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Novel lipid-based formulations enhancing the in vitro dissolution and permeability characteristics of a poorly water-soluble model drug, piroxicam

Sunil Prabhu *, Maru Ortega, Chan Ma

College of Pharmacy, Western University of Health Sciences, Pomona, CA 91766, USA

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Abstract

Lipid-based delivery systems are becoming increasingly popular as carriers of drugs due to their ability to overcome barriers to oral absorption. The purpose of this study was to prepare novel lipid-based formulations of a model drug, piroxicam (PXCM), a poorly water-soluble non-steroidal anti-inflammatory drug (NSAID) using 1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine (DMPC) phospholipid alone, and in combination with polyethylene glycol (PEG 4600). Lipid-based drug delivery systems were prepared using conventional methods of preparation and the following aspects were evaluated (1) in vitro dissolution behavior, (2) absorption via Caco-2 cell monolayers and (3) stability of formulations over a 12-month period. In addition, physical characterization studies using differential scanning calorimetry (DSC) were also performed. Formulations of PXCM were prepared using DMPC in the following combinations (A) 1:1 and (B) 2:1 and a mixture of DMPC and PEG 4600 (C) 2:1:1, respectively. Dissolution studies conducted in phosphate buffered saline (PBS, pH 7.4, $37 \pm 0.5\,^{\circ}C$) using the USP type II (paddle) dissolution apparatus showed an increase in dissolution rate and extent of the PXCM from all solid dispersion formulations when compared to the control. As such, the rate of drug release was observed to be fastest with formulation (C) showing the greatest increase of over two-fold compared to the control. Release of PXCM from formulations (A) and (B) was intermediate with the latter showing superior dissolution behavior despite containing lower amounts of the carrier lipid than the former. This observation indicates a possible existence of threshold levels for phospholipids carriers beyond which dissolution could be adversely affected. DSC studies further confirmed the dissolution behavior of these formulations demonstrating different levels of amorphous to crystalline nature. Results of HPLC analysis from Caco-2 cell culture studies showed increase in transport of PXCM from all formulations, with formulation (C) showing the maximum increase followed by formulations (B) and (A), when compared to control. The apparent permeability coefficients (P_{app}) were calculated to be 7.92×10^{-6}, 9.48×10^{-6}, 9.2×10^{-6} and 5.6×10^{-6} cm/s for formulations (A)–(C) and control, respectively. Overall, permeation appeared to improve for all formulations over the control. Stability studies at various temperatures showed all formulations to have good stability for the first 6 months;

* Corresponding author. Tel.: +1 909 469 5550; fax: +1 909 469 5600.
E-mail address: sprabhu@westernu.edu (S. Prabhu).

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then a decline in dissolution rates was observed, especially for PEG-based lipid carrier systems, attributed to the increase in crystalline content of the solid dispersions upon storage.

Keywords: Lipid-based drug delivery systems; Poorly water-soluble drugs; Dissolution; Permeability; DSC; DMPC; PEG 4600; Stability

1. Introduction

Oral drug delivery remains the most popular route of administration. However, limitations in the physical–chemical properties of the drug sometimes prevents a successful therapeutic outcome. Specifically, problems of poor solubility and chemical stability in the gastrointestinal tract, poor permeability and sensitivity to metabolism are often causes that result in the rejection of potential drug candidates as commercial products. Lipid-based delivery systems are becoming increasingly popular as carriers of drugs because of their ability to bypass some of the more resistant chemical and physical barriers associated with poorly absorbed drugs. Examples of such lipid-based drug delivery systems include conventional emulsions and microemulsions and more recently liposomes, microspheres, solid-lipid nanoparticles, cubosomes, etc. (Bummer, 2004). There is evidence in literature that lipid-based systems have been most successful in enhancing the bioavailability of Class II molecules that are poorly water-soluble but highly permeable drug molecules (Amidon et al., 1995). Some proposed mechanisms of action of lipid-based systems to enhance oral bioavailability of compounds include enhanced wetting of hydrophobic solids resulting in enhanced dissolution, increased rate of dissolution into aqueous environment from oil droplets of high surface area, promotion of absorption via intrinsic lipid pathways, enhanced thermodynamic activity via supersaturation of the aqueous environment of the GI tract, particle size reduction to molecular size yielding a solid-state solution within the carrier (Serajuddin, 1999; Brigger et al., 2002; Vauthier et al., 2003).

