Osmotic Flow Through Asymmetric Membrane: A Means for Controlled Delivery of Drugs With Varying Solubility

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ABSTRACT

A nondisintegrating, controlled release, asymmetric membrane capsular system of flurbiprofen was developed and evaluated for controlled release of the drug to overcome some of its side effects. Asymmetric membrane capsules were prepared using fabricated glass mold pins by phase inversion process. The effect of different formulation variables was studied based on 2^3 factorial design; namely, level of osmogen, membrane thickness, and level of pore former. Effects of polymer diffusibility and varying osmotic pressure on drug release were also 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. Differential scanning calorimetry studies showed no incompatibility between the drug and the excipients used in the study. In vitro release studies for all the prepared formulations were done ($n = 6$). Statistical test (Dunnett multiple comparison test) was applied for in vitro drug release at $P > .05$. The best formulation closely corresponded to the extra design checkpoint formulation by a similarity ($f2$) value of 92.94. The drug release was independent of pH but dependent on the osmotic pressure of the dissolution medium. The release kinetics followed the Higuchi model and the mechanism of release was Fickian diffusion.

KEYWORDS: asymmetric membrane, factorial design, extra design, polymer diffusibility.

INTRODUCTION

There has been an increasing interest in the development of osmotic devices in the past 2 decades, and various osmotic pumps have been reviewed.[1] The elementary osmotic pump (EOP) was first introduced by Theeuwes in the 1970s.[2] 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 2 disadvantages: (1) the tablet core was prepared by compressing 2 kinds of compartments together, a complex technology as compared with that of EOP, and (2) after coating, a complicated laser-drilling technology was used to drill the orifice next to the drug compartment.[3] Osmotic tablets with an asymmetric membrane coating, which can achieve high water fluxes, have been described.[4] The asymmetric membrane capsule (AMC)[5,6] is also an example 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. In spite of 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.[7]

Flurbiprofen (FLU) [(+/−)- 2-(2-fluoro-4-biphenylyl) propionic acid is an important nonsteroidal anti-inflammatory drug (NSAID), effectively used in the treatment of rheumatoid arthritis,[8] osteoarthritis, and mild to moderate pain,[9] sore throat,[10] and ocular inflammatory conditions.[11] Because of its short elimination half-life (4 hours), multiple dosing is required to achieve and maintain therapeutic concentration, and adverse gastrointestinal (GI) reactions can occur.[12] Therefore, development of oral sustained-release formulations of this drug is highly desirable in order to achieve improved therapeutic efficacy and patient compliance.

Therefore, the aims of this work were (1) to develop and evaluate asymmetric membrane capsules (AMCs) to deliver drugs with varying solubility, like FLU, in a controlled manner, and (2) to evaluate the influence of variables based on 2^3 factorial design apart from evaluating the effect of polymer diffusibility and different osmotic pressure on the drug release from the prepared AMCs. Because the drug solubility was expected to be a decisive factor for the success of AMC, the drug release mechanism from AMC was

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further studied by examining the influence of citric acid, considered to be a solubility enhancer for the drug.

Materials and Methods

Materials

FLU was obtained from Sun Pharmaceuticals Pvt Ltd, Gujarat, India. Sodium di-hydrogen phosphate and di-sodium hydrogen phosphate (both analytical reagent grade) were purchased from S. D. Fine Chemicals, Mumbai, India. Ethylcellulose (EC, 50 cps), acetone, glycerin, and ethyl alcohol were procured from Qualigens Pvt Ltd, Mumbai, India, and mannitol from Merck, India, was purchased from C. N. Chemicals, Uttar Pradesh, India. Solvents of reagent grade and double-distilled water were used in all experiments.

Methods

Solubility and Dose/Solubility Ratio Studies

The kinetics of osmotic drug release is directly related to the solubility of the drug within the formulation. Assuming the capsule formulation to consist only of the pure drug, the fraction of drug that will be released with zero-order kinetics is given by Equation 1.[13,14]

$$F(Z) = 1 - S/\rho, \quad (1)$$

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. Drugs with a solubility of 0.05 g/cm^3 would be released with 95% zero-order kinetics according to Equation 1. However, the zero-order release rate would be slow owing to the small osmotic pressure gradient. Conversely, highly water-soluble drugs would demonstrate a high release rate that would be zero-order for a small percentage of the initial drug load. The dose/solubility (D/S) ratio was also ascertained because FLU is listed in class II in the biopharmaceutical classification system (BCS). Therefore, to assess the solubility of the drug in various dissolution mediums, saturated solutions of the drug were prepared in 0.1N HCl, double-distilled water, and phosphate buffer pH 7.4 in a closed container at 37°C. Excess amounts were added to ensure saturation, and solutions were equilibrated for 24 hours. The saturated solutions were filtered, and the concentration was determined by UV spectrophotometer at 247 nm after suitable dilutions. The density of the drug was determined by pycnometer (Jindal Scientific Industries Pvt Ltd, Ambala, India).

Drug Analysis and Preparation of Calibration Curve

A double-beam UV spectrophotometer (Shimadzu-1700, Kyoto, Japan) was used for drug analysis. A known detectible amount of FLU (10 μg/mL) was taken and dissolved in the dissolution medium and analyzed at 247 nm. Standard concentrations in the Beer-Lambert's range of 2 to 16 μg/mL were prepared and studied for 3 days for interday and intraday variations. Statistical test (linearity test) was applied to authenticate the standard curve.

