PHYSICOCHEMICAL INTERACTION OF NAPROXEN WITH ALUMINIUM HYDROXIDE AND ITS EFFECT ON DISSOLUTION

SHWETA PAROHA[1], RAVINDRA DHAR DUBEY[2], SUBRATA MALLICK[1*]

[1]*School of Pharmaceutical Sciences, Siksha O Anushandhan University, Bhubneswar-751003, Orissa, India.*
[2]*Indian Institute of Integrative Medicine (CSIR), Jammu, Jammu & Kashmir -180001, India.*
[*]*corresponding author: s_mallickin@yahoo.com*

Abstract

The interaction study of naproxen with aluminium hydroxide has been carried out in both solid state and wet state. Naproxen was co-milled in the solid state and co-lyophilized after overnight wet equilibration in different weight ratios. Physicochemical characterization of the interaction was carried out using analytical techniques such as Fourier transform infrared spectroscopy (FTIR), Differential scanning calorimetry (DSC) and Scanning electron microscopy (SEM) studies. *In vitro* dissolution studies were also performed to examine the effect of interaction on dissolution. FTIR spectra suggested an acid–base interaction between the carboxylic acid containing naproxen and aluminium hydroxide. DSC thermogram confirmed the absence of complexation between naproxen-aluminium hydroxide and SEM study indicated the presence of stabilized microparticles containing cluster of nano- and submicron drug crystals associated with aluminium hydroxide. *In vitro* dissolution studies revealed the improved release of naproxen from formulated powder samples

Rezumat

A fost studiată intercțiunea naproxenului cu hidroxidul de aluminiu, în stare solidă şi umectată. Caracterizarea fizico-chimică a interacțiunii s-a realizat prin metode analitice, spectroscopie în infraroşu cu transformată Fourier şi studii de calorimetrie diferențială şi microscopie de baleiaj electronic.

S-au desfăşurat studii *in vitro* de dizolvare pentru a evidenția efectul interacțiunii asupra eliberării substanței active.

Rezultatele obținute au demonstrat existența unei interacțiuni de tip acid-bază, absența fenomenului de complexare, prezența microparticulelor stabilizate, iar studiile de dizolvare au evidențiat o cedare favorabilă a naproxenului din probele sub formă de pulbere.

Keywords: naproxen, aluminium hydroxide, solid state interaction, wet state interaction, *in vitro* dissolution

Introduction

Naproxen is a nonsteroidal anti-inflammatory drug (NSAID) and is used to relieve pain and inflammation in a wide range of musculoskeletal

conditions, including various forms of arthritis, gout, muscle sprains and strains, and tendinitis. Chemically, naproxen is a propionic acid derivative and is usually better tolerated than aspirin or indomethacin. NSAIDs can occasionally cause serious side effects, such as ulceration, bleeding or perforation of the stomach and doctors advise to use antacids concomitantly. It is understood from the literature that the effect of different antacids on the absorption rate and extent of NSAIDs administered orally depends on the nature of the antacid. For example, aluminium hydroxide, unlike magnesium oxide, usually reduces the rate of absorption of some anti-inflammatory drugs [1]. Concomitant ingestion of an antacid without aluminium may be recommended for rapid onset of the analgesic effect of ibuprofen [2]. Galeazzi [3] from his researches suggested that aluminium hydroxide and other magnesium containing antacids decrease the bioavailability of indomethacin. Concurrent antacid administration had no significant effect on the droxicam pharmacokinetics and the study suggested that antacids do not significantly alter the oral absorption of single-dose droxicam [4, 5]. Upon concomitant administration of naproxen and aluminium hydroxide it can be assumed that the solubility of the weak acid naproxen (pKa 4.5 - 5) will increase because the pH of the stomach content increases. Hence, the absorption of naproxen could be influenced by interactions between naproxen and aluminium ions (if there any). Extensive literature survey revealed no report on naproxen-aluminium hydroxide interaction and its effect on dissolution of drug.

In this study, we explored the feasibility of physicochemical interaction between naproxen and aluminium hydroxide in the solid state and wet state. Solid state interaction was studied after co-milling; and co-lyophilization was performed after wet mass equilibration for the wet state interaction study. Unlike other techniques, milling is economically and environmentally desirable; it does not require toxic solvents [6] and sophisticated equipments [7]. FTIR, DSC and SEM techniques were used to examine the interaction of drug in the milled and lyophilized powder. Finally, *in vitro* dissolution was performed to investigate the effect of interaction on dissolution.

