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Research Article

Osmotically Regulated Floating Asymmetric Membrane Capsule for Controlled Site-Specific Delivery of Ranitidine Hydrochloride: Optimization by Central Composite Design

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Abstract. A nondisintegrating, floating asymmetric membrane capsule (FAMC) was developed to achieve site-specific osmotic flow of a highly water-soluble drug, ranitidine hydrochloride (RHCl), in a controlled manner. Solubility suppression of RHCl was achieved by the common ion effect, using optimized coated sodium chloride as a formulation component. The capsular wall of FAMC was prepared by the phase inversion process wherein the polymeric membrane was precipitated on glass pins by dipping them in a solution of cellulose acetate followed by quenching. Central composite design was utilized to investigate the influence of independent variables, namely, level(s) of membrane former, pore former, and osmogen, on percent cumulative drug release (response). The release mechanism of RHCl through FAMC was confirmed as osmotic pumping. The asymmetry of the membrane was characterized by scanning electron microscopy that revealed a dense nonporous outer region of membrane supported by an inner porous region. Differential scanning calorimetry indicated no incompatibility between the drug and excipients. *In vitro* drug release in three biorelevant media, pH 2.5 (low fed), pH 4.5 (intermediate fed), and pH 6.5 (high fed), demonstrated pH-independent release of RHCl ($P>0.05$). Floating ability for 12 h of the optimized FAMC9 was visually examined during the *in vitro* release studies that showed maximal drug release with zero-order kinetics ($r^2=0.9991$). Thus, a novel osmotically regulated floating capsular system was developed for site-specific delivery of RHCl.

KEY WORDS: asymmetric membrane capsule; central composite design; floating system; osmotic delivery; ranitidine hydrochloride.

INTRODUCTION

Osmotic devices are considered as a promising strategy for the controlled delivery of drugs. Since its elementary inception in 1970, osmotic delivery devices have been sequentially developed to eliminate their limitations (1,2). This led to the introduction of an asymmetric membrane concept that relies on drug delivery by an osmotic driving force. Asymmetry of the membrane refers to vertically nonsimilar regions—the outer surface has a smooth, thin, dense, and nonporous region to resist mass transfer, while the inner region is rough, thicker, and porous to provide support and mechanical strength to the outer region (3–5). Incorporation of pore former (a water-soluble excipient) in the coating composition of the membrane results in *in situ* and *ex situ* pore formation when the asymmetric membrane comes in contact with aqueous media. Thus, the asymmetric membrane capsule (AMC) can be considered as a versatile device for the delivery of drugs.

Research inputs from our group have resulted in the successful development of AMCs of drugs with varying water solubility, namely, ketoprofen (6,7), flurbiprofen (8), promethazine hydrochloride (9), phenylephrine hydrochloride (10), and triprolidine hydrochloride (11), thus proving the efficacy of the system for both poorly water-soluble and highly water-soluble drugs. The present work aims at exploring the feasibility of AMCs for site-specific osmotically regulated delivery of ranitidine hydrochloride (RHCl) as a floating single-unit device. As the primary requirement for an osmotically regulated system is that it should not lose its structural integrity until complete drug release, so the system will be formulated by water-insoluble polymers such as cellulose acetate/cellulose acetate butyrate/ethyl cellulose. Thus, a buoyant AMC with a density less than that of gastric fluid (1.004–1.01 g/cm^3) was aimed for the controlled release of RHCl in the gastric cavity.

RHCl is a competitive and reversible inhibitor of H_2 receptor indicated in the treatment of gastric ulcer, duodenal ulcer, Zollinger–Ellison syndrome, gastroesophageal reflux disease, and erosive esophagitis (12). RHCl is given generally in 150 mg twice a day or 300 mg once a day as an oral dosage form. But this dose needs to be increased to 150 mg four to five times a day for treating the endoscopically diagnosed

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erosive esophagitis (13). Clinically, oral treatment of gastric disorders with RHCl in combination with antacids promotes the local delivery of RHCl to the receptors of the parietal cell and increases the efficacy of the drug (14). The conventional dose (150 mg) inhibits acid secretion for 4–5 h, requiring frequent dosing that causes fluctuations in plasma levels of the drug. Colonic metabolism, intestinal degradation of RHCl (15), and absorption window in the proximal part of the gut makes it a suitable candidate for a gastroretentive drug delivery device.

Accordingly, various research reports on the gastroretentive system of RHCl can be found in the literature (16–18), but, to the best of our knowledge, no work has been undertaken on development of an osmotically regulated, pH-independent, single-unit capsular system of RHCl. Hence, optimization of an osmotically regulated floating asymmetric membrane capsule (FAMC) of the highly water-soluble drug RHCl (>550 mg/mL) was aimed, with the help of central composite design (CCD). Development of the formulation was mediated through solubility modulation by common ion effect. Evaluation was focused on physiochemical characterization and *in vitro* performance efficacy in biorelevant media. The efficacy of the experimental design was validated.

MATERIALS AND METHODS

Materials

RHCl was obtained as a gift sample from Siemen Laboratories, Gurgaon, India. Cellulose acetate (CA 398-10) was procured from Sigma Aldrich Chemical, St. Louis, Missouri, USA. Potassium dihydrogen orthophosphate, sodium dihydrogen phosphate, acetone, ethanol (95%), and glycerol were purchased from S.D. Fine Chemicals, Mumbai, India. Sodium chloride (NaCl) and sodium hydroxide were purchased from Qualigens Pvt. Ltd., Mumbai, India. Solvents and reagents of analytical grade and double-distilled water were used in all experimental work.

Methods

Differential Scanning Calorimetry

The differential scanning calorimetry (DSC) profiles of pure and physical mixtures of RHCl were recorded on Pyris Diamond DSC-4 (PerkinElmer, Wellesley, Massachusetts, USA). Thermal behaviors were studied using perforated and sealed quartz pans and under a nitrogen gas flow of 400 mL/min. The samples (RHCl, CA 398-10, NaCl, and physical mixture(s) of RHCl with NaCl and CA 398-10) were heated at 5°C/min over a temperature range of 50–300°C. The reference sample used in all the determinations was alumina.

