Research Paper

International Journal of Pharmaceutical Sciences and Nanotechnology
Volume 10 • Issue 3 • May – June 2017
MS ID: IJPSN-3-7-17-KIRAN KUMAAR

Development and Clinical Evaluation of Mucoadhesive Gastroretentive Tablets of Dipyridamole

Kiran Kumar[1]*, S. Gurunath[1], P. Srikanth[1], M. Ajitha[2] and Y. Madhusudan Rao[1]

[1]Department of Pharmaceutics, Vaagdevi Institute of Pharmaceutical Sciences, Bollikunta, Warangal 506005, Telangana State. India, and [2]R & D Cell JNTUH Kukatpally Hyderabad-500085, India.

Received March 7, 2017; accepted April 8, 2017

ABSTRACT

The present research work was focused to develop a mucoadhesive tablet dosage form for dipyridamole, which shows pH dependent solubility, it is highly soluble in acidic pH and as the pH increases the solubility of the drug decreases. Hence it was selected as the drug candidate for the present research. Mucoadhesive tablets of Dipyridamole were successfully prepared by using polymers like HPMC K4M, Chitosan and Isabgul husk by wet granulation method. FT-IR studies showed that there is no incompatibility between drug, polymer and various excipients used in the formulations. Formulated tablets have shown satisfactory results for physical parameters and complied with the pharmacopeial limits. The *ex-vivo* mucoadhesive strength and mucoadhesive force was found to be in the range of 16.15- 21.20 g and 1.56-2.06 N. Total *ex-vivo* mucoadhesive time was observed in between 10 to 12 hours. The *in-vitro* drug release was found to be more than 90% for the formulations FMD2, FMD3 and FMD8, up to 12 hours. Based on *in-vitro* drug release and mucoadhesive properties, formulation FMD2 was selected as optimized formulation. The dissolution data were further characterized by fitting the data into various kinetic models. The drug release from the matrices followed zero order with non-fickian release (diffusion + erosion controlled) for the optimized formulation. The optimized formulation was further subjected to swelling studies, which showed a swelling index of 286% up to 24 hours. The results indicated that the selected polymers were of swellable type. The stability studies were carried for 6 months as per ICH and WHO guidelines and the results of the stability study revealed that the optimized formulation is stable during the storage period. The *in-vivo* radiographic studies in fed condition, for the Mucoadhesive tablets (FMD2) showed a gastric residence time of more than 6 hours. When the radiographic images were taken at different time intervals and it was found to be in a particular location, which suggested that the retention of the dosage form might be due to the adhesion of dosage form to the gastric mucosa.

KEYWORDS: Dipyridamole; Mucoadhesive; Radiographic Studies; Chitosan and Isabgul husk.

Introduction

Drugs experience a pH range of 1-8 across the G.I tract and need to be in solubilized form to successfully cross the biological membrane. Most of the drugs are passively absorbed in their un-ionized form and the extent of ionization at different pH values depends on solubility, stability and ionization by changing the physical properties of the drug in different portions of the G.I tract, can lead to regional variability in absorption of drugs. Several approaches have been emmerged to prolong the residence time of the dosage forms at the absorption site and one of these is the development of oral controlled release mucoadhesive system. In the early1980's, Professor Joseph R. Robinson at the University of Wisconsin pioneered the concept of mucoadhesion as a new strategy to prolong the residence time of various drugs on the ocular surface (Bhupinder et al., 2006).

Various gastrointestinal mucoadhesive dosage forms, such as discs, microspheres, and tablets, have been prepared and reported by several research groups. Adhesion can be defined as the bond produced by contact between a pressure-sensitive adhesive surface (Agaiah et al., 2011 and Amish et al., 2012).

The American Society of Testing and Materials has defined it as the state in which interfacial forces, which may consist of valence forces, interlocking action, or both, hold two surfaces together. A bioadhesive is defined as a substance that is capable of interacting with biological materials and being retained on them or holding them together for extended periods of time (Moes 1993). According to Good defined mucoadhesion as the state in which two materials, atleast one biological in nature, are held together for an extended period of time by interfacial forces. It is also defined as the ability of a material (synthetic or biological) to adhere to a biological tissue for an extended period of time (Gupta et al., 1993).

