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Wet Process-Induced Phase-Transited Drug Delivery System: A Means for Achieving Osmotic, Controlled, and Level A IVIVC for Poorly Water-Soluble Drug

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A phase-transited, nondisintegrating, controlled release, asymmetric membrane capsular system for poorly water-soluble model drug flurbiprofen was developed and evaluated both in vitro and in vivo for osmotic and controlled release of the drug. Asymmetric membrane capsules (AMCs) were prepared using fabricated glass mold pins through wet phase inversion process. Effect of varying osmotic pressure of the dissolution medium on drug release was studied. Membrane characterization by scanning electron microscopy showed an outer dense region with less pores and an inner porous region for the prepared asymmetric membrane. In vitro release studies for all the prepared formulations were carried out (n = 6). Statistical test was applied for in vitro drug release at $p > .05$. Predicted in vivo concentration from in vitro release data closely matched the minimum effective concentration (in vivo) level achieved by the drug from its release through phase-transited AMC in rabbits for the first hour. The drug release was found to be independent of the pH but dependent on the osmotic pressure of the dissolution medium. In vivo pharmacokinetic studies showed level A correlation ($R^2 > .99$) with 42.84% relative bioavailability compared to immediate release tablet of flurbiprofen. Excellent correlation achieved suggested that the in vivo performance of the AMCs could be accurately predicted from their in vitro release profile.

Keywords phase transited; osmotic; correlation; in vivo; mold pins

INTRODUCTION

There has been increasing interest in the development of osmotic devices in the past two decades, and various osmotic pumps have been reviewed (Santus & Baker, 1995). The elementary osmotic pump (EOP) was first introduced by Theeuwes in the 1970s (Theeuwes, 1975). However, this type of EOP was only suitable for the delivery of water-soluble drugs. To overcome the limit of EOP, a push-pull osmotic tablet was developed in the 1980s. The push-pull osmotic tablet had two disadvantages: (a) the tablet core was prepared by compressing two kinds of compartments together, a complex technology as compared with that of EOP, and (b) after coating, a complicated laser-drilling technology was used to drill the orifice next to the drug compartment (Theeuwes, Saunders, & Mefford, 1978). To avoid sophisticated techniques of all osmotic tablet systems, monolithic osmotic tablet system was proposed and studied (Lu, Jiang, Zhang, & Jiang, 2003). Osmotic tablets with an asymmetric membrane coating, which can achieve high water fluxes, have been described (Herbig, Cardinal, Korsmeyer, & Smith, 1995). The asymmetric membrane capsules (AMCs) prepared either by wet or by dry process (Philip & Pathak, 2006, 2007) are also examples of a single-core osmotic delivery system, consisting of a drug-containing core surrounded by an asymmetric membrane. One of the advantages of an asymmetric membrane is the higher rate of water influx, allowing the release of drugs with a lower osmotic pressure or lower solubility. Despite this advantage, there are many instances where the solubility of the drug is too low to provide a reasonable driving force for water ingress. The capsule shell of an AMC is made from a water-insoluble polymer such as cellulose acetate (CA) or ethylcellulose (EC). Capsule shells with a range of membrane permeability properties can be prepared. Asymmetric membrane coatings have been developed for osmotic drug delivery that offers significant advantages over the membrane coatings used in conventional osmotic systems (Lin & Ho, 2003). The advantages of offering nonsteroidal anti-inflammatory drug [known to cause gastrointestinal (GI) disturbances] through osmotic delivery from AMC is that the release of the drug is primarily controlled by the difference in osmotic pressure between the external fluid and the drug-containing core of the capsule. The volume of drug solution delivered from the AMC will be roughly equal to the volume of water imbibed within a given time interval. This means that the GI mucosa will not be in contact with any undissolved drug, which could have resulted in GI disturbance. Moreover, sustained zero-order drug releases can be achieved using AMCs.

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Flurbiprofen [(+/−)-2-(2-fluoro-4-biphenylyl) propionic acid] is an important nonsteroidal anti-inflammatory drug, effectively used in the treatment of rheumatoid arthritis (Marsh, Schuna, & Sundstorm, 1986), osteoarthritis, mild to moderate pain (Gilman, Rall, & Taylor, 1990), and ocular inflammatory conditions (Thaller, Kulshrestha, & Bell, 2000). Because of its short elimination half-life (4 h), multiple dosing is required to achieve and maintain therapeutic concentration, and adverse GI reactions can occur (Chandran, Roy, & Saha, 2005).

Therefore, development of oral sustained release formulations with a high level of in vitro in vivo correlation (IVIVC) of this drug is highly desirable to achieve improved therapeutic efficacy and patient compliance. Therefore, the aims of this work were (a) to develop AMCs to deliver flurbiprofen with and without its solubility enhancer in a controlled manner and (b) to evaluate the in vivo performance of the prepared AMC.

