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Research Paper

Formulation development of gastroretentive tablets of lamivudine using the floating-bioadhesive potential of optimized polymer blends

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Keywords
bioavailability; experimental design; formulation by design; in-vitro/in-vivo correlation; scintigraphy

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Abstract

Objectives The current studies entail successful formulation of optimized gastroretentive tablets of lamivudine using the floating-bioadhesive potential of carbomers and cellulosic polymers, and their subsequent in-vitro and in-vivo evaluation in animals and humans.

Methods Effervescent floating-bioadhesive hydrophilic matrices were prepared and evaluated for in-vitro drug release, floatation and ex-vivo bioadhesive strength. The optimal composition of polymer blends was systematically chosen using central composite design and overlay plots. Pharmacokinetic studies were carried out in rabbits, and various levels of in-vitro/in-vivo correlation (IVIVC) were established. In-vivo gamma scintigraphic studies were performed in human volunteers using ^{99m}Tc to evaluate formulation retention in the gastric milieu.

Key findings The optimized formulation exhibited excellent bioadhesive and floatational characteristics besides possessing adequate drug-release control and pharmacokinetic extension of plasma levels. The successful establishment of various levels of IVIVC substantiated the judicious choice of in-vitro dissolution media for simulating the in-vivo conditions. In-vivo gamma scintigraphic studies ratified the gastroretentive characteristics of the optimized formulation with a retention time of 5 h or more.

Conclusions Besides unravelling the polymer synergism, the study helped in developing an optimal once-a-day gastroretentive drug delivery system with improved bioavailability potential exhibiting excellent swelling, floating and bioadhesive characteristics.

Introduction

Development of oral controlled release products is precluded by their inability to retain and localize the drug delivery system (DDS) within the desired region of gastrointestinal tract.[1] Considerable research, therefore, has poured over the last a few years into the plausibility of controlled and site-specific delivery to the gastrointestinal tract.[2]

Among the myriad approaches used to improve the gastric residence time (GRT) of DDSs, the vital ones include floating DDSs (FDDSs), bioadhesive systems, swelling and expanding systems and high-density systems.[3] With a bulk density less than that of gastric fluids, an FDDS remains buoyant in the stomach for a prolonged period of time without reducing the gastric emptying rate.[4] While the system floats on the gastric contents, the drug is released slowly at the desired rate from the system, resulting in an increased GRT and a better control over the fluctuations in plasma drug levels.[5] Nevertheless, an FDDS is effective only when the fluid level in the stomach is sufficiently high. As the stomach empties and the tablet moves to the pylorus, the buoyancy of the dosage form may be impeded. This serious limitation can largely be overcome by enabling the FDDS to adhere to the mucous lining of stomach wall.[6] Floating and bioadhesive DDSs, therefore, greatly

improve the possibility of increasing the residence time of DDSs in the stomach, resulting in more effective absorption and increased bioavailability of drugs.[6,7]

Hydrophilic matrices are considered ideal for achieving extension of drug release, coupled with bioadhesive and/or floatational characteristics. A single cellulosic polymer, like hydroxypropylmethylcellulose (HPMC) or sodium carboxymethylcellulose (CMC), has been employed to attain floatational properties although they cannot usually fulfill all the requisites of gastroretentive floating-bioadhesive controlled release systems, hence calling for the rational use of a synergistic blend of two or more polymers. A combination of ionic (e.g. carbomers) and non-ionic (e.g. celluloses) polymers, in this regard, has extensively been employed to attain sustained release and/or gastroretention.[8–15] Accordingly, it is a challenging task to attain the desired floating-bioadhesive potential and sustained release characteristics of the DDS using a blend of these diversely behaving polymers. Systematic studies using Design of Experiments (DoE) could efficiently surmount this hiccup of balancing floatation and bioadhesion employing optimized polymer blends.[13] DoE optimization is a well-documented means of developing 'the best possible' formulation under a given set of conditions, circumventing unnecessary experimentation and thus saving considerable time, money and effort.[16,17] Application of such DoE techniques for the development of optimized drug delivery products, lately termed as Formulation by Design (FbD),[18] is known to provide an in-depth understanding and ability to explore and defend the ranges for varied formulation and processing factors.

Lamivudine, a BCS Class I synthetic nucleoside analogue, is commonly employed as a part of highly active antiretroviral therapy (HAART). Prescribed in a dose of 100–150 mg twice a day, the drug is well absorbed in the upper gastrointestinal tract with a short biological half-life of 5–7 h.[19] Decreasing the frequency of medication to a once-a-day regimen tends to decrease systemic side effects and improve patient convenience and compliance to HAART in HIV-infected patients.[20]

The objective of this study, therefore, was to employ a systematically FbD-optimized blend of polymers to develop effervescent floating-bioadhesive controlled release tablet formulations of lamivudine, to evaluate their in-vitro and in-vivo performance in animals, establish their in-vitro/in-vivo correlation (IVIVC), and verify their gastroretentive potential using in-vivo scintigraphic studies in humans.

Materials and Methods

Materials

Lamivudine was provided *ex gratis* by M/s Zydus Cadila Healthcare Ltd (Ahmadabad, India). Methocel K15M (HPMC) and Carbopol (CP) 971P were obtained as gift samples from M/s Dow Chemical Company (Findlay, USA) and M/s B.F. Goodrich Ltd (Brecksville, OH, USA), respectively. Polyethylene oxide (PEO, Sentry Polyox WSR 303) was obtained from Union Carbide Corporation (Texas City, TX, USA). Avicel PH 101 (microcrystalline cellulose, MCC) and magnesium stearate (MS) were obtained from M/s Signet Chemical Corporation (Mumbai, India) and M/s Loba Chemie Ltd (Mumbai, India), respectively. The HPLC-grade solvents, acetonitrile and methanol, were obtained from M/s Merck Ltd (Mumbai, India). All other chemicals used in the studies were of analytical grade. All the reagents were used as received. Porcine gastric mucosa for bioadhesion studies was obtained from the local slaughterhouse in the suburbs of Chandigarh, India.

Screening of polymers and their levels

Five polymers, CP 971P, PEO 303, HPMC K100LV, HPMC K4M and HPMC K15M, were chosen for formulating oral controlled release floating-mucoadhesive matrices, with the ratios of lamivudine to polymer ranging between 1 : 0.5 and 1 : 3. The polymer blend containing the two polymers CP 971P and HPMC K15M was selected for further investigation. Various formulations were prepared using different ratios of these two polymers, to embark upon the pragmatic range of the levels of the two polymers for further evaluation.

Formulation of tablets as per experimental design

Different compressed matrix tablet formulations of lamivudine were formulated using varying amounts of the polymers (i.e. CP 971 P and HPMC), and MCC as an inert diluent, along with a fixed quantity of sodium bicarbonate (12.0%) as an effervescing agent, and MS (1.0% w/w) as glidant and lubricant. Lamivudine and the polymers, CP 971P and HPMC, were screened through an #80 mesh sieve (180 μm), and MCC and MS were screened through a #120 mesh sieve (125 μm) before use. All the materials were accurately weighed and mixed intimately in a polythene bag for 10 min. The blended mix was subsequently compressed into tablets using bi-concave, oblong punches (20 × 10 mm diameter), fitted to a single-punch electric compression machine (M/s Cadmach, Ahmadabad, India).

Experimental design

A central composite design (CCD) for two factors at three levels each (with $\alpha = 1$) was selected to optimize varied response variables.[21] The two factors, CP 971P (i.e. polymer X_1) and HPMC (i.e. polymer X_2), were varied in the polymer blend as required by the experimental design, and the factor levels suitably coded (Table 1). The amount of MS was kept constant at 1% w/w, while MCC was employed in a sufficient quantity to maintain a constant tablet weight of 1200 mg.

