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ORIGINAL ARTICLE

Colon targeting of fluticasone propionate inclusion complex: a novel approach in inflammatory bowel disease

Naveen K. Thakral · Alok R. Ray · Jette Jacobsen · Daniel Bar-Shalom · André Huss Eriksson · Dipak K. Majumdar

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Abstract Purpose of the present research was to present fluticasone propionate, a glucocorticoid, as a novel formulation exhibiting improved aqueous solubility, and targeting the drug directly to colon for the treatment of inflammatory bowel disease. Inclusion complex of the drug with hydroxypropyl betacyclodextrin were prepared by solvent evaporation and subsequently the granules of the inclusion complex were coated with Eudragit S100, in order to achieve colon targeting. Inclusion complex was characterized by FTIR, DSC, XRD and ^{1}H-NMR studies. In vitro drug release from coated granules and the drug transport across excised rat colon using modified Ussing chamber were also attempted. The drug was found to be present in amorphous form, when included in HPβCD cavities. Furthermore, intrinsic dissolution of the drug was found to increase by $\sim$18 times. Coated granules exhibited no drug release in 0.01 N HCl as dissolution medium, indicating gastro-resistance, while 92 % of the drug was released in 120 min, in phosphate buffer (pH 7.4) as dissolution medium. The drug transport studies with rat colon led to more drug transport and concentration in target tissue, when presented as inclusion complex. The formulation releases the drug with improved aqueous solubility in colonic region, and thus concentrating the drug at the target tissue itself.

Keywords Fluticasone propionate · Inclusion complex · Colon targeting · Ussing chamber · Eudragit S100

N. K. Thakral · A. R. Ray
Centre for Biomedical Engineering, Indian Institute of Technology Delhi, Hauz Khas, New Delhi 110016, India
e-mail: nk_thakral@rediffmail.com

A. R. Ray
Biomedical Engineering Unit, All India Institute of Medical Science, New Delhi 110029, India

J. Jacobsen · D. Bar-Shalom
Department of Pharmaceutics and Analytical Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark

A. H. Eriksson
Bioneer:FARMA, Department of Pharmaceutics and Analytical Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark

D. K. Majumdar (✉)
Department of Pharmaceutics, Delhi Institute of Pharmaceutical Sciences and Research (Formerly College of Pharmacy), University of Delhi, Pushp Vihar, Sector III, New Delhi 110017, India

Introduction

Inflammatory bowel disease (IBD) is a group of disorders that cause the sections of the gastrointestinal tract to become inflamed and ulcerated. An abnormal response from the body's immune system plays a role in each of the two main forms of IBD namely Crohn's disease and ulcerative colitis. These are usually lifelong disease and require ongoing medication [1]. Glucocorticoids constitute a major part of medical treatment of inflammatory bowel disease [2], but conventional glucocorticoids (cortisone, prednisone) produce troublesome systemic side effects, such as acne, moon-face, hypertension, dyspepsia, mood disturbances, insomnia and impaired glucose tolerance [3]. To avoid these side effects, the efforts are on to develop steroids with improved topical action. Hydrocortisone and prednisolone retention enemas and foam preparations have been developed for distal ulcerative colitis and proctitis [4–6], but these proved to be of little or no use due to effect

on adrenal gland functions and other systemic side effects [7–9]. Fluticasone propionate (FP), a new generation glucocorticoid, exerts a potent anti-inflammatory action when administered topically (e.g. in the lower airways). The drug has been evaluated for use in different IBD conditions with encouraging results [10]. However, when administered orally, almost 100 % of the drug is subjected to first pass metabolism and metabolized via the cytochrome P-450 system in liver [2, 11]. Moreover, its poor aqueous solubility does not allow its transport to mucosal surface, which eventually leads to poor absorption with oral administration. The poor absorption of the drug along with high first pass metabolism leads to the negligible oral bioavailability (<1 %) of the drug [12, 13]. First pass metabolism is the fundamental reason for the safe use of potent topical glucocorticoids in IBD conditions [14]. The local action of the drug can be improved by facilitating local transport of the drug to colonic mucosal surface, which in turn can be achieved by improving the aqueous solubility of the drug.

The cyclodextrins (CDs) are water-soluble macrocyclic polymers. Chemically they are cyclic oligosaccharides containing at least 6 D-(+) glucopyranose units attached by α-1,4 glucosidic linkage. Molecules with suitable size and shape can be held within the cavity of cyclodextrin to form inclusion compounds [15]. The lipophilic cavity of cyclodextrin molecules provides a microenvironment into which appropriately sized non-polar moieties can enter to form inclusion complexes [16]. No covalent bonds are broken or formed during formation of inclusion complexes [17]. The main driving force for the complex formation is the release of enthalpy-rich water molecules from the cavity of CD. Water molecules are displaced by more hydrophobic guest molecules to attain apolar–apolar association and decrease of cyclodextrin ring strain resulting in a more stable lower energy state [18]. Inclusion in cyclodextrins exerts a strong effect on the physicochemical properties of guest molecules, for instance in the form of solubility enhancement of highly insoluble guests, as they are temporarily caged within the host cavity [19]. The 3 natural CDs, viz. α, β and γ CDs (with 6, 7, and 8 glucose units respectively) differ in their ring size and solubility. The cavity size of α-CD (4.7–5.3 Å) is insufficient for most drugs and that of γ-CD (7.5–8.3 Å) is sufficient but very expensive. Because of ready availability and optimum cavity size, β-CD (6.0–6.5 Å), has been widely used in the pharmaceutical applications, but poor aqueous solubility and reported nephrotoxicity of β-CD [20], has led to a search for more soluble and safer derivatives of cyclodextrins [21]. Hydroxypropyl betacyclodextrin (HPβCD), hydroxypropyl derivative of cyclodextrin, is reported to possess enhanced aqueous solubility and reduced nephrotoxicity [22, 23], and hence was selected for the present study in order to improve aqueous solubility of FP.