EXPERIMENTS AND METHODS

Materials

Flurbiprofen was obtained from Sun Pharmaceuticals Pvt. Ltd. (Gujarat, Baroda, India). 2-(4-biphenylyl)-propionic acid used as internal standard for high-performance liquid chromatography was synthesized in our laboratory from flurbiprofen. Sodium dihydrogen phosphate and disodium hydrogen phosphate (both analytical reagent grade) were purchased from S. D. Fine Chemicals (Mumbai, India). EC (50 cps), acetone, glycerin, and ethyl alcohol were procured from Qualigens Pvt. Ltd. (Mumbai, India). Mannitol from Merck India (New Delhi, India) was purchased from C. N. Chemicals (Uttar Pradesh, Mathura, India). Acetonitrile and methanol [high-performance liquid chromatography (HPLC) grade] were procured from (Ranbaxy Fine Chemicals, Mumbai, India). Solvents of reagent grade and double-distilled water were used in all experiments.

Methods

Solubility Studies

The kinetics of osmotic drug release is directly related to the solubility of drug within the formulation. Assuming the capsule formulation to consist only of the pure drug, the fraction of drug released with zero-order kinetics is given by Equation 1 (McClelland, Sutton, Engle, & Zentner, 1991; Zentner, McClelland, & Sutton, 1991).

$$F(z) = \frac{1-S}{\rho}, \tag{1}$$

where $F(z)$ is the fraction released by zero-order kinetics, S is the drug's solubility (g/cm^3), and ρ is the density (g/cm^3) of the drug. In general, drugs with a solubility of 0.05 g/cm^3 would be released with 95% zero-order kinetics according to Equation 1. However, zero-order release rate would be slow because of the small osmotic pressure gradient. Conversely, highly water-soluble drugs would demonstrate a high release rate that would be zero order for only a small percentage of the initial drug load. Therefore, to assess the solubility of the drug in various dissolution mediums, saturated solutions of the drug were prepared in 0.1 N HCl, phosphate buffer pH 7.4, and double-distilled water without and with 10, 15, 20, and 25 mg citric acid in a closed container at 37°C. Excess amounts of the drug were added to ensure saturation, and the solutions were equilibrated for 24 h. The saturated solutions were filtered and concentration determined by UV spectrophotometer (Shimadzu 1700, Tokyo, Japan) at 247 nm after suitable dilutions. Density of the drug was determined by pycnometer (Jindal Scientific Industries Pvt. Ltd., Ambala, India).

Preparation of AMCs

AMCs were prepared by using the wet phase inversion process. The membranes were precipitated on glass mold pins (having a diameter of 5.52 ± 0.05 mm and 6.1 ± 0.022 mm for the body and cap, respectively) by dipping the glass mold pins in a coating solution of 10 and 15% wt/vol of EC and varying amounts of glycerol (8and 20% wt/vol) dissolved in acetone (50% vol/vol) and ethanol (25% vol/vol for 8% wt/vol glycerol and 30% vol/vol for 20% wt/vol glycerol), and air dried for 15 s. After this, the pins were immersed in an aqueous quenching solution (10% wt/vol of glycerol) for 10 min. Immersion of EC-coated glass mold pins in a quench bath helped in the generation of asymmetric membranes. Asymmetric membranes in shape of the body and cap of conventional capsules were then stripped after removal from the quench bath and dried at ambient temperature for at least 8 h. The body and the cap were then trimmed to fit inside each other for formation of AMC. Drug loading of 200 mg after passing through 100-μm mesh sieve and having particle size 130 μm was mixed with or without mannitol (50 mg) in a polythene bag and the AMCs were filled manually. Mannitol was used as an osmogen as flurbiprofen was found to be osmotically inactive (Martin, 1999). The filled AMCs were then sealed with ethanolic solution of EC. The composition of all the AMCs formed and AMC with a solubility enhancer for the drug (AMC 9) is summarized in Table 1. For administration to rabbits, the optimized formulation was filled with a drug loading of 0.278 mg of flurbiprofen/kg body weight of the rabbit (US FDA recommends using a factor of 12 for converting human dose to equivalent rabbit dose), mixed with 0.315 ± 0.36 mg mannitol and 0.155 ± 0.024 mg citric acid in a polythene bag, and were manually filled inside the capsule.

Scanning Electron Microscopy

Scanning electron microscopy (SEM) asymmetric membranes obtained before and after complete dissolution of core contents were examined for their porous structure using Jeol 6100 SEM (Jeol, Tokyo, Japan). After dissolution, asymmetric membrane structures were dried at 50°C for 8 h and stored in

TABLE 1
Composition of the Eight Asymmetric Membrane Capsule (AMC) Formulations Along with the Solubility Enhancer for the Drug (AMC 9)

		AMC								
No.	Variable	1	2	3	4	5	6	7	8	9[a]
1	Ethylcellulose (% wt/vol)	10	15	10	15	10	15	10	15	10
2	Mannitol (mg)	0	0	50	50	0	0	50	50	50
3	Glycerol (% wt/vol)	8	8	8	8	20	20	20	20	8
4	Quenching concentration[b] (% wt/vol)	10	10	10	10	10	10	10	10	10
5	Quenching time (min)	10	10	10	10	10	10	10	10	10
6	Acetone (% vol/vol)	50	50	50	50	50	50	50	50	50
7	Ethanol (95%) (% vol/vol)	30	30	30	30	25	25	25	25	30
8	Water (mL)	90	90	90	90	90	90	90	90	90
9	Citric acid (mg)	0	0	0	0	0	0	0	0	25

AMC, Asymmetric membrane capsule.
[a]AMC 9 that contains citric acid.
[b]10% wt/vol of glycerol in water.

desiccator before examination. Asymmetric membranes were sputter coated for 5–10 min with gold by using fine coat ion sputter and examined under SEM.