Equilibrium Solubility

To assess the solubility of the drug in various dissolution media, an excess amount of RHCl was added to double-distilled water (pH 6.0) and phosphate buffer (pH 2.5, 4.5, and 6.5) in a closed container at 25±0.5°C (19). The solutions were equilibrated for 72 h in water bath shaker (Hicon Enterprises, New Delhi, India). The saturated solutions were filtered, ensuring temperature maintenance at 25±0.5°C by the use of specially designed temperature regulator boxes. The concentration of RHCl in double-distilled water (pH 6.0) and phosphate buffer (pH 2.5, 4.5, and 6.5) was determined spectrophotometrically at the wavelength maxima of 314.5, 315, 312.5, and 312 nm, respectively, after suitable dilutions using a double-beam UV spectrophotometer (Shimadzu Pharmaspec-1700, Kyoto, Japan).

Preparation and Characterization of Coated Sodium Chloride Crystals

Coating of NaCl crystals was done using CA 398-10 as hydrophobic polymer in a laboratory-fabricated fluidized bed drier (FBD Lab Model No. 1). Ten grams of NaCl crystals sifted through sieve no. 16 was spray coated with varying strengths (1–4% *w/v*) of coating solutions of CA 398-10 (Table I) for 10 min at 30°C and 16–18 kg/cm^2 pressure. Coated NaCl crystals were characterized for angle of repose, Hausner's ratio, and Carr's index (20). Surface morphology of coated and uncoated NaCl was compared by scanning electron microscopy (methodology described later). For the selection of optimized coated NaCl, preliminary *in vitro* drug release studies were carried out to determine $t_{50\%}$. For this study, four prototype AMC formulations (F1–F4), each containing 200 mg of RHCl and 100 mg of coated NaCl (varying coat strengths of 1%, 2%, 3%, and 4% *w/v* CA 398-10; Table I), were prepared and evaluated for *in vitro* release in phosphate buffer pH 4.5.

Solubility Modulation of RHCl by Common Ion Effect

An excess amount of RHCl was suspended in 2 mL of phosphate buffer(s) pH 2.5 and pH 6.5. Selected coated NaCl, in different molar concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 M, was separately incorporated in the test media so that a total of 12 flasks were utilized for the study. The flasks were shaken for 72 h in a water bath shaker and maintained at 25± 0.5°C. Suspensions were then filtered through 0.22 μ nylon filter and analyzed spectrophotometrically. This study helped in the selection of levels of coated NaCl crystals that can assure desired solubility modulation.

Experimental Design for FAMCs of RHCl

CCD was used as an experimental design for the optimization of FAMCs. The independent variables in the study

Table I. Prototype AMC Formulations (F1–F4) of RHCl Designed for the Selection of Optimized Coated NaCl to Determine $t_{50\%}$ and Coating Compositions Used for Coating of NaCl Crystals (C1–C4)

	Formulation code			
(A) Excipient	F1	F2	F3	F4
Ranitidine HCl (mg)	200	200	200	200
Coated sodium chloride crystals (mg)	100	100	100	100
(B) Coating	C1	C2	C3	C4
Cellulose acetate (% *w/v*)	1	2	3	4
Glycerol (mL)	1	1	1	1
Ethanol 95% *v/v* (mL)	3	3	3	3
Acetone (mL) q.s.	10	10	10	10

were concentration of CA 398-10 (X_1), concentration of glycerol (X_2), and content of coated NaCl (X_3). For each of these variables (factors), an experimental range in terms of levels was selected based on the results of preliminary experiments. Each factor was taken at five equally distanced levels that were coded as +1, +*a*, 0, −*a*, and −1 to denote high, intermediate high, central, intermediate low, and low levels, respectively. The response parameters were percent cumulative drug release in 12 h ($\%CDR_{12\ h}$) and floating ability of AMCs. The composition of the formulations (n=15), along with an extra design checkpoint formulation with coded values, is tabulated in Table II.

Preparation of Asymmetric Membrane Capsules of RHCl

FAMCs were prepared by the wet phase inversion process, in which the polymeric membrane was precipitated on fabricated glass mold pins with diameters of 7.22±0.05 and 7.73±0.02 mm for the body and cap, respectively. The glass mold pins were dipped in coating solution of CA 398-10 dissolved in acetone and mixed with glycerol in ethanol (95% *v*/*v*). The precipitated membrane on pins was air-dried for 15 s and immersed in an aqueous quenching solution (10% *w*/*v* of glycerol) for 10 min to get the asymmetric shell. The body and cap from the pins were stripped in accordance to the length of conventional hard gelatin capsules (HGC) after removal from the quench bath and dried at ambient temperature (25–30°C) for at least 8 h. The body and the cap were then trimmed to fit each other for the formation of FAMCs. Drug loading was done by mixing RHCl with coated NaCl (the amount of osmogen for individual FAMC is shown in Table II) in polythene bag and each AMC was filled manually. The filled FAMCs were then sealed with 10% *w*/*v* sealing solution of CA 398-10. FAMCs were characterized for appearance, surface, and dimensions and compared with conventional HGC.

In Vitro Drug Release

In vitro drug release studies of FAMC formulations were performed using the USP paddle-type dissolution apparatus (Hicon Enterprises, New Delhi, India) for 12 h (rotating speed of 50 rpm at 37±0.5°C in 900 mL drug release media). As a floating formulation was intended, the formulations were evaluated in three simulated fed state conditions, namely, low fed state (phosphate buffer pH 2.5), intermediate fed state (phosphate buffer pH 4.5), and high fed state (phosphate buffer pH 6.5) on drug release. One milliliter of the sample was withdrawn at 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 h. The same volume of fresh media was added to maintain sink condition after each sampling. The samples were suitably diluted by fresh media and analyzed UV spectrophotometrically at λ_{max} 315 nm for phosphate buffer, pH 2.5; 312.5 nm for phosphate buffer, pH 4.5; and 312 nm for phosphate buffer, pH 6.5. The cumulative amount of drug released at each time point was plotted against time. Additionally, during the study, each FAMC formulation was observed for its floating behavior throughout the study period.

