits its therapeutic application by delaying rate of absorption and finally the onset of action [1]. Cyclodextrins are commonly used in drug formulations as solubility enhancers because of their ability to form water-soluble inclusion complexes with poorly water-soluble drugs [2,3]. Various anti-inflammatory drugs have been complexed with cyclodextrins, obtaining in this case further advantages such as dose lowering, reduction of side effects (particularly gastric irritation) and taste masking [4,5]. Both the nature of the cyclodextrin (native or chemically modified, crystalline or amorphous) and the method of complexation may play a role in drug solubilization [6]. Therefore, it seemed of interest to extend our investigation to a series of binary systems of valdecoxib with crystalline native β-cyclodextrin (βCd) and its amorphous and highly soluble derivatives, hydroxypropyl β-cyclodextrin (HPβCd) and sulfobutyl ether 7 β-cyclodextrin (SBE7βCd).

The drug–cyclodextrin complexes were prepared by methods such as kneading and coevaporation. The influence of the method of complexation on the physico-chemical properties of the drug–Cd complex was investigated in order to select the most effective system for improving valdecoxib dissolution properties and in vivo performance. Drug cyclodextrin interactions in solution were investigated by phase solubility analysis supported by ^{1}H nuclear magnetic resonance and circular dichroism spectroscopy. Differential scanning calorimetry, infrared spectroscopy and powder X-ray diffractometry were used to characterize the solid

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state of all binary systems, whereas their dissolution properties were evaluated according to USP XXI/XXII paddle method. Furthermore, an additional objective of the present work was to study the anti-inflammatory activity of these Cd-based formulations.

2. Materials and methods

2.1. Materials

Valdecoxib (VALD) was a gift sample from Ajanta Pharma (Mumbai, India). β-Cyclodextrin, and hydroxypropyl-β-cyclodextrin (average degree of substitution; 5.5) was kindly provided by S.A. Chemicals (Mumbai, India) and sulfobutyl ether-7-β-cyclodextrin was generously donated by Cydex, Inc. (Overland Park, KS). All reagents and solvents used were of analytical grade.

2.2. Phase solubility studies

An excess of drug was added to 10 ml of water or Cd aqueous solutions (0.003–0.015 M) in 25 ml stoppered conical flasks and shaken at constant temperature (37 °C). At equilibrium after 2 days, aliquots were withdrawn, filtered (0.22 μm pore size) and spectrophotometrically assayed for drug content at 244.5 nm (Shimadzu-UV 160A Spectrophotometer). Each experiment was carried out in triplicate (coefficient of variation (CV) $<3\%$). The apparent 1:1 stability constants of the VALD–Cd complexes were calculated from the phase-solubility diagrams.

2.3. Preparation of solid inclusion complexes

The inclusion complexes of valdecoxib with βCds were prepared by using the following two methods.

Kneading method: Kneaded products (KN) were prepared in a 1:1 molar ratio by wetting physical mixtures in a mortor with the minimum volume of ethanol–water 1:1 (by volume) mixture and kneading thoroughly with a pestle to obtain a paste which was then dried under vacuum at room temperature and stored in a dessicator until further evaluation.

Coevaporation method: Coevaporated products (COE) were prepared by coevaporation of equimolar drug–Cd ethanol–water (1:1 v/v) solutions on a water bath at 50 °C.

Each solid product was sieved through 80# and same fraction was used for the following tests. Physical mixtures (PM) were obtained by tumble mixing equimolar amounts of 80# fractions of respective simple components for 10 min.

2.4. Differential scanning calorimetry (DSC)

DSC analysis was performed using Shimadzu-Thermal Analyzer DT 40 on 2–8 mg samples (Sartorius BP 210 S electronic microbalance). Samples were heated in an open aluminium pans at a rate of 10 °C min^{-1} in a 30–300 °C temperature range under a nitrogen flow of 40 ml/min.

2.5. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra were recorded on samples prepared in KBr disks using Jasco FTIR-5300 spectrophotometer. Samples were prepared in KBr disks (2 mg sample in 200 mg KBr) by means of hydrostatic press at a force of 5.2 τ cm^{-2} for 3 min. The scanning range was 450–4000 cm^{-1} and the resolution was 4 cm^{-1}.

