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Research Article

Preparation and Characterization of Cyclodextrin Inclusion Complexes for Improving Solubility and Dissolution of Nimesulide

Ajay Semalty[1*], Yuveraj Singh Tanwar[2]

[1]Department of Pharmaceutical Sciences, H.N.B. Garhwal University, Srinagar (Garhwal) 246 174, Uttarakhand, India
[2] B.N. P.G. College of Pharmacy. Udaipur 313002, Rajasthan, India

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ABSTRACT

Nimesulide (4'-nitro-2'-phenoxy methane sulfonanilide) is a selective cyclooxygenase-2 inhibitor and one of the potent non steroidal anti-inflammatory drugs (NSAIDs). It is practically insoluble in water and hence has a low bioavailability. To improve solubility and dissolution of nimesulide, its β-cyclodextrin complex were prepared. Nimesulide was complexed with β-cyclodextrin in 1:1, 1:2 and 1:3 molar ratios using solvent evaporation method with the addition of freeze drying. The prepared inclusion complexes were evaluated for solubility, scanning electron microscopy (SEM), differential scanning calorimetry (DSC), X ray powder diffraction (XRPD) and *in vitro* dissolution study. All the complexes showed about up to 12 fold increase in solubility. The complex prepared in 1:3 ratios showed the greatest improvement in solubility (from 9.67 to 108.60 μg/ml). In SEM, the complexes showed irregular disc shaped non-porous surface. XRPD data indicated that maximum amorphization was induced in the complex prepared with 1:2 ratio. The DSC data confirmed the formation of inclusion complex. The dissolution of the drug in the complexes was also found to be improved. Complex prepared by solvent evaporation method in 1:2 molar ratio showed a marked improvement in dissolution profile (complete release in just 30 minutes) than that of pure drug which showed just 60.02 % drug release at the end of 3 hour. It was concluded that the β-cyclodextrin complex made in 1:2 molar ratio showed good solubility and the dissolution profile as compared to the complex made in 1:1 and 1:3 molar ratio. It was concluded that the complex prepared by solvent evaporation method with 1:2 molar ratio showed the best performance with respect to great improvement in solubility, the best amorphization and the best in vitro dissolution profile as compared to other complexes.

Keywords*: BCD complex; Solubility; in vitro dissolution, Differential Scanning Calorimetry, X-RPD, Scanning Electron Microscopy, Solubility behavior*

INTRODUCTION

For the Biopharmaceutical Classification System (BCS) class II drugs (low solubility, high permeability) drug solubility (aqueous) is the rate-limiting step for the absorption and hence the bioavailability of the drugs. The solubility of a drug is one of the most critical factors in developing a drug into a dosage form or delivery systems. The aqueous solubility governs the amount of drug that will diffuse and be available for dissolution subsequently. Therefore, the diffusion (of the drug in gastrointestinal fluid) and hence the solubility is the rate limiting step in the dissolution process. The drugs which have the water solubility less than the 10 mg/ml (over the pH range of 1 t-7 at 37 ºC) show the potential bioavailability problems [1]. Therefore, orally administered drug must have a fair solubility in gastro intestinal medium for the good bioavailability. Dissolution and solubility are the two important properties which play an important role in formulation development of the drugs [2, 3]. Poor solubility and the dissolution of drugs is the major challenge for formulation scientists. Various techniques like solid dispersion, solvent deposition, micronization etc. have been investigated for resolving solubility issue in pharmaceutical product development. Each of these techniques has its own merits and some demerits. Out of these, the complexation technique has been

***Corresponding Author Address:** Department of of Pharmaceutical Sciences, H. N. B. Garhwal University, Srinagar (Garhwal) 246 174, Uttarakhand, India; Tel: +91 1346 252174; Fax: +91 1346 252174; E mail: semaltyajay@gmail.com*

employed more precisely to improve the solubility and the dissolution of poorly water soluble drugs [4-10].