The purpose of this research was to use lipid-based drug delivery systems to study the dissolution, permeability and stability of a poorly water-soluble model drug, piroxicam (PXCM). Piroxicam is a non-steroidal anti-inflammatory drug, classified in the Biopharmaceutic Drug Classification (BCS) system as a Class II drug with low solubility and high permeability. It demonstrates a slow and gradual absorption via the oral route and has a long half-life of elimination, rendering a prolonged therapeutic action and a delayed onset of anti-inflammatory and analgesic effect (Tagliati et al., 1999). Previous studies have demonstrated that piroxicam, when prepared in polyethylene glycol (PEG) 4000 solid dispersion system, gave a faster dissolution than its corresponding mixtures (Pan et al., 2000). Other studies using solid dispersions of piroxicam in polyvinylpyrrolidone (PVP) showed significant increase in dissolution (Tantishaiyakul et al., 1999) over the pure drug formulation. However, not many studies have reported the effect of lipid-based carriers on the in vitro and in vivo behavior of piroxicam drug. A recent study used gelucire 44/14 (surfactant)-based solid dispersions to enhance the bioavailability of piroxicam in humans (Yuksel et al., 2003). Our group has previously shown the increase in dissolution rates of a poorly water-soluble drug, ethopropazine, using combinations of phospholipids and polyethylene glycol (Prabhu et al., 2001).

The present study proposes the use of a phospholipid 1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine (DMPC) alone and its combination with polyethylene glycol 4600 (PEG 4600). DMPC was selected due to its lower phase transition temperature (T_c) of 23.5 °C enabling quicker hydration at room temperature compared to phospholipids with higher T_c's. In addition, phospholipids with polar head groups such as phosphatidylcholine (PC) are more preferable due to their low toxicity, availability in pure form, stability and lower cost (Lasic, 1993). The selection of PEG 4600 was based on its excellent solubility in aqueous and most organic solvents; furthermore, PEG's demonstrate favorable kinetics and tissue distribution in the body as well as lack of toxicity and immunogenicity (Zaplisky and Harris, 1997) Lipid-based formulations containing drug and DMPC carrier were prepared in the following ratios: (A) 1:1, (B) 2:1 and a combination

of drug, DMPC and PEG 4600, and (C) 2:1:1, respectively. Samples were prepared using conventional methods. All formulations were studied for in vitro dissolution behavior, permeability using Caco-2 cell monolayers and a 12-month physical storage stability test at temperatures of 4, 25 and 60 °C. Further characterization of the drug/carrier combination(s) was conducted using differential scanning calorimetry.

2. Materials and methods

2.1. Materials

Piroxicam and magnesium stearate were obtained from Spectrum Chemicals (Irvine, CA). The phospholipid, 1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine (DMPC), was acquired from Genzyme Pharmaceuticals (Cambridge, MA). Polyethylene glycol 4600 was obtained from Union Carbide Corp. Microcrystalline cellulose (Avicel) was supplied by FMC Corp. (Newark, DE). Chloroform (HPLC grade), buffer salts, hydrochloric acid were purchased from Fisher Scientific Co. (Pittsburgh, PA). Nitrogen (N_2) gas was supplied by Praxair Incorporated (Pomona, CA).

2.2. Methods

2.2.1. Lipid-based formulations: preparation and manufacture

Three novel formulations were prepared as follows: (A) 1 part of PXCM to 1 part of the phospholipids, DMPC (1:1), (B) 2 parts of PXCM to 1 part of the phospholipids, DMPC (2:1) and (C) 2 parts of PXCM to 1 part of DMPC and 1 part of PEG 4600. Formulations of piroxicam blended with microcrystalline cellulose (Avicel) and magnesium stearate were prepared as controls. Using the solvent method of evaporation (Chiou and Riegelman, 1971), accurately weighed amounts of PXCM and carriers were dissolved in minimum quantities of chloroform. Samples were dried under current of N_2 gas for a period of 6 h. The solid dispersion lipid-based formulations containing the drug and carrier(s) was then sieved through a 60-mesh sieve and stored until further use.