Targeted delivery to the colon leads to delivery of the drug specifically to the colon to achieve a high local concentration, and thus reduce the dose and undue side effects. The specific pH conditions of the colonic region can be exploited to deliver the drug to the region by developing a pH- dependent system [24–27]. A polymer that would release the drug at colonic pH i.e. pH >7 appear suitable for the same. Eudragit S100 is an anionic polymer synthesized from methacrylic acid and methacrylic acid methyl ester, and has solubility in the region of the digestive tract where juices are neutral to alkaline, i.e. pH >7. Thus, the purpose of the present research was to increase the aqueous solubility of FP by preparing inclusion complex with HPβCD and subsequently to achieve colon targeting of the drug by coating the granules of FP-HPβCD inclusion complex with Eudragit S100.

Materials and methods

Materials

Fluticasone propionate (FP), Eudragit S100 and hydroxypropyl betacyclodextrin (HPβCD) were kind gifts from Nexus Pharmachem Pvt. Ltd. (Ahmadabad, India), Evonic Industries (Mumbai, India) and Gangwal Chemicals (Mumbai, India), respectively. The following ingredients, were of analytical grade: Span 80 (Loba Chemie Pvt. Ltd., Mumbai, India), liquid paraffin and polyvinyl pyrrolidone (Molecular weight 40,000) (Merck Specialties Pvt. Ltd. Mumbai, India), petroleum ether (RFCL Ltd. New Delhi, India), ethanol anhydrous (Kemetyl A/S, Denmark), ammonium dihydrogen phosphate, isopropyl alcohol, sodium hydroxide (NaOH), hydrochloric acid (HCl), di sodium hydrogen phosphate, potassium bromide (KBr), sodium dihydrogen phosphate and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (Sigma-Aldrich Labochemikalien GmbH), deuterated dimethyl sulfoxide (DMSO) (Sigma-Aldrich USA), magnesium chloride, calcium chloride, potassium chloride, sodium bi-carbonate and sodium chloride (Merck K GaA, Darmstadt, Germany). Methanol and acetonitrile (Sigma-Aldrich Labochemikalien GmbH) were of HPLC grade. Ringer solution [each 1,000 ml contains di-sodium hydrogen phosphate (Na_2HPO_4), 227.1 mg; sodium di-hydrogen phosphate (NaH_2PO_4), 47.9 mg; magnesium chloride ($MgCl_2{\cdot}6H_2O$), 243.9 mg; calcium chloride ($CaCl_2{\cdot}2H_2O$), 176.4 mg; potassium chloride (KCl), 372.7 mg; sodium chloride (NaCl), 6545.6 mg; sodium bi-carbonate ($NaHCO_3$), 2100.1 mg; Glucose, 1999.8 mg; HEPES, 5957.6 mg; de-ionized water up to 1,000 ml; pH adjusted to 7.4 with NaOH (5 M).] was used.

HPLC analysis of FP

FP was analyzed as per the method described in British Pharmacopoeia 2009, using a Merck Hitachi Instrument having pump (L-7100), autosampler (L-7200), interface (D-7000), UV detector (L-7400), column oven (L-7350) and column C 18(2) of the size 250 × 4.6 mm, containing stationary phase octadecylsilyl silica gel for chromatography R (5 µm) (Luna 136527-21). Mobile phase used for the analysis contained 15 volumes of acetonitrile, 35 volumes of 1.15 g/l solution of ammonium dihydrogen phosphate, adjusted to pH 3.5, and 50 volumes of methanol. An injection volume of 20 µl was injected maintaining a flow rate of 1.5 ml/min, and a temperature of 40 °C, and FP was detected at 239 nm.

Phase solubility studies

Phase solubility studies were carried out as per the method described by Higuchi and Connors [28]. Thus, an excess amount of FP (30 mg) was added to 5 ml of aqueous solutions containing varying concentrations of HPβCD (0–12 mM) in sealed glass tubes. The suspensions were placed in an incubator shaker for 24 h at 37 °C and 50 rpm. After removal from the incubator shaker, the tubes were left to stand for 2 h to allow the un-dissolved matter to settle. The solutions were filtered through a 0.45 µm filter membrane and the concentration of FP dissolved was determined by HPLC analysis. The apparent stability constant K_s for the complex was calculated from the phase solubility diagram according to the following equation:

$$K_S = \text{Slope}/S_o(1 - \text{Slope})$$

where S_o is the intrinsic solubility of FP. The complexation efficiency (CE) for the complex [29] was calculated as follows:

$$\text{CE} = \frac{[\text{D/CD}]}{[\text{CD}]} = S_o \cdot K_s = \frac{\text{Slope}}{(1 - \text{Slope})}$$

where [D/CD] is the concentration of dissolved complex, [CD] is the concentration of dissolved free cyclodextrin and Slope is the slope of phase solubility profile.

Preparation of FP inclusion complex (FPIC)

FP was dissolved in boiling ethanol (78 °C), and HPβCD was added to achieve 1:1 molar ratio. The solution was evaporated in a rotary evaporator at 45 °C and vacuum at 70 mbar (BÜCHI, Switzerland, Rotavapor R-210) to obtain powder of inclusion complex (FPIC).

Preparation and encapsulation of the FPIC granules

Polyvinyl pyrrolidone (500 mg) was dissolved in 8 ml of isopropyl alcohol. The solution was mixed with the FPIC powder (4 g) and the resultant mass was passed through a sieve (#20) and air dried to achieve granules of FPIC.