Cyclodextrins (CDs) are important class of compounds that form inclusion complexes. These complexes are prepared when the cyclodextrin envelops any drug partially or fully inside it. The inclusion complexes of a drug improve its various properties like solubility, dissolution, stability, bioavailability etc. [11].

Cyclodextrins (CDs) are produced in the bacterial degradation of cellulose. These are amphiphilic molecules (cyclic oligosaccharides) which consist of (α-1,4) linked α-D-glucopyranose units with lipophilic central cavity and a hydrophilic outer surface. [12]. It has been found that for the solubility or dissolution rate limited drugs (specially BCS class II drugs; and class IV drugs in some cases) cyclodextrins complexation may be a potential approach to improve the dissolution, absorption and the bioavailability [13-18].

Nimesulide (4'-nitro-2'-phenoxy methane sulfonanilide) is a selective cyclooxygenase-2 (COX-2) inhibitor and is 5-16 folds selective for COX-2 than COX-1 (Fig. 1). It is one of the potent non steroidal anti-inflammatory drugs (NSAIDs) [19-21]. It is very sparingly soluble in water (about 0.01 mg/mL)**,** due to which its dissolution in gastro intestinal fluid is very low, which in turn adversely affects its bioavailability. It also shows hepatic and gastrointestinal toxicity in long term use [22-24]. Due to the poor aqueous solubility and wettability of nimesulide it is always a challenge to formulate its oral or parenteral dosage forms. As the absorption of nimesulide is dissolution (and hence solubility) rate limited, increasing the aqueous solubility of nimesulide may be a potential approach to improve its dissolution and hence the bioavailability. Therefore, the present study aims to develop of inclusion complex of nimesulide with ß-cyclodextrins (BCD) for improving its aqueous solubility and the dissolution. The complexes (N-BCD) thus prepared were evaluated for solubility, thermal behaviour (by DSC), crystallinity (by X-RPD), surface morphology (by SEM) and in-vitro dissolution study.

MATERIALS AND METHODS

Materials: Nimesulide (98%) was obtained from Panacea Biotech, Delhi (India). ß-cyclodextrin was obtained from Sigma Aldrich, Mumbai India. All other chemical and reagents were of analytical grade.

Method of Preparation of Inclusion Complexes of Nimesulide: In the present study, nimesulide-β cyclodextrin (β-CD) complex in the different molar ratio 1:1, 1:2 and 1:3 were prepared by solvent evaporation methods.

The nimesulide-β CD complexes - NBCD1:1, NBCD1:2 and NBCD1:3 were prepared by taking three different molar ratios of drug: β-CD as 1:1, 1:2 and 1:3, respectively with the solvent evaporation method. The desired molar weight of nimesulide (dissolved in minimum quantity of methanol: water in 1:1) and β-CD (which was pretreated with hot water) was mixed. The resultant solution was evaporated for 2 hour under vacuum at 45°C in rotary vacuum evaporator (Perfit 5600, India). When the solution remained to 3-4 ml, the solution was freeze dried at – 45°C and a compression pressure of 0.5 torr. The dried products were collected and placed in vacuum desiccator overnight.

Characterization of Prepared Inclusion Complexes of Nimesulide

Solubility Study: The change in solubility due to complexation was determined in distilled water at 25 ± 0.1 °C solubility for drug and the complex [25, 26].

SEM: SEM of the complex was performed using a Scanning Electron Microscope (JEOL JSM 5600).

X-RPD: To study the change in crystallinity of nimesulide post complexation X-ray powder diffractometry of the different powder samples was performed on a Bruker AXS-D8 Discover Powder X-ray diffractometer, Germany [26].

DSC: Differential Scanning Calorimetry of nimesulide, β-CD and the N-BCDs were performed using a 2910 Modulated Differential Scanning Calorimeter V4.4E (TA Instrument, USA). Each sample weighing 5.0 ± 0.2 mg was heated in a covered sample pan under nitrogen gas flow over the temperature range 20-300°C at a heating rate of 10 °C min^{-1}. Individual thermograms were recorded and studied.