Statistical Analysis

The release profiles of RHCl from all formulations (n= 15) in release media of different pH were statistically compared with the release data of the theoretical formulation (predicted release data) that were determined from the polynomial equations. The statistical significance was tested at $P<$ 0.05 and correlation was calculated between the predicted and experimental maximal release of the drug from all formulations (n=15) by plotting data in the form of a best-fit line. Design Expert Software 8.0.2, USA was used for data analysis and for constructing the polynomial equations. The interac-

Table II. Composition of FAMC Formulations of RHCl Based on CCD Along with an Extra Design Checkpoint Formulation (FAMC16) and Quenching Solution (10% *v*/*v* of Glycerol in Water)

	Formulation composition						
Formulation code	RHCl (mg)	CA 398-10 (% *w*/*v*) X_1	Glycerol (% *v*/*v*) X_2	Coated NaCl (mg) X_3	Ethanol (95%) (% *v*/*v*)	Acetone (% *v*/*v*), q.s.	Dependent variable
FAMC1	200	10 (−1)	3 (−1)	132 (−1)	30	100	
FAMC2	200	18 (+1)	3 (−1)	132 (−1)	20	100	
FAMC3	200	10 (−1)	15 (+1)	132 (−1)	20	100	
FAMC4	200	10 (−1)	3 (−1)	158 (+1)	30	100	Percent cumulative
FAMC5	200	18 (+1)	15 (+1)	132 (−1)	20	100	drug release at 12 h
FAMC6	200	10 (−1)	15 (+1)	158 (+1)	20	100	
FAMC7	200	18 (+1)	3 (−1)	158 (+1)	20	100	
FAMC8	200	18 (+1)	15 (+1)	158 (+1)	20	100	
FAMC9	200	12 (−*a*)	9 (0)	145 (0)	25	100	Buoyancy[a]
FAMC10	200	16 (+*a*)	9 (0)	145 (0)	25	100	
FAMC11	200	14 (0)	6 (−*a*)	145 (0)	30	100	
FAMC12	200	14 (0)	12 (+*a*)	145 (0)	25	100	
FAMC13	200	14 (0)	9 (0)	138.5 (−*a*)	25	100	
FAMC14	200	14 (0)	9 (0)	151.5 (+*a*)	25	100	
FAMC15	200	14 (0)	9 (0)	145 (0)	25	100	
FAMC16[b]	200	11 (−0.75)	13.5 (+0.75)	154.75 (+0.75)	30	100	

[a] Qualitative dependent variable
[b] Extra design checkpoint formulation

tion between variables and surface response graph of variables on drug release was also determined.

Validation of Experimental Design

The experimental design was validated by preparing an extra design checkpoint formulation (FAMC16), the composition of which is shown in Table IV. Polynomial equations were generated by applying the Design Expert Software 8.0.2, USA on the release data of CCD batch formulations (n=15) for simulated fed state pH. Thus, three polynomial equations were generated that were reduced to retain significant coefficients. The predicted value of the response parameter at each pH was determined and compared with the experimental value at 5% level of significance. Additionally, the value of α was 1.97, which indicates relatively low and flat error around the center points.

Selection of Optimized Formulation

Optimized FAMC was selected based on cumulative percent drug release at 12 h with coefficient of determination (r^2) for zero-order release and its floating ability.

Scanning Electron Microscopy

Asymmetric membranes were examined for their outer dense and inner porous morphology before and after *in vitro* drug release study by scanning electron microscope (Jeol 6000, Tokyo, Japan). Membranes were air-dried for 12 h and stored between sheets of wax paper in a desiccator before examination. The asymmetric membrane samples were sputter coated for 5–10 min with gold using the fine-coat ion sputter and examined under SEM at suitable magnification.

Kinetics of Drug Release

Release of the drug from an osmotic system may get affected by many factors like thickness of the membrane, osmotic gradient, pore size, etc. Various mathematical equations have been proposed to describe the kinetics of drug release from drug delivery systems, namely, zero-order, first-order, Higuchi, and Peppas and Korsmeyer models (20). The criterion for the best model was based on maximum linearity of the data to fit with the model when incorporated in PCP Disso Software (PCP Disso Version 2.08 Software, Pune, India).

Determination of Osmotic Pressure as Driving Force for Drug Release

The optimized formulation was introduced in phosphate buffer, pH 2.5, and variation in osmotic pressure was accomplished by controlling the amount of coated NaCl in the capsule and in the surrounding environment. Condition A: FAMC9 containing 200 mg of RHCl without coated NaCl inside and outside the capsule that represented zero osmotic gradient; condition B: FAMC9 containing 200 mg RHCl and 150 mg coated NaCl inside the capsule and 0 mg coated NaCl outside the capsule to demonstrate the effect of common ion on the drug release; condition C: 0 mg of RHCl with 150 mg of coated NaCl inside and 75 mg of coated NaCl outside the capsule; condition D: 200 mg RHCl and 150 mg coated NaCl inside and 300 mg coated NaCl outside the capsule. Conditions C and D were intended to analyze the effect of increasing the osmotic pressure (26.45 and 105.82 mmHg, respectively, for C and D) of the external media on drug release.

RESULTS AND DISCUSSION

Differential Scanning Calorimetry

The DSC thermograms suggested no interaction between the drug and excipients used in the formulation. Endothermic peaks were observed at 134±0.3°C, with ΔH of 65.5 mJ/mg corresponding to the melting point of RHCl (Fig. 1a), and at 244±1.13°C (ΔH=81.5 mJ/mg) for CA 398-10 (Fig. 1b). No endothermic peak was observed for NaCl in the experimental scanning temperature range (Fig. 1c). The endothermic peak of the drug was retained in the physical mixture of RHCl with NaCl (Fig. 1d), and its mixture with CA 398-10 (Fig. 1e) displayed a slight depression in the enthalpies due to the presence of excipients (6). Thus, no incompatibility was detected between the drug and proposed excipients.

Equilibrium Solubility

The solubility of RHCl was determined as 554.69±3.51 mg/mL in double-distilled water, 503.15±3.27 mg/mL in phosphate buffer, pH 2.5, 547.48±1.79 mg/mL in phosphate buffer, pH 4.5, and 595.87±5.76 mg/mL in phosphate buffer, pH 6.5. Clearly, the solubility of the highly water-soluble drug, RHCl, was not significantly (P>0.01) dependent on pH. This high solubility of RHCl may cause the entire dose to be readily released in solution that would lead to erratic gastric absorption and side effects associated with high drug plasma concentration. For developing a sustained/controlled release system, the solubility of the drug needs to be reduced to a level that is suitable for effective osmotic release. Hence, a solubility modulator (coated NaCl) was used. This approach (based on the common ion effect) has been utilized by us in the development of AMCs of highly water-soluble drugs—phenylephrine hydrochloride (10) and triprolidine hydrochloride (11).