Materials and Methods

Materials

Naproxen was received as a gift sample from Ranbaxy Research Laboratories, Gurgaon, India. Aluminium hydroxide was purchased from CDH, New Delhi, India (minimum assay 47% (Al_2O_3); pH of solution not

more than 10.0; maximum limits of impurities chloride 0.5%, sulfate 0.25%, arsenic 0.0005%). All other chemicals were of analytical grade.

Solid-state co-milling

The physical mixture of naproxen and aluminium hydroxide was prepared by blending physically the components in the solid state, in weight ratios as mentioned in Table I. The physical mixture was placed into a cylindrical vessel of ball mill (Labotech Company Ltd, India) and 1 h period of constant milling was performed at lab ambient temperature of ~25°C. No significant increase in temperature of the milled material was detected at the end of the process. The ball mill was operated at 100 rpm and ball charge in the vessel allowed smooth cascading motion and significant attrition and impact during milling. After 1 h of running the milled material was removed and passed through mesh 44 (opening ~350 μm) and used for further analysis.

Preparation of wet mass and co-lyophilization

A wet mass (slurry) was prepared by kneading the blends with a small amount of water and left overnight for a period of 12 hrs at laboratory ambient condition followed by lyophilization in a freeze dryer (Lark, Penguin Classic Plus, India) for 10 hrs or until it was dried completely. The temperature was maintained approximately at -40° C and the pressure at 15-20 Pa. The freeze dried sample was packed in a polythene sachet and preserved in the desiccator for further analysis.

Table I

Composition code of co-milled and co-lyophilized powdered sample of naproxen with aluminium hydroxide

Sample code	Naproxen (wt %)	Aluminium Hydroxide (wt %)	Drug-Excipient Ratio	Processing
NC	100	0	Bulk naproxen	--
NA(PM)	50	50	1:1	Physical blend
N2A1(M)	66.7	33.3	2:1	Co-milled
N1A1(M)	50	50	1:1	Co-milled
N1A2(M)	33.3	66.7	1:2	Co-milled
N2A1(F)	66.7	33.3	2:1	Co-lyophilized
N1A1(F)	50	50	1:1	Co-lyophilized
N1A2(F)	33.3	66.7	1:2	Co-lyophilized

Physicochemical characterization

Fourier transform infrared spectroscopy

Infrared spectra of pure naproxen, milled and lyophilized samples were collected using a FTIR Spectrophotometer (JASCO FT/IR-4100typeA,

Japan). Standard KBr disk method was used to obtain the FTIR spectra. The sample containing approximately 1mg of drug and 200 mg of dried KBr was blended uniformly in an agate mortar and pressed into a translucent disk. The samples were scanned from 400 to 4000 cm^{-1} with 80 accumulations per spectrum and a resolution of 4 cm^{-1} and scanning speed of 2 mm s^{-1}. The spectra were analyzed with the Spectrum software: Spectra Manager. The optimum points smoothing was performed on all the spectra to remove spikes and negative peaks. The relative peak intensities at the area of interest were further taken for the calculation of the interaction.

Differential scanning calorimetry

DSC thermograms of powder samples of naproxen were obtained on a Differential scanning calorimeter (model: DSC -60 Shimadzu, Japan.) connected to a computerized thermal analyzer. Approximately 16 mg of samples were sealed in the aluminium pan, and the measurement was performed at a heating rate of 10° C min^{-1} using dry nitrogen flow (30 mL min^{-1}). The temperature was calibrated with pure indium, with a melting point of 156.60° C. An empty pan was used as a reference. It was carried out to investigate the drug interaction with formulation aluminium hydroxide.

Scanning electron microscopy

The surface morphology of selected powdered samples was visualized by a Scanning electron microscope (JSM, 6100, JEOL, Tokyo, Japan) at 10 Kv. Scanning electron microscopy was used to evaluate the surface texture, shape, and size of the particles. The samples for SEM were prepared by lightly sprinkling the particles on a double adhesive tape, which struck to an aluminium stub. They were coated with gold for 10 min under vacuum by using SPI Sputter coating unit (SPI supplies, division of structure probe Inc., USA).