Table 1 Factor combinations as per the chosen experimental design

Formulation code	Experimental trial No.	Coded factor levels	
		X_1	X_2
A	1	–1	–1
B	2	–1	0
C	3	–1	1
D	4	0	–1
E1	5	0	0
E2	10	0	0
E3	11	0	0
E4	12	0	0
E5	13	0	0
F	6	0	1
G	7	1	–1
H	8	1	0
I	9	1	1
Translation of coded levels in actual units			
Coded level	–1	0	1
X_1: CP (mg)	75	150	225
X_2: HPMC (mg)	150	300	450

Extent of release until 16 h (Q_{16h}), buoyancy time (T_b) and bioadhesive strength (ρ) were taken as the response variables.

In-vitro drug release studies

Dissolution studies were carried out on all the tablet formulations in triplicate, employing the USP XXX paddle method (Apparatus 2; M/s Pharma Test Apparatebau AG, Hainburg, Germany) at 50 rpm and 37 ± 0.5°C using simulated gastric fluid (SGF) pH 1.2 as the dissolution medium.[22] Samples were withdrawn periodically at suitable time intervals and replaced with an equivalent volume of plain dissolution medium. Samples were analysed spectrophotometrically at 280 nm by employing a UV-visible spectrophotometer (Geaesys 6; M/s Thermospectronic, Rochester, NY, USA). Drug release data obtained during in-vitro dissolution studies were analysed using ZOREL software,[23] with in-built provisions for applying the correction factor for volume and drug losses during sampling.[24] Drug release data were fitted into Korsemeyer–Peppas model for swellable compressed matrices, as described by Equation 1.[25,26]

$$\frac{M_t}{M_\infty} = k_1 \cdot t^n + k_2 \cdot t^{2n} \ldots \quad (1)$$

where, M_t is amount of drug released at time 't', M_∞ is amount of drug released at an infinite time and n is the Fickian diffusion coefficient. The symbols k_1 and k_2 are the magnitudinal contribution of diffusion and polymer relaxation mechanism. Based on the phenomenological analysis, the type of release (i.e. whether Fickian, non-Fickian (anomalous) or zero-order) was predicted.[25,26] The value of $T_{60\%}$ was calculated using Stineman interpolation option of the GRAPH 2.0 software (M/s Micromath Inc., St Louis, USA).

Ex-vivo bioadhesion studies

Porcine gastric mucosa was used as the model membrane for ex-vivo determination of the bioadhesive strength of the various formulations. The mucosal membrane was excised by removing the underlying connective tissue and was placed on the base of a Texture Profile Analyzer (TAX TEE 32; M/s Stable Microsystems, Godalming, Surrey, UK). A tablet was attached to the stainless-steel probe fixed to the mobile arm of the texture analyser. The area of contact of mucosa was moistened with 50 µl of SGF. The mobile arm was lowered at a rate of 0.5 mm/s until a contact with the membrane was made. A contact force of 10 g was maintained for 300 s, after which the probe was withdrawn from the membrane. The peak attachment force, determined in triplicate was recorded, as reported in literature.[27]

Determination of duration of buoyancy

The duration for which the formulation floated in the dissolution medium, in the upper one-third of the dissolution vessel, was determined periodically after every 15 min by careful visual observation during the dissolution run.[28] To rule out any possibility of the tablet sticking to the paddle or vessel wall, the tablets were allowed to float in a beaker and the time of floatation was noted, taking every care to avoid its contact with the wall of the beaker.

Optimization data analysis and validation of optimization model

The response variables that were considered for systematic DoE optimization included Q_{16h}, T_b and ρ. For the studied design, the multiple linear regression analysis (MLRA) method was applied using Design Expert 6.0.10 (M/s Stat-Ease, Minneapolis, USA) software to fit a full second-order polynomial equation with added interaction terms to correlate the studied responses with the examined variables. The polynomial regression results were demonstrated for the studied responses. Finally, the optimum formulation was chosen using overlay plots, drawn using the Design Expert software. Eight formulations were selected as the confirmatory check-points to validate the DoE optimization results.[13,16,28] Two among these check-point formulations (Float-Bioad 1 and Float-Bioad 2), yielding the highest plausible values of Q_{16h}, T_b and ρ, were selected for further studies. The observed and predicted responses from these validation check-points were critically compared and linear correlation plots were constructed, forcing the line through the origin.[29] The residual graphs between predicted and observed responses were also constructed separately and the percent

bias (= prediction error) was calculated with respect to the observed responses.

Drug release comparison with marketed brand

Drug release profiles of the two check-point formulations (Float-Bioad 1 and Float-Bioad 2) were compared with a conventional marketed brand (Lamivir; M/s Cipla Pharma Ltd, Mumbai, India) containing 300 mg of lamivudine.

In-vivo pharmacokinetic studies in rabbits

Two check-point formulations (Float-Bioad 1 and Float-Bioad 2), along with the marketed brand, Lamivir, were subjected to in-vivo evaluation in rabbits. Taking cognizance that the research work adheres to the guidelines for care and use of the laboratory animals, all the animal investigations were performed as per the requisite protocol approved by the Institutional Animal Ethic Committee of Panjab University, Chandigarh, India (letter no. 2621). The committee is duly approved for the purpose of control and supervision of experiments on the animals by the Government of India. A single-dose parallel design study was carried out using unisex New Zealand white rabbits, 2.35–2.70 kg. The rabbits were divided into three groups of six animals. Group I and Group II received the Float-Bioad 1 and Float-Bioad 2 formulations, respectively, while Group III was administered with Lamivir. The dose of lamivudine for rabbits was calculated employing Equation 2, taking K_m factor for humans and rabbits as 37 and 12, respectively.[30,31]

$$Human\ Dose = Animal\ Dose \times \frac{Animal\ K_m}{Human\ K_m} \ldots \qquad (2)$$

The rabbits were fasted for 12 h before drug administration. All the rabbits were allowed free access to water throughout the study. Following drug administration, rabbits were kept in their cages, and free access to food and water was allowed after 6 h. Serial blood samples (1 ml) were withdrawn from the marginal ear vein of the rabbit at 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 10, 12, 14, 16 and 24 h post-dosing and placed in the heparinized tubes. Plasma was harvested by centrifugation (3000 rpm, 5 min), and stored at −20°C until analysis.

The content of lamivudine in plasma samples was analysed by reversed-phase high-performance liquid chromatography (RP-HPLC) employing a slightly modified procedure as reported by Kano *et al.*[32] The mobile phase consisted of an aqueous solution of sodium dihydrogen phosphate monohydrate (10 mM), methanol and acetonitrile, in the ratio of 94 : 3 : 3. A volume of 50 µl of internal standard (stavudine 10 µg/ml in methanol) and 25 µl of 0.2 M ammonium acetate solution (to increase HPLC peak resolution) were added to 500 µl plasma sample. This mixture was agitated using a vortex mixer (M/s Remi Equipment Pvt. Ltd, Mumbai, India) for 30 s, followed by addition of 2 ml of acetonitrile to precipitate the plasma proteins. The resultant mixture was centrifuged (3 500 rpm, 10 min) and the supernatant layer was filtered (0.45 µm membrane filter; M/s Millipore, Bangalore, India). Further, the sample was reconstituted with 500 µl of mobile phase and agitated again for 30 s. The reconstituted sample was transferred to the vial (Type I Class A borosilicate; M/s Borosil glass works, Ahmedabad, India) and 25 µl of each sample was injected into the liquid chromatographic system (M/s Shimadzu Scientific Instruments, Kyoto, Japan), composed of an LC-10ADVP pump, an SPD-10ADVP variable wavelength detector and a SIL-10ADVP sampler with 50 µl loop. The analytical column was a µBondapak reverse phase C18 column (300 mm × 3.9 mm i.d., 10 µm particle size, Part no. WAT27324; M/s Waters Corporation, Milford, PA , USA). Before analysis, the mobile phase was filtered through a 0.22-µm membrane filter (M/s Millipore, India), and degassed for 15 min. The liquid chromatographic analysis was conducted keeping a flow rate of 1.2 ml/min at a spectrophotometric λ_{max} of 277 nm.