Characterization and Optimization of Coated NaCl

In osmotic systems, the release rate of an excipient relative to the release rate of the drug is an important factor that determines the duration of drug release. Thus, to achieve osmotically regulated controlled delivery of the highly water-soluble drug RHCl (>550 mg/mL), solubility modulation was attempted by the common ion effect. NaCl containing the common ion Cl^- was selected to achieve a target solubility of 30–150 mg/mL. At the same time, it was desirable to achieve solubility modulation for the target duration of 12 h. Therefore, NaCl crystals were coated with CA 398-10, an insoluble polymer. Coated and uncoated NaCl crystals were comparatively characterized and visualized by micrographs. The micrographs of NaCl crystals showed sharp-edged crystals (Fig. 2a), whereas the coating of crystals with polymer resulted in the disappearance of the sharpness of the crystals' edges (Fig. 2b). Apparently, no change in the size of particles after coating could be deduced. Analysis of

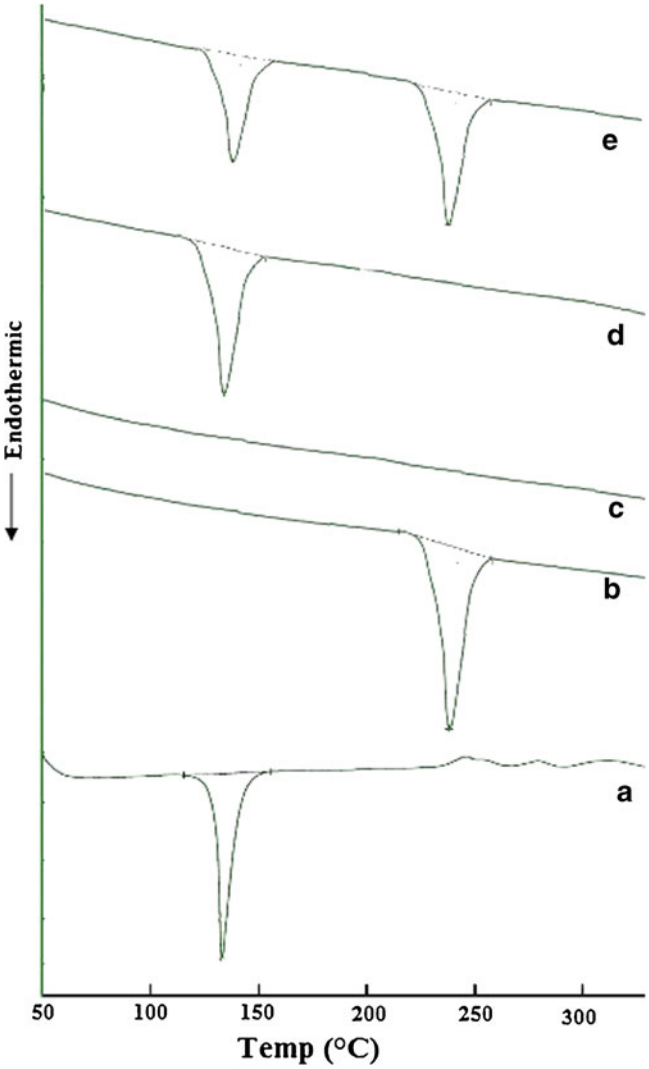

Fig. 1. DSC thermographs of *a* RHCl, *b* CA 398-10, *c* NaCl, *d* physical mixture of RHCl and NaCl, *e* physical mixture of RHCl, NaCl, and CA 398-10

the flow properties revealed excellent flow characteristics for NaCl crystals (Table III), whereas the CA 398-10-coated NaCl crystals showed passable flow properties. However, the Hausner's ratio value of 1.2 indicated appreciable flow characteristics (20) of coated NaCl. It was thus deduced that coated NaCl could be used for experimental work as easy handling was assured.

For the selection of optimum coated NaCl, *in vitro* drug release of four prototype FAMCs was carried out for 6 h. The capsular composition containing NaCl crystals coated with 3% *w*/*v* of CA 398-10 that released 49.2% of RHCl was selected as optimized coated NaCl (Fig. 3). As the drug delivery system is expected to release 100% drug in 12 h, we were looking for a formulation component that would help in achieving 50% drug release in 6 h. Based on this preliminary experimentation, NaCl coated with 3% *w*/*v* CA 398-10 as solubility-modulating agent in the AMC formulation would accomplish a drug release approximating 100% in 12 h. Other coat strengths displayed a %CDR either above or below 50% in 6 h. Predicatively, either the entire drug may get released before 12 h (in case of 1% *w*/*v* and 2% coat strength) or it may exceed 12 h with 4% coat strength. This suggests the importance of coat thickness on the solubility modulator crystals for achieving the desired release (11).

Solubility Modulation of RHCl

For a gastroretentive system, drug release in the fed state condition is one of the important optimization criteria, hence solubility modulation of RHCl in varying molar environments of CA 398-10-coated NaCl crystals at pH 2.5 (low fed) and pH 6.5 (high fed) was assessed(21). The aim of solubility modulation was to achieve the target solubility range of 30–150 mg/mL that was presumed to be sufficient for osmotically regulated delivery of RHCl. As evident from Fig. 4, the common ion Cl^- was able to reduce the solubility of RHCl to 91 mg/mL at pH 2.5 and 120 mg/mL at pH 6.5 in the molar environment of 2.5 M NaCl and to 45 mg/mL at pH 2.5 and 68 mg/mL at pH 6.5 in the molar environment of 3.0 M NaCl that approximated target solubility. This guided the selection of levels of coated NaCl in the experimental design utilized for the development of AMCs. Hence, the low level of the amount of coated NaCl was set as 2.5 M and the high level was recognized at 3.0 M, and other levels were intermediate values. The solubility modulation was attempted at extreme low and extreme high fed state conditions, as any effect in these values could be extrapolated to the

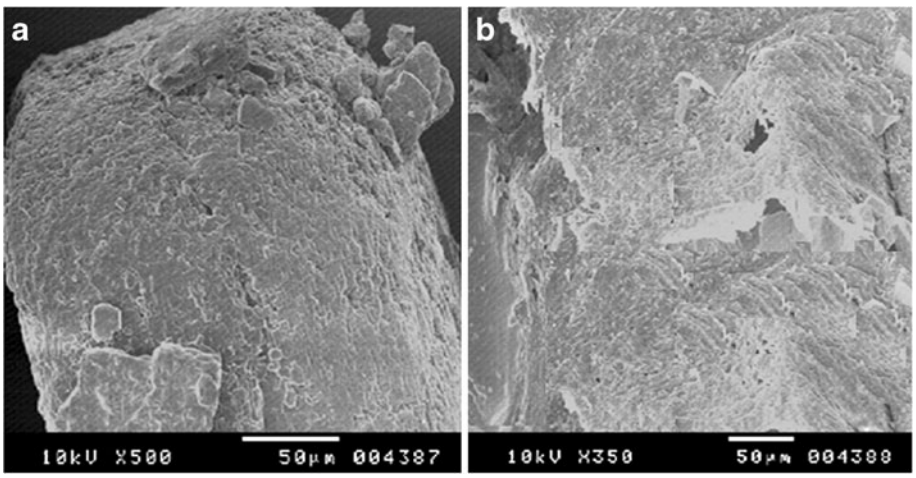

Fig. 2. Scanning electron micrographs of **a** NaCl crystal and **b** CA 398-10-coated NaCl crystal