In Vitro Drug Release

In vitro cumulative drug release from the prepared formulations ($n = 6$) was studied by using British Pharmacopoeia (BP) paddle-type apparatus (rotating speed 75 rpm at 37°C ± 0.5°C). The dissolution medium was 0.1 N HCl as simulated gastric fluid (SGF) (900 mL, pH 1.2) for the first 2 h, followed by phosphate buffer as simulated intestinal fluid (SIF) (900 mL, pH 7.4) for the rest of the experiment. One milliliter of the sample was withdrawn at specified time intervals and suitably diluted by fresh dissolution medium and analyzed at 247 nm.

Statistical Analysis

The release profiles up to $t_{50\%}$ of flurbiprofen from all the formulations ($n = 6$) in the dissolution medium was statistically compared by Dunnett multiple comparison t test (Instat software, Graphpad Software Inc., San Diego, CA, USA) with the release rate profiles of the marketed formulation. The statistical significance was tested at $p > .05$. Best formulation among the formulations was chosen after pair-wise comparison using dissimilarity factor (f_1) (PCP Disso Software version 2.01, Pune, India), and the formulation with the lowest f_1 value but with zero-order kinetics was selected as the best formulation.

In Vivo Prediction of Flurbiprofen Concentration Using In Vitro Release Data

A fundamental theory of the time course of action of drugs relies on the knowledge of the effective concentration at the ultimate site of action, but this is not accessible. One approach is that of compartmental analysis. Based on one compartment model, the prediction of drug concentration in blood after 1 h (effective concentration) following in vitro dissolution of AMCs was done by using Equation 2. The pharmacokinetic parameters required for calculations were taken from the relevant literature (Bennett & Brown, 2004).

$$C_b = \frac{D_o}{V_d} \times e^{-kt}, \quad (2)$$

where C_b is the drug concentration in blood, D_o is the total amount of dose given, V_d is the volume of distribution, k is the first-order elimination rate constant, and t is the time at which the drug concentration in blood has to be calculated.

Effect of Varying Osmotic Pressure

To confirm the mechanism of flurbiprofen release, release studies of the optimized formulation were conducted in a media providing greatest sink condition but of different osmotic pressure. To increase the osmotic pressure of the dissolution medium (SIF), mannitol (osmotically effective solute) was added and the pH was adjusted to 7.4 ± 0.5. Release studies were performed in 900 mL of phosphate buffer pH 7.4 using BP dissolution apparatus II (75 rpm). Two methods were used, the first was the direct measurement of the flurbiprofen in the dissolution medium at predetermined time intervals and the second was residual analysis method (to reduce the effect of any chance interference of the flurbiprofen by mannitol). In residual analysis method, the formulation undergoing dissolution was withdrawn from the vessel at predetermined intervals and cut open to dissolve the contents into 250 mL of SIF.

One milliliter of the sample was taken and suitably diluted and analyzed at 247 nm to determine the residual amount of drug in each AMC. Results were found to be comparable with both the methods.

In Vivo Study Conditions

Healthy male and female domestic rabbits (IAEC/RAP/1561) between 2 and 3 years and weighing 4 and 5 kg were taken from Rajiv Academy for Pharmacy (Mathura, India). The experiment followed European Community guidelines and the use of rabbits in this study was approved by the institutional animal ethical committee of Rajiv Academy for Pharmacy. The rabbits were housed individually in polypropylene cages and maintained under standard conditions (12 h light and 12 h dark cycle, 25 ± 30°C, 35–60% humidity) in the animal room of Rajiv Academy for Pharmacy. The animal experiment was processed following the internationally accepted ethical guidelines for the care of laboratory animals. Before the experiments, the rabbits were fed with standard food for 1 week, to adapt to the laboratory conditions, and then kept on standard food till the end of the study. Sixteen hours before the experiments, they were fasted overnight, but allowed free access to water. Twenty-two rabbits were included in the study, which were divided into three groups. Group 1 consisted of 10 rabbits (five males and five females) for pooled blood sample collection. Group 2 had six rabbits (three male and three female) for in vivo pharmacokinetic studies of the test formulation, and group 3 had six rabbits (three male and three female) for in vivo pharmacokinetic studies of the reference formulation. The body weights of rabbits were determined before the start of the experiment.

Preparation of Stock and Standard Solution for Flurbiprofen

Flurbiprofen stock solution was prepared by spiking 10 mg/mL concentration in rabbit plasma. The sample was then centrifuged at 3,000 rpm for 1 min. The plasma sample was then dissolved in methanol : acetonitrile : phosphate buffer (pH 7.4) (40:20:40) and then filtered and suitably diluted to make 1 mg/mL solution. From this stock solution, dilutions were made to prepare 0.3, 2, 4, 6, 8, and 10 μg/mL solutions for preparation of the standard. 2-(4-biphenylyl)-propionic acid, internal standard for flurbiprofen was dissolved in acetonitrile to prepare 1 mg/mL stock solution. Both stock solutions were stored at 4°C. The analysis was done by setting the UV detector at 247 nm.