Pharmacokinetic data analysis and in-vitro/in-vivo correlation

Pharmacokinetic data analysis and modelling of plasma level–time data of lamivudine were carried out employing Model 3 (i.e. one-compartment open-body model (1-CBM) following peroral administration)[33] option of Win-Nonlin software (version 5.0; M/s Pharsight Corporation, Sunnyvale, CA, USA). Data analysis was accomplished using nonlinear function minimization employing Gauss–Newton algorithms built into the software. Statistical validity of the results was discerned on the basis of minimization of various model fitness parameters such as Akaike Information criterion (AIC), Schwartz Bayesian criterion (SBC), sum of squares due to residuals (SSR) and maximization of Pearsonian correlation coefficient (R). The in-vivo plasma level data were compared with the corresponding in-vitro data, and attempts were made to establish various levels of IVIVC. For establishing Level A IVIVC, percent absorbed data was obtained at various time points using the Wagner–Nelson method, and was plotted versus percent drug release data[34,35] Levels B, C and Multiple Level C were attempted using standard techniques.[36]

In-vivo scintigraphic studies in man

The gastric retention of the optimized formulation among the two chosen floating-bioadhesive formulations (Float-Bioad 1 and Float-Bioad 2) was studied vis-à-vis control formulation employing γ-scintigraphy in young healthy volunteers fasted overnight ($n = 6$). The control formulation contained the drug and excipients in equal proportion as in the optimized formulation, except the two release-

controlling polymers (CP 971P and HPMC). The non-alcoholic and non-smoking subjects (four male, two female) were in the close young age group of 24–29 years, with their body weight ranging between 50 and 59 kg and body surface area ranging between 1.45 to 1.61 m^2. The study subjects had a standard light morning breakfast of about 380 calories with around 50% of calories due to fat content.

The scintigraphic study was performed at the Department of Nuclear Medicine at the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India. The investigation followed the tenets of the Declaration of Helsinki,[37] duly approved by the Institutional Human Experimentation Committee of PGIMER (letter no. 2674). Prior informed consent was obtained from each participating volunteer. The control and optimized formulation were labelled with 500–700 μCi (18.5–25.9 MBq) of ^{99m}Tc sulfur colloid, by incorporating a volume of 100 μl of the colloid solution into the granular mix and then compressing it in the centre of the tablets. The tablets were administered orally to each individual with 200 ml of potable water. Following oral administration of the radiolabelled preparation, anterior and posterior static images (2 min/image) were acquired (in 256 × 256 matrix) under the dual-head Ecam gamma camera (M/s Siemens, Erlangan, Germany) at time intervals of 0, 60, 150, 225, 300 and 360 min for the test formulation and 0, 30, 60, 80 and 110 min for the control formulation. The study subjects were instructed to keep medically mobile and had free access to water until the completion of the radiographic acquisition. The scintigraphic images obtained with the control and the optimized formulation were subjected to analysis using the in-built computer software.

Accelerated stability analysis

Accelerated stability studies of the optimized formulation were carried out at 40 ± 2°C and 75 ± 5% relative humidity. Tablets were packaged in round 40 cm^3 high-density polyethylene bottles with tamper-proof child-resistant closures (M/s Mark Pack, Hyderabad, India). Samples were withdrawn at predetermined periodic intervals of 0, 1, 2, 3, 4, 5 and 6 months, and were analysed for drug content, dissolution performance, buoyancy and bioadhesive strength during storage at accelerated conditions.

Results

Screening of polymers and excipients

Excellent controlled release abilities were shown by polymer blends of CP 971P+HPMC K15M ($t_{60\%}$ = 9.92 h), PEO 303 + HPMC K100LV ($t_{60\%}$ = 8.43 h) and HPMC K15M+HPMC K4M ($t_{60\%}$ = 7.07 h). However, the HPMC K15M+HPMC K4M and PEO 303 + HPMC K100LV blend showed poor bioadhesive strength. All the systems showed excellent buoyancy up to 24 h.

In-vitro drug release studies

Table 2 lists various dissolution parameters computed for all the controlled release bioadhesive formulations. The values of release rate exponent (n), calculated as per the algorithm proposed by Peppas and Sahlin,[38] ranged between 0.618 and 0.789. The values of n, by and large, showed an increasing trend with increase in the content of either polymer. The values of Fickian diffusion constant (k_1) varied between 1.042 and 1.128, while those of polymer relaxation constant (k_2) varied between 0.034 and 0.058. The values of Q_{16h} ranged between 73.22 and 93.00%. An almost linear descending trend was observed in Q_{16h} with an increase either in CP 971P or HPMC fraction. Drug release rate from all the formulations portrayed a noticeable initial burst release.

Ex-vivo bioadhesive strength determination

Bioadhesive strength increased with rising in polymer levels (Table 2). Distinct augmentation in the bioadhesive strength

Table 2 Overall dissolution and floatation parameters for all the gastroretentive tablet formulation of lamivudine prepared as per the experimental design

Formulation code	Release exponent (n)	Kinetic constant (k)	Fickian diffusion constant (k_1)	Polymer relaxation constant (k_2)	Release till 16 h (Q_{16h}, %)	$T_{60\%}$ (h)	Buoyancy time (T_b, h)	Bioadhesive strength (ρ, g)
							Mean ± SD	Mean ± SD
COPT1	0.651	0.152	1.128	0.0461	93.00	7.79	16.00 ± 0.24	35.1 ± 2.85
COPT2	0.679	0.121	1.089	0.0453	79.78	9.87	23.94 ± 0.86	105.4 ± 3.42
COPT3	0.618	0.135	1.116	0.0341	73.22	12.61	23.80 ± 0.80	110.4 ± 4.8
COPT4	0.701	0.131	1.104	0.0496	91.80	8.40	6.06 ± 0.08	90.5 ± 2.52
COPT5	0.710	0.106	1.061	0.0513	79.54	10.77	11.02 ± 0.12	105.9 ± 4.78
COPT6	0.785	0.087	1.042	0.0554	78.07	11.73	10.67 ± 0.10	169.6 ± 3.89
COPT7	0.781	0.104	1.067	0.0581	84.90	8.71	4.33 ± 0.04	151.2 ± 0.93
COPT8	0.789	0.091	1.046	0.0583	81.51	10.31	5.02 ± 0.08	161.3 ± 3.65
COPT9	0.711	0.104	1.067	0.0470	77.26	12.63	9.39 ± 0.14	208.0 ± 2.57

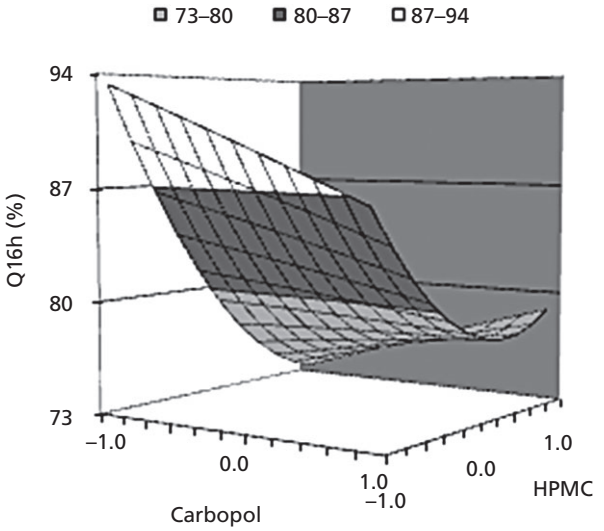

Figure 1 Response surface plot showing the influence of Carbopol and HPMC on the values of Q_{16h} of floating-bioadhesive tablet formulations of lamivudine.