Dipyridamole, a non-nitrate coronary vasodilator that also inhibits platelet aggregation (Singh et al., 1994 and Kim et al., 2004), is combined with other anticoagulant

drugs, such as warfarin, to prevent thrombosis in patients with valvular or vascular disorders (Kong et al., 2003). Dipyridamole is also used in myocardial perfusion imaging, as an antiplatelet agent, and in combination with aspirin for stroke prophylaxis. Dipyridamole was considered as ideal drug candidate due to its maximum solubility in the lower pH environment compared to higher pH environment. Earlier Katakam et al (2012) and several others developed single and multiple units of dipyridamole, but very little work was carried in the field of mucoadhesion technology. In this study, we attempted to develop optimized mucoadhesive dosage form for dipyridamole.

Materials and Methods

Materials

Dipyridamole Gift sample from AET Labs. Hyderabad. Chitosan was Gift sample from Zydus Cadila, Ahmedabad. $HPMCK_4M$ was Gift sample from Signet Chemical Corporation, Mumbai. Isabgul husk (psyllium husk) was procured from Keyur industries, Gulab Park, Sidhpur, Microcrystalline cellulose PH102, Conc. Hydrochloric acid, Magnesium stearates, Talc, Isopropyl alcohol & PVP K30 are from local suppliers.

Preparation Method of Dipyridamole Mucoadhesive Tablets

Accurately weighed quantities of drug along with polymers and microcrystalline cellulose (MCC PH102) as shown in Table 1 were taken in a motor and mixed thoroughly by geometric dilution method. Non-aqueous granulation was carried out by using 10 % of PVP K30 in isopropyl alcohol. Wet mass was prepared by adding quantity sufficient of PVP solution and passed through 10 mesh. Wet granules were dried at 50 - 60°C for 30 min. Dried granules were passed through the 18 mesh and lubricated with talc and Magnesium stearate. Finally, the powder blend was mixed well in a polybag. Final blend was compressed into tablets using 10 mm size round flat bevelled punches and corresponding dies on 16-station rotary compression machine (Cemach, India).

In this work Isabgul husk was grinded in mixer grinder and passed through 60 mesh prior to its utilization in the formulation.

Evaluation of the Developed Formulations

Drug-excipient compatibility studies by fourier transform infrared (FTIR) spectroscopy: Infrared spectra were taken by using KBr pellet technique using a Bruker Alpha FT-IR Spectrophotometer in the wavelength region of 400 to 4000 cm−1. The procedure consisted of dispersing a sample (drug alone or mixture of drug and excipients) in KBr and compressing into discs by applying a pressure of 5 tons. The Fourier transform infrared (FTIR) spectra of samples were obtained using FTIR spectroscopy.

Evaluation of Mucoadhesive Tablets of Dipyridamole

The compressed tablets were studied for their physicochemical properties like weight variation, hardness, thickness, friability, drug content, mucoadhesive strength, *ex-vivo* mucoadhesion time and *in-vitro* drug release.

Weight Variation Test

To study the weight variation, twenty tablets were taken and their weight was determined individually and collectively on a digital weighing balance. The average weight of one tablet was determined from the collective weight. The weight variation test would be a satisfactory method of determining the drug content uniformity. The percent deviation was calculated using the following formula.

% Deviation = (Individual weight – Average weight / Average weight) × 100

Tablet Hardness

Hardness of tablet is determined as the force applied across the diameter of the tablet in the order to break the tablet. The resistance of the tablet to chipping, abrasion or breakage under condition of storage transformation and handling before usage depends on its hardness. For each formulation, the hardness of 10 tablets was determined using Monsanto hardness tester and the average is calculated and presented with standard deviation.

TABLE 1

Composition of Mucoadhesive Tablets of Dipyridamole.

