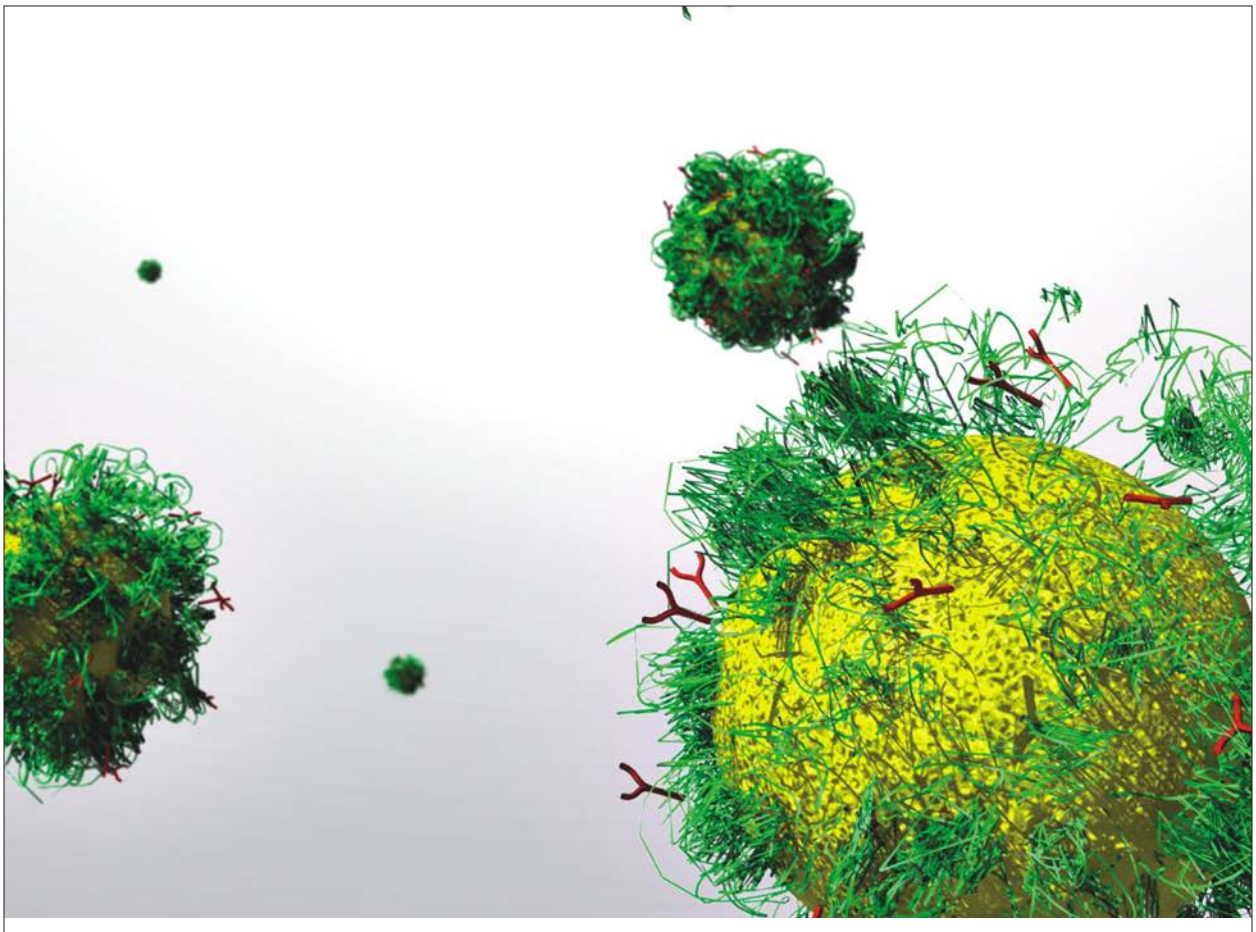

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Nimesulide-phosphatidylcholine Complex for Improvement of Solubility and Dissolution