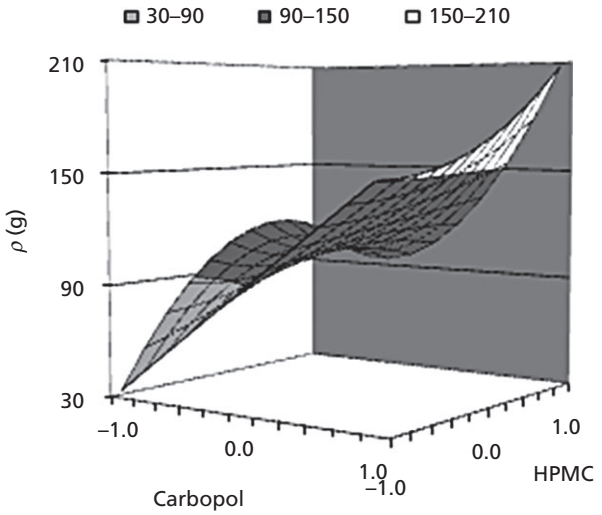

Figure 2 Response surface plot showing the influence of Carbopol and HPMC on the values of bioadhesion of floating-bioadhesive tablet formulation of lamivudine.

was discernible with an increase in the amount of either polymer (CP 971P or HPMC). Maximum bioadhesive strength was observed at the highest levels of both the polymers.

Buoyancy time

Buoyancy time (T_b) of the tablets increased in a linear fashion with increase in the HPMC content. With increase in CP 971P content (Table 2), on the contrary, buoyancy time tended to show a linear declining trend.

Response surface analysis

The coefficients of the polynomial equation (Equation 3), generated using MLRA for Q_{16h}, ρ and T_b of the polymer blend, formed excellent fits to the data, with the value of R^2 ranging between 0.9851 and 0.9994.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \beta_4 X_1^2 + \beta_5 X_2^2 + \beta_6 X_1 X_2^2 + \beta_7 X_2 X_1^2 \ldots \quad (3)$$

Figure 1 reveals a decline in the value of Q_{16h} with increase in the concentration of each of the polymers (i.e. CP 971P and HPMC), the influence of HPMC being much more noticeable. Maximum value of Q_{16h} was observed at the lowest levels of both the polymers, especially those of CP 971P.

Figure 2 shows nearly curvilinear ascending patterns for the values of bioadhesive strength, as the content of either polymer is increased. Although the maximum value of bioadhesive strength was observed at the highest levels of both the

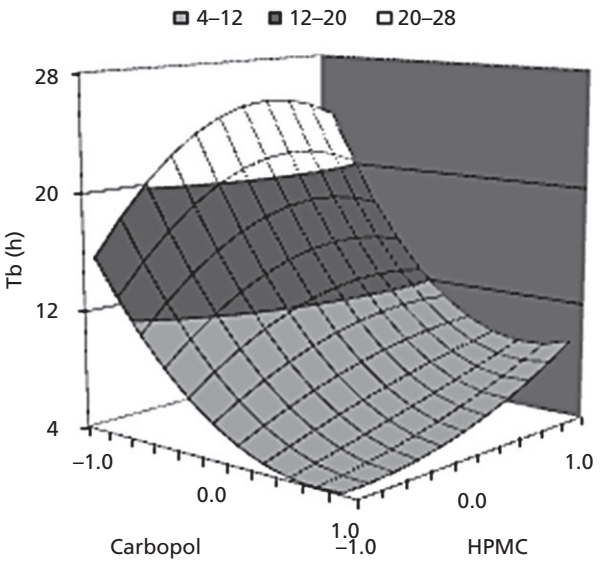

Figure 3 Response surface plot showing the influence of Carbopol and HPMC on the values of buoyancy time of floating-bioadhesive tablet formulation of lamivudine.

polymers, the effect of CP 971P was found to be much more significant than that of HPMC.

Figure 3 shows a slightly positive influence of HPMC in attributing floatational characteristics to the formulated matrices. The influence of CP 971P, on the other hand, was distinctly negative, the effect being more pronounced at the lower levels of CP 971P. At higher levels of HPMC and lower levels of CP 971P, an asymptote was observed. Hence, the

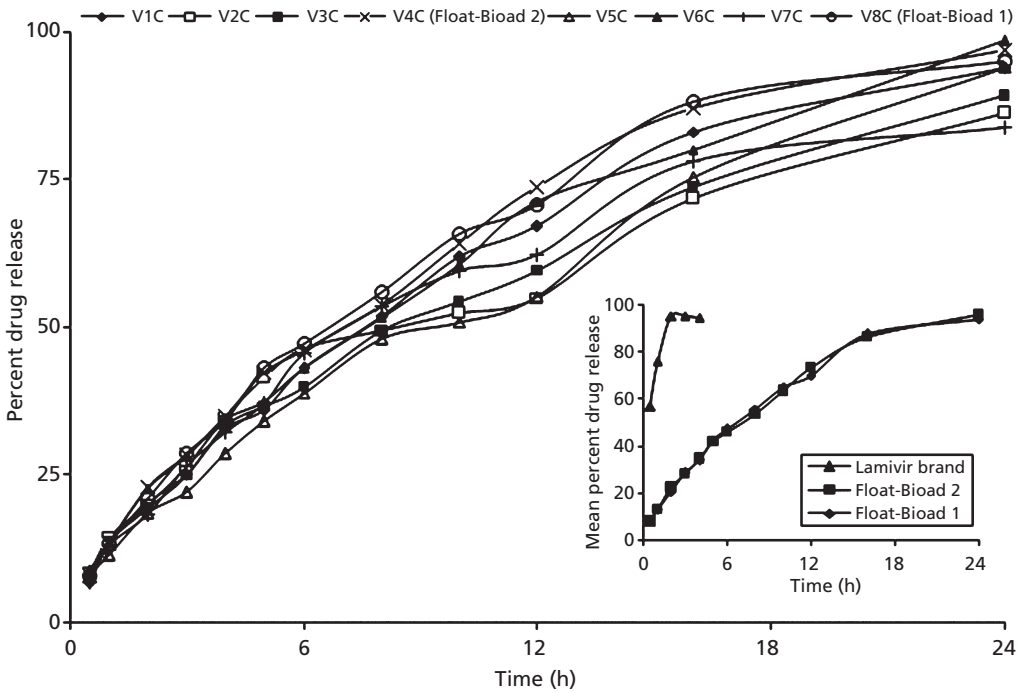

Figure 4 Dissolution profiles of various check-point floating-bioadhesive formulations of lamivudine (V1C-V8C). The inset shows drug release profile of the marketed brand of lamivudine and the floating-bioadhesive formulations Float-Bioad 1 and Float-Bioad 2.

higher levels of CP 971P were counter-productive to imparting buoyancy characteristics to the formulation system.