Formulation Code	Dipyridamole (mg)	Chitosan (mg)	$HPMCK_4m$ (mg)	Isabgul husk (mg)	MCC PH 102 (mg)	Talc (mg)	Mg. Stearate (mg)
FMD1	50	100	200	_	135	10	5
FMD2	50	200	100	_	135	10	5
FMD3	50	150	150	_	135	10	5
FMD4	50	-	200	100	135	10	5
FMD5	50	_	100	200	135	10	5
FMD6	50	_	150	150	135	10	5
FMD7	50	_	300	_	135	10	5
FMD8	50	300	_	_	135	10	5
FMD9	50	_	_	300	135	10	5

Total Weight of each Tablet: 500 mg.

Tablet thickness

Tablet thickness is an important characteristic in reproducing appearance. Five tablets were taken and their thickness was recorded using Vernier calipers and screw gauge. The average thickness for tablets was calculated and presented with standard deviation.

Friability

It is the measure of mechanical strength of tablets. Roche friabilator (Electrolab, Mumbai, India) was used to determine the friability by following procedure. Preweighed tablets (20 tablets) were placed in the friabilator. The tablets were rotated at 25 rpm for 4 minutes (100 rotations). At the end of test, the tablets were reweighed; loss in the weight of tablet is the measure of friability and was expressed in percentage as

$$\% \text{ Friability} = [(W_1 - W_2) / W_1] \times 100 \quad .....(1)$$

Where W_1 = Initial weight of 20 tablets

W_2 = Weight of the 20 tablets after testing

Drug Content (Assay)

The drug content of the tablets was determined according to in-house standards and it meets the requirements if the amount of the active ingredient in each of the 3 tested tablets lies within the range of 90% to 110% of the standard amount. The prepared tablets were tested for their drug contents. Ten tablets were finely powdered and the powder equivalent to 100mg of Dipyridamole was accurately weighed and transferred to 100mL volumetric flask. Sufficient amount of 0.1N HCl was added and made up to 100mL and sonicated for 30 minutes to dissolve the drug completely. The solution was filtered and from the filtrate 0.5mL and 1.0mL were taken and diluted to 10mL with 0.1N HCl. The absorbance of these resulting solutions was measured at 284nm.

In-Vitro Drug Release Characteristics

The *in vitro* drug release study was performed for the mucoadhesive tablets using USP Type II dissolution apparatus under the following conditions (Deshmukh et al., 2009).

Dissolution Test Parameters

Medium	:	900mL of 01.N HCl
Rotation speed	:	50 rpm
Temperature	:	37 ± 0.1ºC
Sampling Volume	:	5mL
Sampling Time	:	0.5, 1, 2, 3, 4, 6, 8, 10, 12 hours

The *in-vitro* drug release from the mucoadhesive tablets was carried by adhering tablet on a glass slide. One drop of molten hard paraffin is taken on the glass slide and tablet was placed on the drop of hard paraffin and was pressed. Glass slide was placed in the dissolution vessel containing 900 mL of 0.1 N HCl. At predetermined time intervals, samples (5 mL) were withdrawn and replenished with same volume of fresh media. The drug content in the samples was estimated using UV-spectrophotometer at 284 nm.

Evaluation of Mucoadhesive Strength of Tablets

Measurement of adhesion force was determined by using goat gastric mucus membrane. The equipment and the membrane were hydrated by pouring 0.1N HCl on the membrane with a dropper. One side of the tablet was adhered to the plastic vial cap and the cap was tied to the nylon thread. Other end of the thread was tied with a plastic cup to contain counter weights and it was made to pass through two pulleys. Now the tablet was placed over the membrane and 50gm weight was placed over the tablet for 15 minutes to induce mucoadhesion. After 15 minutes increments of 0.5 gm of weight was placed in the cup and the counter weight at which the tablet detaches from the membrane was determined. From the mucoadhesive strength, the force of adhesion was calculated using the formula as given below.