In vitro dissolution studies

Dissolution tests of all the naproxen powder samples were performed using USP XXIV type II dissolution apparatus (Electrolab, dissolution tester USP TDT 06L) with a rotation speed of 100 rpm and temperature 37º C. The dissolution study was first attempted in distilled water. These powder samples did not wet easily and stuck to the wall of the vessel and to the paddle because of their strong hydrophobic nature. Addition of 0.1 % of sodium lauryl sulfate (SLS) in distilled water the wettability of the drug was sufficient to avoid these undesirable facts and to observe differences among the dissolution profiles of the drug powder formulations. The final volume in all cases was 900 ml. Aliquots were withdrawn at appropriate time intervals and replaced by the fresh dissolution medium. The samples were

filtered through a 0.45 μm syringe filter and absorbance data were recorded at 230.2 nm using UV visible Spectrophotometer (JASCO V-630 spectrophotometer, Software: Spectra Manager). The respective concentration was estimated using a standard calibration curve. The mean of three determinations was used to calculate the amount of drug released from the samples and the error expressed as standard deviation (mean ± sd, n = 3). These data were used to calculate the cumulative percent of drug released from different formulations.

Results and Discussion

Naproxen was co-milled with aluminium hydroxide in the solid-state at laboratory ambient temperature (~25°C). Also wet mass equilibrated overnight and freeze dried was prepared for the wet-state interaction study. The solid state ball-milling and wet mixing and subsequent drying (as in wet granulation technique of tablet processing) are effective, simple and scalable. The extent of physicochemical interaction of naproxen in solid and wet state with aluminium hydroxide using FTIR, DSC and SEM has been detailed below. Solid state ball-milling produced slightly moist powder whereas, dried powder material was obtained after co-lyophilization.

Characterization by FTIR

FTIR technique can be extensively used for drug –drug and drug –excipient interaction studies. The intermolecular hydrogen bonding in crystalline naproxen as well as its solid dispersions with PVP [8-10] has been well documented. The crystal structure of naproxen reveals that instead of cyclic dimeric congener as ibuprofen it exists as a trimolecular catemer [11]. FTIR spectrum of naproxen is mainly characterized by vibrations at 1725 cm^{-1} and 1684 cm^{-1} attributed to non-hydrogen-bonded –C=O stretching and hydrogen-bonded -C=O stretching of the catemer, respectively. As most of the naproxen molecules are not engaged in hydrogen bonding the intensity of the vibrational band at 1725 cm^{-1} is more dominant. The FTIR spectra of the physical mixture, milled and lyophilized materials are given in Figure 1**.** The carbonyl stretching region was more sensitive to the consequences of drug-aluminium hydroxide interaction. The carboxylic acid peak intensity at 1725 cm^{-1} and 1684 cm^{-1} has been significantly decreased in the ball milled and freeze dried powders. Considering the acidic nature of the carboxylic acid group of naproxen, the possibility of an acid–base interaction between the drug and aluminium hydroxide was investigated. This type of acid–base reaction explains the changes in the FTIR spectra of some reports of comilled powders [12-14]. Additionally loss of intermolecular hydrogen bonding involved by carboxylic acid group can also be suggested. An acid–

base reaction between the carboxylic acid-containing drugs and aluminium hydroxide explains the changes in the FTIR spectra of both the milled and freeze dried powders. The carboxylate peak in the range 1600–1575 cm^{-1} gradually increased in the milled and freeze dried powder as the weight contribution of aluminium hydroxide has been increased in the sample with the consequent decrease of carboxyl peak intensity. The carboxylate peak was more intense in the freeze dried sample.