Table III. Comparative Flow Properties of NaCl and CA 398-10-Coated NaCl Crystals

Parameters	NaCl crystals	CA 398-10-coated NaCl crystals
Angle of repose	26°9′ (good)	36°5′ (passable)
Hausner's ratio	1.1 (excellent)	1.2 (good)
Carr's index	9.8 (excellent)	17.1 (passable)

intermediate fed state pH 4. Theoretically, the expected osmotic delivery of RHCl over 12 h at zero order was possible once the desired solubility modulation is achieved.

In Vitro Drug Release

A floating drug delivery system is administered in fed state conditions and the pH can vary from 2.5 to 6.5, depending on the meal taken (22). The system aimed in this project is the osmotically regulated floating delivery system of RHCl; therefore, it is anticipated that drug release from the system must be independent of environmental conditions and should not be affected by variation in simulated pH conditions of the stomach in the fed state. Thus, *in vitro* drug release studies were conducted in pH 2.5, 4.5, and 6.5, corresponding to low, intermediate, and high fed state pH, respectively. Comparative %CDR profiles from the formulations, along with a marketed formulation (MF; Zantac 150 mg), are shown in Fig. 5a (pH 2.5), b (pH 4.5), and c (pH 6.5). The *in vitro* release profiles in all experimental simulated fed state conditions showed no significant difference ($P>0.05$) in the release of RHCl from FAMCs, suggesting pH-independent release. However, the MF showed a distinct release pattern when compared to the release of RHCl from AMCs. More than 95% of the drug was released from the MF in the first 3 h of study at all three simulated pH media that modeled first-order release. As a result, most of the drug will reach the blood for systemic action after absorption through the GIT that may lead to fluctuations in plasma drug levels and undesirable side effects. But this type of burst release was not observed from FAMC formulations rather the release fitted zero-order model.

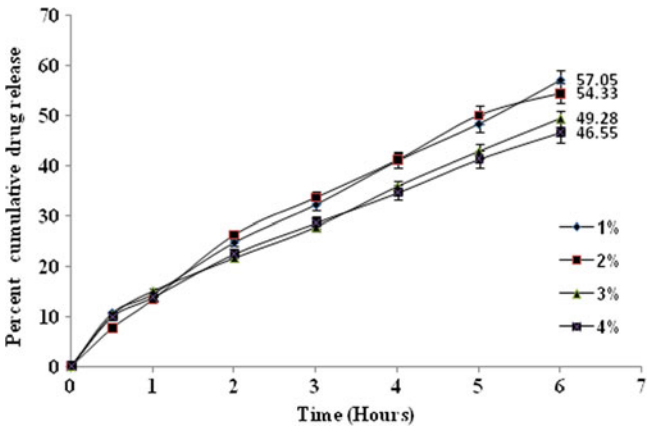

Fig. 3. *In vitro* drug release profiles of prototype RHCl AMC formulations (1%=F1, 2%=F2, 3%=F3, 4%=F4) in phosphate buffer pH 4.5 for 6 h for the selection of optimized CA 398-10-coated NaCl crystals ($n=3$)

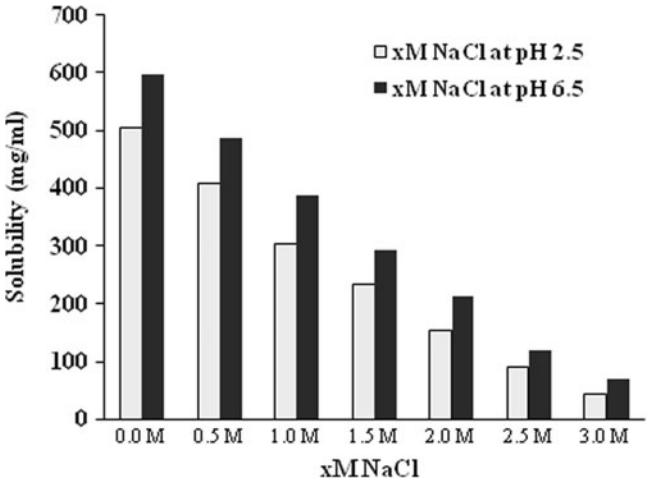

Fig. 4. Solubility modulation bar charts of RHCl in variable molar concentrations of coated NaCl in pH 2.5 and 6.5 ($n=3$)

The effect of formulation variables (concentration of CA 398-10, glycerol, and amount of coated NaCl) were investigated by careful observation of the release profiles in different simulated pH values and are described in present study with reference to a study at phosphate buffer pH 2.5 because the release profiles were similar in pH 4.5 and 6.5. The effect of any variable can be clearly analyzed when the remaining two variables in the formulation are kept the same. The incorporation of higher amount of coated NaCl (FAMC4, FAMC6, FAMC7, and FAMC8) resulted in the development of significant osmotic pressure inside the capsular core, and at the same time, the presence of the common ion Cl^- reduced the solubility of RHCl that could have resulted in decreased release of RHCl. However, the generation and maintenance of the osmotic gradient for a longer duration due to the coated NaCl crystals counteracted the suppression in release (11), and hence, higher drug release was observed at 12 h from these formulations (~93%) when compared to the formulation made with lower levels of coated NaCl (FAMC1, FAMC2, FAMC3, and FAMC5) that displayed release to the order of ~87% in 12 h.

The effect of incorporating pore former on the release of RHCl from FAMCS was analyzed in FAMC3, FAMC5, FAMC6, and FAMC8 (formulations with high levels of glycerol) and in FAMC1, FAMC2, FAMC4, and FAMC7 (formulations with lower levels of glycerol). The formulations with higher levels of glycerol showed higher %CDR (average release, ~92%) than those with lower levels of glycerol (~88%). When CA was at higher levels (FAMC2, FAMC5, FAMC7, and FAMC8), the drug release reduced (average release, ~83%) in comparison to formulations with lower levels of CA 398-10 (FAMC1, FAMC3, FAMC4, and FAMC6, with average release, ~96%). This effect was due to the increased diffusional path length across the membrane as a result of the increased thickness of the capsular wall that presented as a barrier to drug release (23).