2.6. X-ray powder diffractometry

X-ray powder diffraction patterns were recorded on a Jeol JDX 8030 X-ray diffractometer using Ni-filtered, Cu Kα radiation, a voltage of 40 kV and a 25 mA current. The scanning rate employed was 1° min^{-1} over the 10–40° diffraction angle (2θ) range.

2.7. Nuclear magnetic resonance (NMR) spectroscopy

^{1}H-NMR spectra were recorded using a Bruker AVANCE 500 DRX (500 MHz) instrument. Samples were prepared in 0.6 ml of 0.1 N NaOD in D_2O. This mixture allows a suitable solubilization of the free drug and complexes for these experiments.

2.8. Circular dichroism (CD) spectroscopy

Circular dichroism spectra were obtained by a Jasco J-600 Spectropolarimeter. Absorbance of the samples was kept below 2 in the whole wavelength range explored (200–300 nm). All the spectra were corrected for the signal exhibited by the βCd, HPβCd and SBE7βCd solution in the absence of the guest. The signal to noise ratio was improved by superposition of five different scans.

2.9. Dissolution rate studies

Dissolution rate studies were performed in distilled water (pH 6.8) at 37 ± 0.5 °C, using USP XXI/XXII apparatus (Electrolab, India) with paddle rotating at 50 rpm. Solid products, each containing 5 mg of drug were subjected to dissolution. At fixed time intervals, samples withdrawn were filtered (pore size 0.22 μm) and spectrophotometrically assayed for drug content at 244.5 nm. Each test was carried out in triplicate (coefficient of variation (CV) $<3\%$). Dissolution efficiency (DE) was calculated from the area under the dissolution curve at time t (measured using the trapezoidal rule) and expressed as percentage of the area of the rectangle described by 100% dissolution in the same time [7].

2.10. Pharmacological studies

The anti-inflammatory study was performed using carrageenan induced rat hind paw edema method on Charles foster rats (180–200 g) [8]. The animals were housed into groups of six and maintained on a standard diet with free access to water. Animals were fasted overnight before experiment. Cd complexes (equivalent to 1 mg VALD kg^{-1}) or plain drug (1 mg/kg^{-1}) were administered orally as aqueous suspensions with 0.25% carboxymethyl cellulose (CMC). An aqueous solution of 0.25% CMC was administered to control group. Edema was induced by injecting 0.1 ml lambda carrageenan (1% w/v) into the plantar tissue of the hind-paw. The volume of the treated paw was measured with a plethysmometer at hourly intervals for a period of 5 h and the percentage inhibition of edema was calculated.

3. Result and discussion

3.1. Solubility studies

Valdecoxib is relatively insoluble in water (i.e. 10 μg/ml at pH 7.0 at 25 °C). The saturation solubility (So) of VALD at pH 6.8, 25 °C was 9.4 μg/ml, which is in good agreement with literature values [1]. Solubility studies showed that the concentration of VALD in distilled water at pH 6.8, 25 °C is notably affected by the presence of Cds. The phase solubility diagram obtained was linear as seen in Fig. 1 and such profiles according to Higuchi and Connors are of A_L type. Because these profiles are characterized by a slope of less than one, it was assumed that the solubility increase is due to the formation of 1:1 complex. Stability constant values obtained for VALD–Cd complexes were in the rank order of SBE7βCd (1422 M^{-1}) > HPβCd (300 M^{-1}) > βCd (149 M^{-1}).

3.2. Solid state studies

3.2.1. Differential scanning calorimetry (DSC)

The DSC curves of pure components and of the different drug–cyclodextrin equimolar systems are shown in Fig. 2.

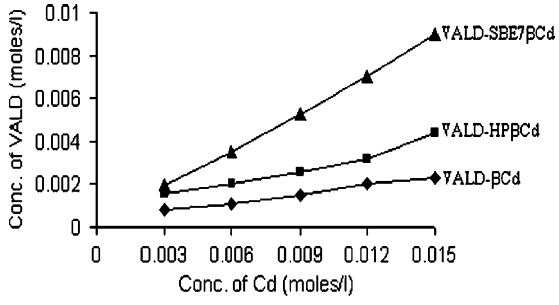

Fig. 1. Phase solubility diagram of VALD–cyclodextrin system. Each point is the mean (±SD) of three determinations.