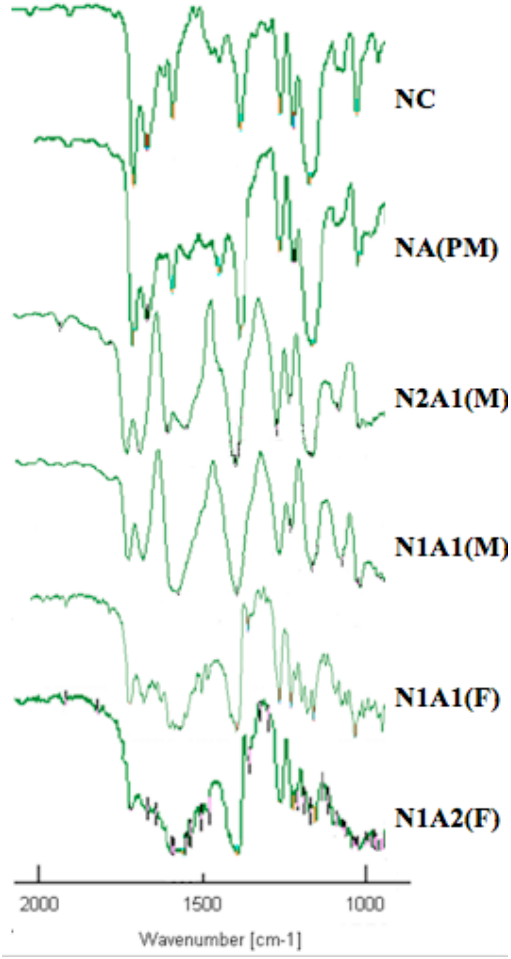

Figure 1

FTIR spectra of (i) pure naproxen crystals**:** NC; (ii) physical mixture of naproxen and aluminium hydroxide at 1:1 ratio**:** NA(PM); (iii) co-milled powder of naproxen and aluminium hydroxide at 2:1 ratio**:** N2A1(M); (iv) co-milled powder of naproxen and aluminium hydroxide at 1:1 ratio**:** N1A1(M); (v) co-lyophilized powder after aqueous state equilibration of naproxen with aluminium hydroxide at 1:1 ratio**:** N1A1(F); and (vi) co-lyophilized powder after aqueous state equilibration of naproxen with aluminium hydroxide at 1:2 ratio**:** N1A2(F).

Characterization by DSC

DSC is a powerful method for the study and detection of complexation between drug-drug and drug-metals. DSC thermograms of pure naproxen, milled and lyophilized materials are shown in Figure 2. Sharp melting point endothermic peak with high intensity at 157.14°C (heat required -42.04 J/g) of naproxen is noticed in thermogram of pure naproxen. The melting endotherm of naproxen has also been observed in all DSC traces of N2A1(M) and N2A1(F); it is slightly shifted (153.53°C and 156.71°C with required heat -37 J/g and -23.79 J/g respectively) because of the presence of aluminium hydroxide. The comparatively smaller endotherm observed at 180.22 and 188.81°C corresponds to the dehydration endotherm in powder containing adsorbed water in aluminium hydroxide. The retained water held within the milled sample is relatively more than in the freeze dried sample because the former sample has not been dried. That is why the heat of dehydration endotherm is more (-17.40 J/g) in milled sample compared to that of freeze dried sample (-10.96 J/g).

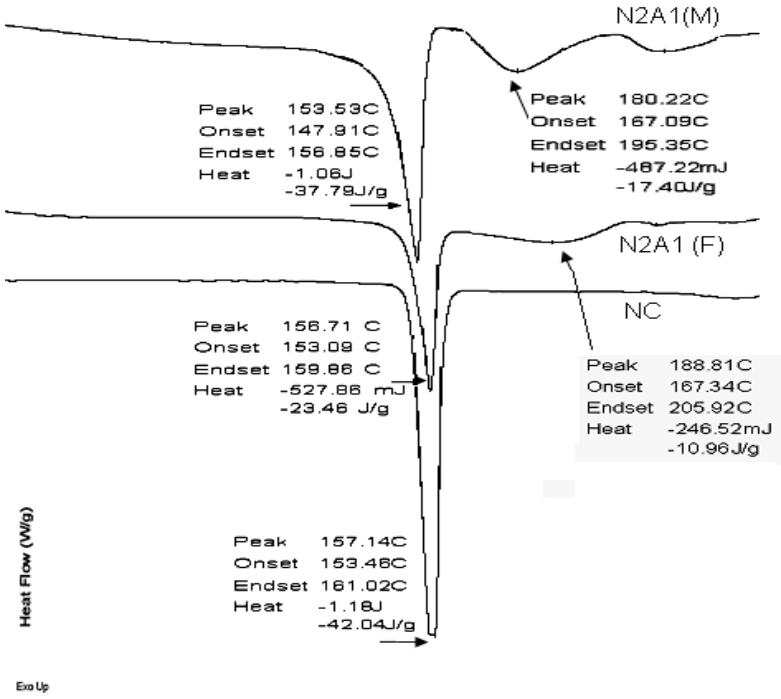

Figure 2

DSC thermograms of (i) pure naproxen crystals**:** NC; (ii) co-milled powder of naproxen and aluminium hydroxide at 2:1 ratio**:** N2A1(M); and (iii) co-lyophilized powder after aqueous state equilibration of naproxen with aluminium hydroxide at 2:1 ratio**:** N2A1(F).