The results concluded that, when osmogen and pore former were at high levels in FAMC6 and FAMC8, higher release (average release, ~92%) was observed than FAMC1 and FAMC2 (average release, ~85%), having lower levels of both variables. The effect is attributable to the combined result of increased perforation of the capsular wall and osmotic gradient that has been documented in our previous studies (8–11).

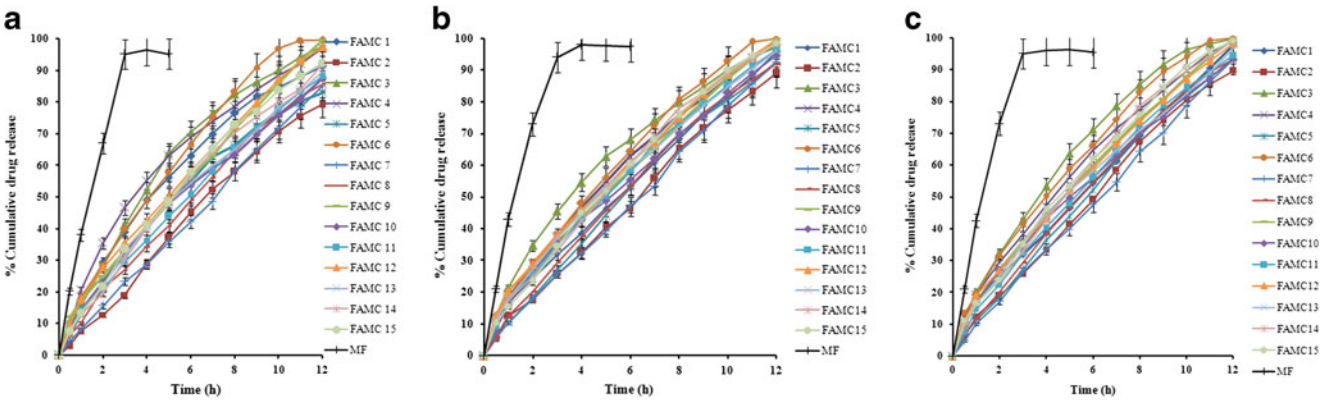

Fig. 5. Comparative *in vitro* drug release profiles of the RHCl from formulations (FAMC1–FAMC15) designed by CCD along with an MF in **a** phosphate buffer pH 2.5, **b** phosphate buffer pH 4.5, and **c** phosphate buffer pH 6.5 (n=3)

On the other hand, at high levels of CA 398-10 and glycerol (FAMC5 and FAMC8), the release of RHCl was constrained (average release, ~84%) due to the thick wall with incomplete pores across it. This was not seen with the formulations FAMC1 and FAMC4 (average release, ~94%). FAMC7 and FAMC8, having high levels of CA 398-10 and osmogen, also gave decreased drug release, again due to increased diffusional path length and reduced solubility of the drug by the presence of more common ion (23).

Formulations with intermediate levels (FAMC9 to FAMC15) showed release >90% on average at all three pH levels (Figs. 6, 7, and 8), but some combinations displayed release above 98% (FAMC9 and FAMC12) also. Among the formulations made using CCD, maximum release was shown by the formulations FAMC6 and FAMC9 (>99%) at all three pH levels in 12 h. FAMC3, FAMC4, and FAMC12 also showed release up to 98%.

In Vitro Buoyancy

Visual monitoring of the formulations during the *in vitro* drug release test in variable simulated gastric fluids revealed buoyancy for 12 h. The capsule shell was composed of polymer CA 398-10 that has a density of about 0.43 g/cm^3 (24), which is much lower compared to the density (1.004 g/cm^3) of gastric fluid (19). The low density of the polymer imparted the buoyant character to the formulation, as most of capsule bulk was made up of cellulose acetate. Except for cellulose acetate, the rest of the excipients were water-soluble, therefore the floating ability is co-relatable to cellulose acetate. To confirm the buoyant character, the true density of the optimized FAMC9 was found to be 0.816 g/cm^3 when determined by liquid displacement method using ethanol (95% *v*/*v*) as the displacement liquid. Accordingly, the FAMC floated in the release test media (Supplementary Figure) for 12 h, realizing a novel vista for the utilization of AMCs as gastroretentive system for a highly soluble drug.

Statistical Data Analysis

The formulations were statistically analyzed by one-way analysis of variance at the 5% level of significance for the effect of fed state conditions on drug release. The release from all formulations indicated no significant difference (P>0.05) and hence the release of the drug from FAMCs can be considered independent of the pH of fed state conditions. In order to validate the design, polynomial equations were constructed to quantify the influence of individual formulation variable and interaction between variables through the coefficients in polynomial equations at each fed state pH. A multivariable linear function was used in the form of $Y=f(X_i)$ as a fitting equation, where Y is the response parameter (%CDR at 12 h) and X_i is the independent variable (i=1, 2, 3) representing the concentration of CA 398-10, glycerol, and amount of coated NaCl, respectively. The transformed polynomial equations for $\%CDR_{12\ h}$ at each pH with significant coefficients (95% confidence level) are:

$$\%CDR_{12h} \text{ at pH } 2.5 = 91.0 - 3.6(X_1) + 5.4(X_2) - 1.2(X_3) \quad (1)$$

$$\begin{aligned}\%CDR_{12h} \text{ at pH } 4.5 = {} & 97.7 - 3.2(X_1) + 0.9(X_2) \\ & + 1.1(X_3) + 0.03(X_1X_2) \\ & - 0.3(X_1X_3) - 0.5(X_2X_3) \\ & - 1.8(X_1^2) + 0.5(X_2^2) - 1.9(X_3^2)\end{aligned} \quad (2)$$

$$\%CDR_{12h} \text{ at pH } 6.5 = 96.3 - 3.6(X_1) + 0.9(X_2) + 0.7(X_3) \quad (3)$$

where X_1, X_2, and X_3 are the concentration of CA 398-10, concentration of glycerol, and content of coated NaCl, respectively. By fitting the coded values for each formulation in the equations, the predicted values were deduced and compared with the experimental values of $\%CDR_{12\ h}$. A fairly good correlation was obtained, as shown in Fig. 6a (r=0.9752). The surface response graph depicts the influence of different levels of the variables on the response parameter $\%CDR_{12\ h}$ (Fig. 6b). It was observed that release was decreased on increasing the level of CA 398-10, but when the level of glycerol increased, the release also increased. The contour plot in Fig. 6c signifies the intensity of the interaction of the levels of the variables on drug release along with the desirability factor and Fig. 6d revealed no interaction of the variables (concentration of CA 398-10 and glycerol) on the response parameter.