The obtained granules were coated with Eudragit S100. A solution of Eudragit S100 was prepared in acetone (2 g/20 ml). Granules of FPIC (500 mg) were suspended in this solution, followed by immediate emulsification in liquid paraffin containing 2 % w/v of Span 80 with the help of a mechanical stirrer (1,500–2,000 rpm) [26, 27]. The stirring at room temperature was continued for 3 h for the complete evaporation of the acetone. The coated granules were filtered and washed with petroleum ether and dried in vacuum desiccators to obtain coated granules of FPIC (cFPIC).

Characterization of FPIC

Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of FP, HPβCD, their physical mixture and FPIC were recorded using an FTIR spectrophotometer (Bruker model Alpha) in the range of 4,000–500 cm^{-1} as KBr pellets.

X-ray diffraction (XRD)

X-ray diffractograms of FP, HPβCD, their physical mixture and FPIC were recorded using an X-ray diffractometer (X'Pert Pro, PW 3050/PW 3071; Lelyweg, The Netherlands) using nickel-filtered CuKα radiation (λ = 1.540598 Å) generated at 40 kV and 30 mA, and a scanning rate of 2°/min over a 2θ range of 10–80°.

Differential scanning calorimetry (DSC)

Thermal analysis of FP, HPβCD, their physical mixture and FPIC was performed using a DSC-TA system (Perkin Elmer). The sample was sealed in a crimped aluminum pan by application of the minimum possible pressure and heated at a rate of 10 °C/min from 20 to 320 °C in a nitrogen atmosphere. An empty aluminum pan was utilized as the reference pan.

^{1}H-nuclear magnetic resonance (^{1}H-NMR)

^{1}H-NMR spectra of FP, HPβCD and FPIC were obtained on Bruker Spectrospin-300 at 300 MHz at 293 K, using DMSO as solvent and tetramethylsilane (TMS) as internal reference.

Estimation of drug content

Accurately weighed FPIC and cFPIC granules equivalent to 10 mg of FP were dispersed in 100 ml ethanol each and subjected to centrifugation at 3,000 rpm for 10 min. The supernatants were withdrawn and diluted with ethanol. The resulting solutions were analyzed for drug content by HPLC as described above.

Intrinsic dissolution of FP and FPIC

Intrinsic dissolution of FP and FPIC powder was carried out to assess the change in rate and extent of dissolution. Accurately weighed FP (200 mg) was transferred into the die cavity of a Wood's apparatus. A punch was placed in die cavity. The powder was compressed in a hydraulic press (Graseby Specac) at a pressure of 2,000 psi for 1 min. Three such pellets were prepared. The same procedure was adopted for the preparation of three pellets of FPIC containing the equivalent of 200 mg of FP. The pellets thus formed were mounted on the shafts of a 7 station dissolution apparatus (ERWEKA-DT 70). The height of the shaft was adjusted so that height of the lower surface of pellet remained 3.8 cm from the bottom of the dissolution vessel. The dissolution medium used was 900 ml of phosphate buffer (pH 7.4), and the experiment was conducted for 3 h at 100 rpm. During the experiment, air bubbles were not allowed to be formed on the compacted pellet or die surface. Samples (5 ml) were withdrawn at suitable time intervals and analyzed for drug content by HPLC. Each withdrawn sample was replaced with fresh dissolution medium.

In vitro drug release profile

Accurately weighed cFPIC granules equivalent to 10 mg of drug were filled into a hard gelatin capsule (size 4) and placed in a dissolution apparatus (USP type 1) containing 900 ml of 0.01 N HCl (pH 2.0) as release medium (ERWEKA-DT 70) at 37 °C and the drug release study was conducted for 2 h at 50 rpm. Sample (5 ml) was withdrawn at the end of the experiment, filtered through a 0.45 μm filter and analyzed for drug contents by HPLC method as described above. All the experiments for studying the in vitro release profile were conducted in triplicate.

In a similar experiment, accurately weighed cFPIC granules equivalent to 10 mg of drug were filled in a hard gelatin capsule (size 4) and placed in a dissolution apparatus containing 900 ml of phosphate buffer (pH 7.4), keeping all other conditions as above. Samples (5 ml) were withdrawn at regular intervals, for up to 3 h, and each withdrawn sample was replaced with an equal volume of fresh release medium. The samples were filtered through a 0.45 μm filter and analyzed by HPLC as described previously. Since the granules has been coated with Eudragit S100, a pH sensitive polymer, which dissolve only at a pH above 7, the formulation was tested for drug release at colonic pH i.e. pH 7.4.

In vitro drug transport across rat colon

Ten to fifteen centimeters section of distal colon from a healthy male Sprague–Dawley rat (Taconic, Lille Skensved, Denmark) was cut along the mesenteric line and cleaned with Ringer solution. Tissues without Peyer's patches were mounted in modified Ussing chamber at 37 °C, 5 % carbogen for stirring, aperture 0.4 cm^2 ($n = 8$) (EM-LVSYS-8, Physiological Instruments, San Diego, Ca, USA) applying 2.0 ml Ringer in donor and receiver chamber, respectively. After 20 min equilibration time the Ringer solution was removed and the transport study was initiated applying 2.1 ml (199.76 nM/ml) of FP in Ringer ($n = 4$) or 2.1 ml of FPIC in Ringer (equivalent to 199.76 nM/ml of FP) ($n = 4$) in donor chamber and 2.1 ml Ringer in receptor chamber. Sampling (100 μl) from both chambers was done at $t = 0$ and $t = 110$ min. The spontaneous transepithelial potential difference [PD(mV)] with reference to serosal bathing solution and the short circuit current [$I_{sc}(\mu amp/cm^2)$] were measured using automatic voltage clamps (Physiologic Instrument VCC600, Precision Instrument Design, CA) at different time intervals and transepithelial resistance (TEER) [$R_t(\Omega\ cm^2)$] was calculated using the resistance graph or Ohm's law ($R_t = PD/I_{sc}$), to monitor the tissue integrity. The drawn samples were analyzed by HPLC as described before. After completion of the experiment, 100 μl of carbachol (100 μM), a drug that binds and activates the acetyl choline receptors (cholinergic agonist) was added to all the receptor chambers to assess the viability of the tissues. At the end of the experiment, estimation of the amount of drug retained in the tissues was conducted by washing the tissues twice with Ringer solution and subsequently air drying and dropping the same in centrifuge vials prefilled with 2 ml acetonitrile. All the centrifuge vials were swirled for 20 min and then centrifuged (Biofuge-15, Heraeus-Sepatech) at 4,000 rpm for 30 min. The supernatant from all the vials was subjected to drug content analysis by HPLC.