Force of adhesion (N) =

$$\frac{\text{mucoadhesive strength in grams}}{100} \times 9.81 \quad .....(2)$$

Evaluation of *Ex-vivo* Mucoadhesion Time

The *Ex-vivo* mucoadhesion time was examined after application of tablet over excised goat mucosa for 5 minutes after earlier being secured on a glass slide. The slide-containing tablet was immersed in the USP paddle type dissolution apparatus containing 900 mL of 0.1 N HCl and the temperature was maintained at 37 ± 0.5 ºC. A distance of 2.5cm was adjusted for the paddle of the dissolution apparatus from the tablet, which was rotated at 50 rpm. The time taken by tablet to detach from the membrane was recorded in minutes (Ranga Rao et al., 1989 and Singh et al., 2010).

Drug Release Kinetic Analysis

The model dependent methods all rely upon a curve fitting procedure. Different mathematical functions have been used to model the observed data. Both the linear and non-linear models are being used in practice for dissolution modeling. Linear models include Zero order, Highuchi, Hixon – Crowell, quadratic and Polynomials, where as the nonlinear models include First order, Weibull, Korsmeyer-Peppas etc (Lingam et al., 2008).

Swelling Studies

Determination of swelling index of tablets: Radial swelling of the matrices was monitored by immersing the tablet in beaker containing dissolution medium (250mL at room temperature). At predefined time interval, an increase in the tablet diameter was determined over a specified period of time. The same measured in at least two different axes perpendicular to each other and their mean value is taken.

Swelling index (SI), expressed as percent, was calculated as per the following equation:

$$SI = \{[Dt - D / Di\} \times 100 \quad .....(3)$$

Where,

Dt = tablet diameter at time t, Di = Initial diameter of tablet

Method

The temperature of beaker containing 250 mL 0.1N HCl (dissolution medium) was maintained at 37.5 ± 0.5 °C. The scale was placed below the beaker so that scale could be easily shown. The diameter of the tablet was initially measured and then it was put inside the beaker. The tablet was placed such that diameter could be measured at specific time interval without taking the tablet out of the medium.

Stability studies

To assess the drug and formulation stability, stability studies were done according to ICH and WHO guidelines (Ritger et al., 1997 and Mathews 1999). Optimized formulation kept in the humidity chamber (Pooja labs, India) maintained at 40 °C and 75% Relative Humidity for 6 months. At the end of studies, samples were analyzed for physicochemical parameters. For the comparison of release profiles of initial and stability samples, "difference factor" f1 and "similarity factor" f2, were calculated (Moore and Flanner1996). The difference factor (f1) measures the percent error between the two curves over all time points and was calculated as follows

$$f_1 = \frac{\Sigma_{j-1}^{n} \left|R_j - T_j\right|}{\Sigma_{j-1}^{n} R_j} \times 100 \qquad \text{.....(4)}$$

Where, n is the number of sampling points, R_j and T_j are the percent dissolved of the reference and test products at each time point j. The two release profiles are considered to be similar, if f_1 value is lower than 15 (between 0 and 15). The similarity factor (f_2) is a logarithmic transformation of the sum of squared error of differences between the test T_j and the reference products R_j over all time points. It was calculated using the following equation:

$$f_2 = 50 \log \left\{ \left[\left[1 + (1/n)^{-0.5} \Sigma_{j-1}^{n} w_j \left|R_j - T_j\right|^2 \right] \right\} \times 100 \qquad \text{.....(5)}$$

Where, w_j is an optional weight factor and other terms are as defined earlier. The two dissolution profiles are considered to be similar, if f_2 value is more than 50 (between 50 and 100).

Determination of *In-vivo* Gastric Eesidence Time in Human Volunteers

The *in vivo* X-ray studies were approved by the institutional ethical committee with approval No.IHEC/ VGOPC/059/2015. Tablets were administered to healthy human volunteers (n=3) aged between 20-25 years and weighing between 50-60kgs were selected for these studies. For these studies, optimized mucoadhesive formulation was modified by replacing 30 mg of Dipyridamole with X-ray grade barium sulfate, which is a radio-opaque substance, keeping all other ingredients constant. The *in-vivo* gastric residence time determination was carried out in fed conditions (Katakam et al., 2012 and Doodipala et al., 2011) In fed state, the tablet was administered to the volunteers after taking a standard fat and protein breakfast with 200mL of water.