HPLC Conditions

Assay validation was done using Cecil 4200® HPLC system. Instrumentation of HPLC system consisted of Cecil CE4100® HPLC dual piston short stroke pump and a Cecil 4200® UV-Visible detector was set at 247 nm for flurbiprofen (Cecil instrument Ltd., England). The mobile phase consisted of methanol : acetonitrile : phosphate buffer (pH 7.4) (40:20:40, by volume), filtered and degassed under reduced pressure and pumped at 1 mL/min through C18 (Thermo Electron Corporation®, England) 250 mm × 4.6 mm column with 5-μ packing, with a typical pressure of 67 ± 0.05 bars.

Validation Parameters

The method of specificity was assessed by comparing the chromatograms obtained from the drug to its respective internal standard with those obtained from the blank. Linearity, range, limit of quantification, and limit of detection (LOD) were obtained from the standard concentrations (0.3, 2, 4, 6, 8, and 10 μg/mL), which in turn were obtained from the stock solution(s). Each concentration was prepared six times ($n = 6$). The limit of quantitation (LOQ) was the lowest concentration assayed where the signal/noise ratio was at the least 10:1, and the LOD was defined as a signal/noise ratio of 3:1. The accuracy, precision, and recovery in plasma assay validation involved quality control (QC) concentrations prepared from newly prepared spiked stock solution of flurbiprofen (1, 5, and 10 μg/mL). The QC samples were divided into 0.1-mL aliquots in centrifuge tubes and stored at –70°C before use. Intraday and interday variability were tested with 12 replicates of each QC control concentration. Means, standard deviations, and coefficient of variation were calculated by standard methods. Recovery test was performed by adding known amounts of respective stock solution of flurbiprofen to the sample with known content and preparing solutions with the respective mobile phase. The percentage of recovery was calculated by comparing the determined amount of these standards with the added amount.

Route of Administration and Withdrawal of Blood Samples

The rabbits were taken as per the phases divided for in vivo pharmacokinetic studies. The rabbits were administered the reference and the test formulations with the help of a gastric feeding tube after placing them in a restraining device (rabbit holder). This feeding tube was of 7 mm diameter which had been bent to approximate the pharyngeal curvature. A mouth speculum was used to administer the capsules. The mouth speculum was a stainless steel rod that acted as a tongue depressor and had a centrally placed hole. Care was taken that the tube did not enter the trachea or puncture the esophagus or stomach which was evident as no violent reaction (coughing or gasping, which usually follows on accidental introduction of the tube into the larynx or trachea) was seen. For withdrawal of blood samples, the rabbits were anesthetized by subcutaneous injection of 25% urethane-physiological saline (4 mL/kg). A fine cannula hypodermic needle (0.8 mm) with syringe attached to it was inserted into a right femoral artery to facilitate the sampling of blood for drug analysis. The syringe was detached from the needle and the cannula closed with the cap to prevent clotting of the blood. To further ensure that clotting of blood did not take place, the cannula before closing was

flushed with 10% vol/vol of heparin/normal saline solution. The needle was kept inside the artery by means of leucoplast tape. At perquisite time periods, 2 mL of blood sample(s) were withdrawn through the cannula into heparinized glass vials and centrifuged at 3,000 rpm for 10 min to obtain 1 mL ± 0.14 mL of the plasma and frozen (–20°C) until analyzed. Blood samples were taken at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 24, and 30 h for the test formulation and reference formulation of flurbiprofen. To maintain homeostasis of the rabbits, an injection of same volume of physiological saline was given through the ear vein.

Flurbiprofen Determination in Rabbit Plasma

The determination of flurbiprofen rabbit plasma was carried out by taking 1 ± 0.14-mL aliquots of plasma, which were pipetted into a 15-mL centrifuge tube. Plasma drug mixture was prepared (100 mcg) by adding 100 µg of acetonitrile, 200 µg of internal standard, 500 µg of 2.5 M *O*-phosphoric acid solution in a 10-mL glass tube and vigorously shaken on a vortex mixer for 20 s. The samples were then centrifuged at 3,000 rpm for 10 min. The organic layer was transferred to a 10-mL centrifuged tube and evaporated to dryness under stream of dry nitrogen at 37°C. The residue was reconstituted in 250 µg of respective mobile phase. An appropriate aliquot (20–75 µg) was then injected directly into the loop injector.

Pharmacokinetics and Statistical Analysis

The plasma concentration time data of flurbiprofen were fitted in Quick-Calc software (Plexus Supporting Services, Ahmedabad, India) and the pharmacokinetic parameters calculated. Mass balance model-dependent technique (Wagner Nelson) was also used to calculate the absorption parameter. The area under the concentration–time curve (AUC_{0-t}) was determined using the trapezoidal method. $AUC_{t-\infty}$ was calculated by dividing the last recordable plasma concentration over elimination rate constant. C_{max} and T_{max} were determined through the observation of individual animal drug concentration versus time curves. Relative bioavailability was determined by using the Equation 3.

$$fr = \left(\frac{AUC_{test}}{AUC_{std}} \times \frac{Dose_{std}}{Dose_{test}} \right) \times 100 \qquad (3)$$

To compare the main parameters of the different dosages forms, a two-sided unpaired *t* test was conducted with Microsoft Excel 2003. Statistical significance was tested at $p < .01$.

In Vitro In Vivo Correlation

The USP Biopharmaceutics subcommittee has established the four categories to define the correlation between in vitro dissolution and in vivo absorption.