Results and discussion

Phase solubility study

The phase solubility diagram of FP, as depicted in Fig. 1, was found to be linear AL type, as per the classification of Higuchi and Connors [28]. This suggests establishment of 1:1 stoichiometry of FP and HPβCD complex over the concentration range (0–12 mM). The solubility of FP was

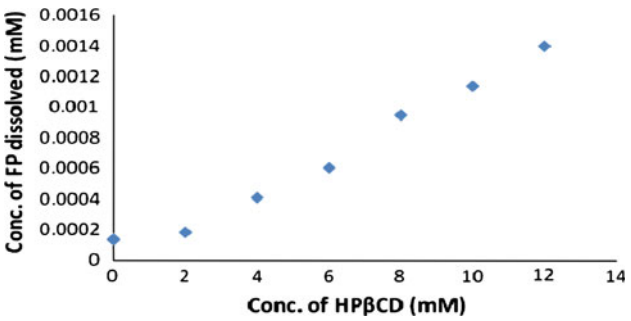

Fig. 1 Phase-solubility profile of FP in presence of HPβCD. The figure depicts a negative deviation at low HPβCD concentrations (AL^-)

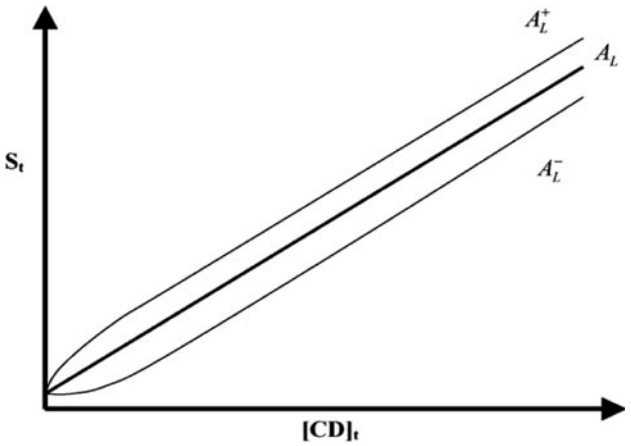

Fig. 2 Linear phase-solubility profiles. A normal profile (AL), a profile with a positive deviation at low cyclodextrin concentrations (AL^+) and a profile with a negative deviation at low cyclodextrin concentrations (AL^-). (Reproduced from 'reference number 30', with permission from Elsevier.)

found to increase up to 10.17-fold in presence of 12 mM of HPβCD. The apparent stability constant (K_S) calculated from phase solubility diagram was used to compare the affinity of drug for the complexing agent. In the phase solubility diagram, the intrinsic solubility (S_o) should ideally be equal to the intercept (S_{int}), but this mainly applies in case of drugs with intrinsic solubility greater than about 1 mM. In the case of drugs with intrinsic solubility below about 0.1 mM, a strong negative deviation is reported to be observed (Fig. 2). The reason for the lower value of S_{int} than S_o may be attributed to the non-ideality of water as a solvent. This negative intercept deviation ($S_o > S_{int}$), results in an AL(−) type of curve, which leads to overestimation of K_S when calculated from slope and intercept [30].

In the present case, K_S was found to be 558 M^{-1} when calculated from S_o and 1,606 M^{-1} when calculated from S_{int} (Fig. 1), clearly indicating overestimation, which infers that, for the present study, phase solubility may not be a reliable method for determination of K_S.

Loftsson et al. [30] reported that average complexation efficiency (CE) value for 28 different drug-HPβCD or drug-randomly methylated betacyclodextrin (RMβCD) complex is about 0.3, indicating that on an average only about one out of every four cyclodextrin molecules in solution are forming water-soluble complex with poorly soluble drug, assuming 1:1 drug/cyclodextrin complex formation. In the present study the CE value for the complex was found to be 0.385, indicating that one out of every three cyclodextrin molecules in solution forms a water-soluble complex with drug. Since CE is dependant only on slope of phase solubility curve and not on S_o or S_{int}, it shows lesser variation than in the case of K_S.

Characterization of FPIC

FTIR spectra

The FTIR spectra of FP, HPβCD, their physical mixture and FPIC are presented in Fig. 3. The FTIR spectrum of FP shows a peak at 3318.65 cm^{-1} of –OH stretching suggesting external hydrogen bonding in crystalline FP. The peak at 1740.33 cm^{-1} is attributed to a carbonyl group (C=O) being attached to aliphatic ring and the one at 1698.29 cm^{-1} is due to a carbonyl group being attached to sulfur (S–C=O). The peak at 1658.25 cm^{-1} indicates C=O stretching and the one at 1608.65 cm^{-1} indicates C=C stretching vibrations in the quinonoid aromatic ring. The peak at 1271.8 cm^{-1} indicates C–F stretching vibrations, while the peak at 1028.96 cm^{-1} is due to F–C-S stretching vibrations. The peak at 882.14 could be attributed to OOC/CCH aromatic deformation [31].