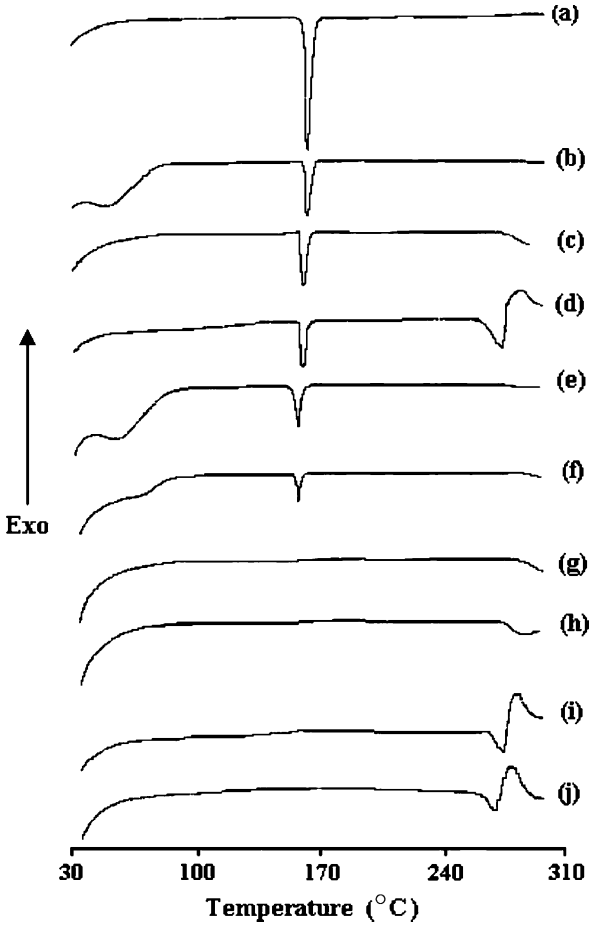

Fig. 2. DSC curves of VALD (a), VALD-βCd equimolar physical mixture (b), VALD-HPβCd equimolar physical mixture (c), VALD-SBE7βCd equimolar physical mixture (d), VALD-βCd kneaded product (e), VALD-βCd coevaporated product (f), VALD-HPβCd kneaded product (g), VALD-HPβCd coevaporated product (h), VALD-SBE7βCd kneaded product (i), VALD-SBE7βCd coevaporated product (j).

The DSC curve of VALD was typical of a crystalline anhydrous substance with a sharp melting endotherm (T_{onset} = 167.8 °C, T_{peak} = 172.8 °C). The characteristic thermal profile of the drug appeared to lower temperature but was still well recognizable in the physical mixtures with all the Cds and eventhough strongly reduced in intensity, in the inclusion compounds with βCd as a consequence of interaction between the components [9]. Total disappearance of drug thermal profile was instead observed in all the complexes with HPβCd and SBE7βCd. This phenomenon is generally considered as indicative of complex formation/drug amorphization and/or stronger interaction in the solid state between VALD and βCd derivatives.

3.2.2. Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra of all drug–Cd physical mixtures did not differ from that of the drug alone in the areas of the main VALD absorption bands and in particular the characteristic sulfonamide-stretching band of valdecoxib (1149 cm^{-1}) was unchanged. A shift to higher frequencies (ranging from

1153 to 1159 cm^{-1}) of this band was, on the contrary, observed for all the other binary products with Cds and was explained by the dissociation of the intermolecular hydrogen bonds associated with crystalline drug molecules [10]. This may be indicative of the drug monomeric dispersion as a consequence of the interaction with the Cds, which could result in its inclusion into the hydrophobic cavity of the carrier [11].

3.2.3. X-ray powder diffractometry

X-ray powder diffraction patterns of VALD and corresponding complexes with Cds are shown in Fig. 3. In the X-ray diffractogram of VALD powder, sharp peaks at a diffraction angle (2θ) of 12.26, 15.88, 19.88, 22.08, 23.92° are present and it suggests that the drug is present as a crystalline material. Diffraction peaks relevant to crystalline VALD were detectable in all the systems with βCd. In the physical mixtures with HPβCd and SBEβCd, the presence of free crystalline drug was revealed by few broad peaks of low intensity which emerged on the diffuse background due to the amorphous carrier, indicating a clear loss of crystallinity of drug. Complete drug amorphization was instead observed in all other products of VALD with each amorphous βCd derivative. A similar behavior was previously reported for ibuproxam [12], ketoprofen and ibuprofen [13].