2.2.2. In vitro dissolution studies

Dissolution studies were conducted using a Type II (paddle) USP Dissolution apparatus (Vankel VK7000). The dissolution medium consisted of 300 ml phosphate buffered saline (PBS) at a pH of 7.4. The temperature of the medium was maintained at 37.5 ± 0.5 °C. Weighed amount of solid dispersion (representing 20 mg drug) was placed into capsules then introduced into the dissolution medium. At predetermined time intervals, 5 ml samples were withdrawn and then replaced with fresh PBS. Samples were filtered and immediately analyzed using a UV–vis recording spectrophotometer (Shimadzu UV-2401PS) at the wavelength of 330 nm. All studies were conducted in triplicate.

2.2.3. Caco-2 cell culture model

The Caco-2 cell line obtained from American Type Culture Collection (ATCC, Rockville, MD) was maintained at 37 °C in a humidified atmosphere of 5% CO_2 and 95% relative humidity in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% penicillin–streptomycin solution and 2 mM glutamine. Cultured media were changed every 2–3 days. For subculturing, the cells were removed enzymatically using 1.0 ml of 0.05% trypsin and 0.02% EDTA in Hanks' Balanced Salt Solution (HBSS).

For the transport experiments, the Caco-2 cells were seeded at a density of 2×10^5 cells/cm^2 in a 24 mm diameter and a 0.4 μm pore size, 6-well Transwell™ culture plate inserts (Costar). Cells were grown for at least 3 weeks before experiments.

2.2.4. Drug transport in Caco-2 cell monolayers

The transepithelial electrical resistance (TEER) was measured using an EVOM™ epithelial volt ohmmeter (World Precision Instrument, Sarasota, FL). Measurements were taken on the apical side of the cells before starting and at the end of the study. The range of resistance before the study was between 100 and 150 Ω.

Donor solutions containing PXCM formulations representing 5 mg of drug was prepared with HBSS medium containing Mg^{2+} and Ca^{2+} and warmed in the water bath at 37 °C. Prior to assay, the confluent Caco-2 cell monolayer containing Transwell™ inserts were equilibrated with HBSS for 15 min at 37 °C. The inserts were then positioned into 6-well plates containing 2.5 ml of HBSS medium with Mg^{2+} and Ca^{2+} (pH at 7.4, basolateral solution). 2.5 ml of the previously prepared donor solution containing the drug were added to

the apical side of the monolayer. Three hundred microliter samples were withdrawn from the basolateral side at regular time intervals and replaced immediately by equivalent amounts of fresh HBSS. Samples were subsequently analyzed by HPLC.

The apical to basolateral permeability (apparent permeability coefficient, P_{app}) of PXCM was calculated according to the following equation (Mainprize and Grady, 1998):

$$P_{app} = \frac{dQ}{dt}(1/A \times C_0)$$

where dQ/dt is the rate of appearance of drugs on the basolateral side (mg/s), calculated from the slope of the regression line describing the cumulative amount versus time, C_0 the initial drug concentration on the apical side (mg/ml) and A is the surface area of the monolayers (cm^2).

2.2.5. HPLC analysis

The HPLC studies were performed using a Hewlett Packard Series 1100 HPLC system with autosampler and a C-8 analytical column with a particle size of 5 μm (Phenomenex Prodigy ODS 3 Column, 4.6 μm × 250 mm). The mobile phase consisted of acetonitrile (55%)–10 mM phosphate buffer pH 2.5 (45%). PXCM was detected at a wavelength of 220 nm, at a flow rate of 1 ml/min. The retention time of PXCM at this flow rate was between 5–7 min. The injection volume was maintained at 100 μl.

2.2.6. Thermal analysis

Differential scanning calorimetry studies were conducted to obtain thermograms of PXCM formulations. A cell base was used with samples weighing about 5 mg with a heating rate of 20 °C/min from 0 to 200 °C. Peak transition temperature and heat of melting were determined by means of a thermal analyzer (Mettler-Toledo, Toledo, OH). Indium was used as reference.

2.2.7. Storage stability studies

All PXCM formulations were stored in closed glass vials for a period of 12 months at temperatures of 4, 25 (RT) and 60 °C. Dissolution testing was conducted at 6- and 12-month intervals to assess changes in drug release characteristics. The dissolution procedure used was the same as described above.