Validation of Experimental Design

The experimental design was validated by formulating an extra design checkpoint formulation (FAMC16). The predicted value for FAMC16 was statistically tested for significant

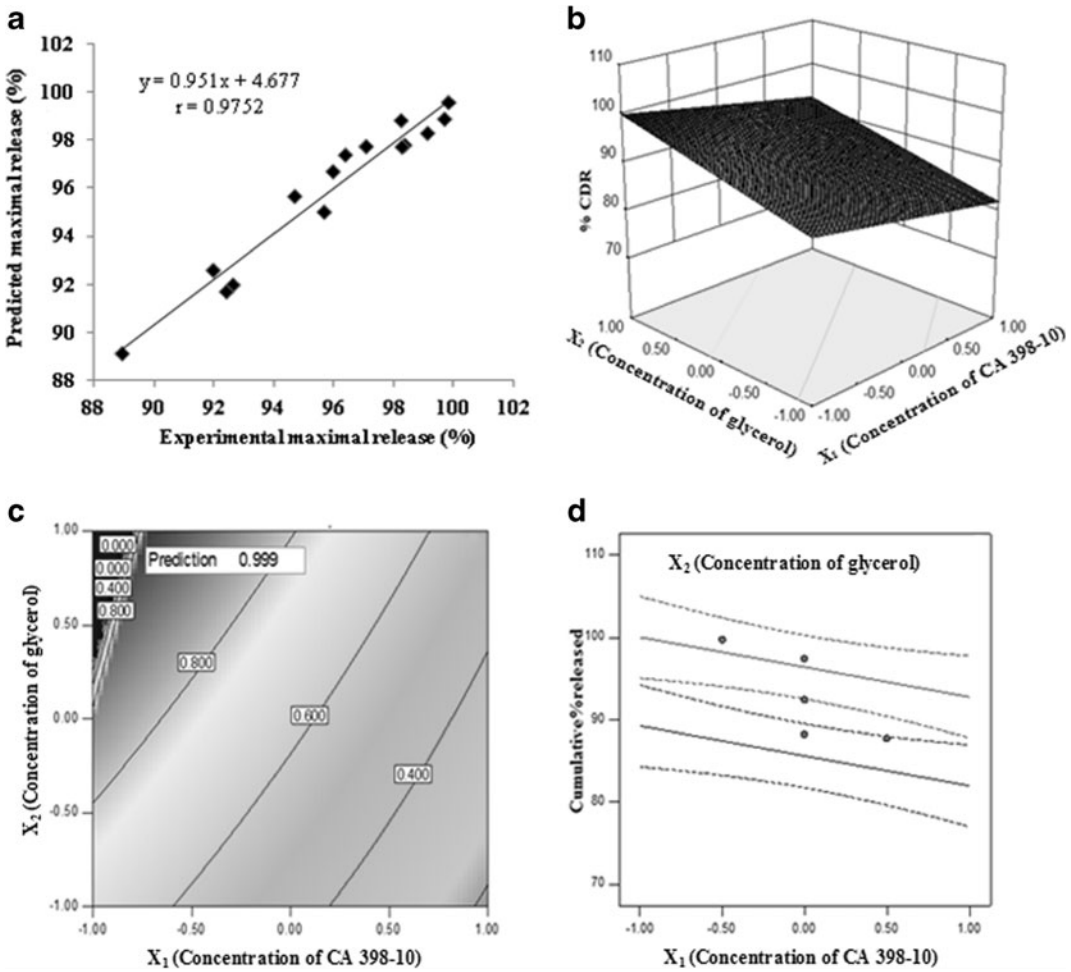

Fig. 6. **a** Correlation plot of the predicted and experimental values of maximum %CDR at 12 h, **b** response surface plot, **c** contour plot with desirability factor, and **d** interaction plots between the experimental variables

difference against the experimental value at all test pH, by Student's *t* test (25). No significant difference was observed at $P>0.05$ (Table IV), thus validating the experimental design.

Selection of Optimized Formulation

A careful investigation of the dependent variables showed that the formulation FAMC9 had a desirability factor of 0.999 at all the simulated fed state pH (Fig. 6c) and was selected as the best formulation because FAMC9 addressed maximal drug release in a controlled manner at all simulated fed state pH as 99.6% (pH 2.5), 99.6% (pH 4.5), and 99.5% (pH 6.5). Though another formulation, FAMC6, also showed a CDR of more than 99%, it did not follow the zero-order release kinetics; hence, FAMC9 was selected over FAMC6. The *in vitro* buoyancy for 12 h in variable conditions and the desirability factor of 0.999 supported the selection of FAMC9 as the optimized formulation.

Scanning Electron Microscopy

Scanning electron microphotographs of the inner and outer surfaces of the capsular membrane of FAMC9 before and after drug release demonstrated its asymmetric character (Fig. 7). The SEM photograph of the outer region of the polymeric membrane before drug release study revealed a dense, nonporous, and smooth membranous structure of CA 398-10, as shown in Fig. 7a, while Fig. 7b depicts the inner porous and rough surface of the capsular membrane. After complete drug release at 12 h, the membrane was exhausted and numerous pores in the outer layer (Fig. 7c) as well as large pores in the inner layer of membrane were developed (Fig. 7d). Generation of pores is attributed to the dissolution of glycerol into the aqueous media during drug release, leaving uniform pores behind it. As the outer region of the membrane provided permselectivity and resistance to mass transfer, therefore as the *in vitro* drug release study proceeded, permselectivity increased (23).