3.3. Circular dichroism (CD) spectroscopy

The interaction of valdecoxib with βCd and its derivatives in aqueous solution was investigated by CD spectroscopy. CD spectra of valdecoxib in the presence of cyclodextrins in distilled water are shown in Fig. 4. VALD alone gave no CD band at experimental conditions studied, because it has no asymmetric carbon atom in a molecule. In the presence of βCd and HPβCd the optical activity of VALD was induced at 245, 230 and 220 nm with positive sign and a very small negative peak at 210 nm as a result of perturbation of the electronic transition of the molecule caused by asymmetric cavity of cyclodextrin following complexation. In the presence of SBE7βCd, valdecoxib showed a similar induced circular dichroism (ICD) spectra but with the shift of positive bands to lower wavelength region. The change in spectral modifications observed in the presence of SBE7βCd can be considered the effect of a stronger interaction of SBE7βCd with VALD and/or to the different conformation of VALD in the cavity of SBE7βCd [14].

It is well known that cyclodextrins have neither CD nor absorption band at wavelengths longer than 220 nm and the inclusion of optically inactive compounds within the cyclodextrin cavity generates extrinsic Cotton effect in the wavelength region of drug chromophores. Thus, the results of CD spectroscopy reveals that VALD is embedded

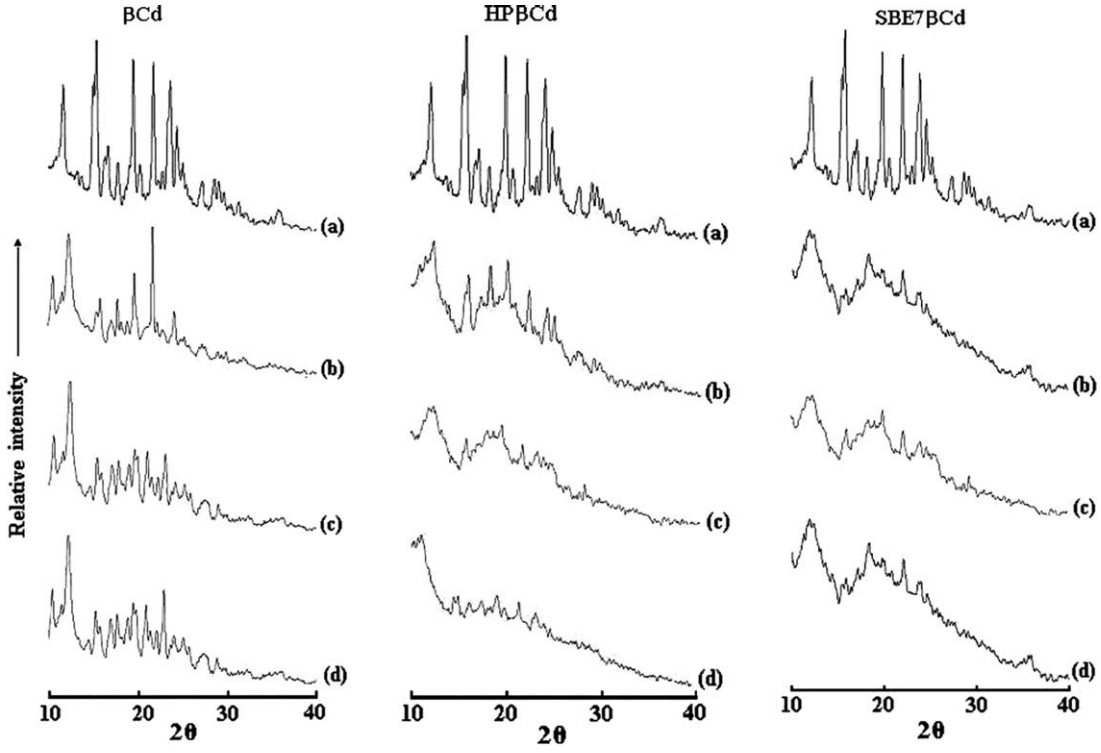

Fig. 3. XRD spectra of plain VALD (a) and of equimolar physical mixtures (b), kneaded (c) and coevaporated (d) complexes with βCd and its amorphous derivatives.