Preparation of Asymmetric Membrane Capsules

AMCs were prepared by using the phase inversion process, in which the membrane was precipitated on glass mold pins—having a diameter of 5.52 mm ± 0.05 and 6.1 mm ± 0.022 for the body and cap, respectively—by dipping the glass mold pins in a coating solution of 10% wt/vol and 15% wt/vol of EC and varying amounts of glycerol (8% wt/vol and 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 seconds. After this, the pins were immersed in an aqueous quenching solution (10% wt/vol of glycerol) for 10 minutes. Immersion of EC-coated glass mold pins in a quench bath helped in 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 hours. The body and the cap were then trimmed to fit inside each other for formation of AMC. The thickness of the coatings was found to be 953.49 ± 0.24 μm and 635 ± 0.39 μm by scanning electron microscopy (SEM) (Figure 1D) for the capsules containing 15% wt/vol and 10% wt/vol of EC, respectively. Drug loading of 200 mg, based on Equation 4, after passing through 100-mesh sieve and having particle size 130 μm, was mixed with or without mannitol (50 mg) in polythene bag, and AMCs were filled manually. Mannitol was used as an osmogen as FLU was found to be osmotically inactive.[15] The filled AMCs were then sealed with ethanolic solution of EC. The physical characterization of AMC with a conventional hard gelatin capsule (HGC) is given in Table 1. The composition of all the AMCs formed, along with the extra design checkpoint AMC (AMC 9) and AMC with a solubility enhancer for the drug (AMC 10), is represented in Table 2.

Scanning Electron Microscopy

Asymmetric membranes obtained before and after complete dissolution of core contents were examined for their porous structure and thickness using Jeol 6100 SEM (Jeol, Tokyo, Japan). After dissolution, asymmetric membrane structures were dried at 50°C for 8 hours and stored in dessicator before examination. Asymmetric membranes were sputter coated for 5 to 10 minutes with gold by using fine coat ion sputter and examined under SEM.

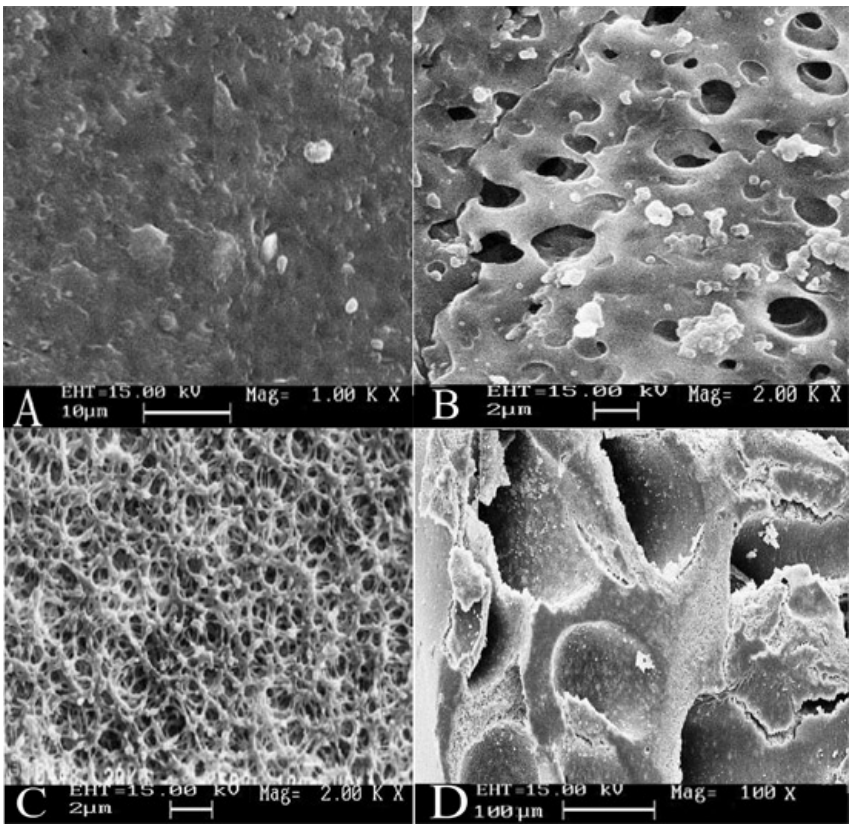

Figure 1. SEM of coating membrane: (A) outer region, 8% wt/wt glycerol (original magnification ×1000); (B) inner region, 20% wt/wt glycerol (original magnification ×2000); (C) after complete dissolution, 20% wt/wt glycerol (original magnification ×2000); (D) cross-section (original magnification ×100).

Differential Scanning Calorimetry

The differential scanning calorimetry (DSC) profiles of pure and physical mixtures of FLU were recorded on Pyris Diamond DSC-4 (PerkinElmer, Wellesley, MA). Thermal behaviors were studied under normal conditions with perforated and sealed quartz pans and with a nitrogen gas flow of 400 mL/min. The samples (8.76 mg for pure FLU, 9.881 mg for FLU and mannitol, and 10.35 mg for FLU, mannitol, and EC) were heated at 5°C/min over a temperature range of 22°C to 200°C, 23°C to 300°C, and 26°C to 300°C, respectively. The reference sample used in all 3 determinations was alumina with a weight of 10.5 mg. Peak temperatures and enthalpies were calculated by calculating the mean of 3 measurements.

Determination of Immediate and Sustained Release Dose

The calculation of the immediate and sustained release dose from the total dose was calculated after running a dosage form (without the polymer) in a medium in which the drug freely dissolved and by using Equation 2.[16]

$$D_t = f \times D_i(1 + 0.693 \times t/t_{1/2}), \qquad (2)$$

where D_t is the total dose required to form the dosage form, f is the fraction of average effective concentration of the drug and the upper limit of drug concentration in the blood, t is the time until desired sustained effect is reached, and $t_{1/2}$ is the half-life of the drug.