3. Results and discussion

3.1. Dissolution studies

As shown in Fig. 1, dissolution of piroxicam pure drug was extremely poor in the PBS media with only about 44% drug going into solution during the 2-h run. Poor solubility of PXCM (<1 mg/25 ml phosphate buffered saline (PBS) solution) can be attributed to its hydrophobic nature, poor wettability (AHFS, 2000) and evidence of particle agglomeration during the dissolution runs. The dissolution rate increased significantly when lipid and polymer-based carriers were added, either individually in different ratios or in combination with each other. Formulation (C) showed the highest increase in dissolution rate with the entire drug amount released within 30 min of the run. The addition of PEG 4600 to the phospholipid DMPC afforded a greater solubilizing effect on the drug thus enhancing the dissolution rate. Overall, the presence of PEG creates a better micro-environment for the dissolution of the drug (Weuts et al., 2005). In this case, the presence of the PEG polymer in combination with DMPC showed over a two-fold increase in dissolution rate when compared to the control formulation. The dissolution of drug from formulation (C) also increased when compared to solid dispersion formulations containing only DMPC phospholipids. Formulations (A) and (B) exhibited intermediate release characteristics and showed no significant difference in release rates. Formulation (A) despite containing twice the amount of

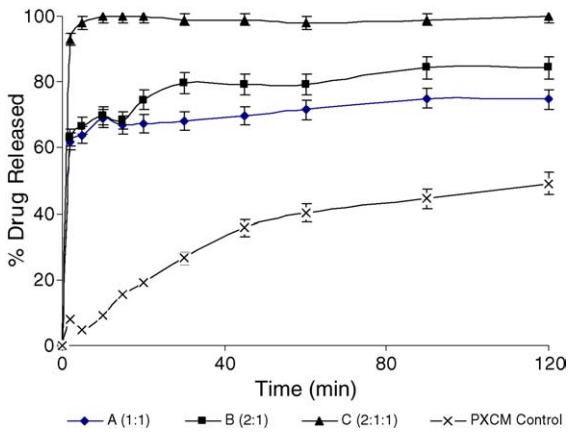

Fig. 1. Dissolution profiles of piroxicam (PXCM) drug alone and in combination with lipid and PEG-based carrier systems.

phospholipid did not represent any distinct advantage over formulation (B). In fact, the extent of dissolution from formulation (A) decreased over that of formulation (B). This observation suggested the possibility of a threshold level for phospholipid content, beyond which dissolution could be adversely affected. Overall, the DMPC-only based formulations increased dissolution rates of PXCM by over 1.5-fold over that of the control formulation.

3.2. Drug transport in Caco-2 cell monolayers

The transport of PXCM through Caco-2 monolayers was the highest from formulation (C) as shown in Fig. 2, followed by formulation (B) and (A). All three formulations demonstrated a higher rate and extent of absorption than the control; however, a comparison of the extent of absorption between the three lipid-based formulations did not demonstrate a significant difference. The absorption of PXCM from formulation (C) was only slightly higher than either (B) or (A). The pattern of absorption followed the in vitro dissolution behavior of the lipid-based formulations reinforcing the role of PEG 4600 as a solubilizer for the piroxicam drug in formulation (C). This observation was expected since the presentation of the drug at the site of absorption is dependent on the carriers. The solubilizing effect of PEG 4600 and the phospholipids, DMPC, allows the drug to quickly associate into the aqueous surrounding perhaps by molecular complexation or formation of a solid-state solution. This enables the solubilized drug to be absorbed quicker than the drug presented at the site of absorption without any carriers. A rank-order correlation of absorption was evident in the following order of formulations, (C) > (B) > (A). The absorption pattern of formulation (B) with respect to (A) further confirmed the possibility of a threshold level for the DMPC lipid carrier in the PXCM formulation.

The permeability coefficients (P_{app}) were calculated from the slope(s) of the absorption profile(s) to be 7.92×10^{-6}, 9.48×10^{-6}, 9.2×10^{-6} and 5.6×10^{-6} cm/s for formulations (A)–(C) and the control, respectively. Previous studies reported in literature categorized the permeability coefficient (P_{app}) score of $1–10 \times 10^{-6}$ cm/s as being moderately absorbed (ranging from 20 to 70%)(Lee, 1997). Based on this observation, the overall trend of absorption of PXCM suggests that the drug is moderately absorbed from the Caco-2 monolayers using lipid-based carriers. However, as seen from Fig. 3, formulation (C) and (B) tend towards the higher range of absorption suggesting a moderate to high absorption behavior compared to formulation (A) and the control. The P_{app} for formulation (C) was calculated to be slightly lower than that of formulation (B) but this was not statistically significant ($p > 0.05$).