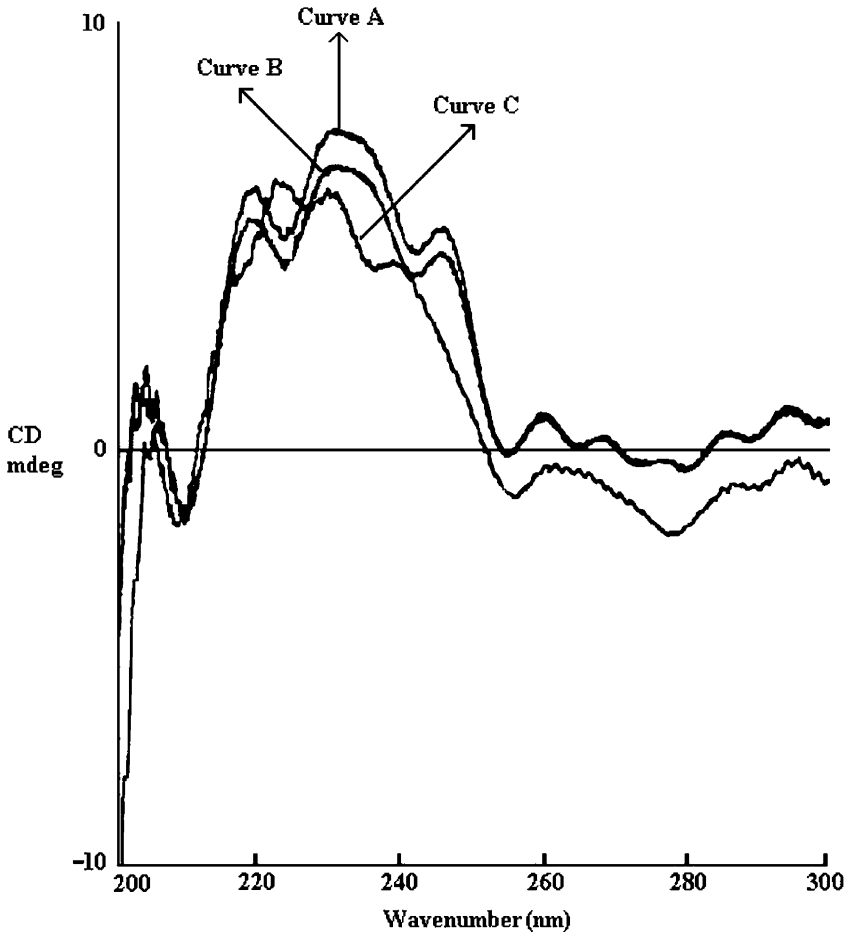

Fig. 4. CD spectra of VALD–cyclodextrin system. Curve A: VALD-HPβCd system. Concentration: VALD (3.2×10^{-5} M). Curve B: VALD-βCd system. Concentration: VALD (3.6×10^{-5} M). Curve C: VALD-SBE7βCd system. Concentration: VALD (3.6×10^{-5} M). Solvent: distilled water.

in the asymmetric locus of the cyclodextrin cavities and the conformation of VALD within the cavity of SBE7βCd is somewhat different from that of βCd and HPβCd.

3.4. Nuclear magnetic resonance (NMR) spectroscopy

Evidence of inclusion complex formation for the VALD–Cd system was obtained by NMR spectroscopy. Valdecoxib is a diaryl substituted isoxazole moiety. All the proton signals except the aromatic protons in the ^{1}H NMR spectrum of pure VALD are essentially located in a narrow range (4.6–4.7), which makes proton assignments difficult. However, it was observed that in the ^{1}H NMR spectrum of VALD–Cd solid complex, as presented in Fig. 5 the signals of the aromatic protons of VALD showed a significant spectral shift as shown in Table 1. This might suggest that the drug molecule interact with the cyclodextrin cavity providing an inclusion complex. The ^{1}H NMR spectra of the VALD phenyl groups in free and complexed form obtained under present conditions show only spectral shift changes of the corresponding signals and as such there are no new peaks that could be assigned to the complexes.