Results

Calibration Curves of Dipyridamole

UV-Spectro-photometric method was used for estimation of dipyridamole. Solution of dipyridamole (10µg/mL) was scanned in the wavelength range of 200-400 nm and found to have maximum absorption (λ_{max}) at 284 nm. The standard plots of Dipyridamole were prepared in 0.1 N HCl. The standard graphs showed good linearity with R^2 values ranging from 0.9990 to 0.9996.

Solubility of Dipyridamole

The quantitative solubility of dipyridamole in different buffers was shown in Table 3. Dipyridamole is highly soluble in 0.1N HCl, having quantitative solubility (45.24 mg/mL). As pH increased solubility decreased drastically, i.e., pH 4.5 acetate buffer (20.22 mg/mL), pH 6.8 phosphate buffer (2.4 mg/mL), and pH 7.4 phosphate buffer (1.6 mg/mL). It shows pH dependent solubility, highly soluble in acidic pH but poorly soluble in alkaline pH. The solubility data is shown in Table 2.

TABLE 2

Solubility of dipyridamole in different buffers.

Buffer	Absorbance	Dilution factor	Regression Equation	Solubility (mg/mL)
0.1N HCl (pH 1.2)	0.552	500	Y = 0.059 + 0.0151	45.24
pH 4.5 acetate buffer	0.472	100	Y = 0.042 + 0.009	20.22
pH 6.8 Phosphate buffer	0.712	10	Y = 0.040 + 0.002	2.4
pH 7.4 Phosphate buffer	0.623	10	Y = 0.036 + 0.003	1.6

Drug-Excipient Compatibility Study by Fourier Transform Infrared (FT-IR) Spectroscopy

Potential chemical interaction between drug and polymers may change the therapeutic efficacy of the drug. To investigate the possibility of chemical interaction between drug and excipients FTIR spectra of pure Dipyridamole and optimized formulation mixture of Dipyridamole were analyzed over the range 400 to 4000 cm^{-1}.

The FT-IR spectrum shown in Figure 1 and peak values in Table 3 indicated good compatibility between drug and polymers used in the formulations. The optimized formulation was subjected to FTIR studies to confirm that there are no drug- polymer interactions. The result indicated the absence of interaction because the major functional groups which were present in the pure drug were also present in the mixtures of drug and polymers without any major shifting in the peak values.