3.3. Thermal analysis: differential scanning calorimetry (DSC) studies

Fig. 4 shows the thermograms for piroxicam drug, formulation (A)–(C). Piroxicam showed a single endothermal peak at 200.3 °C, DMPC at 65 °C and

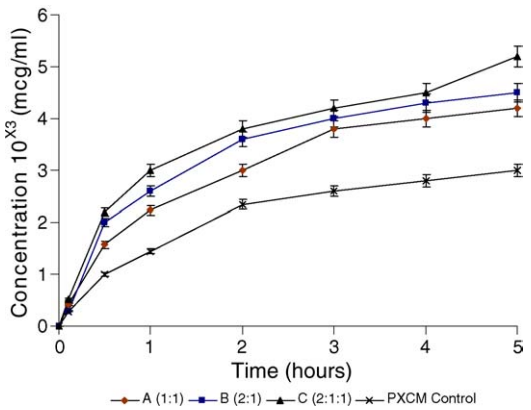

Fig. 2. HPLC analysis of samples from Caco-2 cell studies evaluating the absorption of piroxicam (PXCM) alone and in combination with lipid and PEG-based carrier systems.

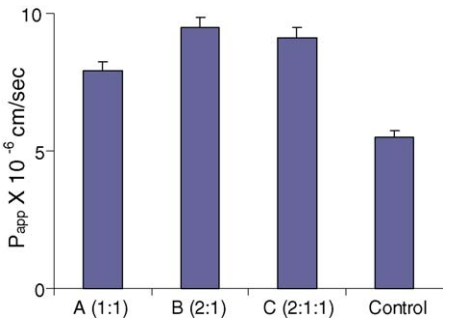

Fig. 3. The apparent permeability coefficients (P_{app}) of lipid-based delivery systems containing piroxicam (PXCM) compared to the control formulation.

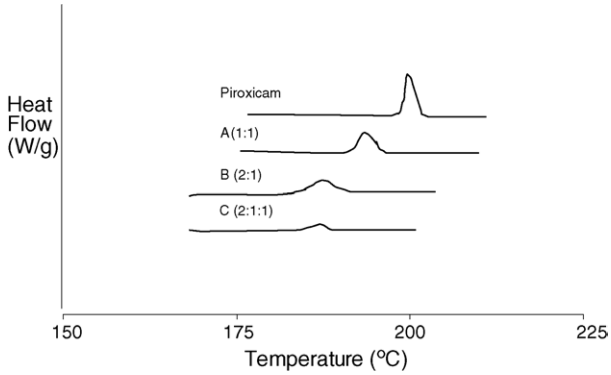

Fig. 4. DSC thermograms of piroxicam (PXCM) alone and the lipid-based formulations (A)–(C).

PEG 4600 at 58.4 °C (data not shown). Of the three lipid-based formulations, formulation (C) showed a significantly lowered height of the endothermal peak from the original PXCM peak. Also, the lower melting temperature of 185 °C showed a shift from the original indicating a significant proportion of the lipid-based formulation to be in amorphous form. During the scan of formulation (C), DMPC showed an endothermal peak at 61.4 °C; however, there was no evidence of an endothermal peak for PEG 4600 indicating its presence in amorphous form. Hence, the presence of PEG 4600 as a solubilizer was further affirmed as the DSC profiles correlated extremely well with the in vitro dissolution behavior of formulation (C). Shifts in endothermal peaks were also observed in formulation (A) and (B) whereby the melting temperatures were lowered to 192.3 and 187.6 °C, respectively, and peak heights were also considerably lowered. However, the height of peaks for both formulations was higher than formulation (C) as observed in Fig. 4, suggesting a crystalline presence but due to the shifts in temperatures and peaks, similar to formulation (C), indicated presence of amorphous content in both the formulations. Interestingly, formulation (B) with lower amounts of DMPC than formulation (A) demonstrated a higher magnitude of shift in peak and temperature than (A) suggesting the presence of increased amorphousness in formulation (B). This last observation was consistent with the in vitro dissolution behavior and further reaffirmed the possibility for a threshold level of phospholipids as carriers in drug delivery.