Characterization by SEM

Scanning electron micrographs (SEM) of pure naproxen, physical mixture, milled and lyophilized powders are shown in Figure 3. Untreated pure drug shows distinctive morphological views of geometric shape of naproxen (Figure 3A) due to the crystalline nature. Naproxen crystal can be identified clearly in the physical mixture of drug and aluminium hydroxide (Figure 3B). Particle size has been reduced significantly upon co-milling of naproxen with aluminium hydroxide [N1A2(M)] and the individual naproxen crystals were submicron sized and agglomerated loosely with aluminium hydroxide in the domain of irregular particles of aluminium hydroxide of the milled sample (Figure 3C). Needle shaped submicron clustered crystals of naproxen attached with aluminium hydroxide were produced (Figure 3D) after co-lyophilization with aluminium hydroxide to form loose aggregates [N1A2(F)].

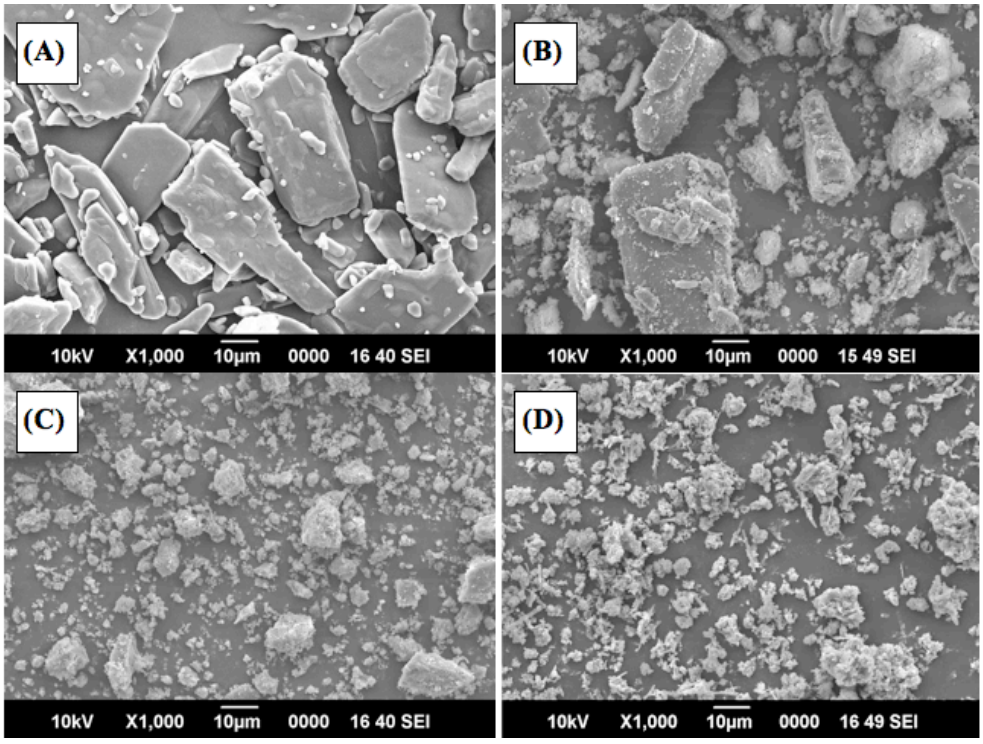

Figure 3

Scanning electron micrograph of samples: (A) pure naproxen crystals, (B) physical mixture of naproxen and aluminium hydroxide at 1:1 ratio, (C) co-milled powder of naproxen and aluminium hydroxide at 1:2 ratio, and (D) co-lyophilized powder after aqueous state equilibration with aluminium hydroxide at 1:2 ratio.