Table 1. Physical Characterization of Prepared AMC With Conventional HGC*

		Dimensions, mm†				
		Cap		Body		
Type of Capsule	Appearance	Length	Diameter	Length	Diameter	Sealed
HGC	Transparent	9.02 ± 0.11	6.10 ± 0.12	16.08 ± 0.14	5.08 ± 0.02	19.03 ± 0.08
AMC	Opaque	9.01 ± 0.13	6.13 ± 0.14	16.07 ± 0.17	5.13 ± 0.15	18.99 ± 0.17

*AMC indicates asymmetric membrane capsule; and HGC, hard gelatin capsule.

†Values are expressed as mean ± SD of 3 readings (n = 3).

Table 2. Composition of the 8 AMC Formulations Along With the Extra Design Checkpoint Formulation (AMC 9) and the Solubility Enhancer for the Drug (AMC 10)*

		AMC									
Sr Number	Variable	1	2	3	4	5	6	7	8	9†	10‡
1	Ethylcellulose (% wt/vol)	10	15	10	15	10	15	10	15	13.75	10
2	Mannitol (mg)	0	0	50	50	0	0	50	50	37.5	50
3	Glycerol (% wt/vol)	8	8	8	8	20	20	20	20	17	8
4	Quenching concentration§ (% wt/vol)	10	10	10	10	10	10	10	10	10	10
5	Quenching time (minutes)	10	10	10	10	10	10	10	10	10	1
6	Acetone (% vol/vol)	50	50	50	50	50	50	50	50	50	50
7	Ethanol (95%) (% vol/vol)	30	30	30	30	25	25	25	25	25	30
8	Water (mL)	90	90	90	90	90	90	90	90	90	90
9	Citric acid (mg)	0	0	0	0	0	0	0	0	0	25

*AMC indicates asymmetric membrane capsule; and Sr, serial.

†Extra design checkpoint batch.

‡AMC 10, which contains citric acid.

§10% wt/vol of glycerol in water.

In Vitro Drug Release

In vitro cumulative drug release from the prepared formulations (n = 6) was studied by using *British Pharmacopeia* (*BP*) paddle type apparatus (rotating speed 75 rpm at 37°C ± 0.5°C). The dissolution medium was 0.1N HCl as simulated gastric fluid (SGF) (900 mL, pH 1.2) for the first 2 hours, 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. The amount of drug released at each time point was calculated and summed to give cumulative amount.

Statistical Analysis

The release profiles up to $t_{50\%}$ of FLU from all formulations (n = 6) in the dissolution medium was statistically compared by Dunnett multiple comparison t test (Instat software, Graphpad Software Inc, San Diego, CA) with release rate profiles of the theoretical formulation (extra design checkpoint batch), which was obtained by using the polynomial equation. The statistical significance was tested at $P > .05$. The best formulation among the nonsignificant pairs of formulations was chosen after pairwise comparison using similarity factor ($f2$) (PCP Disso Version 2.08 Software, Pune, India), and the formulation in the factorial design batch with the highest value of $f2$ was selected as the best formulation. Other parameters calculated using PCP Disso Version 2.08 software were ratio of mean dissolution time, area under the curve, and dissolution efficiency (% DE).

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 hour (effective concentration) following in vitro dissolution of AMCs was done by using Equation 3.[16] The pharmacokinetic parameters required for calculations were taken from the relevant literature.[17]

$$C_b = D_0/V_d \times e^{-kt}, \tag{3}$$

where C_b is the drug concentration in blood, D_0 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

In order to confirm the mechanism of FLU release, release studies of the optimized formulation were conducted in media 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 media using *BP* dissolution apparatus II (75 rpm). Two methods were employed: the first was the direct measurement of the FLU 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 FLU 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 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 of both the methods were compared and they suggested that both methods were similar in analysis of the drug.

Effect of Polymer Diffusibility

The diffusibility of a drug molecule through the rate-controlling membrane of a polymer membrane permeation controlled drug delivery system from the optimized formulation was studied using both the formulation stored in a dessicator for 24 hours and also that from a freshly fabricated drug delivery device. In vitro dissolution for 1 hour was done with a sampling time of 10 minutes. One milliliter of the sample was withdrawn and suitably diluted and analyzed at 247 nm. The effect of polymer diffusibility was calculated[18] using Equation 4 for AMCs that were freshly fabricated and Equation 5 for those stored for 24 hours.

$$D_p = h_p^2/6t_l, \tag{4}$$

where D_p is the polymer diffusibility, h_p is the thickness of the polymer membrane, and t_I is the time axis intercept of the back extrapolation through the steady-state drug release data.

$$D_p = h_p^2/3t_b, \tag{5}$$

where D_p is the polymer diffusibility, h_p is the thickness of the polymer membrane, and t_b is the negative time axis intercept of the back extrapolation through the steady-state drug release data.