1. Level A: The in vitro dissolution curve corresponds to the in vivo absorption curve in a 1:1 manner and the two curves completely overlap each other.
2. Level B: The mean dissolution time (MDT) in vitro, calculated by moment analysis, shows correlation with the mean residence time (MRT) in vivo.
3. Level C: One of the dissolution parameters ($t_{50\%}$, $t_{90\%}$, etc.) shows correlation with one of the pharmacokinetic parameters (*AUC*, C_{max}, T_{max}, etc.).
4. Level D: The disintegration correlates qualitatively with the in vivo behavior, or the in vitro data correlates qualitatively with the in vivo data.

Level A correlation is the highest category of correlation and is most applicable to modified release systems. With this correlative procedure, the specially designed AMCs in vitro dissolution curve was compared with the in vivo absorption rate after suitable deconvolution. The correlation was demonstrated after plotting the fraction absorbed in vivo versus the fraction release in vitro.

RESULTS AND DISCUSSION

Solubility Studies

Solubility studies showed that flurbiprofen had varying solubility in the different mediums studied, 0.1 N HCl (8.9×10^{-6} g/cm^3), phosphate buffer pH 7.4 (11.62×10^{-3} g/cm^3), double-distilled water (8.665×10^{-3} g/cm^3), and with 10 mg citric acid (26.98×10^{-3} g/cm^3), 15 mg citric acid (33.32×10^{-3} g/cm^3), 20 mg citric acid (48.11×10^{-3} g/cm^3), and 25 mg citric acid (52.65×10^{-3} g/cm^3). The increase in flurbiprofen solubility with increasing citric acid amount could be attributed to the ability of the hydroxy acid's solubility in water (they are solvated through their hydroxyl groups) in creating increased microenvironmental pH with increased concentration. The density of flurbiprofen was found to be 0.3045 g/cm^3.

The experimental values of the $F(z)$ suggested that, to increase the rate at which zero-order release kinetics is achieved by the fraction of drug undergoing dissolution, an external agent (buffering agent) needs to be incorporated in the formulation. The increase in the solubility of flurbiprofen was achieved by the inclusion of citric acid in the formulation because, unlike a conventional dose, the formulations without citric acid were not able to achieve therapeutic concentrations within the first hour, probably owing to the lower solubility of flurbiprofen in the acidic medium. The incorporation of citric acid in the formulation provided an increased microclimate pH of stagnant diffusion layer around the drug particle, which was around the pKa of flurbiprofen (~4) (Avdeef, 1998). This stagnant diffusion layer was at a higher pH than the bulk of the dissolution medium (SGF, pH 1.2). Because higher pH favors the dissolution of weakly acidic drugs, the solubility of flurbiprofen increased in the stagnant diffusion layer at a higher pH, thereby resulting in a higher release from

the formulation as compared with other formulations without citric acid.

Scanning Electron Microscopy

Various proportions of EC membranes with varying proportions of pore-forming agent, glycerol, were obtained before and after complete dissolution and studied by SEM. EC concentration varied with different glycerol levels. Membrane (8% wt/vol glycerol) obtained before dissolution showed outer, dense, nonporous region (Figure 1A) and an inner, lighter, porous region. After complete dissolution, the exhausted membrane showed a large number of pores similar to a net-like structure (Figure 1C), and the formulation prepared with this membrane did not show swelling or rupturing. Membranes containing 20% wt/vol of glycerol showed similar nonporous and porous regions (Figure 1B). The formulation with this membrane showed slight swelling or elongation but no rupture. Membranes containing higher proportion of glycerol (25% wt/vol) showed larger pores. The formulation prepared with this membrane caused bursting. So, it could be assumed that more than 20% wt/vol of glycerol would cause rupturing of membrane during dissolution. The SEM study suggested that 20% wt/vol of glycerol can be used as an optimum concentration to obtain maximum release rate of drugs without rupturing of coating membrane for the core composition given in this study.

In Vitro Drug Release

In vitro studies were performed for all the prepared formulations. The results showed that incorporation of mannitol (AMC 3) resulted in development of significant osmotic pressure inside the capsular system, which increased the release rate of flurbiprofen. This effect was also evident while studying the individual effect of the mannitol, which caused a decrease of 49.07 min in achievement of $t_{50\%}$ from AMC 3 (Figure 2) When the pore former (glycerol) was at a higher concentration, the release from this formulation was more probably owing to increased pore formation on the membrane during dissolution, causing burst release. Glycerol caused a decrease of 34.89 min (Figure 2). When EC concentration was at a higher level, the release of flurbiprofen from the capsular membrane was constrained when compared with AMC 1 formulation. The decreased flurbiprofen release from AMC 2 might be because of the increased diffusional path for the drug to transverse before being released into the dissolution medium. Individual effect of EC concentration at higher level (Figure 2) showed an increase of 87.22 min. Interactive studies surprisingly showed a delayed achievement of $t_{50\%}$ in AMC 8. A careful analysis of the interactive study between the three variables showed that there was an increase of 9.03 min. This finding may be owing to the increased drug-holding capacity for the polymer at a higher concentration coupled with the swelling of the asymmetric membrane because of higher glycerol content, which suggested that the membrane thickness still had a prominent role in constraining the release of flurbiprofen though this delay was greatly reduced by the burst release of flurbiprofen resulting from individual effects of the other two variables.