Dissolution Study: *In vitro* dissolution studies for NBCD complexes as well as plain drug were performed in triplicate in a USP XXIII six station dissolution test apparatus (Veego Model No. 6DR India) at 100 rpm and at 37°C. An accurately weighed amount of the complex equivalent to 50 mg of drug was put into 900 ml of phosphate buffer solution (pH 6.8). Samples of dissolution fluid were withdrawn at different intervals and replaced with the equal volume of fresh media. Withdrawn samples were filtered, diluted suitably and then analysed spectrophotometrically.

Statistical analysis: Results were expressed as mean values and standard deviations (± SD).

RESULTS AND DISCUSSION

In the present study, nimesulide-β cyclodextrin complex in the different molar ratio 1:1, 1:2 and 1:3 were prepared by solvent evaporation methods to improve solubility and dissolution of the drug.

Solubility Study: The pure nimesulide showed aqueous solubility of 9.67±0.902 µg/ml. On the other hand all the complexes showed about up to 12 fold increase in solubility (Table 1). The complex NBCD1:3 showed the greatest improvement in solubility (from 9.67 to108.60 µg/ml).

Surface Morphology (SEM): The scanning electron microscopy (SEM) of nimesulide and the complexes are given in fig. 2. Nimesulide was seen as tabular-shaped crystals with smooth surfaces (Fig. 2a). The β-CD appeared as irregular shaped crystals (Fig. 2b). The complexes showed a clear and significant change in the morphology and shape of particles. All the complexes showed irregular disc shaped non-porous surface (Fig. 2c, 2d and 2e). But there was no clear difference between the complexes prepared in different ratio. In particular, with NBCD 1:2 and 1:3 the particles were more irregular with relatively rough surface.

The irregular and rough surface of the complex prepared in 1:2 and 1:3 ratio (NBCD1:2 and NBCD 1:3) might be responsible for the better improvement in solubility. Though SEM does not provide much information regarding complexation it helps in assessing the probable nature of the complex with respect to solubility and the presence of single component in the complex. It was also evident that in the SEM of NBCD 1:2 it was not possible to differentiate crystals of both components and this indicated the better interaction of drug particles with cyclodextrin.

Crystallinity Study (XRPD): The X-Ray diffraction pattern of the N, β-CD and the complexes revealed the changes in crystallinity of the drug in the complex (Fig. 4). In the X-ray diffractogram, nimesulide showed sharp diffraction peaks indicating drug as the crystalline material (Fig 3a). The crystalline peaks of drugs were predominant in the thermogram of the physical mixture (Fig 3c). On the other hand, X-ray diffraction pattern of NBCD complexes exhibited reduction (in NBCD 1:1 and NBCD 1:3) and disappearance (in NBCD 1:2) in the intensity of large diffraction peaks of drug. Evidently it was not possible to distinguish the characteristic peak of the drug. This indicated the decrease in crystallinity or the partial amorphization of the drug. The result confirmed that nimesuilide was no longer present as a crystalline material in its β-CD complex and existed in the amorphous state [27-29].

It was also evident that the rank order of crystallinity in the comoplexes was NBCD1:1> NBCD1:3> NBCD 1:2. The maximum amorphization was observed in NBCD 1:2 and this was evident due to disappearance of crystalline peaks corresponding to the drug in the particular thermogram (Fig 3e). While the other two complexes showed intermediate crystallinity and hence the partial amorphization was confirmed. These results are well supported by previous studies reported for other drugs with amorphous β-CD and its derivatives [30-31].

Thermal Analysis (DSC): Differential scanning calorimetry (DSC) was performed to indicate the formation of the inclusion complex. The complex formation is indicated by any significant change (elimination of endothermic peaks, the appearance of new peaks, change in peak, shape and its onset, peak temperature, melting point and relative peak area or enthalpy) in characteristic peak of the drug and or the complexation agent.