Kinetics of Drug Release

In general the release of drug from an osmotic system depends on many factors such as osmotic pressure, pore size, and coating thickness. The in vitro release from conventional dosage form of 50 mg (without the polymer) exhibited a fast release, with over 80% release in the first hour. The release from the formulations containing polymer was more controlled, with $t_{50\%}$ being more than 10 hours. In order to describe the kinetics of drug release from controlled release formulation, various mathematical equations have been proposed. The zero-order rate[19] (Equation 6) describes systems where drug release is independent of its concentration and is generally seen for poorly water-soluble drug in matrix, transdermals, etc. The first-order equation[20] (Equation 7) describes systems in which the release is dependent on its concentration (generally seen for water-soluble drugs in porous matrix). The Higuchi model[21] describes the release of the drug from an insoluble matrix to be linearly related to the square root of time and is based on Fickian diffusion (Equation 8). The Hixson-Crowell cube root law[22] (Equation 9) describes the release of drug from systems where it depends on the change in surface area and diameter of the particles or tablets with time and mainly applies in the case of systems that dissolute or erode over time. In order to authenticate the release model, dissolution data can further be analyzed by Peppas and Korsmeyer equation[23] (Equation 10).

$$Q_t = k_0 t \tag{6}$$

$$ln\ Q_t = ln\ Q_0 - k_1 t \tag{7}$$

$$Q_t = k_{\mathrm{H}} t^{1/2} \tag{8}$$

$$Q_0^{1/3} - Q_t^{1/3} = k_{HC} t \tag{9}$$

$$\frac{M_t}{M_\infty} = k t^n, \tag{10}$$

where Q_t is the amount of drug released at time t; Q_0 is the initial amount of the drug in the formulation; k_0, k_1, k_H, and k_{HC} are release rate constants for zero-order, first-order, Higuchi model, and Hixson-Crowell rate equations. In Equation 10, M_t is the amount of drug released at time t, and M_∞ is the amount released at time ∞; k is the kinetic constant, and n is the diffusional coefficient. The criteria for the best model were based on goodness of fit and residual sum of squares (SSQ).

Results and Discussion

Solubility Studies and Dose/Solubility Ratio

Solubility studies showed that FLU had varying solubility in different mediums studied (0.1N HCl (8.9×10^{-6} g/cm^3), double-distilled water (8.665×10^{-3} g/cm^3), and phosphate buffer pH 7.4 (11.62×10^{-3} g/cm^3). The density of FLU was found to be 0.3045 g/cm^3. The D/S ratio of 22.471 in 0.1N HCl meant that a dose of 200 mg would require 22.471 L to fully dissolve, which if not available could result in side effects usually associated with the drug. The fabricated AMC will overcome these side effects, as only the amount of drug that dissolves in the SGF will come out of the capsular system, and the rest of the drug will not be in contact with the gastric mucosa. The D/S ratio in double-distilled water showed improved solubility (0.023 L) with the maximum reached in phosphate buffer pH 7.4 (0.0172 L), which meant that if the drug delivery system were to partially or fully dissolve in the SIF, it could result in an

unprecedented and unexpected plasma variations of the drug. However, the ability of the fabricated AMCs to remain intact in the SIF will result in the release of FLU in a controlled manner.

The experimental values of the F(z) suggested that, in order 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 FLU 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 FLU 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 pK_a of FLU (~4).[24] 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 FLU 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.

Drug Analysis and Preparation of Calibration Curve

The drug solution in phosphate buffer pH 7.4 showed a λ_{max} of 247 nm and an absorbance of 0.554 ± 0.01. Calibration curves (2-16 μg/mL) were made using freshly prepared solutions for 3 consecutive days to study the reproducibility of the standard curve. Precision value coefficient of variation (CV) of 0.01% suggested that the standard curve was reproducible. A high degree of correlation was observed between the concentrations taken and the respective absorbances obtained (R^2 = 0.9998). Linearity test was applied to check whether the obtained regressed line was a straight line or a curve. The test showed perfect linearity for the regressed line at 95% confidence interval (P = .3571).

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. Membrane 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. Membrane containing higher proportion of glycerol (25% wt/vol) showed larger pores. The formulation prepared with this membrane caused bursting. So, it can 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 presented in this study.

Differential Scanning Calorimetry

The thermal behavior of pure FLU and in mixture was investigated by heating the respective samples at 5°C/min (Figure 2). For the first sample (pure FLU) an endothermic peak was observed at 111°C ± 0.2°C with an enthalpy of 85.4 ± 1.2 mJ/min. The second sample (FLU and mannitol) had 2 endothermic peaks at 111°C ± 0.25°C and 161°C ± 0.32°C with enthalpies of 43.5 ± 1.2 mJ/min and 93.6 ± 1.1 mJ/min, respectively. The third sample (FLU, mannitol, and EC) had 3 endothermic peaks at 110°C ± 0.21°C, 159°C ± 0.22°C, and 248°C ± 0.21°C with enthalpies of 48.8 ± 1.5 mJ/min, 54.7 ± 2.1 mJ/min, and 21.9 ± 0.98 mJ/min, respectively. This clearly showed that there was no interaction between the drug and other excipients used in the study.

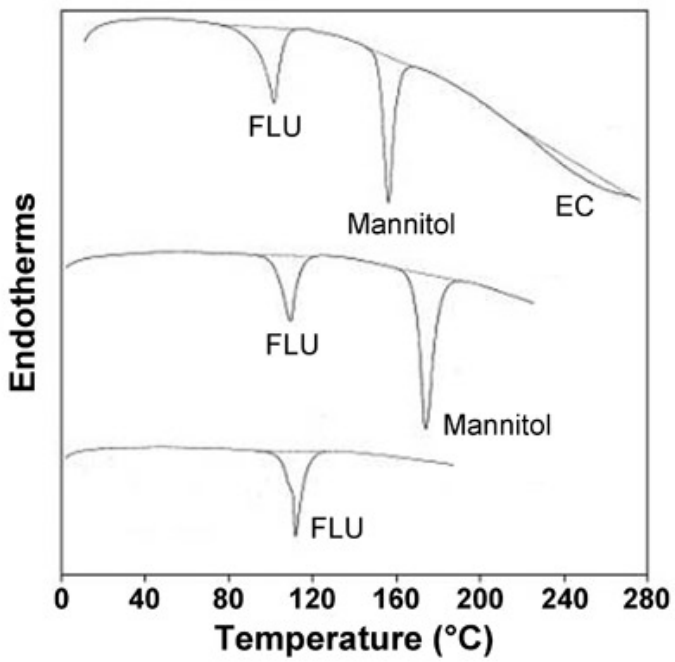

Figure 2. DSC thermograms for FLU and its mixtures.