The FTIR spectrum of HPβCD exhibits a broad band of hydroxyl group at 3319.6 cm^{-1} and vibration bands of C–O and O–H groups at 1150.48 and 1005.97 cm^{-1}. Furthermore, a peak at 1644.50 cm^{-1} indicates H–O–H bending.

The FTIR spectrum of FPIC exhibits a broad absorption band at 3311.67 cm^{-1} for hydroxyl group, due to complexation of FP with HPβCD (intermolecular hydrogen bonding). FTIR of inclusion complex exhibits the peaks of FP at 1,741, 1,697, 1,658, 1,609 and 1,027 cm^{-1}, suggesting incorporation of the drug.

XRD

The powder XRD pattern of FP, HPβCD, their physical mixture and FPIC are shown in Fig. 4. The XRD indicated an amorphous structure of HPβCD while FP was crystalline as confirmed by presence of principal peaks at 21.02°, 24.70°, 15.86°, and 14.81° 2θ. The diffractogram of physical mixture of FP and HPβCD had crystalline peaks, which appears to be contributed by FP. The FPIC appears to be amorphous in nature, as a characteristic halo pattern for the amorphous form is present in diffractogram and the characteristic peaks of FP were not observed indicating loss of crystallinity.

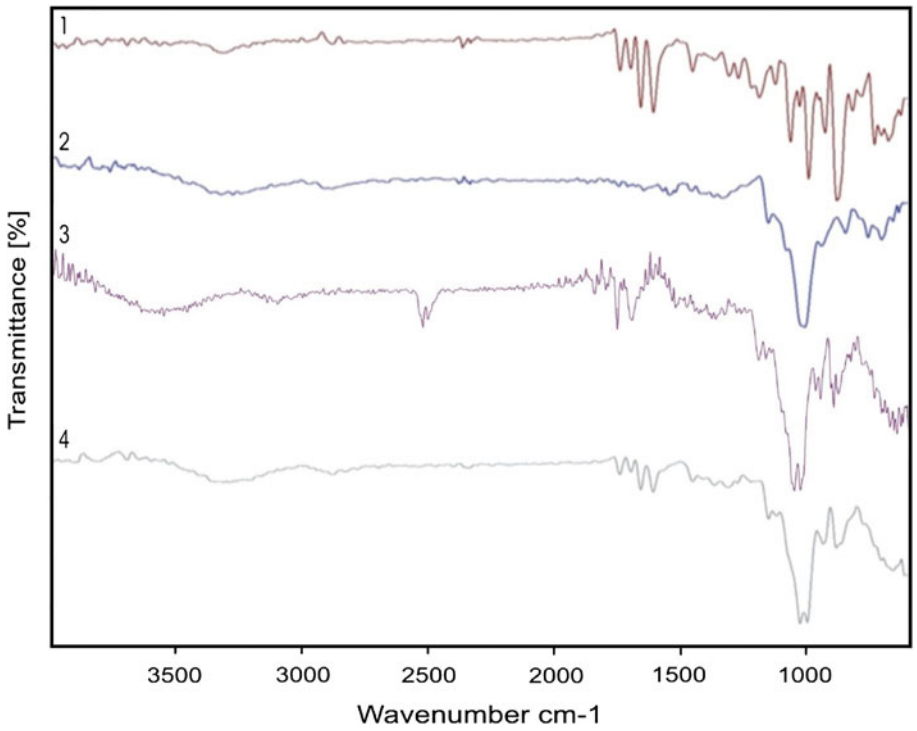

Fig. 3 FTIR spectra of *1* FP, *2* HPβCD, *3* Physical mixture of FP and HPβCD in 1:1 molar ratio, and *4* FPIC

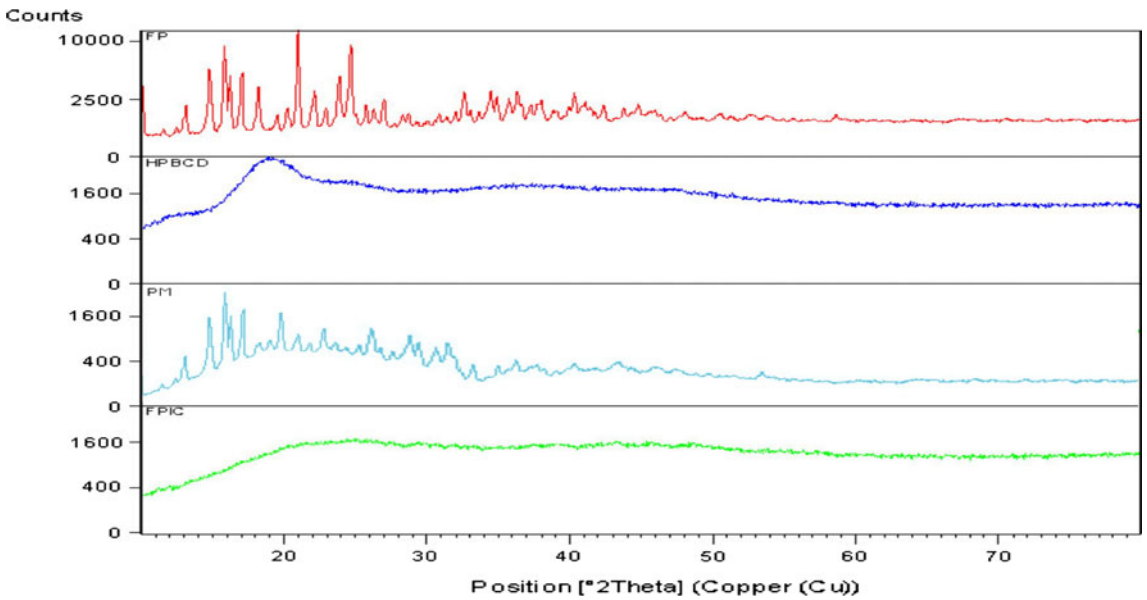

Fig. 4 Powder X-ray diffractograms of FP, HPβCD, physical mixture of FP and HPβCD in 1:1 molar ratio, and FPIC

DSC

DSC thermograms of the drug FP, HPβCD, their physical mixture and the inclusion complex are presented in Fig. 5. Pure FP exhibited sharp melting endotherm at 294.91 °C, indicating the crystalline nature of the drug. However, in the thermogram of FPIC, the melting endotherm of drug was replaced by a broad depressed endotherm, which may be attributed to loss of crystallinity of the drug when included in HPβCD host.