However, results from ^{1}H NMR study suggest that large spectral changes of VALD were observed in the mono-substituted aryl ring, where as the unsubstituted phenyl ring showed no considerable shifts. These results suggest that Cd cavity includes preferably the mono substituted phenyl ring of the valdecoxib.

3.5. Dissolution rate studies

The results in terms of dissolution efficiency and percent of active ingredient dissolved at 15 min are collected in Table 2. The improvement of dissolution rate obtained with physical mixtures can be attributed to both, improved drug wettability due to presence of hydrophilic cyclodextrin which can reduce the interfacial tension between poorly soluble drug and dissolution medium and formation of readily soluble complexes in dissolution medium [15]. As for the other systems, in case of combinations with βCd, both methods, kneading and coevaporation found to be

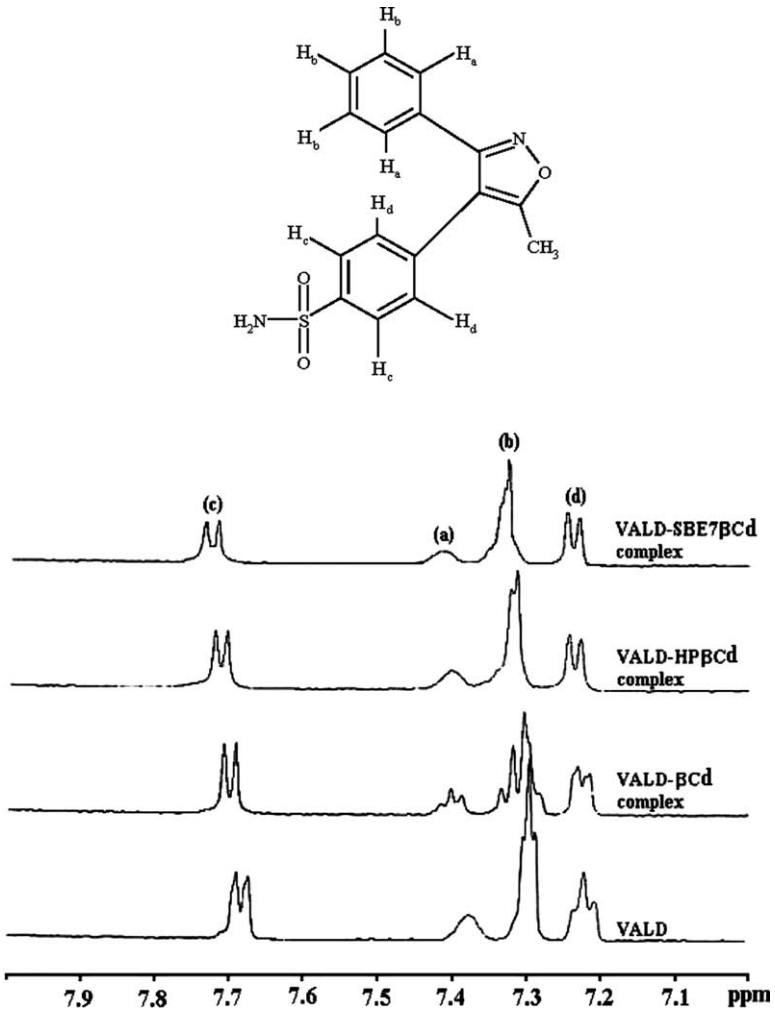

Fig. 5. ^{1}H NMR spectra of free VALD and VALD–Cd complexes.

similar in achieving the enhancement of drug dissolution rate (about 50% of drug dissolved at 15 min). On the contrary, for preparations with HPβCd and SBE7βCd, kneading method showed the greatest improvement of drug dissolution rate (about 90% of drug dissolved at 15 min) followed by coevaporation. The dissolution efficiencies of kneaded and coevaporated products were two to three times higher than those of the corresponding physical mixtures and five to eight times higher than that of plain drug.