Determination of Immediate and Sustained Release Dose

The calculation of the immediate and sustained release dose from the total dose was calculated after running dissolution of a conventional dose (50 mg), without the polymer in a medium in which the drug freely dissolved and by using Equation 2. The result obtained suggested that ~41.5 mg (immediate release) of FLU from the AMCs should be released within the first hour like a conventional dose, and then the sustained release dose (158.5 mg) should be released at a constant rate for the next 23 hours for which the total dose (200 mg) had been originally calculated (24 hours). AMC 10, which contains the solubility enhancer for the drug (citric acid), released 42.1 mg within the first hour and achieved the therapeutic concentration required and then gave sustained release for the next 23 hours.

In Vitro Drug Release

In vitro studies were performed in 2 groups for the factorial design batches. The first group (group 1) consisted of AMC 1 formulation with all the variables at lower level and AMC 2, AMC 3, AMC 5 with 1 variable at a higher level and 2 at lower levels. 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 FLU. This effect is also evident while studying the individual effect of the osmogen (mannitol causes a decrease of 49.07 minutes in achievement of $t_{50\%}$ from AMC 3) (Figure 3). 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 causes a decrease of 34.89 minutes (Figure 3). When, EC concentration was at a higher level, the release of FLU from the capsular membrane was constrained as compared with AMC 1 formulation. The decreased FLU release from AMC 2 might be towing to the increased diffusional path for the drug to transverse before being released into the dissolution medium. Yates analysis for the individual effect of EC concentration at higher level (Figure 3) showed an increase of 87.22 minutes.

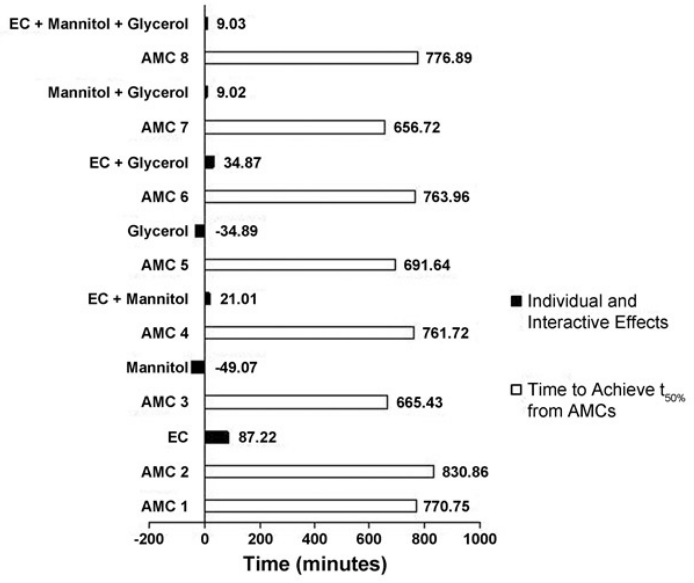

Figure 3. Comparative graphical representation of dissolution data: black squares = individual and interactive effects of the variables; white squares = time taken by AMCs to achieve $t_{50\%}$.

Yates analysis for calculation of interactive studies in group 2 composed of AMC 4, AMC 6, AMC 7 in which 2 variables were at a higher level and 1 at a lower level, and AMC 8 in which all the variables were at high level. Surprisingly, in AMC 8 there was a delayed achievement of $t_{50\%}$. A careful study of the Yates analysis for interactive study between the 3 variables showed that there was an increase of 9.03 minutes (Figure 3). 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 due to higher glycerol content, which suggested that the membrane thickness still had a prominent role in constraining the release of FLU; though this delay was greatly reduced by the burst release of FLU resulting from individual effects of the other 2 variables also at higher level. The other formulations in the order had a $t_{50\%}$ as expected with the interactive study favoring the release data.

As the study was statistically designed, it was possible not only to authenticate the validity of the factorial design but also to provide the formulator with a wide choice of formulation components. A polynomial equation was constructed that would relate the effect of individual factor and the interactions between the factors through coefficients in the polynomial equation generally calculated for a response, in this case $t_{50\%}$. The reduced model for $Yt_{50\%}$ with significant coefficient values at 95% confidence level is as follows:

$$Yt_{50\%} = \mathrm{B}_0 + \mathrm{B}_1(X_1) + \mathrm{B}_2(X_2) + \mathrm{B}_3(X_3), \qquad (11)$$

where Y is the measured response; B_0 is the intercept or in other terms the arithmetic mean response of 8 runs; and B_1, B_2, B_3 represent significant coefficients computed from responses of the formulations in the design at 95% confidence interval.

Yates algorithm was used for calculation of the predicted response ($Yt_{50\%}$). Having predicted to have a $t_{50\%}$ of 669.5 ± 1.43 minutes, the extra design checkpoint batch was found to have a $t_{50\%}$ value of 676.06 ± 2.01 minutes (R^2 between the observed and predicted $t_{50\%}$ was 0.9995).