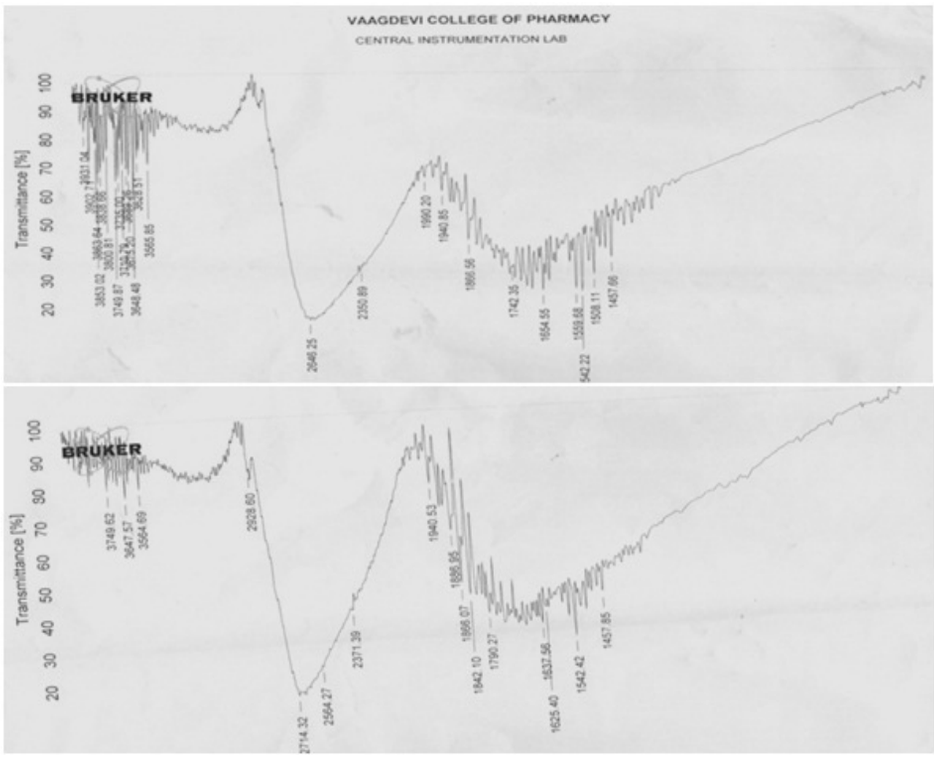

Fig.1. FT-IR spectrum of dipyridamole pure drug and optimized formulation of dipyridamole.

TABLE 3

Functional groups and Range for Dipyridamole and optimized formulation.

Functional group	Absorption range	Pure drug Peak	Optimized formulation
Alcohols (O-H)	3650-3580	3648.48	3291.24
Pyrimidine	1600-1300	1542.22	1530
Aromatic amines	1360-1180	1342.44	1359.24

Evaluation of Physical Parameters of Mucoadhesive Tablets of Dipyridamole

All the prepared formulations were tested for Physical parameters like Hardness, thickness, Weight Variation, Friability and were found to be within the Pharmacopeial limits. The results of the tests were tabulated in Table 4. The drug content of all the formulations was determined and found to be within the permissible limit. This study indicated that all the prepared formulations were good. The results of the physical tests of many of the formulations were within the limits and complied to pharmacopeial limits.

Effect of Mucoadhesive polymers on Mucoadhesive strength, Mucoadhesive force and *Ex-vivo* Mucoadhesive time

All the formulations were tested for mucoadhesive strength, mucoadhesive force and mucoadhesive time. All the batches showed good *ex-vivo* mucoadhesive properties. The mucoadhesive strength and mucoadhesive force was found to be in the range of 16.30- 21.76 g and 1.59-2.13 N. Total mucoadhesive time was observed in between 10 to 12 hours. The results of the *ex-vivo* mucoadhesive properties were shown in Table 5 and the method developed to determine the mucoadhesive strength is shown in Figure 2.

TABLE 4

Physical Parameters of Mucoadhesive Tablets of Dipyridamole.

Formulation code	Weight variation (mg) Mean ± SD (n=20)	Hardness (kg/cm^2) Mean ± SD (n=10)	Thickness (mm) Mean ± SD (n=5)	Friability (%) (n=20)	Assay (%) Mean ± SD (n=3)
FMD1	500.2 ± 2.5	4.5 ± 0.34	5.38 ± 0.05	0.38	97.27 ± 0.52
FMD2	498.90 ± 2.73	4.1 ± 0.54	4.92 ± 0.06	0.26	99.81 ± 0.51
FMD3	495.80 ± 2.50	4.2 ± 0.48	5.45 ± 0.03	0.24	99.45 ± 0.82
FMD4	502.09 ± 2.13	4.5 ± 0.35	4.97 ± 0.04	0.39	100.22 ± 0.71
FMD5	499.05 ± 3.48	4.4 ± 0.26	5.26 ± 0.06	0.46	100.48 ± 1.24
FMD6	497.37 ± 2.32	4.2 ± 0.42	4.87 ± 0.06	0.21	99.34 ± 0.62
FMD7	500.15 ± 1.19	4.1 ± 0.55	4.59 ± 0.05	0.31	100.44 ± 0.42
FMD8	500.60 ± 2.27	4.4 ± 0.25	4.99 ± 0.25	0.25	100.46 ± 1.18
FMD9	498.15 ± 3.84	4.3 ± 0.50	4.96 ± 0.04	0.32	99.35 ± 1.03

SD = Standard deviation

TABLE 5

Effect of mucoadhesive polymers on mucoadhesive strength and mucoadhesive force.