Search for optimized formulation

While locating the optimized formulation using overlay plots, the following criteria were taken into consideration to achieve the highest possible values of bioadhesive strength, complete and controlled drug release and excellent floatation:

$Q_{16h} > 87\%$; $\rho > 34$ g; $T_b > 7.5$ h

Two short-listed formulations had the following characteristics: Float-Bioad 1, with the polymer levels of 99.0 mg of CP 971P and 150.0 mg of HPMC, showed Q_{16h} as 92.26%, T_b as 12.02 h and ρ as 52.98 g; Float-Bioad 2, composed of 135.0 mg of CP 971P and 156 mg of HPMC, exhibited Q_{16h} as 90.74%, T_b as 7.85 h and ρ as 85.32 g. Both of these formulations were later investigated for the pharmacokinetic studies.

Validation of response surface methodology results

Linear correlation plots between the predicted and observed responses demonstrated high values of r, in the range 0.9888–0.9999, indicating excellent goodness of fit ($P < 0.001$). The residual plots were also found to exhibit quite uniform and randomized scatter of the residual points, when plotted against the observed values of the response variables. Comparison of the observed responses with the anticipated responses revealed that the values of prediction error varied normally between −4.45% and 5.42% with overall mean ± SEM being quite insignificant (i.e. −1.35% ± 1.34). Figure 4 shows diverse dissolution profiles for all the eight check-point formulations (V1C-V8C).

Comparison of release performance with marketed brand

Strikingly different drug release profiles in Figure 4 (inset) show that the release of lamivudine from the floating-bioadhesive formulations is much more sustained than that of the conventional marketed formulation, Lamivir. The $T_{60\%}$values for the formulations were 8.32 h and 8.74 h for Float-Bioad 1 and Float-Bioad 2, respectively, whereas that of the marketed immediate-release brand, Lamivir, was just 1.00 h.

In-vivo pharmacokinetic studies in rabbits

The mean plasma concentration–time profile observed in rabbits is depicted in Figure 5. The two floating-bioadhesive formulations (Float-Bioad 1 and Float-Bioad 2) exhibited markedly better extension of plasma drug levels as compared with the marketed formulation.

The method of analysis (i.e. RP-HPLC) was found to be quite sensitive, selective and reproducible for measuring concentrations of lamivudine with limit of quantification (LOQ)

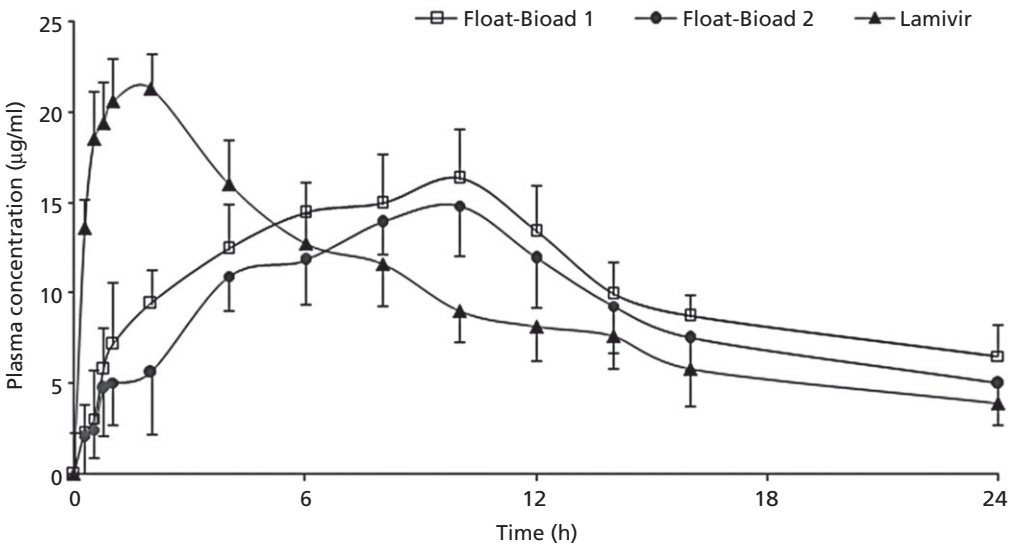

Figure 5 Plasma drug levels of lamivudine obtained in rabbits ($n = 6$) at varied time points for formulations, Float-Bioad 1 and Float-Bioad 2 and their comparison with the marketed formulation. Each point represents the mean and each crossbar indicates 1 SEM.

as 68 ng/ml and limit of detection (LOD) as 25 ng/ml. Float-Bioad 1 and Float-Bioad 2 formulations exhibited almost identical extension of drug release, as is apparent from similar values of T_{max}. Compared with the marketed formulation, Float-Bioad 1 and Float-Bioad 2 formulations exhibited nearly 30.7% and 12.9% increase in the extent of oral bioavailability, as discerned from the corresponding values of the AUC. However, higher values of AUC obtained with formulation Float-Bioad 1 than formulation Float-Bioad 2 demonstrated significantly better oral bioavailability of the former as illustrated in Figure 6a ($P < 0.05$). The formulation Float-Bioad 1, therefore, was finally chosen as the optimized formulation and subjected to subsequent studies.

Level A IVIVC was established for Float-Bioad 1 and Float-Bioad 2 (Figure 7) formulations (r^2 as 0.9790 and 0.9710, respectively, $P < 0.005$ each). Simple log and log-log transformation Level A IVIVC was also executed as in Table 3. No statistically significant correlation, however, could be established for the marketed formulation. Level B correlation was established between mean non-compartmental parameters *viz*. mean dissolution time (MDT) and mean residence time (MRT) of the three formulations (i.e. Float-Bioad 1, Float-Bioad 2 and Lamivir). The various correlations of multiple Level C could be observed for pooled mean data between absorption rate parameters, listed in Table 3.

Table 3 Statistically significant Level B and multiple Level C in-vitro/in-vivo correlations (IVIVC)

In-vitro parameter	In-vivo parameter	R^2	*P* value
Level A IVIVC (Float-Bioad 1)			
% Dissolved	% Absorbed	0.979	<0.005
Log % Dissolved	Log % Absorbed	0.972	<0.005
Log % Dissolved	% Absorbed	0.888	<0.05
% Dissolved	Log % Absorbed	0.857	<0.05
Level A IVIVC (Float-Bioad 2)			
% Dissolved	% Absorbed	0.971	<0.005
Log % Dissolved	Log % Absorbed	0.970	<0.005
Log % Dissolved	% Absorbed	0.867	<0.05
% Dissolved	Log % Absorbed	0.857	<0.05
Level B IVIVC			
MDT (h)	MRT (h)	0.9910	<0.1
Multiple Level C IVIVC			
$T_{50\%}$ (h)	Log T_{max}	0.9952	<0.05
$T_{60\%}$ (h)	Log T_{max}	0.9946	<0.05
$T_{70\%}$ (h)	Log T_{max}	0.9951	<0.05
$T_{80\%}$ (h)	Log T_{max}	0.9998	<0.01
$T_{90\%}$ (h)	Log T_{max}	0.9949	<0.05
Log $T_{70\%}$	Log AUC	0.9999	<0.005
Log $T_{70\%}$	Log C_{max}/AUC	0.9963	<0.05
Log $T_{80\%}$	Log C_{max}/AUC	0.9971	<0.05

In-vivo γ-scintigraphy studies

Figures 8a and 8b refer to the gamma scintigraphic images in human subjects following oral intake of the control and optimized formulation, respectively. The individual scintigraphic data in each volunteer are presented in Table 4, including the GRT data from visual observations and $t^1/_2$ values from raw data and linear fit, respectively. The subjects who ingested the optimized formulation showed gastro-retention for around 5 h. In none of the cases, even at 6 h imaging, were the tablets found to go beyond the duodenum. The optimized formulation remained intact during the entire course of study, as was evident from the localized presence of radioactive material.