Effect on dissolution

The dissolution profiles of crystalline naproxen and milled and lyophilized materials have been evaluated for 90 min and depicted in Figure 4 and Figure 5. Because of its poor aqueous solubility, naproxen crystalline powder (NC) exhibited a slow rate of dissolution of 66.27 % at the end of a 90 min period even in the medium containing 0.1% of SLS. N1A2(F) showed the highest percentage of dissolution (94.38 %) amongst all the wet equilibrated freeze dried samples and N2A1(M) exhibited maximum release of 85.43 % amongst all solid state comilled samples of naproxen. The dissolution profile of total systems improved in the order: naproxen< N1A1(M)< N2A1(F)< N1A2(M)< N2A1(M)< N1A1(F)< N1A2(F). Freeze dried samples relatively better improved in drug release rather than the milled samples because of intimate interaction occurred in the wet equilibrated freeze dried samples. Conversion into salt form in the wet state brought about better dissolution. On the contrary slow interaction in the dry state due to poor molecular mobility resulted in a less improvement in dissolution of the drug in the milled samples. Extent of dissolution of drug of N1A1(M) and N1A2(M) has slightly been decreased compared to N2A1(M). No significant difference in dissolution was observed with these samples. Excess of aluminium hydroxide remained uninteracted in the sample N1A1(M) and N1A2(M) have slightly affected the release of drug. Gradual enhancement of drug release was observed with the increase of aluminium hydroxide content in the wet state of interaction.

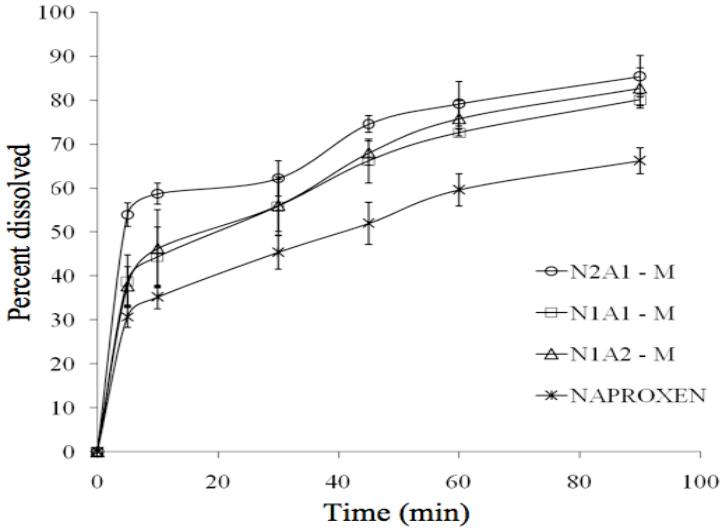

Figure 4

Cumulative percentage of naproxen released in *in vitro* dissolution studies from samples of pure naproxen and powders co-milled with aluminium hydroxide. Each point represents the mean ± SD, n=3.

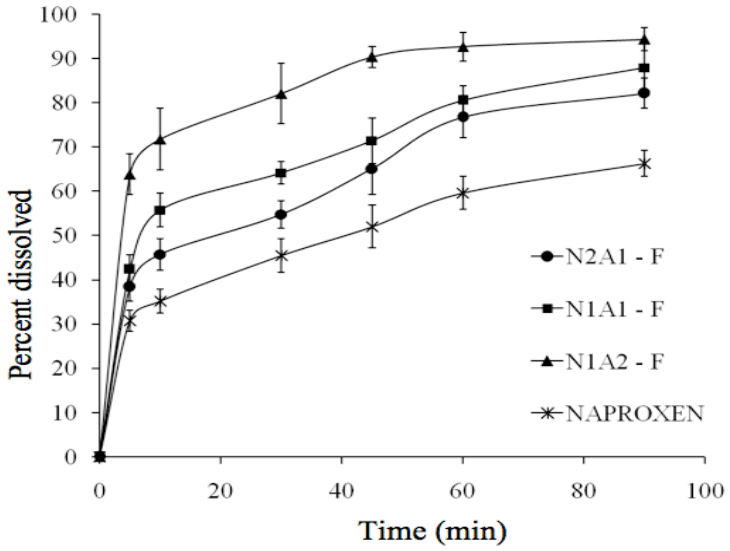

Figure 5

Cumulative percentage of naproxen released in *in vitro* dissolution studies from samples of pure naproxen and powders obtained by co-lyophilization after aqueous state equilibration with aluminium hydroxide. Each point represents mean ± SD, n=3.