3.4. Storage stability studies

A 12-month storage stability study was conducted on all three lipid-based formulations. Dissolution runs were conducted on all stored samples at 6- and 12-month intervals to assess any changes in release behavior of the PXCM, compared to freshly prepared samples (control). Samples from formulations (A)–(C) were each stored at 4, 25 and 60 °C in closed glass vials.

At the end of the first 6-months of storage at 4 °C, maximum storage stability was observed in formulation (B) with only a 4% decline in dissolution rate whereas formulation (C) was the least stable with a 6% loss in rate of dissolution (Fig. 5a). The dissolution rate for formulation (A) was lowered by 5% indicating intermediate stability. At room temperature (25 °C), formulation (B) again was again most stable with an overall drop in dissolution rates of only 3% compared to formulation (C) which was lowered by 6% and formulation (A) by 5% (Fig. 5b). At a storage temperature of 60 °C (Fig. 5c), the stability of formulation (C) was shown to be the least with a drop of 8% in percent drug dissolved compared to 5% for formulation (B) and 5% for formulation (A), at the end of 6 months.

At the end of 12 months of storage at 4 °C, the maximum stability was observed in formulation (B) with a reduction in dissolution rates correlating to only 5% compared to an 18% drop in formulation (C). Formulation (A) demonstrated a 9% drop in dissolution rate (Fig. 5a). At room temperature (25 °C), the maximum drop in drug released was again observed in formulation (C) with 18% compared to formulation (B) at 6%

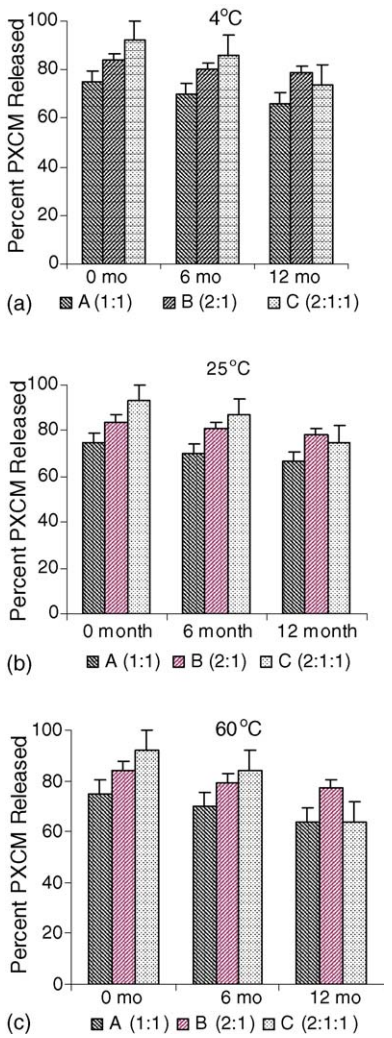

Fig. 5. Storage stability studies (6 and 12 months) at different temperatures (a) 4 °C, (b) 25 °C and (c) 60 °C showing percent piroxicam released from lipid-based formulations.

and formulation (A) at 8% loss (Fig. 5b). Finally, at elevated temperatures of 60 °C, formulation (C) showed a drop of 22% in dissolution rates whereas formulation (B) only dropped by about 7% and (A) by about 11%, at the end of 12-months of storage.

The decrease in stability as a result of decreasing dissolution rates, particularly in formulation (C), could be attributed to the formation of a crystalline mass upon storage for extended periods of time beyond 6-months. Overall, from these observations it appears that over a 12-month period, formulation (C) is the least stable at any given temperature whereas formulation (B) is the most stable. Thus, a rank order correlation in terms of stability would indicate formulation (B) > formulation (A) > formulation (C). Interestingly, all formulations at the end of 6-month storage period demonstrated good stability. For each of the formulations (A, B and C) stored at the three different temperatures (4, 25 and 60 °C), the resultant decrease in dissolution rates was statistically insignificant, thus suggesting that overall all formulations were relatively stable at 6 months and that the degradation of the formulations quickened only after the 6 month storage period. Thus, the ideal period of storage for these formulations could be concluded to be of 6-month duration. However, addition of appropriate stabilizers (e.g., polyvinyl alcohols) could potentially increase the storage stability of all of these formulations for longer periods of time. For this particular study, no additional stability enhancers were used.