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ABSTRACT

Nimesulide, a potent non steroidal anti-inflammatory drug, is a highly selective cyclooxygenase-2 (COX-2) inhibitor. Being a class II drug (according to biopharmaceutical classification system or BCS) its poor aqueous solubility results in low bioavailability. Moreover, its absorption is dissolution rate limited. It also shows hepatic and gastrointestinal toxicity in long term use. Therefore, to improve solubility, dissolution (hence the bioavailability) and to reduce toxic effects of nimesulide, its phospholipid complex was prepared. The prepared phospholipid complex was evaluated for drug loading, solubility, Scanning Electron Microscopy (SEM), Infrared absorption (FT-IR), Differential Scanning Calorimetry (DSC), X-ray powder diffractometry (X-RPD) and *in vitro* dissolution study. The aqueous solubility of nimesulide was improved significantly in the complex. In the SEM phospholipid complex was found to be fluffy and porous with rough surface morphology. FT-IR, DSC and X-RPD data confirmed the formation of the complex. The prepared phospholipid complex showed significantly improved dissolution profile. It was concluded that the phospholipid complexation of nimesulide like BCS class II drugs may be a very effective, reliable and safe approach to improve the solubility and dissolution of drugs.

Key words: Nimesulide, phosphatidylcholine, FT-IR, differential scanning calorimetry, X-RPD, scanning electron microscopy, solubility

INTRODUCTION

Dissolution of drug is directly dependent on the aqueous solubility of the drug. The drugs which have the water solubility less than the 10 mg mL^{-1} (over the pH range of 1-7 at 37°C) show the potential bioavailability problems. Dissolution and solubility are the two important properties which play an important role in formulation development of the drugs (Wells, 2006; Pose-Vilarnovo *et al*., 2001; Ozkan *et al*., 2000). The bioavailability of the drugs which show the dissolution rate limited absorption may be improved by improving their aqueous solubility. Various techniques and dosage forms or drug delivery systems have been designed and adopted for improving the solubility and dissolution of drugs. These techniques include supercritical fluid process, micronization, solid dispersion, cyclodextrin complexes and phospholipid complexes etc. (Perrut *et al*., 2005; Semalty *et al*., 2011; Pralhad and Rajendrakumar, 2004; Babu and Pandit, 2004; Rawat and Jain, 2004; Sajeesh and Sharma, 2006; Semalty *et al*., 2010b).

Out of these, the complexation technique has been employed more precisely to improve the solubility and the dissolution of poor water soluble drugs (Semalty *et al*., 2009a; Bhati *et al*., 2012).

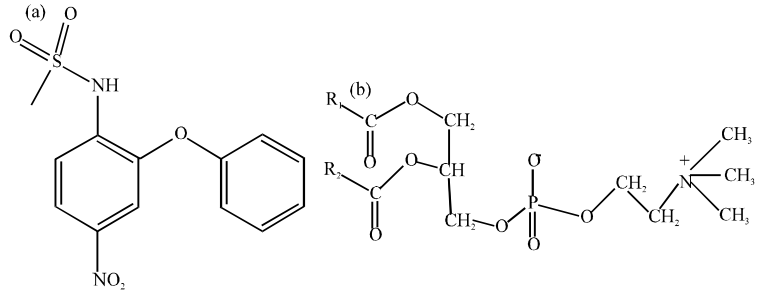

Fig. 1(a-b): (a) Nimesulide and (b) Phosphatidylcholine, R_1 and R_2 = Long chain of fatty acid

Among the complexation techniques the phospholipid complexation and cyclodextrin complexation are the two most widely investigated approached for improving the solubility. In the various previous studies it was reported that developing the drugs as lipid complexes (also called pharmacosomes) may prove to be a potential approaches to improve solubility and to minimize the GI toxicity of drugs (Semalty *et al.*, 2009b, 2010a) In the phospholipid complexation the drug and a phospholipid (Fig. 1b) are treated in certain molar ratio (generally 1:1 or 1:2) to yield an amphiphilic complex with improved solubility, permeability and dissolution profile.