Statistical Analysis

Dunnett multiple comparison test compared all the factorial design batches with a control batch (extra design checkpoint batch). If the value of t was found to be greater than 2.701, then comparison test would have run at a significance value less than .05 or below 95% confidence level and would have been considered to be statistically significant. However, the multiple comparison test, when all the formulations were compared with the standard (AMC 9), resulted in F value of 0.1484 with a *P* value of .9965. The t values of all the formulations was found to be below 2.701, which meant that the test was run at 95% confidence level and that the difference between all the formulations as compared with extra design checkpoint batch were statistically insignificant. AMC 3 showed the least t value of 0.06148 among all the formulations in the study. Similarity factor (f2) of 92.94 (Figure 4) showed that the 2 profiles (AMC 3 and AMC 9) had comparable dissolution profiles, with $t_{50\%}$ reached in 665.43 minutes and 676.06 minutes, respectively. Other parameters calculated were ratio of mean dissolution time for AMC 3 and AMC 9 (1.013) and area under the curve (1.005) at the end of 720 minutes. The dissolution efficiency (%DE) for AMC 9 was 29.26% and for AMC 3, 29.70%, which was better than the other formulations in the factorial design batches. Therefore, AMC 3 was taken as the best formulation for the factorial design batch.

The formulation AMC 10, which had all the composition of the best formulation (AMC 3) and citric acid (25 mg) was run along with AMC 3 to see the effect of increased solubility of FLU owing to the incorporation of citric acid in the formulation. The results of the dissolution studies showed a definite increase in the release of FLU reaching the therapeutic concentration (3.552 μg/mL) like a conventional dose in the first hour and then giving a sustained release, which was not observed in AMC 3 (Figure 5). The AMC 10 formulation showed increased dissolution efficiency of 34.41% and had a mean dissolution time (MDT) of 213.12 minutes. FLU release from AMC 10 suggested that an increased microclimate pH (~4) due to citric acid inside the capsular membrane not only increased the solubility of FLU but also created a concentration gradient, which coupled with the osmotic pressure inside the AMC increased FLU release from AMC 10.

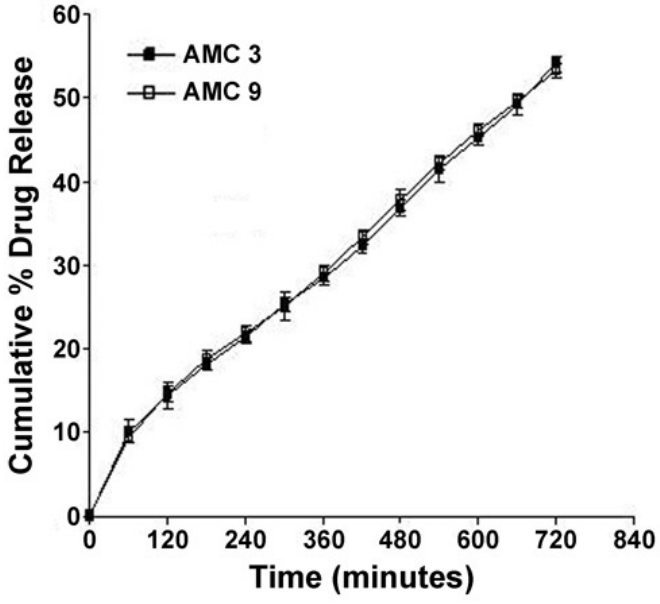

Figure 4. Comparative dissolution profiles (n = 6) for AMC 3 and extra design batch formulation, AMC 9: black squares = AMC 3 (EC, 10%; mannitol, 50 mg; glycerol, 8%); white squares = AMC 9 (EC, 13.75%; mannitol, 37.5 mg; glycerol, 17%).

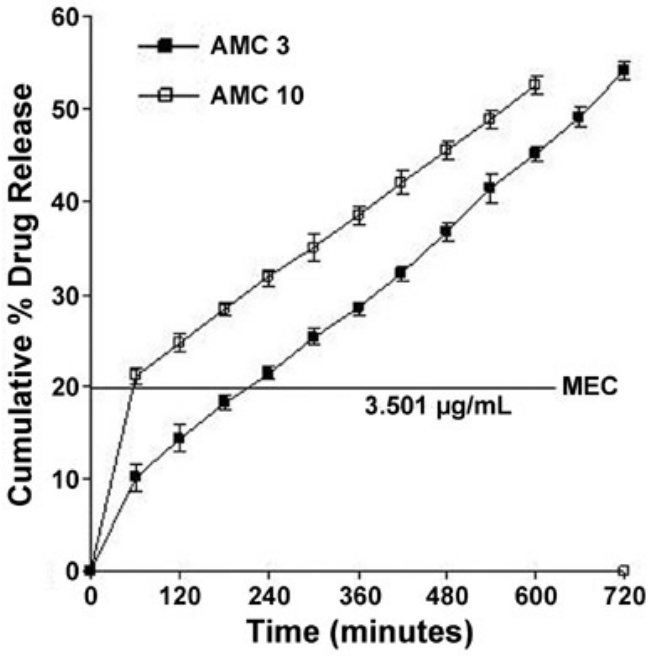

Figure 5. Comparative dissolution profiles (n = 6) for AMC 10 and AMC 3: white squares = AMC 10 (EC, 10%; mannitol, 50 mg; glycerol, 8%; citric acid, 25 mg); black squares = AMC 3 (EC, 10%; mannitol, 50 mg; glycerol, 8%) in achieving the minimum effective concentration (MEC).