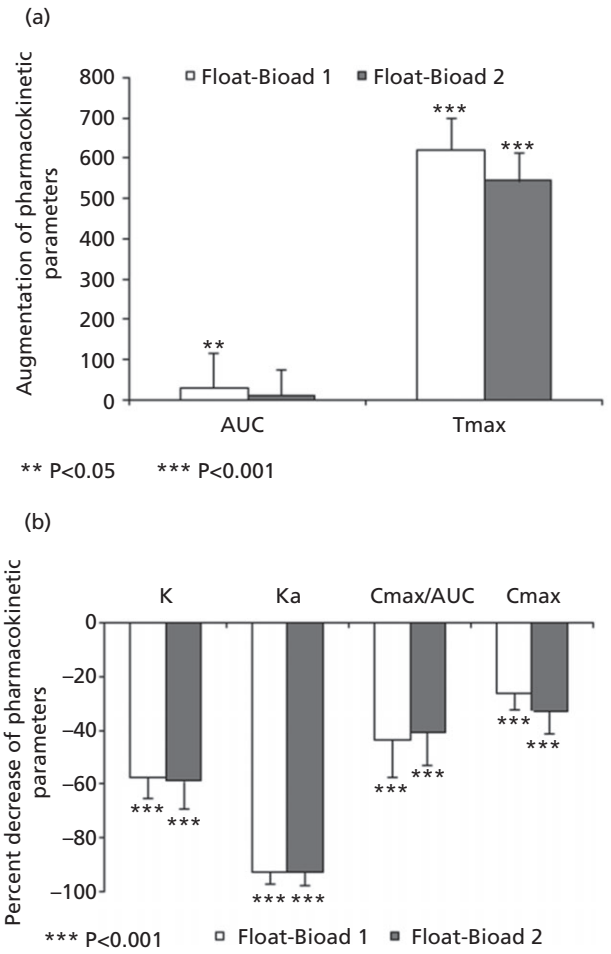

Figure 6 (a) Percent augmentation in the pharmacokinetic parameters. Each error bar indicates 1 SD. (b) Percent decrease in the pharmacokinetic parameters. Each error bar indicates 1 SD.

The control formulation tablet, on the other hand, disintegrated into several pieces within 30 min of its ingestion in all the volunteers.

Accelerated stability studies

Non-significant variation ($P > 0.1$) was observed in the parameters, such as assay, buoyancy time and bioadhesive strength, during the six months of storage of optimized formulation tablets in the accelerated stability studies. The values of all the parameters remained quite well within the desirable limits, showing negligible and random variation over the six months of stress conditions. The values of f_2 for investigating analogy of drug release profiles ranged between 83.28 and 91.97 at various time points during the period of storage.

Discussion

The present investigation describes the development of optimized effervescent floating-bioadhesive controlled release tablet formulations of lamivudine, optimized systematically employing the benefits of DoE methodology.

Five polymers, CP 971P, HPMC K100LV, HPMC K4M, HPMC K15M and PEO 303, were selected for the preliminary pre-optimization studies, owing to their excellent potential for bioadhesive strength, release rate controlling ability, non-toxicity, non-irritancy and stability at gastrointestinal pH. Among these, Methocel (i.e. HPMC) is known to hold good floating potential too.[39] Select grades of CP have already been reported to yield excellent extension in drug release and bioadhesion.[40,41] Hence, based on the preliminary findings, CP 971P was considered as an ideal choice for drug release regulation and bioadhesion. Further, a combination

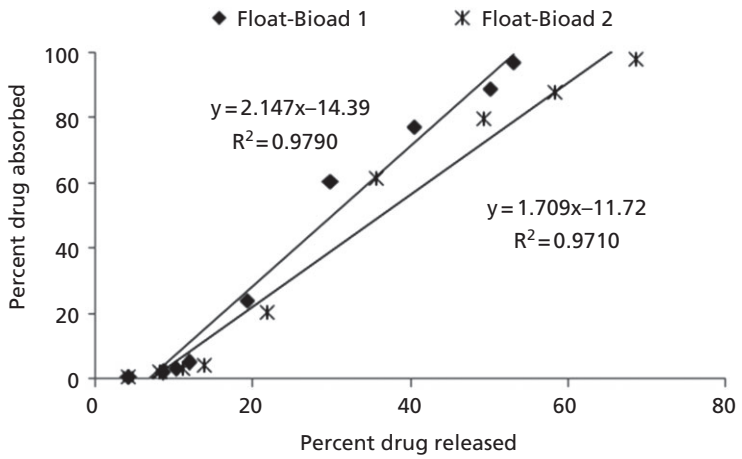

Figure 7 Level A in-vitro/in-vivo correlation (IVIVC) for floating-bioadhesive formulations Float-Bioad 1 and Float-Bioad 2.