In the acid–base reaction, the free acid carboxyl peaks in the milled samples of N2A1(M) and N1A1(M) decreased whereas, in freeze dried samples N1A1(F) and N1A2(F) almost disappeared. The peak for the carboxylate ion appeared in all milled and freeze dried samples and to a maximal extent in N1A2(F). The changes in the FTIR spectra indicate an acid–base interaction between the carboxylic acid containing naproxen and aluminium hydroxide to form their salt. This confirmed the interaction and ultimately facilitated the release of drug. FTIR data showed the presence of a carboxyl peak at 1725 cm^{-1} in the spectrum of pure naproxen. Disappearance of the carboxyl peak and appearance of the carboxylate peak in the FTIR spectra of N1A2(F) suggest salt formation. Aluminium hydroxide has an isoelectric pH of 11.4 [15] and the inherently bound water associated with aluminium hydroxide has an alkaline pH of about 10. Thus, the surface of the aluminium hydroxide will be positively charged and naproxen, being a weak acid, will electrostatically be attracted to aluminium hydroxide. In this study, we observed the changes in carboxylic acid group in naproxen–aluminium hydroxide co-milled and co-lyophilized powder. The moisture present in aluminium hydroxide (8–10%) brought about the molecular mobility in the solid state milling. In the wet state, interaction was more significant due to the presence of excess water and intimate

contact of naproxen and aluminium hydroxide in co-lyophilization. The decrease in the carboxylic acid group seems to correspond to an increase in the carboxylate ion of naproxen, supporting the conversion from the acid to a salt on milling and lyophilization. We found that the DSC thermogram of milled and lyophilized samples showed the melting endotherm similar [16, 17] to the endotherm of pure naproxen. This is the evidence of no indication of complexation between naproxen-aluminium hydroxide. Due to co-milling of naproxen and aluminium hydroxide [N1A2(M)] particle size significantly decreased and the naproxen crystals were found as submicron and clustered with aluminium hydroxide. Naproxen has been precipitated after freeze drying and yielded needle shaped submicron naproxen clustered particles attached with aluminium hydroxide to form loose aggregates [N1A2(F)]. Slightly moist particles produced after comilling of naproxen with aluminium hydroxide may be due to the interaction of aluminium hydroxide and naproxen after squeezing out of inherently bound water associated with aluminium hydroxide whereas, co-lyophilized samples removed even residual bound water of aluminium hydroxide after freeze drying. This suggested that the interaction force between naproxen and aluminium hydroxide systems is stronger than the water held in aluminium hydroxide.

Although FTIR provides evidence for salt formation, DSC data suggested the absence of complexation between naproxen-aluminium hydroxide and SEM analysis confirms the presence of stabilized microparticles containing cluster of nano- and submicron drug crystals. In this study, both of solid-state comilling and co-lyophilization after wet-state equilibration have shown improved dissolution than pure crystalline drug. The release of a drug from a delivery system involves factors of both dissolution and diffusion. The release rate of a drug like naproxen is only important where it is the rate-limiting step in the absorption process and consequent onset of action. The most improved dissolution of drug associated with N1C2(F)-freeze dried powder is due to aluminium salt formation and transformation of crystalline drug to nano- and microparticulate form.

Conclusions

It can be concluded that co-milling and co-lyophilization after wet-state equilibration of drug with commonly used antacid, aluminium hydroxide can improve dissolution. Techniques can be industrially viable and economical. Analytical methods of characterization: FTIR, DSC and SEM scientifically supported the possible improved dissolution, finally proved by *in vitro* dissolution testing. FTIR spectroscopy showed the

gradual decreased intensity of carboxylic acid peak and appearance of carboxylate peak in the co-milled and freeze dried powder of naproxen and aluminium hydroxide. DSC thermogram of the samples resulted in the absence of complexation between naproxen-aluminium hydroxide. SEM method supported that the individual naproxen crystals were submicronical and cylindrical, and attached with the microfine aluminium hydroxide. Dissolution studies revealed the increased percentage release of naproxen from milled and freeze dried powder. Intimate interaction of the wet equilibrated freeze dried samples brought about more improved dissolution of freeze dried samples compared to milled samples. Studies related to interaction of other drugs containing carboxyl acid group with aluminium hydroxide or other antacids could be a potential area of research in the future.

Acknowledgments

The authors are very much grateful to Prof. Manoj Ranjan Nayak, President, Siksha O Anusandhan University for his encouragement and facilities.