Formulation code	Mucoadhesive strength (grams) Mean ± S.D (n= 5)	Mucoadhesive force (N)	*Ex-vivo* Mucoadhesive time (hours)
FMD1	20.66 ± 0.28	2.02	> 12
FMD2	21.76 ± 0.25	2.13	> 12
FMD3	19.33 ± 0.56	1.89	> 12
FMD4	18.66 ± 0.30	1.83	> 12
FMD5	16.30 ± 0.36	1.59	> 12
FMD6	16.31 ± 0.30	1.60	> 10
FMD7	18.56 ± 0.20	1.82	> 12
FMD8	19.26 ± 0.060	1.88	> 12
FMD9	16.63 ± 0.45	1.63	> 10

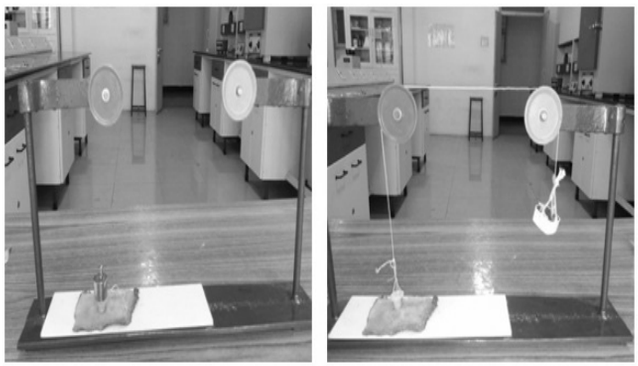

Fig. 2. Method developed to determine the mucoadhesive strength of the mucoadhesive tablets.

In- vitro Drug Release Studies

The *In-vitro* drug release study for the formulations FMD1 to FMD9 was studied. FMD2, FMD3 and FMD8 released more than 90% of the drug in 12 hours and remaining formulations were unable to release the drug completely and sustained for more than 12 hours. This is because of improper wetting of the matrix as high concentration of polymer was selected in order to achieve better mucoadhesion.

FMD2 was selected as optimized formulation based on *in-vitro* drug release and mucoadhesive properties. In the selection of optimized formulation, the *in-vitro* drug release of more than 90% with good mucoadhesion was considered. Formulation FMD2 has shown *in-vitro* drug release of more than 90% at 12 hours and good mucoadhesive strength among the other formulations. The drug release mechanism for formulations FMD1 to FMD9 was found to be zero order except FMD4 and FMD7 which followed first order. When all the formulations were subjected to Higuchi model, the r^2 values were found to be nearer to one, which indicated diffusion based drug release from the matrix. The diffusional exponent values suggested that the drug release from the formulations obeyed both the type of fickian (n<0.45, diffusion controlled) and non fickian (n>0.45, diffusion+ erosion controlled). However, the optimized formulation followed non-fickian mechanism as evident from release exponent (n=0.66). The release kinetics is tabulated in Table 6.

TABLE 6

Regression coefficient (R^2) values of mucoadhesive tablets for different kinetic models.

Formulation Code	R^2				Peppas (n)
	Zero	First	Higuchi	Korsmeyer &Peppas	
FMD1	0.995	0.972	0.950	0.784	0.498
FMD2	0.997	0.933	0.953	0.953	0.661
FMD3	0.997	0.954	0.955	0.907	0.552
FMD4	0.973	0.987	0.992	0.653	0.321
FMD5	0.996	0.984	0.972	0.762	0.455
FMD6	0.992	0.974	0.982	0.853	0.402
FMD7	0.990	0.992	0.988	0.762	0.433
FMD8	0.993	0.957	0.953	0.903	0.529
FMD9	0.995	0.973	0.970	0.806	0.412

R^2 = Correlation coefficient values n = Diffusional exponent values

Mathematical Modeling of Dissolution Profiles

Swelling study: The swelling index of the optimized formulation has shown a good swelling behaviour in 0.1N HCl. The dosage form gradually increased in radial diameter with respect to time and 286.7% swelling index was observed for 24 hours. The swelling index indicates good water uptake by the polymer matrix and good hydration of the polymer matrix, which is desired for the mucoadhesion to the stomach mucosa.