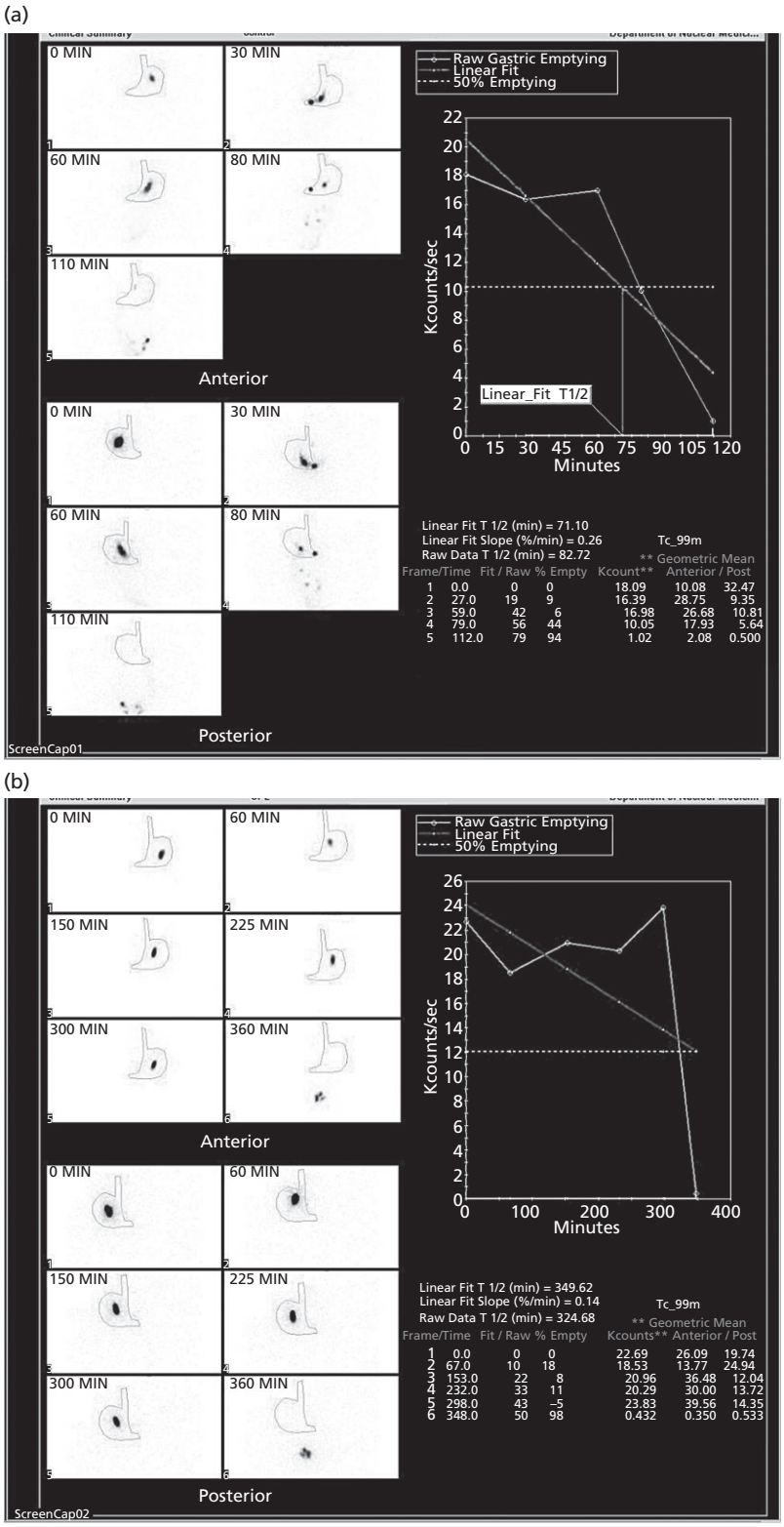

Figure 8 (a) Anterior and posterior static scintigraphic images of stomach in a human volunteer following oral administration of 99mTc-labelled control drug formulation. Also shown is the linear fit time activity curve depicting gastric emptying time of the radio-labeled drug from the stomach. (b) Anterior and posterior static scintigraphic images of stomach in a human volunteer following oral administration of 99mTc-labelled optimized drug formulation. Also shown is the linear fit time activity curve depicting gastric emptying time of the radio-labeled drug from the stomach.

Table 4 Gastric retention time of the optimized (OPT) and control formulations observed during in-vivo γ-scintigaphy

Study	Volunteer code	Visual GRT (min)	Raw data $t^1/_2$ (min)	Linear fit $t^1/_2$ (min)
Control	SA	95	71.10	82.72
	BG	115	115.32	111.87
	VS	160	162.91	166.59
	RK	145	152.81	155.34
	AS	157	161.27	168.35
	RB	124	127.62	122.99
Mean ± SD		132.67 ± 25.68	131.84 ± 35.36	134.64 ± 34.45
OPT	SA	280	291.48	283.56
	BG	330	349.62	324.68
	VS	330	324.50	321.68
	RK	312	310.31	315.63
	AS	329	322.76	326.34
	RB	331	330.42	328.71
Mean ± SD		318.67 ± 20.27	321.52 ± 19.53	316.77 ± 16.88

GRT, gastric retention time.

of ionic polymer (like CP) and nonionic polymer (like HPMC) is known to provide the formulation with controlled drug release and/or desired mucoadhesive properties.[12,14,42,43] Such has already been proved in a number of literature reports on bioadhesive compressed matrices, such as buccoadhesive tablets of metoprolol tartrate,[44] hydrophilic matrices of hydralazine hydrochloride,[14] and hydrodynamically balanced bioadhesives of metoprolol.[45] Particularly, the successful use of the synergistic polymer combination of CP 971P and HPMC has already been documented in various literature reports in attaining controlled release.[13] Thus, out of the five polymers screened, the rational blend of CP 971P and HPMC K15M was selected owing to its better synergistic potential for controlled release, bioadhesive and buoyancy characteristics. Studies on the relative ratio of these polymers required to attain the optimum $T_{60\%}$, Q_{16h}, ρ and T_b were embarked upon, employing DoE. The effervescing agent (i.e. sodium bicarbonate) was added to reduce the lag time in floatation.[46,47]

Following attempts at modelling the respective dissolution data to the Korsmeyer–Peppas model for swellable compressed matrices,[48] an excellent degree of fit ($r^2 > 0.9724$, $P < 0.001$) was obtained in all the cases. In general, all the controlled release floating-bioadhesive hydrophilic matrix formulations, containing the polymer blends of CP 971P and HPMC, showed non-Fickian drug release behaviour throughout. Overall, these results seem to be in close agreement with the findings of Nokhodchi *et al*.,[49] indicating the ambiguous relationship of n with change in polymer composition. As is evident from Table 2, the values of kinetic constant, k, showed an ambiguous trend with increase in the amount of either polymer. The magnitudes of k_1 and k_2 clearly showed that the drug release was predominantly governed by Fickian diffusion, with the contribution of polymer relaxation being nearly negligible. This is in consonance with the earlier findings that a mixture of HPMC with CP 971P results in the reduction of polymer viscosity due to reduced hydration.[12] The initial burst release portrayed in drug release rate curves of the check-point formulations (Figure 4) is a characteristic feature of hydrophilic matrices.[12,13] The high values of Q_{16h} indicate that the major amount of the drug would be released before the device is finally eliminated from the gastrointestinal tract.

The increase in the values of bioadhesive strength with an increase in the amount of either polymer is discernible from the fact that the hydrogels swell readily when they come into contact with hydrated mucous membrane. The results are in consonance with various literature findings.[12,13,50,51] Water sorption reduces the glass transition temperature below the ambient conditions and the hydrogels become progressively rubbery due to uncoiling of polymer chains and subsequent increased mobility of the polymer chains.[12,13] This glass-rubbery transition provides hydrogel plasticization, resulting in a large adhesive surface for maximum contact with mucin and flexibility to the polymer chains for interpenetration with mucin. Increasing the amount of polymer may provide more adhesive sites and polymer chains for interpenetration with mucin, resulting in an augmentation of bioadhesive strength.

The increase in buoyancy time with increasing HPMC content is owed ostensibly to the swelling and hydration of the hydrocolloid particles on the tablet surface, which, in turn, results in an increase in the bulk volume.[28,52] The balance between polymer swelling and water acceptance has already been documented in literature as the vital factor to ensure floatation.[53] The gas-generating agent (i.e. sodium bicarbonate) induces carbon dioxide in the presence of the acidic dissolution medium, thereby increasing polymer hydration and decreasing tablet density. Owing to the air entrapped in it, the swollen polymer maintains a density less

than unity, thus conferring it with buoyancy characteristics. Further, the declining trend of buoyancy time with increasing CP : HPMC ratio vouches the higher swelling tendencies of cellulose derivatives (i.e. HPMC) *vis-à-vis* carbomer derivatives (i.e. CP). This may also be ascribed to higher density of CP (1.76 g/cc) than that of HPMC (1.30 g/cc). Even, the low density of tablets (i.e. <1.004 g/cc) fulfilled the major criterion for a dosage form to float.[54]

In a CCD, all the factors are studied at all the plausible combinations, as it is considered to be most efficient in estimating the influence of individual variables (main effects) and their interactions, using minimum experimentation[31,55,56] This design has an added advantage of determining the quadratic response surface, not estimable using a factorial design at two levels.[57,58] In this study, fitting a cubic model was considered to be a better alternative, as the values of the response surfaces were not known from the previous findings. Hence, a CCD for two factors at three levels with $\alpha = 1$ was chosen. The high values of R^2 exhibited by the polynomial relationships vouched high statistical validity ($P < 0.001$) of Equation 6 for fitting to the experimental data. The higher-order quadratic interaction model, in other words, could describe the data sufficiently well to navigate the design space. As is revealed from Table 2, the amounts of CP and HPMC have a positive influence on the values of coefficients of $T_{60\%}$, the effect being more apparent with HPMC. On the other hand, the positive effect of CP is vividly far more pronounced than that of HPMC in regulating the values of ρ.[59] The negative effect of CP on buoyancy time of tablets can be ascribed to the low swelling properties of carbomers as compared with HPMC, in good agreement with literature.[60]

Further, the comparison of release performance of the two floating-bioadhesive formulations (Float-Bioad 1 and Float-Bioad 2) *vis-à-vis* the conventional marketed formulation, Lamivir, vividly ratified the marked sustained release performance of both the optimized formulations.