^{1}H-NMR

The ^{1}H-NMR spectrum of the inclusion complex between FP and HPβCD showed changes in chemical shifts with respect to that of chemical shifts of FP for the aliphatic and aromatic protons (Table 1; Fig. 6). The effect of HPβCD on the ^{1}H-NMR chemical shift of FP can be divided in two groups, viz. upfield shift and downfield shift. A downfield shift of drug proton indicates its proximity to an

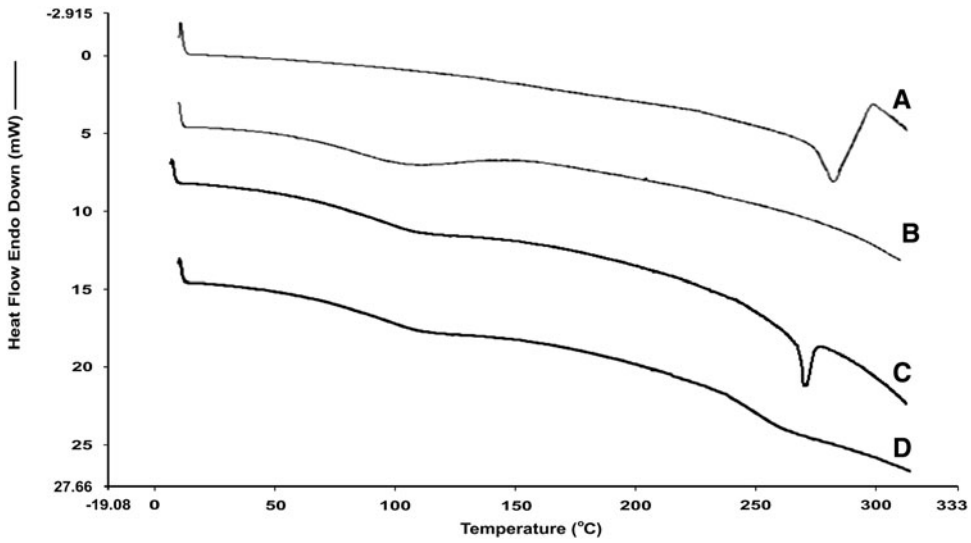

Fig. 5 DSC thermograms of FP (**A**), HPβCD (**B**), Physical mixture of FP and HPβCD (**C**) and FPIC (**D**)

electronegative atom [32]. On the other hand, an upfield shift of drug proton may be due to weak interaction with hydrogen atoms of HPβCD [33]. As shown in table, the positive value of $\Delta\delta$ and its magnitude as compared to other $\Delta\delta$, at position 33, 16 and 36 indicate their location near to an oxygen atom and interaction in the HPβCD cavity (Fig. 6).

The ^{1}H spectra of HPβCD and inclusion complex between FP and HPβCD (Table 2) showed no change in the chemical shift of hydrophilic group –OH, which is there on periphery of the molecule, indicating non involvement of the hydrophilic groups in inclusion complex.

The results suggest that the inclusion process involves mostly the hydrophobic part of FP and the hydrophobic cavity of HPβCD.

Table 1 ^{1}H- Chemical shift of FP in the presence and absence of HPβCD

Position of proton	δ FP	δ FPIC	$\Delta\delta$ (δ FPIC–δ FP)
1	7.269	7.267	−0.002
6	7.236	7.234	−0.002
3	6.118	6.114	−0.004
29	5.576	5.578	−0.002
23	4.215	4.207	−0.008
33	2.252	2.272	0.02
16	2.088	2.094	0.006
9	1.882	1.874	−0.008
15	1.487	1.485	−0.002
19	1.264	1.261	−0.003
25 & 34	1.043	1.020	−0.023
36	1.001	1.020	0.019

Drug contents

The drug contents in FPIC were found to be 23.21 % w/w and in that of coated granules of FPIC 4.083 % w/w.

Intrinsic dissolution

In order to calculate, the intrinsic dissolution of FP and FPIC, a plot of cumulative drug released versus time (in minutes) was plotted, as depicted in Fig. 7. The slope of the plot was determined (after regression) until 10 % of the drug was dissolved, and intrinsic dissolution was calculated by dividing the slope with the exposed surface area. Thus, with pellet diameter being 8 mm, the intrinsic dissolution of FP was calculated to be 21.02 µg/min/cm^2 (slope 16.4) and that of FPIC was calculated to be 387.43 µg/min/cm^2 (slope 302.2).

The results exhibit almost 18 times increase in intrinsic dissolution of drug after complexation with HPβCD. As intrinsic dissolution rate is influenced by the solid state properties of the drug, for instance, crystallinity and amorphism, this sharp increase again confirms the conversion of the drug from crystalline to amorphous form upon complexation.

In vitro drug release profile

When cFPIC granules filled in hard gelatin capsules were exposed to 0.01 N HCl, as release medium, at 37 °C for 2 h, no drug release was observed, indicating gastro-resistance of the drug granules.