The DSC curve of nimesulide (Fig. 4a) exhibited sharp endothermic peak at 150.50 ºC, caused by the melting of nimesulide. The β-CD (Fig. 4b) showed a broad endothermal peak between 55°C to 150°C. This broad peak might be due to the loss of water.
All the complexes showed significant change in the endothermic peak corresponding to the drug (Fig 4d, 4e and 4f). Among the prepared complexes, the complex NBCD 1:2 showed almost complete disappearance of the endothermic peak (corresponding to that of nimesulide). This indicated that the inclusion complex was formed and it was not having any free nimesulide. On the other hand, the physical mixture did not show any change in the characteristic peak of drug and cyclodextrin (Fig. 4c).

Due to complete disappearance of the endothermic peak of nimesulide in NBCD 1:2, it was confirmed to be an inclusion complex without any free nimesulide. This data was well supported by the XRPD studies in which NBCD 1:2 also showed the most significant change in the crystallinity of the drug. In a previous study, nimesulide-β-CD (N-β-CD) complex were prepared. In almost all the complexes prepared with α-, β-, and γ-CDs with kneading and coevaporation method, there was a marked reduction in intensity (and/or broadening) of the nimesulide's endothermic peak at around 150°C and this indicated partial inclusion of

nimesulide in the CD. In the case of N-β-CD, 1:2 M the endothermic peak of nimesulide completely disappeared [32]. Hence the complete disappearance of the endothermic peak of the drug in the prepared complexes indicated the formation of a true inclusion complex. Other studies also well supported the results [33-35]. .

Dissolution Study: The nimesulide-β-CD complex (NBCD1:1, NBCD 1:2 and NBCD1:3) showed better dissolution profile than the nimesulide (Fig. 5). The pure nimesulide showed a total of only 60.02% drug release at the end of 120 minutes. On the other hand the complexes released the complete drug in 30 to 40 minutes only. NBCD 1:2 released the complete drug in just 30 minutes.

NBCD 1:2 being the best in amorphous nature (as confirmed by the XRD data) might have shown the better dissolution. SEM, dissolution and DSC data also well supported the dissolution study. SEM showed more irregular and rough surface in NBCD 1:2 which resulted in improved solubility and enhanced dissolution rate as compared to pure drug. In various previous studies beta-cyclodextrin (β-CD) has been reported to form water soluble complex with many lipophilic water insoluble drug and thereby significantly improving the dissolution of the said drugs [26, 36, 37].

CONCLUSION

Nimesulide is a BCS Class II drug which shows dissolution rate limited absorption. Therefore, to improve its solubility and dissolution profile various β-cyclodextrin complexes were prepared in three different molar ratios (1:1, 1:2 and 1:3). The complexes were evaluated for solubility, SEM, XRD, DSC and in vitro dissolution study. The study concluded that the complex prepared by solvent evaporation method with 1:2 molar ratios showed the best performance with respect to great improvement in solubility, the best amorphization and the best in vitro dissolution profile as compared to other complexes.

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Table 1: Solubility Study of Nimesulide and Its β-CD Complexes at 25 ^{o}C

Drug/ Complex	Solubility in water (µg/ mL)*
Nimesulide	9.67 ± 0.902
NBCD1:1	96.42 ± 0.24
NBCD1:2	104.68± 1.26
NBCD1:3	106.60 ± 1.42

* Data expressed as mean values and standard deviations (± SD); *n=3*

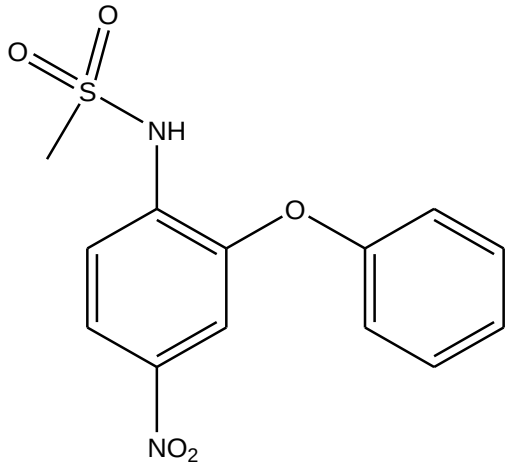

Figure 1: Chemical structure of nimesulide.