The lipid complexes are prepared with phosphatidylcholine (PC, Fig. 1b). PC is an integral part of the cell membrane exists in zwitterionic form. PCs are studied extensively due to their interaction with several physiologically active compounds. PCs are amphiphilic molecules which yield the product of improved solubility and permeability when complexed with drugs with poor solubility and/or permeability. PC is not only a passive carrier in drug delivery but is itself a natural component with well investigated and reported clinical efficacy for various liver diseases (Semalty *et al.*, 2010b; Kidd, 1996, 2002).

Nimesulide (4'-nitro-2'-phenoxy methane sulfonanilide) is one of the potent non steroidal anti-inflammatory drugs (NSAIDs). It is a selective cyclooxygenase-2 (COX-2) inhibitor and is 5-16 folds selective for COX-2 than COX-1 (Fig. 1a) (Bishnoi *et al.*, 2005; Ferrari *et al.*, 1993; Singla *et al.*, 2000). It is very sparingly soluble in water (about 0.01 mg mL^{-1}). The low solubility leads to very low dissolution and hence the poor bioavailability. Nimesulide is also associated with gastrointestinal disturbances and hepatic toxicity as the most frequent side effects on its long term administration (Tan *et al.*, 2007; Davis and Brogden, 1994; Bjarnason and Thjodleifsson, 1999). 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 solubility (and hence the dissolution) rate limited, increasing the aqueous solubility of nimesulide may be a potential approach to improve its dissolution and hence the bioavailability.

Various studies have reported that phospholipid complexes of NSAIDs improve the permeation across the bio membranes, reduced toxicities and thereby improve their bioavailability and GI safety (Khazaeinia and Jamali, 2003). When used in drug dosage form or delivery system, the phospholipid covers the surface of the mucus as a hydrophobic protective coat and hence protects the GI tissues (Goddard *et al.*, 1990; Lichtenberger *et al.*, 1983).

Therefore, the preparing the lipid complex may not only improve the aqueous solubility and dissolution rate (and hence the bioavailability) but also reduce its local gastrointestinal toxicities. For fulfilling these objectives nimesulide-phospholipid complex was prepared in the present study. The complex thus prepared was evaluated physico-chemically for drug content, chemical interaction (FT-IR), thermal analysis (DSC), crystallinity (X-RPD), Surface Morphology (SEM), solubility and dissolution study.

MATERIALS AND METHODS

Materials: Nimesulide (98%) was obtained from Panacea Biotech, Delhi (India). Soya phosphatidylcholine (LIPOID S-80) was obtained as a gift sample from LIPOID, Germany. All other chemical reagents were of analytical grade.

Method of preparation: To prepare the complex nimesulide and Soya phosphatidylcholine (PC) were taken in 1:1 molar ratio and dissolved in 30 mL of dichloromethane in a 100 mL round bottom flask. The solvent was evaporated off in a rotary vacuum evaporator (Perfit 5600, India) under vacuum at 40°C. The dried residue obtained is the resultant complex which was stored in vacuum desiccators.

Drug content: Nimesulide-PC complex equivalent to 50 mg of nimesulide was weighed. To the weighed complex 100 mL of pH 6.8 phosphate buffer was added in a volumetric flask. After the continuous stirring on a magnetic stirrer (Remi, 5MLH, India) for 24 h at room temperature samples were taken, filtered, diluted suitably and then analyzed spectrophotometrically (Lambda 25, Perkin Elmer, USA) at 390 nm to determine the drug content.

Infrared spectroscopy (FTIR): The IR spectra were recorded on a Perkin Elmer FT-IR, RX-1 spectrophotometer in KBr pellets.

Differential scanning calorimetry (DSC): DSC study was performed for the samples of nimesulide, phosphatidylcholine and the prepared complex using a 2910 Modulated Differential Scanning Calorimeter V4.4E (TA Instrument, USA). The investigations were carried out over the temperature range 0-300°C (@ 10°C min^{-1}).