When the release medium was replaced with phosphate buffer pH 7.4, there was no drug release for the first 15 min, which appears to be due to non-dissolution of Eudragit S100 coat. In the next 15 min, a burst in release of

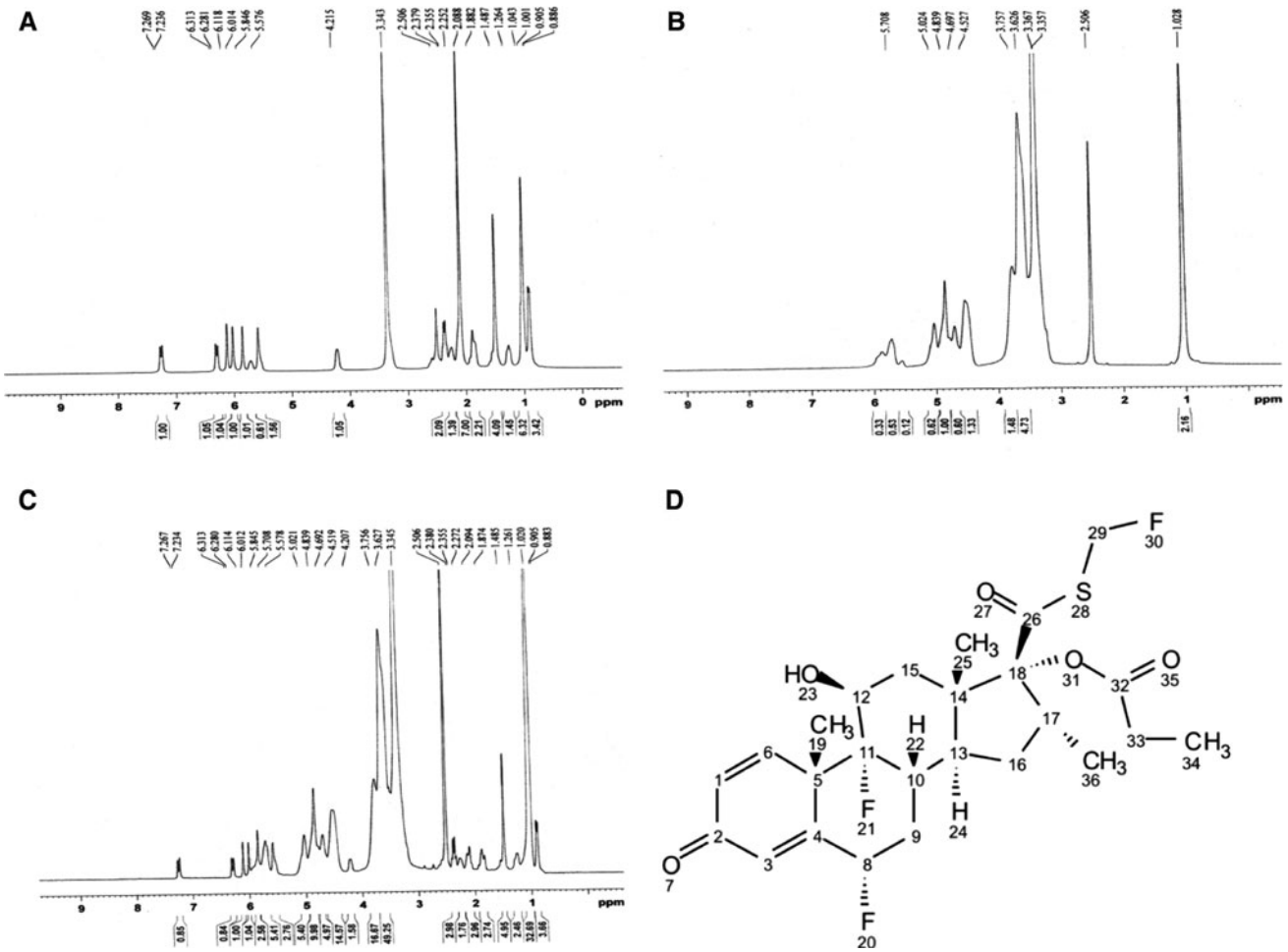

Fig. 6 [1] H-NMR spectra of **A** FP, **B** HPβCD, **C** FPIC, **D** molecular structure of FP

Table 2 [1]H- Chemical shift of HPβCD in the presence and absence of FP

Proton	δ HPβCD	δ FPIC	$\Delta\delta$ (δ FPIC–δ HPβCD)
–CH	5.024	5.021	−0.003
–OH	4.839	4.839	0
–CH	4.697	4.692	−0.005
–CH	3.757	3.756	−0.001
$-CH_2$	3.626	3.627	0.001
$-CH_3$	1.028	1.020	−0.008

drug (~80 %) was observed with a maximum drug concentration (92 %) after 120 min, as depicted in Fig. 8.

In vitro drug transport across rat colon

The integrity of the epithelial cell layer of the colonic tissue was monitored by measuring the transepithelial electrical resistance (TEER). The TEER values were found to be between 89.2 and 135.2 Ω cm^2 and remained steady throughout the experiment, indicating integrity of the epithelial layer. A sharp increase in resistance was observed after addition of 100 µl of carbachol (100 µM), on the receptor side, indicating that tissue was alive. On neither side of the chamber were any changes in pH values observed at the start and completion of the experiment.