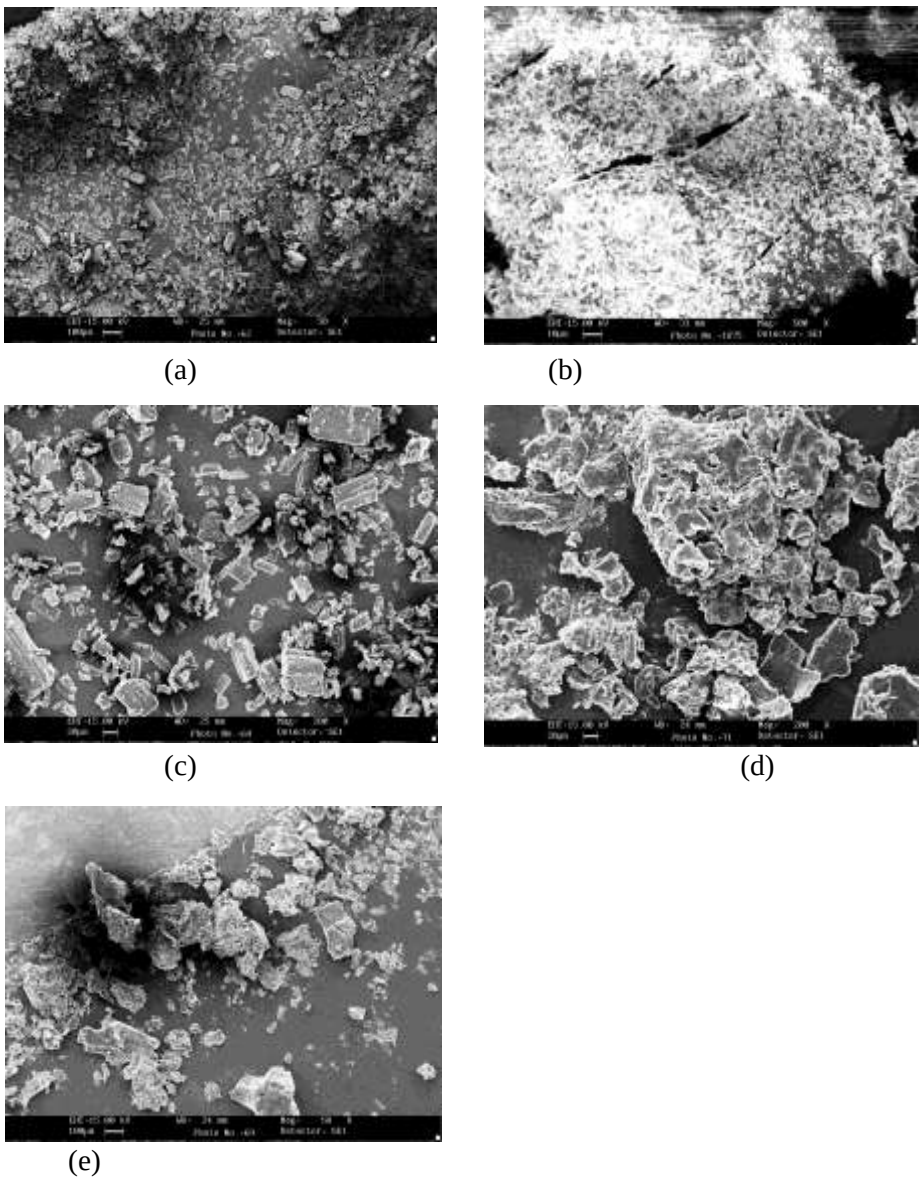

Figure 2: Scanning Electron Micrographs of nimesulide (a); β-CD (b); and its β-CD Complexes NBCD 1:1 (c); NBCD 1:2 (d) and NBCD 1:3 (e)