X-ray powder diffractometry (XRPD): To assess the crystallinity, XRPD of all the samples were performed using Bruker Axs-D8 Discover Powder X-ray diffractometer (Germany). The scanning was performed in the range of 5-50° of 2θ in step scan mode (step width 1°min^{-1}).

Scanning electron microscopy (SEM): To assess the surface morphology of the prepared complex as compared to its components SEM of the complex was performed using JEOL JSM 5600.

Solubility study: To determine the effect of complexation on solubility, solubility of nimesulide, PC and its complex was determined in distilled water at 25±0.1°C (Semalty *et al.*, 2013b).

Dissolution study (*in vitro* drug release): The dissolution studies were carried out in a USP XXIII, eight station dissolution test apparatus, type II (8DR, VEEGO, India) at 100 rpm and at 37°C using pH 6.8 phosphate buffer (900 mL) as media. The complex equivalent to 50 mg of nimesulide was taken for the study and its comparison was done with the dissolution of plain nimesulide (50 mg). 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.

RESULTS AND DISCUSSION

In the present study the complex showed 92% w/w drug content of nimesulide. High loading of drug in the complex make the use of complex practically possible to deliver the therapeutic dosage effectively.

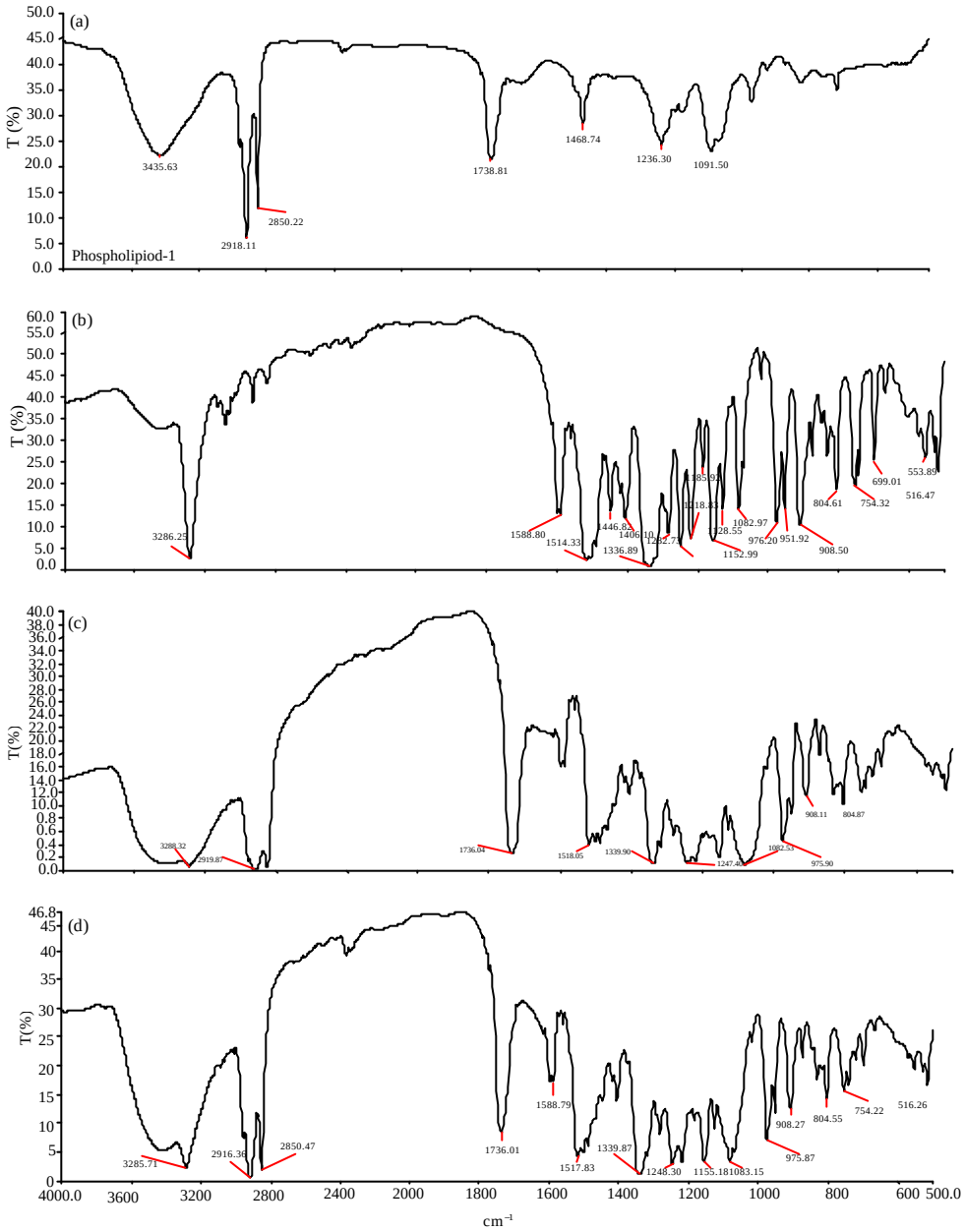

Fig. 2(a-d): IR Spectra: (a) Phospholipid, (b) Nimesulide, (c) Nimesulide-phospholipid complex and (d) Physical mixture

FTIR: The possible interaction between nimesulide and PC (phosphatidylcholine) in the phospholipid complex was studied by FTIR (Fig. 2). PC showed characteristic peaks at 3435 cm^{-1} (Hydroxyl stretching); 2918 and 2850 cm^{-1} (C-H stretching of long fatty acid chain); 1738 cm^{-1} (carbonyl stretching of the fatty acid ester); 1236 cm^{-1} (P = O stretching band); 1091 cm^{-1} (P-O-C stretching) and 970 cm^{-1} ($N^{+}(CH_3)_3$ stretching). Nimesulide showed the characteristic peaks at 3286 cm^{-1} for the amino (N-H stretching and at 1514 cm^{-1} for N = O stretching.

The FTIR of the complex showed significant changes in the characteristic absorption peaks of nimesulide. The peaks of amino (N-H) and nitro (N = O) group at 3286 cm^{-1} and 1514 were shifted to higher wave number in the complex. On the other hand the characteristic absorption peaks of