The entire in-vivo pharmacokinetic study on lamivudine was carried out using parallel design, as it was quite impracticable to carry out crossover studies in animals like rabbits. The pharmacokinetic profiles following oral administration were analysed using the biexponential one-compartment open-body model (1-CBM), wherein the first exponential term represented the absorption process and the second one represented the elimination process.[61] The initial parameter estimates, furnished by preliminary data analysis using MS-EXCEL spreadsheet package, were transported into the milieu of Win-Nonlin pharmacokinetic software, chiefly for comprehensive pharmacokinetic modelling based on iterative routines. Statistically significant and relatively low magnitudes of Akaike Information Criteria (ranging between −54.93 and −68.87) and Schwartz Criteria (ranging between −54.08 and −67.18) indicate excellent credibility of the pharmacokinetic computations during the current work.

Phenomenally high escalation of the values of T_{max} observed with formulations Float-Bioad 1 and Float-Bioad 2 (i.e. 621.7% and 544.8%, respectively; $P < 0.001$ each *vis-à-vis* Lamivir) undisputedly vouches the extended release potential of these two formulations (Figure 6a). Significant augmentation in the values of AUC observed with Formulation Float-Bioad 1 (Figure 6a, $P < 0.05$) can be ascribed to the longer retention of the formulation in the drug absorption region of gastrointestinal tract. Significant reduction in the value of C_{max} (Figure 6b), which is known to be a composite parameter indicative of both rate and extent of drug absorption, was also observed. This decline in C_{max} signifies an ostensible reduction in the rate of drug absorption from both the formulations, as the values of AUC tend to exhibit a somewhat ascending trend. The observation was further fortified by a reduction in the values of K_a and C_{max}/AUC, both being indicative of the rate of absorption.[62] There is strong evidence that the ratio metric C_{max}/AUC should be preferred to C_{max} for assessment of comparative absorption rates following the administration of a single dose, as it tends to have smaller variation than that of C_{max}.[63,64] Significantly lower values of C_{max}/AUC observed with the Float-Bioad 1 and Float-Bioad 2 formulations (i.e. 0.046 $h^{-1} \pm 0.003$, 0.048 $h^{-1} \pm 0.002$, respectively) *vis-à-vis* Lamivir (i.e. 0.081 $h^{-1} \pm 0.002$) in the current studies ($P < 0.001$ each), therefore, corroborate their reduced rate of absorption and improved sustained release potential. The same has also been substantiated by a highly significant increase in the values of T_{max}. Significant reduction in the values of K ($P < 0.001$) points towards the propensity of extensive retention of drug in the body.

Excellent Level A correlations were observed with the two floating-bioadhesive formulations (i.e. Float-Bioad 1 and Float-Bioad 2) but not with the marketed formulation. This could be attributed to the immediate-release characteristics of the Lamivir formulation, which exhibit an in-vitro drug release rate much faster than the in-vivo absorption rate. Thus, a point-to-point correlation between the drug release *in vitro* and drug absorbed *in vivo* could not be established with this immediate-release formulation. Further, high values of r^2 (i.e. 0.9910) observed on plotting the inverse of mean absorption parameters and inverse of mean dissolution parameters corroborate that a significant Level B correlation existed between in-vitro release rate and in-vivo absorption rate (Table 3). Analogously, high values of r^2, ranging between 0.9946 and 0.9998, significantly vouched the establishment of multiple Level C correlations between various in-vitro dissolution and in-vivo pharmacokinetic parameters too (Table 3).

Gamma scintigraphy is a technique whereby the transit of a dosage form through its intended site of delivery can be

imaged *in vivo* non-invasively via judicious introduction of an appropriate short-lived γ-emitting radioisotope.[65] The observed transit of the dosage form can then be correlated with the rate and extent of drug absorption.[66] Based on the results of in-vivo pharmacokinetic studies in rabbits, the optimized formulation was selected for γ-scintigraphy studies in human volunteers owing to its better bioavailability potential. The optimized tablets, in all the subjects, were found to be either in the stomach or duodenum, even at 5 h sampling. Hence, the drug is quite likely to show maximum absorption from its absorption window (i.e. stomach and the anterior parts of small intestine).[19] The volunteers ingesting the control formulation were radiographed more frequently at shorter intervals, as it the formulation was anticipated to exhibit a relatively smaller GRT. This was later confirmed through studies on the control tablets, which sank rapidly to the base of the stomach in all the volunteers within a span of less than an hour. The optimized formulation, on the other hand, retained the drug for 5–6 h, significantly higher than the control formulation, which retained only for less than one hour.

Drug release parameters during the accelerated stability studies unambiguously showed that the dissolution performance of the optimized formulation was negligibly altered even under the stressed conditions. Various dissolution parameters (*viz.* $T_{60\%}$ and Q_{16h}), obtained during various time points of stability studies carried out at 40 $\pm$ 2°C and 75 $\pm$ 5% relative humidity, remained almost unaffected during the studies, suggesting the robustness of the optimized formulation with respect to dissolution characteristics. The values of f_2, ranging between 83.28 and 91.97 at various periodic intervals – well within the pragmatic limits of 50–100, corroborate the analogy of various dissolution profiles during the accelerated stability studies. Only miniscule and random difference in the values of assay content, floatation time and bioadhesive strength further establishes the stability of formulation under stressed conditions. Hence, the optimal gastroretentive tablet formulation was considered to be quite stable during shelf-life storage.

Conclusions

Integration of floatational characteristics with bioadhesion is considered ideal for gastroretention, as the house-keeping waves tend to force gastric emptying.[67,68] Gastroretentive systems, in this regard, are preferred due to their ability of retaining the DDS in the gut and improving bioavailability, especially for drugs that exhibit a specific absorption window in the gastrointestinal tract. Owing to high frequency of administration of most anti-retroviral drugs, extended release HAART is highly advisable to manage various HIV disorders effectively. This work, accordingly, employed the floating-bioadhesive principle to formulate gastroretentive systems of lamivudine for localizing the formulation in the stomach and upper intestine, the preferred site of absorption of lamivudine. During the current studies, rational blends of effective and cost-effective polymers like carbomers and methylcelluloses were found to act synergistically to yield maximal extension of drug release, coupled with excellent floating and bioadhesive properties. Systematic studies employing DoE helped to balance optimal floatation with bioadhesion using a rational combination of CP and HPMC. The choice of design (i.e. CCD) was found to be quite appropriate, as it could detect any non-linearity in the factor–response relationship with minimal expenditure of developmental effort and time. The in-vitro drug release, as well as the in-vivo pharmacokinetic and scintigraphic, studies vouched the successful controlled release and gastroretentive propensities of the optimized formulation. Consequent establishment of various levels (A to C) of IVIVC demonstrated that the in-vitro dissolution performance tested in SGF correlates well with the in-vivo absorption parameters. Apart from being an excellent product development tool, IVIVC can also be exploited for obtaining biowaivers of such systems. Excellent bioadhesion at acidic, as well as alkaline, pH indicates its potential as a gastrointestinal therapeutic system too, for the drug absorption beyond the realms of gastric environment. In a nutshell, the studies could be judiciously extrapolated to develop suitable platform floating-bioadhesive technology(ies) for preparing gastroretentive and gastrointestinal therapeutic system controlled release formulations of lamivudine in combination with other BCS class I drugs, like stavudine and/or zidovudine, for the treatment of HIV infective disorder(s), especially HAART.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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