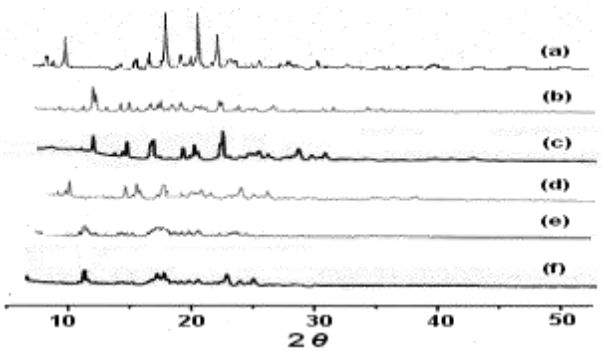

Figure 3: X-ray powder diffraction (XRPD) study of nimesulide (a); β-CD (b); physical mixture (c) and its β-CD Complexes F1 (d); F2 (e) and F3 (f)

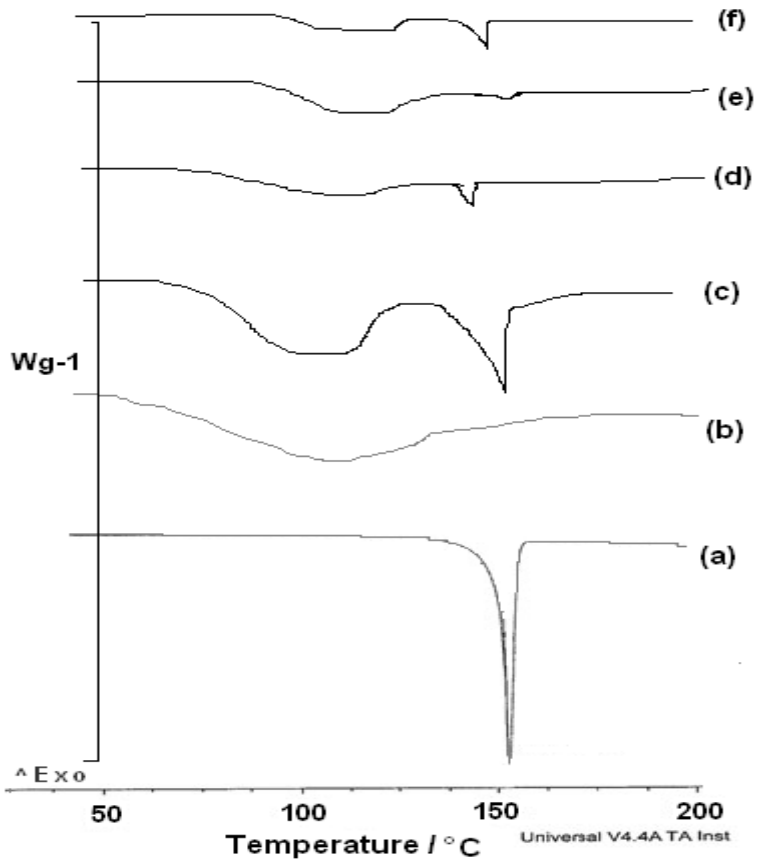

Figure 4: DSC curves of nimesulide (a); β-CD (b); physical mixture (c) and its β-CD Complexes F1 (d); F2 (e) and F3 (f)