In Vivo Prediction of Flurbiprofen Concentration Using In Vitro Release Data

The prediction of drug concentration in blood after 1 hour (effective concentration) following administration of AMCs was done using Equation 3. The first-order elimination rate constant for FLU (0.17 $hour^{-1}$), when incorporated in Equation 3, resulted in the finding that a dose of 42.1 mg released with in the first hour from AMC 10 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 5).

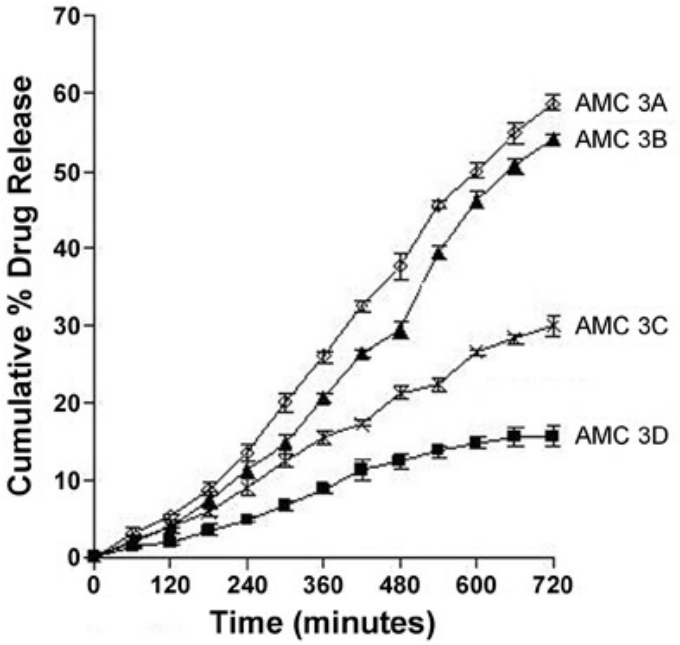

Figure 6. Release profiles (n = 6) from AMC 3 in dissolution medium of different osmotic pressures: white squares = AMC 3a (1.178 mmHg); black triangles = AMC 3b (2.356 mmHg); black cross = AMC 3c (3.535 mmHg); black squares = AMC 3d (4.713 mmHg).

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. FLU release from AMC 3 decreased as the osmotic pressures of the drug release medium increased (Figure 6). When the initial release rates were plotted against external osmotic pressure, a linear relationship was obtained (R^2 = 0.9868) (Figure 7). Similarly, when the release rate was plotted against osmotic pressure difference (the osmotic pressure inside the formulation was found to be 5.891 mmHg), a linear relationship was again obtained (R^2 = 0.9869) (Figure 8). Therefore, it was concluded that the primary mechanism governing the drug release from the developed formulations was osmotic pumping.

Effect of Polymer Diffusibility

The effect of polymer diffusibility on drug release (since drug release results from diffusion of drug through asymmetric membrane barrier) from the optimized formulation (AMC 10) was studied (Figure 9) using the formulation that was stored in a dessicator for 24 hours and also from a freshly fabricated drug delivery device. Polymer diffusibility from freshly prepared formulation and that stored in a dessicator for 24 hours was calculated to be 3733.56 μm/min and –11200.69 μm/min, respectively. A positive value

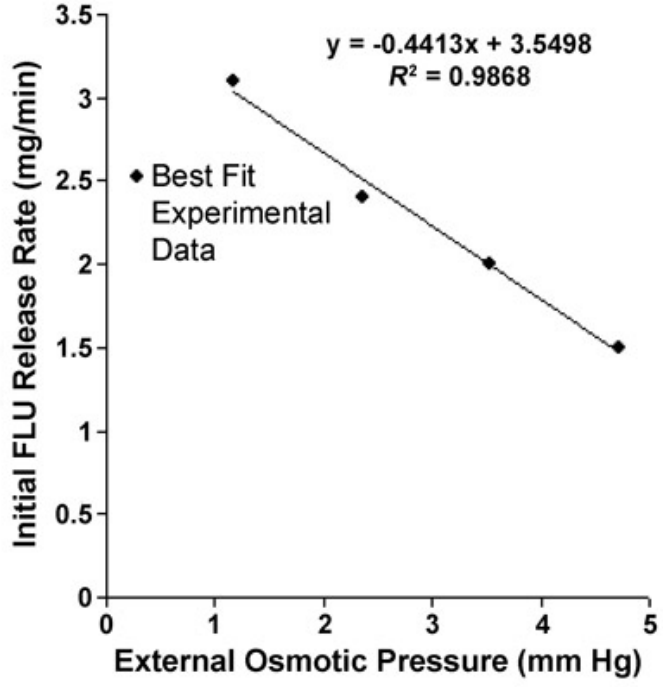

Figure 7. Demonstration of osmotic release from asymmetric membrane capsule (AMC 3) represented by black squares = initial release rate of FLU from AMC 3 (EC, 10%; mannitol, 50 mg; glycerol, 8%).