Though the amount of the drug (FP) or its equivalent in the form of inclusion complex (FPIC) incorporated in the donor chambers was 399.52 nM, average initial concentration of FP in donor chamber containing FP pure drug was found to be 8.55 nM as compared to that of 335.22 nM in case of solution containing FPIC. This may be attributed to the fact that FP pure drug was dissolved up to its saturation solubility, while in the case of FPIC, increased solubility of FP, owing to complex formation led to higher initial concentration. The determination of drug contents in all the receptor chambers at the end of experiment showed that no drug had reached the receptor chamber in the case of pure FP, which indicates absence of any drug transport across the tissue for the pure drug. However, in cases, where FPIC was incorporated in the donor chamber, the

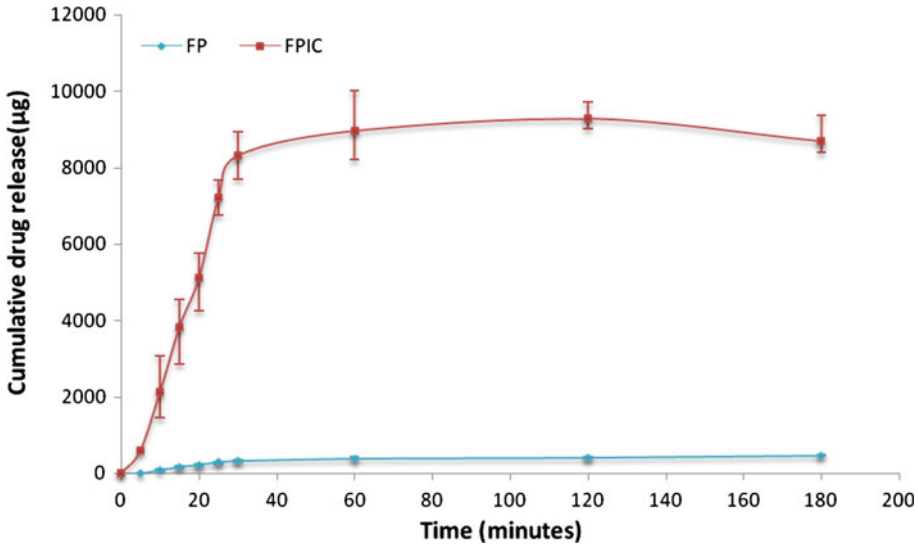

Fig. 7 Intrinsic dissolution of FP and FPIC

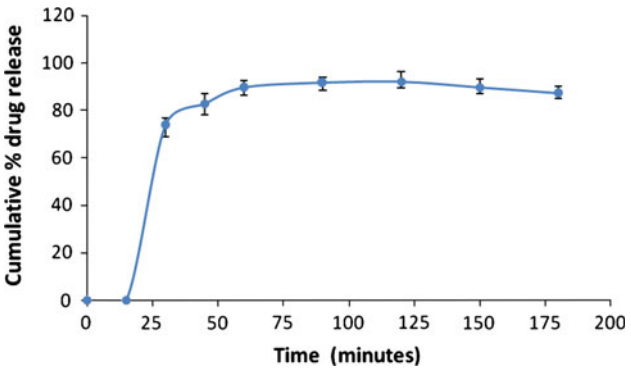

Fig. 8 In vitro drug release from capsules of cFPIC in phosphate buffer (pH 7.4)

average drug contents in receptor chamber were found to be 10.3 nM, indicating that drug has indeed moved across the tissue from donor to the receptor chamber, ostensibly driven by passive diffusion across concentration gradient. Furthermore the drug concentration in all the tissues was also determined at the end of the experiment, to study the drug uptake by the tissue, and the same was found to be 2.03 nM in case of FP pure drug and 254.19 nM in case of FPIC. The surprisingly high localization of drug in tissues in case of FPIC can be attributed to increased availability of the solubilized form of the drug at the mucosal surface, when presented in the form of inclusion complex. The result clearly indicate that by presentation of the drug in the form of inclusion complex it was possible to concentrate the drug in the tissue, which in real life situation is the actual site of action of the drug.

Most of the biomembranes or biological membrane barriers are lipophilic in nature with an aqueous exterior. When this structured water layer at the membrane surface - which is also referred to as the unstirred diffusion layer - is the rate-limiting step of drug permeation through barrier, cyclodextrins can enhance the permeation, by their solubilizing effect. However, it is only the free form of drug, which is in equilibrium with the drug/cyclodextrin complex, that is capable of penetrating lipophilic membranes [34]. The chemical structure of cyclodextrins (i.e. a large number of hydrogen donors and acceptors), their molecular weight (>970 Da) and their very low octanol/water partition coefficient (approximately 3) make them practically impermeable to lipophilic membranes [35, 36]. Studies have also shown that excess cyclodextrin can reduce drug permeability through biological membrane. Therefore it is of the utmost importance to optimize formulations containing cyclodextrin with regard to drug delivery from the formulation [37].

Conclusion

The present study was aimed at targeting FP, a potent anti-inflammatory glucocorticosteroid, to colonic region in the treatment of inflammatory bowel disease. A novel formulation was developed in order to circumvent the issues with respect to poor aqueous solubility of the drug. Thus drug was presented in the form of inclusion compounds with HPβCD, to improve the aqueous solubility of the drug. The drug was demonstrated to be present in amorphous form and intrinsic dissolution of the drug was found to increase $\sim$18-fold when included in the HPβCD. Subsequently, the inclusion compound granules were enteric coated through a pH-sensitive polymeric system, Eudragit S100, so that the formulation releases the drug in colonic region and concentrates the drug at the target tissue itself. This was confirmed through drug transport studies on rat colon using a modified Ussing chamber. These studies allow us to envisage development of a novel formulation of FP with

improved solubility and targeted delivery of drug to colonic region. Extensive first pass metabolism of the drug in the liver is expected to limit its systemic toxicity. Authors believe that further research efforts are warranted to assess the potential of the developed delivery system targeted to release the drug in colon and eventually to ascertain the efficacy of the formulation in a real-life situation.

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