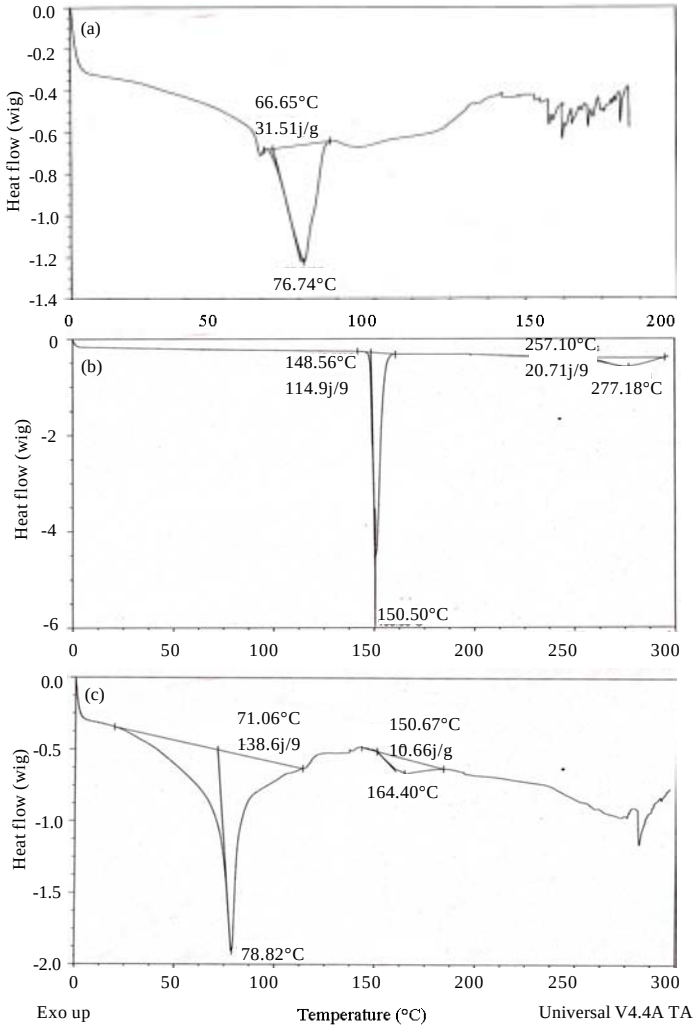

Fig. 3(a-c): DSC Thermograms: (a) Phospholipid, (b) Nimesulide and (c) Nimesulide-phospholipid complex

P = O and P-O-C of the phosphatidylcholine were broadened and also changed their position in the FTIR spectra of the complex. The FTIR spectra of the physical mixture was showing the same characteristic peaks of nimesulide and PC without any significant change and this indicated no interaction in between them.

Therefore, the formation of nimesulide-PC complex was indicated due to interaction of amino and nitro group of nimesulide with polar end of PC. The results of FTIR are well supported by the previous studies done with various PC complexes of drugs (Singh *et al.*, 2011; Semalty *et al.*, 2012).

DSC: DSC of all the samples (nimesulide, PC and its complex) were performed to investigate the thermal behavior of the drug. In the DSC study nimesulide (Fig. 3) exhibited sharp endothermic peak at 150.50°C (ΔH_f = 114.9 J g^{-1}) corresponding to the melting of nimesulide. PC showed an endothermic peak at 76.74 °C (ΔH_f = 31.51 J g^{-1}). The nimesulide-PC complex exhibited a sharp new peak at 78.82°C (ΔH_f = 138.6 J g^{-1}) which showed interactions between nimesulide and PC and confirming the formation of a complex. Various studies also support these results in which complex do not show the peak corresponding to the components of the complex and rather show a