Statistical Analysis

Dunnett multiple comparison test compared all formulations with the marketed formulation (AMC 10). If the value of t was found to be greater than 2.689, then comparison test would have run at a significance value less than 0.05 or below 95% confidence level and would have been considered to be statistically significant. The multiple comparison test, when all the formulations were compared with the AMC 10, resulted in F value of 2.989 (ANOVA) with a p-value of .0023.

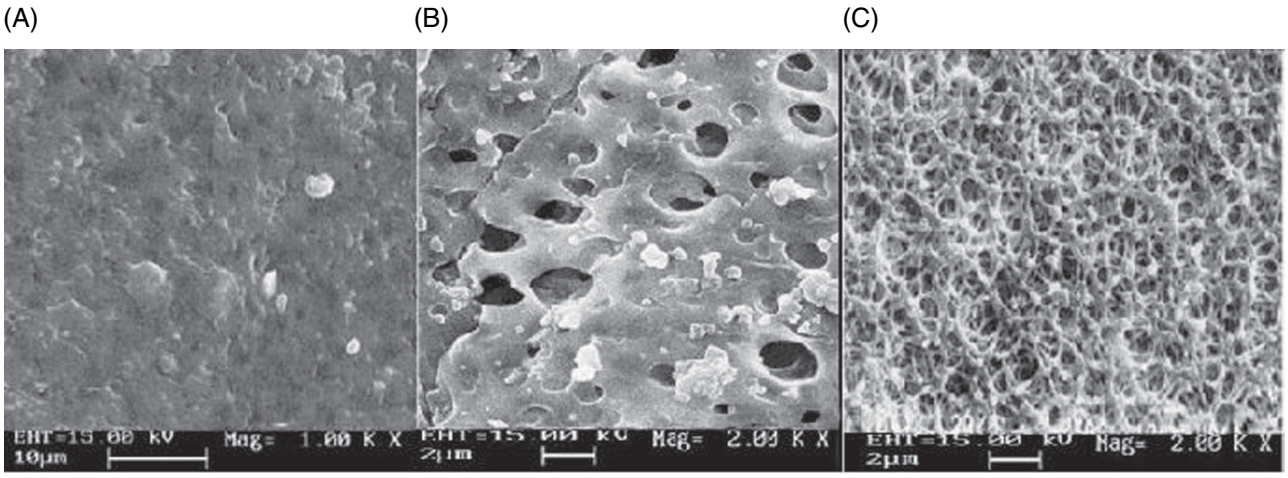

FIGURE 1. Scanning electron microphotographs of coating membrane obtained (A) before dissolution showing outer dense nonporous region and containing 8% wt/vol glycerol at 1,000 ×, (B) before dissolution showing large inner porous region and containing 12% wt/vol glycerol at 2,000 ×, and (C) after complete dissolution showing net-like structure and containing 8% wt/vol glycerol at 2,000 ×.

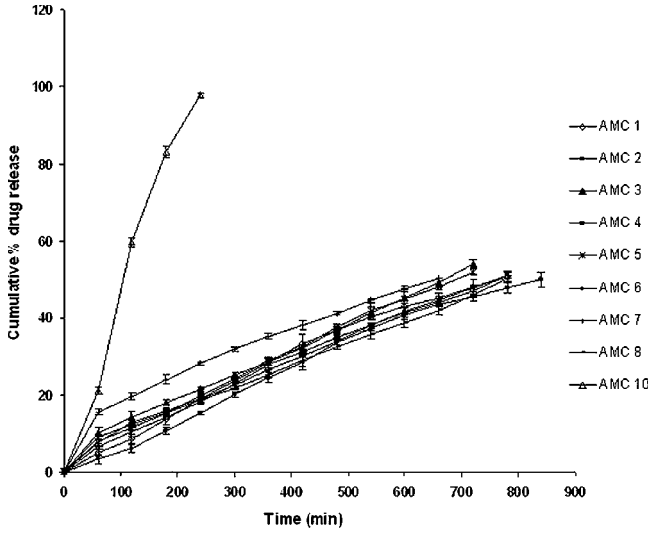

FIGURE 2. Comparative in vitro dissolution profiles (n = 6 along with standard deviations) for all formulations from asymmetric membrane capsule 1 (AMC 1) to AMC 8 along with the marketed formulation (AMC 10).

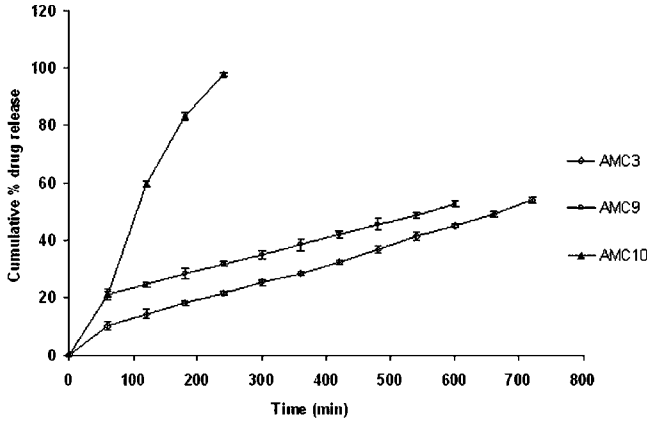

FIGURE 3. Comparative in vitro dissolution profiles (n = 6 along with standard deviations) for asymmetric membrane capsule 3 (AMC 3), formulation with solubility enhancer for the drug (AMC 9) and marketed formulation (AMC 10).