Table 1
Chemical shifts (ppm) for the aromatic protons of VALD in the free state and in the inclusion complex with βCd, HPβCd and SBE7βCd

H valdecoxib	δ_{free}	$\delta_{complex}$			$\delta_{complex} - \delta_{free}$		
		βCd	HPβCd	SBE7βCd	βCd	HPβCd	SBE7βCd
a	7.378	7.403	7.404	7.417	0.025	0.026	0.039
b	7.298	7.319	7.323	7.327	0.021	0.025	0.029
c	7.691	7.709	7.722	7.736	0.018	0.031	0.045
d	7.223	7.232	7.245	7.247	0.010	0.022	0.024

Table 2
Dissolution parameters of valdecoxib (VALD) alone and its equimolar physical mixtures (PM), kneaded (KN) and coevaporated (COE) products with βCd, HPβCd and SBE7βCd

Sample		DP_{15}	DE_{60}
VALD	–	10.3	11.8
βCd	PM	18.4	22.8
	KN	50.7	54.5
	COE	50.7	55.8
HPβCd	PM	27.7	30.5
	KN	91.9	88.1
	COE	75.4	74.6
SBE7βCd	PM	27.7	33.0
	KN	93.4	91.4
	COE	89.2	87.0

DP_{15}, percent drug dissolved at 15 min; DE_{60}, dissolution efficiency at $t=60$ min (calculated from the area under the dissolution curve at $t=60$ min and expressed as % of the area of the rectangle described by 100% dissolution in the same time). Each value is the average of three determinations (coefficient of variation CV < 2.5%).

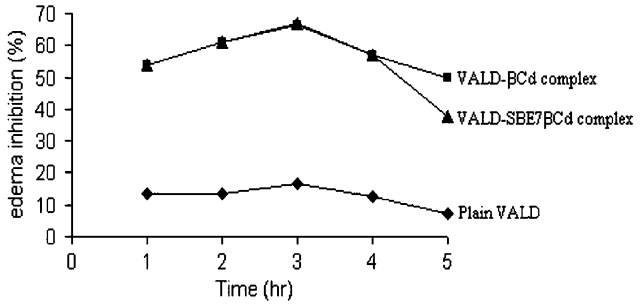

Fig. 6. Anti-inflammatory activity of valdecoxib and its complexes with βCds prepared by kneading.

The effectiveness of βCd derivatives can also be explained on the basis of their greater hydrosolubility, wetting, and solubilizing effect towards VALD. SBE7βCd can be considered as the most effective carrier for enhancing VALD solubility and dissolution properties, according to the results of phase solubility studies. However, also HPβCd and natural βCd are not to be discarded as VALD solubilizing carriers, considering that excessively large stability constants of inclusion complexes have been reported to reduce the drug in vivo absorption rate [16,17].

3.6. Pharmacological studies

To examine whether the notable increase in dissolution rate observed with Cd-based formulations may lead to differences in pharmacological effects, we explored the anti-inflammatory profiles of VALD, VALD–βCd and VALD–SBE7βCd complexes using the rat paw edema method.

As shown in Fig. 6, VALD alone showed slow in vivo absorption giving maximum % inhibition of edema (16%) after a period of 3 h. In contrast, valdecoxib included in the cavity of both the cyclodextrins showed high absorption rate in vivo achieving more than 50% inhibition of edema in the 1 h and maximum percentage of inhibition of edema (66%) after a period of 3 h. These results only partially agreed with those obtained from phase solubility studies. In fact, the significant difference observed for the aqueous solubility and the stability constant of the VALD–SBE7βCd complex in comparision to the natural βCd, was not reflected in pharmacological response of the respective binary systems. In particular, no significant difference ($P > 0.1$) was found between the maximum inhibition of edema for products with βCd and SBE7βCd and this may be attributed to large stability constant of VALD–SBE7βCd system which might have reduced drug in vivo absorption rate.

4. Conclusion

It was shown that the properties of binary systems of VALD with βCd and amorphous βCd derivatives are influenced by the type of cyclodextrin. The use of several different physicochemical characterization methods enabled us to fully characterize and evaluate the products and compare their properties in depth. Kneading technique seemed to be of great interest and utility in order to obtain an improvement in the physicochemical properties of VALD. Pharmacological evaluation in rat indicated that the VALD–Cd complexes might be used in developing a new solid oral formulation with an in vivo performance much better than that of VALD alone. Since there is a direct relationship between the rate of absorption and pharmacological effect, the implications of the in vivo data are that the Cd based formulations can be considered for developing fast dissolving formulation of valdecoxib for quick relief of pain.

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