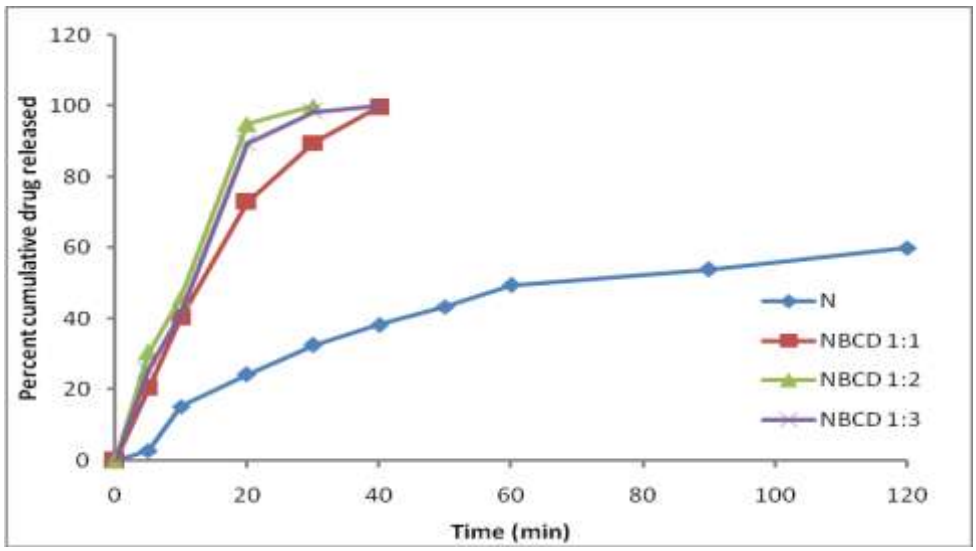

Figure 5: Dissolution study of nimesulide and its complexes

REFERENCES

1. Wells J. Pharmaceutical preformulation: the physicochemical properties of drug substances. In: Pharmaceutics: The Science and Dosage Form Design, 2nd ed, Aulton ME, Ed; Churchil Livingstone: Edinburg; 2006; pp. 113-116.
2. Vilarnovo BP et al. Improvement of water solubility of sulfamethizole through its complexation with β and HP-β-CD characterization of the interaction in solution and in solid state. Eur J Pharm Sci 2001; 13:325-331.
3. Ozkan Y et al. Improvement of water solubility and in vitro dissolution rate of gliclazide by complexation with β- cyclodextrins. Pharm Acta Hel 2000; 74:365-370.
4. Semalty A et al. Essentials of Pharmaceutical Technology, 1st ed.; Pharma Med Press: Hyderabad, 2011; pp. 4-5.
5. Pralhad T, Kumar R. Study of freez-dried quercetin- cyclodextrin binary system by DSC, FTIR, X-ray diffraction and SEM analysis. J Pharm Biomed Ana 2004; 34**:**333-339.
6. Babu RJ, Pandit JK. Effect of cyclodextrin on the complexation and transdermal delivery of bupranolol through rat skin. Int J Pharm 2004; 271**:**155-165.
7. Rawat S, Jain SK. Solubility enhancement of celecoxib using β- cyclodextrin inclusion complexes. Eur J Pharm Biopharm 2004; 51**:**263-267.
8. Sajeesh S, Sharma CP. Cyclodextrin – insulin complex encapsulated polymethacrylic acid based nanoparticles for oral insulin delivery. Int J Pharm 2006;325:147-154.
9. Chen X et al. The preparation and stability of the inclusion complex of astaxanthin with β-CD. Food Chem 2007; 101**:**1580-1584.
10. Semalty A et al. Preparation and characterization of phospholipid complexes of naringenin for effective drug delivery. J Incl Phenom Macrocycl Chem 2010; 67:253-60.
11. Bhati LK et al. Enhancement of complexation efficiency of meloxicam using binary and ternary solid systems: formulation considerations. Am J Drug Discov Dev 2012; 2(1):17-31.
12. Magnusdottir M, Lofisson T. Cyclodextrins. J Incl Phenom Macroc Chem 2002; 44:213-218.
13. Rasheed A, Kumar A. Cyclodextrin as drug carrier molecule. Sci Pharm 2008; **7**6:567-598.
14. Loftsson T, Jarho P, Masson M, Jarvinen T. Cyclodextrin in drug delivery. Expert Opin Drug Deliv 2005; 2**:**335-351.
15. Irie T, Uekama K. Pharmaceutical application of cyclodextrin, Toxicological issues and safety evaluation. J Pharma Sci 1997; 85:147-162.
16. Del VE. Cyclodextrins and their uses: a review. Process Biochem 2004; 39**:**1033-46.
17. Rinaki E, Valsami G, Macheras P. Quantitative biopharmaceutical classification system: the central role of dose/solubility ratio. Pharma Res 2003; 20:1917-19.
18. Uekama K. Design and evaluation of cyclodextrin based drug formulation. Chem Pharm Bull 2004; 52:900-15.