Kinetics of Drug Release

The release profiles of the formulations (FAMC1–FAMC15) were modeled and the results showed that the best-fit models for most of formulations were the zero-order model (which describes that release is independent of its concentration and is generally seen for systems containing poorly water-soluble drug in matrix), first-order model (describes that release is dependent on its concentration and is generally seen for water-soluble drug in porous matrix), Higuchi model (describes release from an insoluble matrix to

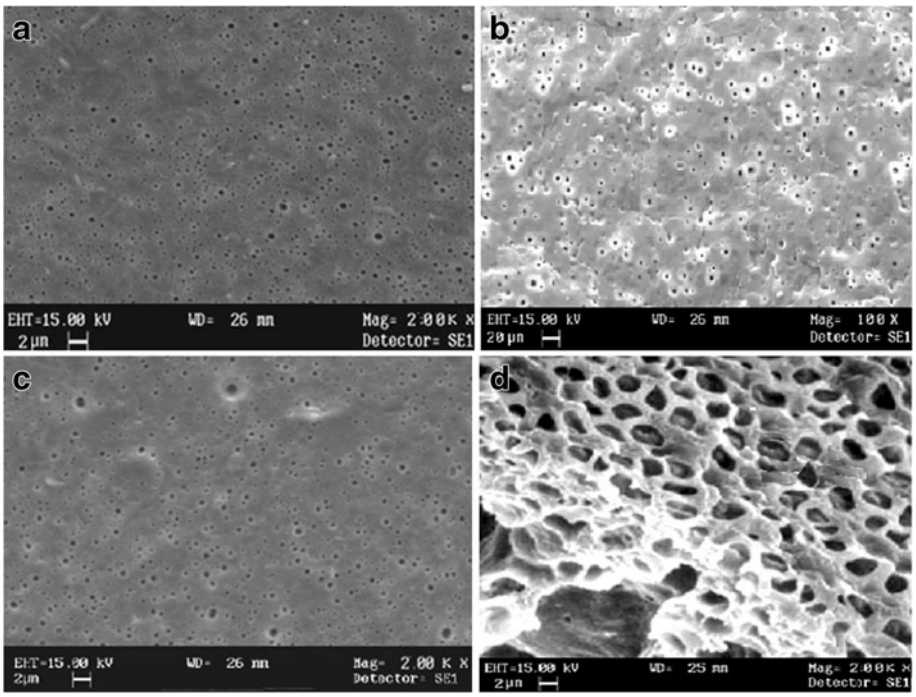

Fig. 7. Scanning electron micrographs of asymmetric membrane depicting **a** outer dense region before drug release study (original magnification at ×2,000), **b** inner porous region before drug release study (original magnification at ×100), **c** outer region after drug release (original magnification at ×2,000), and **d** inner porous region after drug release study (original magnification at ×2,000)

be linearly related to the square root of time), and in order to authenticate the release model, drug release data were further analyzed for the Peppas and Korsmeyer model. The highest coefficient of determination $r^2 \geq 0.9991$ was identified for FAMC9 for zero-order fit, suggesting controlled drug release. Cellulose acetate membranes are reported to generate semipermeable membranes of controlled porosity that have been utilized for osmotic pump-based controlled release systems for pseudoephedrine (26). The results of the present work are consistent with those cited in the literature wherein the cellulose acetate membrane was able to produce osmotically regulated controlled release system of RHCl.

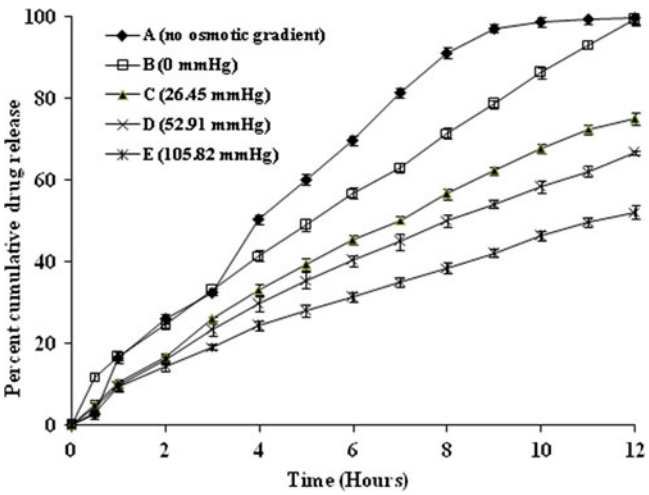

Fig. 8. Comparative *in vitro* drug release profiles of FAMC9 containing 200 mg drug with coated NaCl, (A) 0 mg inside and outside; (B) 150 mg inside and 0 mg outside; (C) 150 mg inside and 75 mg outside; (D) 150 mg inside and outside; and (E) 150 mg inside and 300 mg outside, for confirming osmotic pressure as the driving force for drug release

Confirmation of Osmotic Gradient as Driving Force

In vitro drug release studies of the optimized formulation (FAMC9) were performed in media with different osmotic pressures and the drug release was evidently, highly dependent on the osmotic pressure of the release media. Release of RHCl in the study (a) with no osmogen both inside and outside the capsule resulted in uncontrolled release of the drug (Fig. 8) that could be controlled by osmotic pressure gradient generated by CA 398-10-coated NaCl inside FAMC9 (b). It was demonstrated (Fig. 8) that, as the osmotic pressure of release media increased from 0 mmHg (b) to 26.45 mmHg (c), 52.91 mmHg (d), and 105.82 mmHg (e), the release of RHCl from FAMC9 decreased (99.51%, 75.14%, 66.76%, and 52.31%, respectively) due to the decrease in osmotic gradient. Thus, the primary mechanism commanding the drug release from the developed system was osmotically governed. As

Table IV. Results of Student's *t* Test at $P>0.05$ Applied on the Predicted and Experimental Values of Extra Design Formulation at Different Fed State Conditions

Phosphate buffer	Predicted %CDR	Experimental %CDR	t_{cal}	t_{tab}
pH 2.5	97.8	97.8	1.91	
pH 4.5	99.7	99.8	0.33	4.30
pH 6.5	100.3	99.7	2.24	

t_{cal} and t_{tab} are the calculated and tabulated values of Student's *t* test, respectively

postulated by Kelbert and Bechard, the drug mass transport occurs mainly within the porous cellulose acetate structure and the mechanism responsible for the release is a combination of molecular diffusion/osmotic pressure *via* water transport into the porous cellulose acetate membrane (27).

CONCLUSION

Osmotically regulated FAMC was successfully developed for site-specific zero-order delivery of a highly water-soluble drug RHCl. Controlled release was assisted by solubility modulation of the drug using the common ion effect that will help in controlling the therapeutic levels of the drug in plasma. The release of the drug from the AMC was independent of fed state pH and was dependent on the formulation variables and osmotic gradient. Thus, a novel gastroretentive single-unit osmotically regulated system with intrinsic floating ability was developed that can be explored for the delivery of highly water-soluble drug. Extensive experiments are underway to improve the system to overcome the challenges of a gastroretentive system.

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