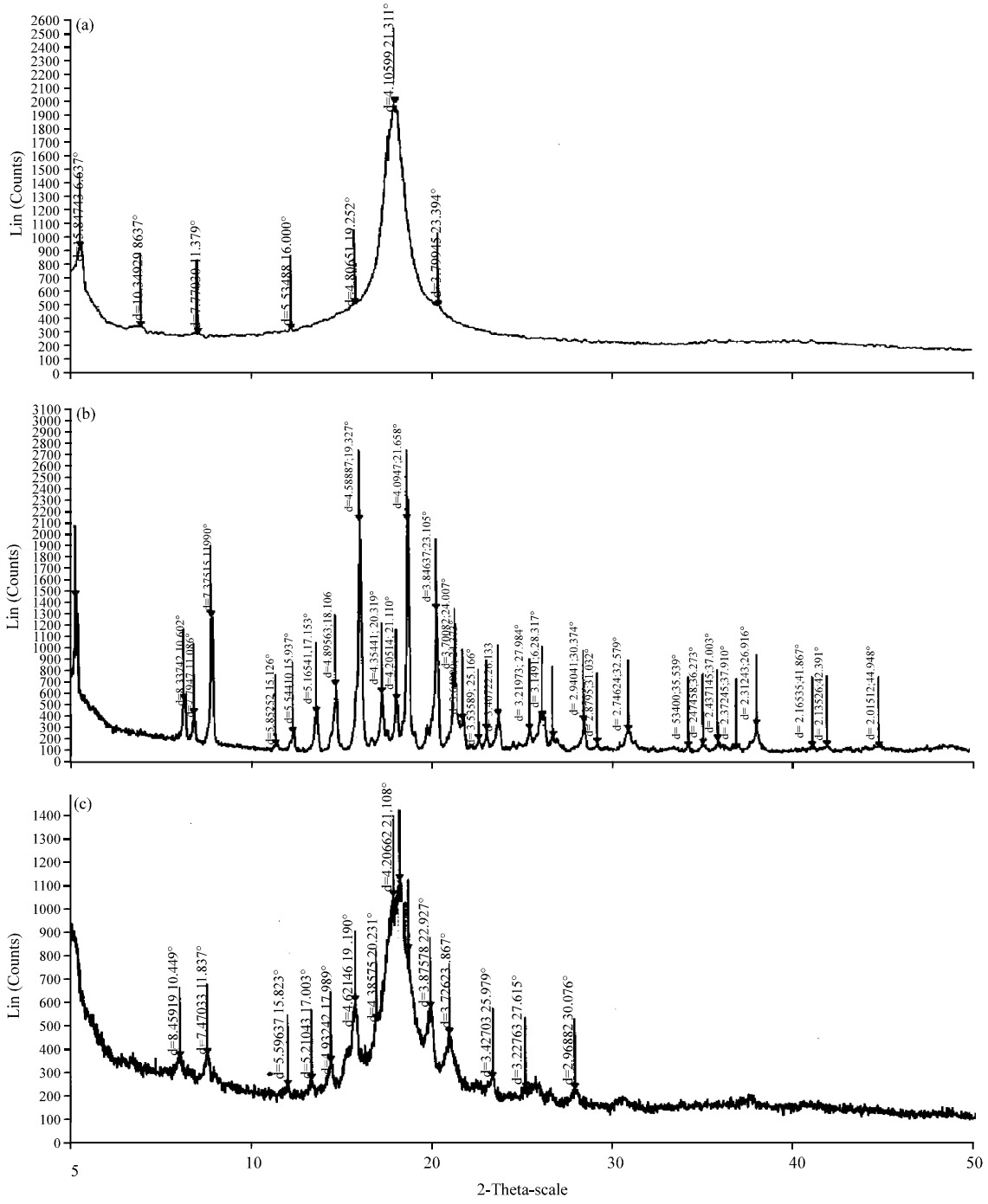

Fig. 4(a-c): X-RPD Pattern: (a) Phospholipid, (b) Nimesulide and (c) Nimesulide-phospholipid complex

entirely new peak (Singh *et al.*, 2011; Semalty *et al.*, 2012; Li *et al.*, 2008; Xiao *et al.*, 2006; Maiti *et al.*, 2007; Kumar *et al.*, 2008).

XRPD: To assess the crystallinity XRPD of nimesulide, phosphatidylcholine and the complex was performed (Fig. 4). Nimesulide showed intense diffraction peaks of crystallinity and suggested that

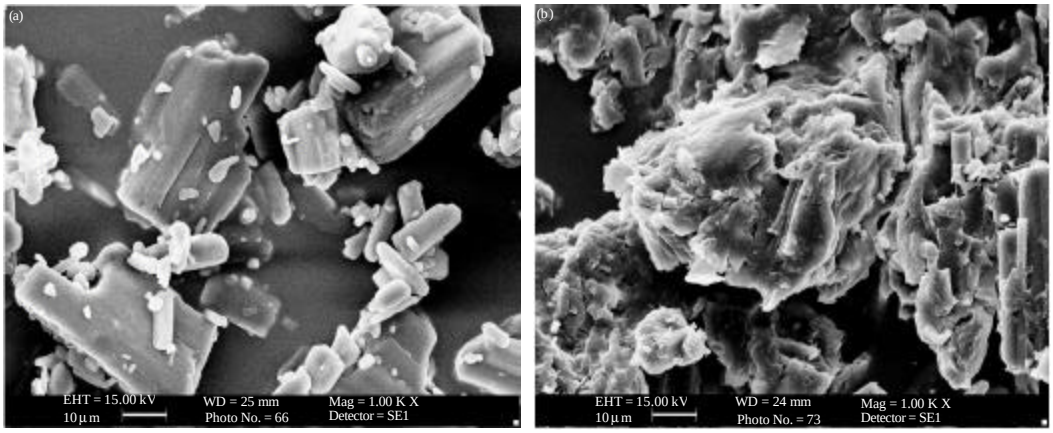

Fig. 5: Scanning electron micrographs, (a) Nimesulide and (b) Nimesulide-Pc complex