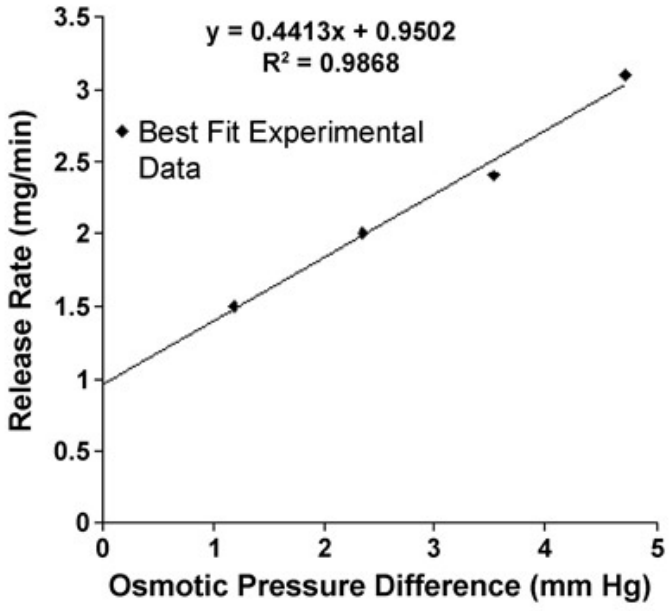

Figure 8. FLU release rate from AMC 3 showing effect of osmotic pressure difference across the membrane represented by black squares = release rate of FLU from AMC 3 (EC, 10%; mannitol, 50mg; glycerol, 8%).

for polymer diffusibility for the freshly prepared formulation suggests a lag time in release of FLU, which means that the drug has not penetrated the membrane (ie, the drug is not released until the dissolution medium has penetrated the membrane barrier) dissolving the drug in the reservoir, whereas a negative value for polymer diffusibility for the formulation stored for 24 hours suggests saturation of FLU at the pores of the AMC. Because of this saturation of the drug at the pores of the membrane, when the dissolution medium enters the AMC the process of drug entering into solution form will be faster, thereby resulting in faster release from the system. Polymer diffusibility studies suggest that the stored formulations may result in burst release before achieving steady-state and can be an important parameter in determining the minimum effective concentration required by the drug.

Kinetics of Release

All the models for selecting the release profile were applied on AMC 3 and AMC 10. The results are summarized in Table 3. The best fit model in case of AMC 3 could have followed first-order, Hixson-Crowell model, and Peppas and Korsmeyer model. While considering the higher correlation coefficient value (R) and lower SSQ value, the release data seem to fit the Peppas and Korsmeyer model better. The drug release data were further analyzed for curve fitting based on Power Law, and the results (AMC 3: n = 0.6617, k = 0.6369, SSQ = 64, and R = 0.9908; AMC 10: n = 0.4053, k = 3.6749, SSQ = 37, and R = 0.9783) confirmed that the formulation AMC 3 followed non-Fickian diffusion (n > 0.5), which meant that the release of FLU from the dosage form AMC 3 is anomalous type (ie, more than 1 type of release phenomena could be involved). This type of release mechanism could be to the result of the poor solubility of FLU in the acidic medium and higher solubility with increasing pH. The only way FLU could have released out of the formulation would have been because of the osmotic pressure developed inside the formulation. The Power Law applied for AMC 10 indicated that the formulation followed Fickian diffusion (n < 0.5) and could be an ideal formulation, giving an initial burst release followed by Fickian diffusion, irrespective of the changes in pH.

Table 3. Different Kinetic Models Applied on AMC 3 and AMC 10*

		Formulations	
Kinetic Model	Parameters	AMC 3	AMC 10
Zero-order	R	0.9795	0.8409
	SSQ	127	668
	k_0	0.0783	0.1009
First-order	R	0.9904	0.9367
	SSQ	68	365
	k_1	−0.0010	−0.0013
Higuchi	R	0.9652	0.9919
	SSQ	214	37
	k_H	1.7448	2.1135
Hixson-Crowell	R	0.9905	0.9115
	SSQ	74	447
	k_{HC}	−0.0003	−0.0004

*AMC indicates asymmetric membrane capsule; and SSQ, sum of squares.

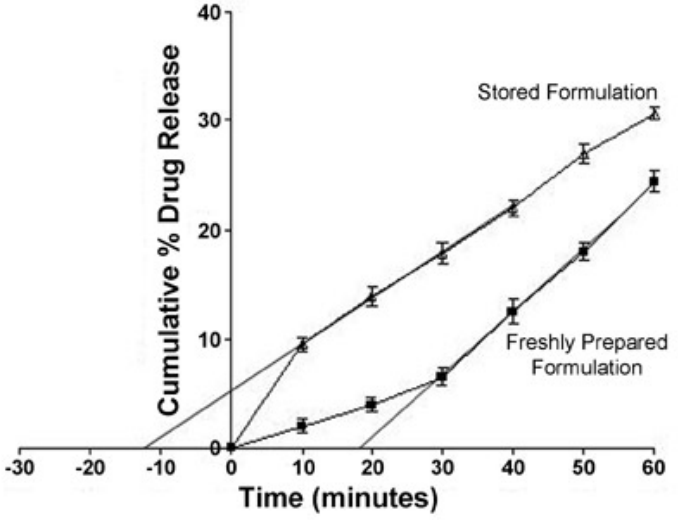

Figure 9. A cumulative percentage drug release versus time profile from stored and freshly prepared AMC 10 (n = 6) by back extrapolation of the data from steady-state drug release: white triangles = stored AMC 10; black squares = immediately prepared AMC 10.

CONCLUSION

Studies showed a pH-independent release for AMC 3 (EC, 10% wt/vol; mannitol, 50 mg; glycerol, 8% wt/vol) and AMC 10 (EC, 10% wt/vol; mannitol, 50 mg; glycerol, 8% wt/vol; citric acid, 25 mg). However, for AMC 3 there was no definite mechanism of release as compared with AMC 10, which gave an initial burst release followed by Fickian diffusion.

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