4. Conclusions

From in vitro dissolution and transport data it is apparent that the presence of PEG 4600 and DMPC together as solubilizers have a significant impact on dissolution characteristics of piroxicam and are better facilitators of drug absorption, over that of only DMPC-based preparations. However, the presence of lipid carriers, DMPC, also seems to enhance the dissolution and absorption of the poorly water-soluble drug, compared to the control. Further studies are warranted on the need to consider threshold levels of lipid carriers for improvement of dissolution characteristics of poorly water-soluble drugs. In terms of storage stability, all formulations exhibited short-term stability of at least 6 months but beyond that seemed to decrease in dissolution rates for formulations containing PEG 4600. For future studies, incorporation of stability enhancers should provide valuable information on long-term storage stability of the solid dispersion formulations.

References

AHFS, 2000. Drug Information. American Society of Health System Pharmacists Inc., Bethesda, MD, pp. 1861–1864.

Amidon G.L., Lennernas H., Shah V.P., Crison J.R., 1995. A theoretical basis for a biopharmaceutical drug classification: the

correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm. Res. 12, 413–420.

Brigger, I., Dubernet, C., Couvreur, P., 2002. Nanoparticles in cancer therapy and diagnosis. Adv. Drug Deliv. Rev. 54, 631–651.

Bummer, P.M., 2004. Physical chemical considerations of lipid-based oral drug delivery—solid lipid nanoparticles. Crit. Rev. Ther. Drug Carrier Syst. 21, 1–19.

Chiou, W.L., Riegelman, S., 1971. Pharmaceutical applications of solid dispersion systems. J. Pharm. Sci. 60, 1281–1302.

Lasic, D.D., 1993. Liposomes: From Physics to Applications. Elsevier, Amsterdam, pp. 63–107.

Mainprize, T., Grady, L.T., 1998. Standardization of an in vitro method of drug absorption. Pharm. Forum 24, 6015–6023.

Pan, R.N., Chen, J.H., Chen, R.R., 2000. Enhancement of dissolution and bioavailability of piroxicam in solid dispersion systems. Drug Dev. Ind. Pharm. 26, 989–994.

Prabhu, S., Brocks, D.R., Betageri, G.V., 2001. Enhancement of dissolution of ethopropazine using solid dispersions prepared with phospholipid and/or polyethylene glycol. Drug Dev. Ind. Pharm. 27, 413–418.

Serajuddin, A.T., 1999. Solid dispersions of poorly water soluble drugs: early promises, subsequent problems and recent breakthroughs. J. Pharm. Sci. 88, 1058–1066.

Tagliati, C.A., Kimura, E., Nothenberg, M.S., Santos, S.R.J.C., Oga, S., 1999. Pharmacokinetics profile and adverse gastric effect of zinx-piroxicam in rats. Gen. Pharmacol. 13, 67–71.

Tantishaiyakul, V., Kaewnopparat, N., Ingkatawornwong, S., 1999. Properties of solid dispersions of piroxicam in polyvinylpyrrolidone. Int. J. Pharm. 30, 143–151.

Vauthier, C., Dubernet, C., Chauvierre, C., Brigger, I., Couvreur, P., 2003. Drug delivery to resistant tumors: the potential of poly(alkyl cyanoacrylate) nanoparticles. J. Control. Release 93, 151–160.

Weuts, I., Kempen, D., Verreck, G., Decorte, A., Heymans, K., Peeters, J., Brewster, M., Van den Mooter, G., 2005. Study of physicochemical properties and stability of solid dispersions of loperamide and PEG 6000 prepared by spray drying. Eur. J. Pharm. Biopharm. 59, 119–126.

Yee, S., 1997. In vitro permeability across caco-2 cells (colonic) can predict in vivo (small intestinal) absorption in man—fact or myth. Pharm. Res. 14, 763–766.

Yuksel, N., Karatas, A., Ozkan, Y., Savaser, A., Ozkan, S.A., Baykara, T., 2003. Enhanced bioavailability of piroxicam using gelucire 44/14 and labrasol: in vitro and in vivo evaluation. Eur. J. Pharm. Biopharm. 56, 453–459.

Zaplisky, S., Harris, J.M., 1997. Introduction to Chemistry and Biological Applications of Poly(ethylene glycol). In: Poly(ethylene glycol) Chemistry and Biological Applications. American Chemical Society, San Francisco, CA, pp. 1–11.