The t values of all the formulations was found to be above 2.689, which meant that the test was run at 95% confidence level and that the difference between all the formulations as compared with the marketed formulation were statistically significant. Dissimilarity factor (f_1) of 5.89 (lowest among all the formulations) showed that the two profiles (AMC 3 and AMC 10) had dissimilar dissolution profiles. Therefore, AMC 3 was taken as the best formulation.

In Vivo Prediction of Flurbiprofen Concentration Using In Vitro Release Data

The prediction of drug concentration in blood after 1 h (effective concentration) following administration of AMCs was done using Equation 3. The first-order elimination rate constant for flurbiprofen (0.17/h), when incorporated in Equation 3, resulted in the finding that a dose of 42.1 mg (approximately 20% cumulative drug release) released within the first hour from AMC 9 would result in a blood concentration of 3.552 µg/mL. The therapeutic blood concentration following a conventional dose of 50 mg was determined to be 3.501 µg/mL (Figure 3).

Effect of Variable Osmotic Pressure

To study the effect of varying osmotic pressure, release studies of the optimized formulation AMC 3 were conducted in media of different osmotic pressures. The results showed that the drug release was highly dependent on osmotic pressure of the release media. Flurbiprofen release from AMC 3 decreased as the osmotic pressures of the drug release medium increased (Figure 4). When the release rate was plotted against osmotic pressure difference (the osmotic pressure inside the formulation

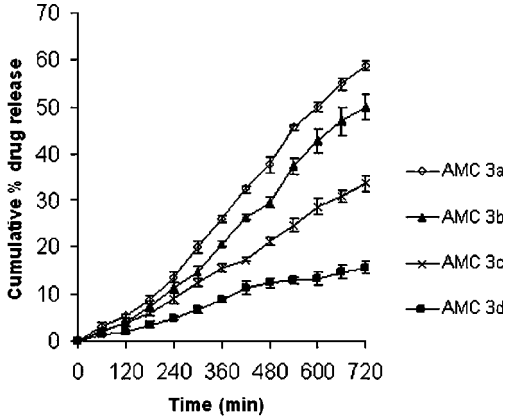

FIGURE 4. Release profiles (n = 6 along with standard deviations) from asymmetric membrane capsule 3 (AMC 3) in dissolution medium of different osmotic pressures. AMC 3a (1.178 mmHg), AMC 3b (2.356 mmHg), AMC 3c (3.535 mmHg), and AMC 3d (4.713 mmHg).

was found to be 5.891 mmHg), a linear relationship was again obtained (R^2 = .9869) (Figure 5) Therefore, it was concluded that the primary mechanism governing the drug release from the developed formulations was osmotic pumping.

Validation Parameters

The analytical performance parameters, namely specificity, linearity, range, precision, accuracy, LOD, and limit of quantification, were validated according to International Conference on Harmonization ICH Q2B guidelines. Specificity was assessed by comparing the chromatograms obtained from the drug to their respective internal standards and with those obtained from the blank that verified the absence of any

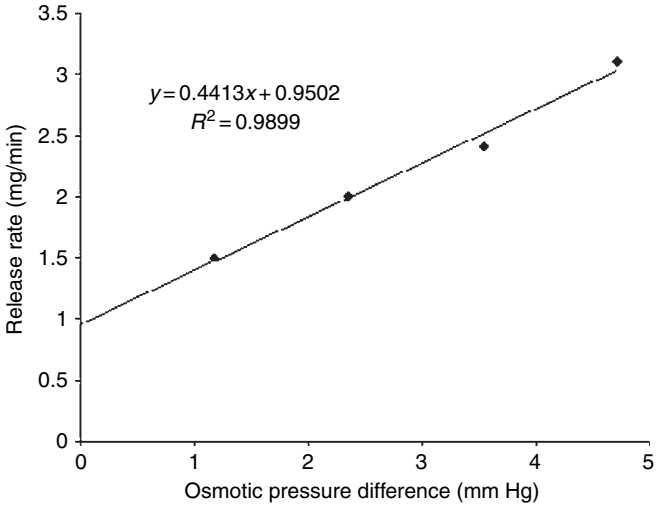

FIGURE 5. Flurbiprofen release rate from asymmetric membrane capsule 3 (AMC 3) showing effect of osmotic pressure difference across the membrane.