References

1. Jukka M, Anneli T, Taneli J, Harri I, Eliisa L, Kristiina P, Pertti JN, Simo T. Magnesium hydroxide in ibuprofen tablet reduces the gastric mucosal tolerability of ibuprofen. *J Clin Gastroenterol*. 2004, 38(1):41-45.
2. Neuvonen PJ. The effect of magnesium hydroxide on the oral absorption of ibuprofen, ketoprofen and diclofenac. *Br J Clin Pharmacol*. 1991, 31(3):263-266.
3. Galeazzi RL. The effect of an antacid on the bioavailability of indomethacin. *Eur J Clin Pharmacol.* 1977, 12(1):65-68.
4. Bartlett A, Costa A, Martínez L, Roser R, Sagarra R, Sánchez J. The effect of antacid and ranitidine on droxicam pharmacokinetics. *J Clin Pharmacol.* 1992, 32(12):1115-1119.
5. Maya MT, Pais JP, Ruas Da Silva J, Morais JA. A comparative bioavailability study to estimate the influence of an antacid on droxicam pharmacokinetics. *Eur J Drug Metab Pharmacokinet.* 1995, 20(4):275-279.
6. Sarkari M, Brown J, Chen X, Swinnea S, Williams RO III, Johnston KP. Enhanced drug dissolution using evaporative precipitation into aqueous solution. *Int J Pharm*. 2002, 243:17–31.
7. Moneghini M, Kikic I, Voinovich D, Perissutti B, Filipovic-Grcic J. Processing of carbamazepine-PEG 4000 solid dispersions with supercritical carbon dioxide: Preparation, characterization, and *in vitro* dissolution. *Int J Pharm.* 2001, 222:129–138.
8. Tomasko DL, Timko MT. Tailoring of specific interactions to modify the morphology of naproxen. *J Cryst Growth.* 1999, 205:233–243.
9. Velazquez MM, Valero M, Rodriguez LJ, Costa S, Santos MA. Hydrogen bonding in a non-steroidal anti-inflammatory drug–Naproxen. *J Photochem Photobio B Biol.* 1995, 29:23–31.
10. Nair R, Nyamweya N, Gönen S, Martinez-Miranda LJ, Hoag SW. Influence of various drugs on the glass transition temperature of poly (vinylpyrrolidone): a thermodynamic and spectroscopic investigation. *Int J Pharm.* 2001, 225:83–96.
11. Bogdanova S, Pajeva I, Nikolova P, Tsakovska I, Müller B. Interactions of poly (vinylpyrrolidone) with ibuprofen and naproxen: experimental and modeling studies. *Pharm Res.* 2005, 22:806–815.

12. Mallick S, Pattnaik S, Swain K, De PK, Saha A, Ghoshal G, Mondal A. Formation of physically stable amorphous phase of ibuprofen by solid state milling with kaolin. *Eur J Pharm Biopharm.* 2008, 68:346–351.
13. Maclean J, Medina C, Daurio D, Alvarez-Nunez F, Jona J, Munson E, Nagapudi K. Manufacture and performance evaluation of a stable amorphous complex of an acidic drug molecule and Neusilin. *J Pharm Sci.* 2011, 100(8):3332-3344.
14. Mallick S, Pattnaik S, Swain K, De PK, Saha A, Mazumdar P, Ghoshal G. Physicochemical characterization of interaction of ibuprofen by solid-state milling with aluminium hydroxide. *Drug Dev Ind Pharm.* 2008, 34(7):726-734.
15. Rus LM, Tomuta I, Iuga C, Maier C, Kacso I, Borodi G, Bratu I, Bojita M. Compatibility studies of indapamide/pharmaceutical excipients used in tablet preformulation. *Farmacia J.* 2012, 60(1):92-101.
16. Kundu SP, Amjad FM, Sultana S, Sultan MZ, Hossain MA, Amran MS. Study of differential scanning calorimetry of complex of magnesium sulfate with aspirin, paracetamol and naproxen. *Bangladesh Pharm J.* 2012, 15(1):7-12.
17. Rinella JV, White JL, Hem SL. Effect of pH on the elution of model antigens from aluminium containing adjuvants. *J Colloid Interf Sci.* 1998, 205:161–165.

Manuscript recieved: September 27th 2012