19. Bishnoi M et al. Protective effect of nimesulide (COX inhibitor), AKBA (5-LOX inhibitor) and their combination in aging associated abnormalities in mice. Methods Find Exp Clin Pharmacol 2005; 27(7): 465.
20. Ferrari PG et al. A controlled clinical study of the efficacy and tolerability of nimesulide vs naproxen in maxillo-facial surgery. Drugs. 1993; 46(1):171-173.
21. Singla AK et al. Review Nimesulide: Some pharmaceutical and pharmacological aspects- An update. J Pharm Pharmacol 2000; 52(5):467-486.
22. Tan HH et al. Nimesulide-induced hepatotoxicity and fatal hepatic failure. Singapore Med J 2007; 48(6):582-585.
23. Davis R, Brogden RN. Nimesulide: An update of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy. Drugs 1994; 48(3):431-454.
24. Bjarnason I, Thjodleifsson B. Gastrointestinal toxicity of non-steroidal anti-inflammatory drugs: the effect of nimesulide compared with naproxen on the human gastrointestinal tract. Rheumatology 1999; 38(1):24-32.
25. Semalty A et al. Development and evaluation of pharmacosomes of aceclofenac**.** Indian J Pharm Sci 2010; 72:571-575.
26. Semalty A et al. Preparation and characterization of cyclodextrin inclusion complex of naringenin and critical comparison with phospholipid complexation for improving solubility and dissolution. J Therm Anal Calorim 2013; 109: DOI 10.1007/s10973-013-3463-y.
27. Singh D et al. Gallic acid-phospholipid complex: drug incorporation and physicochemical characterization. Lett Drug Design Disc 2011; 8(3):284-291.
28. Singh D et al. Emodin–phospholipid complex: A potential of herbal drug in the novel drug delivery system. J Therm Anal Calorim 2012; 108(1):289-298.
29. Semalty A et al. Phyto-phospholipid complex of catechin in value added herbal drug delivery. J Incl Phenom Macrocycl Chem 2012; 69:253-60.
30. Sathigari S et al. Physicochemical characterization of efavirenz–cyclodextrin inclusion complexes, AAPS PharmSciTech, 2009; 10(1): DOI: 10.1208/s12249-008-9180-3
31. Rajendrakumar K et al. Cyclodextrin complexes of valdecoxib: Properties and anti-inflammatory activity in rat. Eur J Pharm Biopharm 2005; 60:39–46.
32. Nalluri BN et al. Physico-chemical characterization and dissolution properties of nimesulide-cyclodextrin binary systems. AAPS PharmSciTech 2003; 4 (1):E2.
33. Wen J et al. Preparation and physicochemical properties of the complex of neringenin with hydroxypropyl-β-CD. Molecule. 2011; 15:4401-4407.
34. Moriwaki C et al. Enhancement of solubility of albendazole by complexation with β-cyclodextrin. Braz J Chem Eng 2008; 25:255-67.
35. Singh D et al. Quercetin-phospholipid complex: An amorphous pharmaceutical system in herbal drug delivery. Current Drug Disc Tech 2012; 9(1):17-24.
36. Tommasini S et al. Improvement in solubility and dissolution rate of flavonoids by complexation with β- cyclodextrin. J Pharm Biomed Ana 2004; 35:379-387.
37. Gibaud S et al. Melarsoprol-cyclodextrin inclusion complexs. Int J Pharm 2005; 306:107-121.