Table 1: Solubility study (H_2O/n-Octanol) at 25°C

Sample	Aqueous solubility (µg mL^{-1})	n-Octanol solubility (µg mL^{-1})
Nimesulide	9.67±0.902	160.608±0.076
Nimesulide complex	0.104±1.025	124.902±0.234

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

the drug is in crystalline state. PC showed a major single diffraction peak. In the nimesulide-phospholipid complex, the sharp and intense peaks characteristic to the crystalline nimesulide were not observed at all. There was only a large diffraction peak s in which it was not possible to distinguish the characteristic peaks of nimesulide. This confirmed the presence of nimesulide in amorphous state in the complex rather than its original crystalline state. It was also evident that XRPD data also well supported the results of DSC studies confirming the interaction resulting in the formation of the complex. Previous studies done with insulin, diclofenac, aceclofenac etc., well supported these results (Cui *et al.*, 2006; Semalty *et al.*, 2009a, 2010c; Singh *et al.*, 2012b).

SEM: To determine the surface morphology SEM micrographs of nimesulide and its complex were obtained (Fig. 5). The pure nimesulide was showing its characteristic small crystals of regular shape with a smooth surface. But the complex showed crystals with blunt faces. The surface of the complex were non porous and rough. The characteristic rough surface morphology might have contributed to the improved solubility and the dissolution of nimesulide from the complex.

Solubility study: Aqueous solubility of nimesulide was found to be improved significantly in the complex (Table 1). This increase in the solubility of the complex may be explained by its amorphous characteristics and reduction in molecular crystallinity of nimesulide.

The amorphous nature of the complex (as confirmed by XRPD); typically rough surface morphology (as confirmed by SEM) and changes brought about due to complexation (as confirmed by FTIR and DSC) might have been responsible for the improvement in solubility (Semalty *et al.* 2013a; Singh *et al.*, 2013).

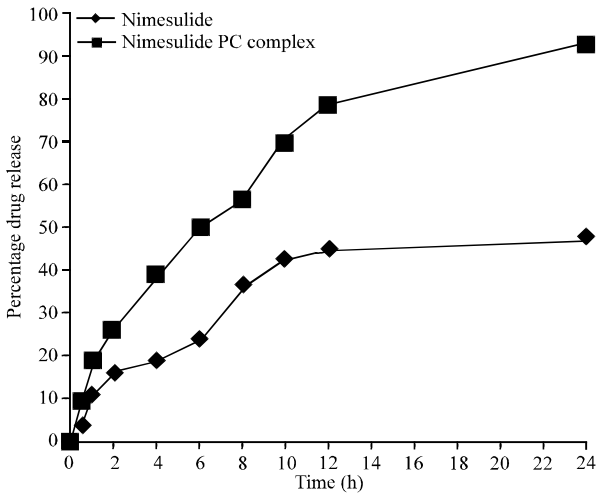

Fig. 6: Dissolution pattern of Nimesulide and its complex

Dissolution study: The complex of nimesulide showed a better dissolution profile than its free state (Fig. 6). Nimesulide showed only 47.65% drug release at the end of 24 h. But the nimesulide PC complex showed 92.76% drug release in dissolution study. The release might have been improved due to complexation which resulted in improved solubility of the drug. The improved surface morphology and amorphization induced by the complexation might have resulted in improved dissolution of nimesulide from its complex (Singh *et al.*, 2012a).

CONCLUSION

To improve the solubility and dissolution of nimesulide its lipid complex was prepared. It was concluded that the lipid complexation of nimesulide can lead to change in its state of crystallinity (amorphizing), its thermal behaviour, solubility and the dissolution profile. The lipid complexes might also be helpful in improving oral absorption of drug (class II drugs) with reduced toxicities and improved gastrointestinal safety (important with respect to NSAIDs).

ACKNOWLEDGMENTS

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