TABLE 2
Various Pharmacokinetic Parameters Calculated Using Quick-Calc Software

Parameters	Reference Tablets for Flurbiprofen	Test AMC for Flurbiprofen
C_{max} (h)	4.06 ± 0.06	3.73 ± 0.02
T_{max} (h)	4.00 ± 0.04	7.00 ± 0.07
$t_{1/2\ (el)}$ (h)	9.95 ± 0.56	18.88 ± 0.34
K_a (h)	0.73 ± 0.76	0.38 ± 0.09
K_{el} (h)	0.07 ± 0.04	0.03 ± 0.05
V_d (L/kg)	1.39 ± 0.52	6.38 ± 0.22
Cl (L/kg h)	0.09 ± 0.08	0.23 ± 0.09
AUC_{0-t^*} (h μg/mL)	68.65 ± 0.19	88.15 ± 0.23
$AUC_{t^*-\infty}$ (h μg/mL)	8.63 ± 0.14	44.28 ± 0.47
Relative bioavailability (%)	–	42.84 ± 0.08

AMC, asymmetric membrane capsule.

interference. The linearity of the method used for the drug was evaluated on a standard curve of the peak area versus the concentration of the analyte. A five-point calibration curve was constructed with working standards and was found linear (R^2 = .9999) in a range 0.3–10 μg/mL (R_t was found to be 4.33 ± 0.09 and 3.56 ± 0.05 min for flurbiprofen and the internal standard, respectively). The LOD and LOQ were found to be 0.10 and 0.30 μg/mL, respectively. The results of determination of accuracy using QC concentrations were 99.98 ± 0.03%. Precision assay showed that the averages of the relative standard deviations within 1 day (intraday) were 0.16–0.88% and among every other day (interday) were 0.87–3.12%. The results showed that the method was accurate. Recovery test was performed again on QC samples and the results, 99.89 ± 1.08 μg/mL for 1 μg/mL, 100.25 ± 0.12 for 5 μg/mL, and 99.93 ± 0.25 for 10 μg/mL, validated the method.

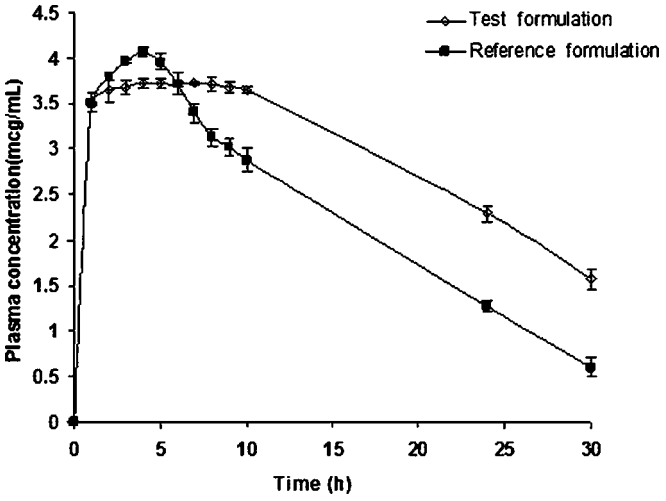

FIGURE 6. Comparative plasma concentration time profile (n = 6 along with standard deviation) of test and reference formulations.

Pharmacokinetic Parameters

The relevant pharmacokinetic parameters are listed in Table 2. From Figure 6, it is apparent that the specially designed dosage form effectively sustained and controlled the release of flurbiprofen and also maintained an elevated plasma concentrations upto the 10th hour. Although the statistical analysis of the C_{max} and T_{max} values for the conventional tablets and the test AMCs were statistically insignificant ($p > .01$) at 99% confidence level, there was statistically significant difference ($p < .01$) between the elimination half-life and the AUC for the two formulations of the drug. This suggested the capacity of the test formulation to sustain the release of drug. The decrease in AUC for the conventional tablet could be interpreted as that the formulation with a rapid rate of drug release tends to attain lower systemic bioavailability. This observation could be attributed to the fact that as the drug releases at a rate that exceeds the rate of absorption, it leads not only to side effects but also to first pass metabolism. Test formulations with increased AUC could be attributed to sustained drug release at a lower rate, thus not only enhancing the bioavailability that meant enhanced absorption (percent relative bioavailability showed an improvement of 42.84 ± 0.08) but also minimizing the first pass metabolism.

In Vitro In Vivo Correlation

The results of IVIVC (Figure 7) demonstrated that a good level A correlation could be achieved with the fabricated AMCs between the fraction of drug release from the dosage

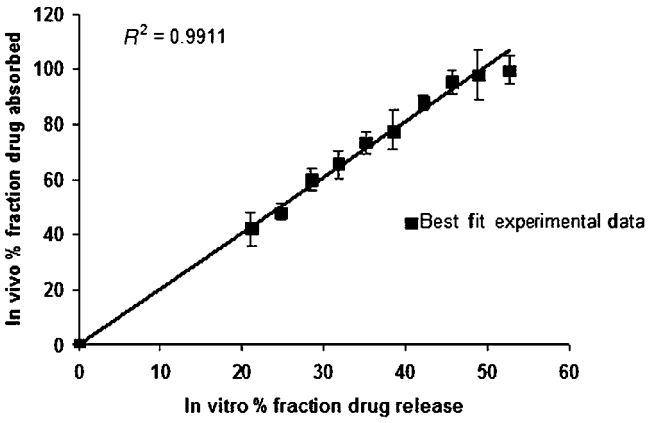

FIGURE 7. In vitro in vivo correlation of asymmetric membrane capsule delivering flurbiprofen.

units and the fraction of drug absorbed. Therefore, the in vitro release profile of flurbiprofen from the fabricated AMCs can be used to accurately predict their in vivo performance.

CONCLUSION

AMCs of flurbiprofen were successfully prepared and tested both in vitro and in vivo. The AMCs not only showed level A correlation but also controlled release with osmotic pumping as the principle mechanism of release. Level A correlation meant that by using in vitro release profile of flurbiprofen from phase-transited AMC can also predict their in vivo performance.

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