Stability studies: The analysis of the dissolution data, of optimized formulation FMD2 after storage at 40°C ± 5°C/75% ± 5% RH for 6 months showed, no significant change indicating the two dissolution profiles are considered to be similar (f_2 value was more than 50 i.e 86.50 at 3rd month and 81.07 at 6th month and f_1 value less than 15 i.e 3.71 at 3rd month and 4.39 at 6th month) which indicate good similarity between dissolution profiles during the stability period. The assay % and mucoadhesive parameters after the stability period have not shown any much variations in the physical strength of the tablets indicating good physical stability. The physical parameters are tabulated in Table 7.

TABLE 7

Stability study of optimized formulation (FMD2) for mucoadhesive tablets of atenolol.

Parameters observed during stability study	0 month	After 3 month	After 6 month
Assay % Mean ± S.D (n= 3)	99.81 ± 0.51	98.76 ± 0.65	98.45 ± 1.73
Mucoadhesive strength (grams) Mean ± S.D (n= 5)	21.76 ± 0.25	22.20 ± 0.65	21.95 ± 0.55
Mucoadhesive force (N)	2.13	2.17	2.14
Ex-vivo Mucoadhesion time	>12	>12	>12

In-vivo X-ray study: The Prepared Barium Sulfate loaded tablets were evaluated for the following parameters and shown in the Table 8. The gastric residence time of optimized Dipyridamole mucoadhesive tablets were evaluated by conducting *in vivo* X-ray studies in healthy human volunteers. From the radiographic images, it was observed that the gastric residence time for the developed Dipyridamole mucoadhesive tablets was up to six hours in fed condition. The behavior of the mucoadhesive tablet in the stomach of human volunteers was observed in real time using radiographic image

made 15 minutes after the administration, the tablets were observed in human stomach. The next pictures were taken at 2nd hour, 4th hour, and 6th hour and no changes were detected. The tablet had not altered its position, which may be due to the adhesion of dosage form to the gastric mucosa. When next image was taken at 8 hour, it was disappeared from the stomach area. The X-ray images are shown in Figure 8.

TABLE 8

Physical parameters of barium sulfate loaded tablets.

Parameters	Optimized batch (FMD2)	Tablets containing $Baso_4$
Hardness (Kg/cm^2) Mean ± S.D (n= 10)	4.11 ± 0.26	5.2 ± 0.32
Thickness(mm) Mean ± S.D (n= 5)	4.92 ± 0.06	4.97 ± 0.02
Ex-vivo Mucoadhession time (Hours)	> 12	> 12
Mucoadhesive strength (grams) Mean ± S.D (n= 5)	21.76 ± 0.25	21.84 ± 0.50

Discussion

The present research work was carried out to develop an optimized mucoadhesive GRDDS of dipyridamole. Earlier, Katakam et al., and several others worked on dipyridamole due to its maximum solubility in stomach environment compared to lower gastrointestinal tract but very little work was carried out in the field of mucoadhesive systems. So an attempt has been made to develop an optimized mucoadhesive system for dipyridamole by wet granulation method. In this research work, several polymers were studied to obtain a good bioadhesion, in this regard chitosan, $HPMCK_4M$ and isabgul husk have shown better results compared to other polymers. When low concentration of polymers was utilized, required mucoadhesion was not achieved and when higher concentrations were utilized drug, release was very poor. Even individual polymers themselves could not show desired mucoadhesion and drug release so polymers in the combination were utilized. Earlier several authors reported the mucoadhesive properties of chitosan and $HPMCK_4M$. Based on the literature review these polymers were selected. Isabgul husk was tried for its tacky property but the drug release was very poor from the formulations made in combinations with isabgul husk.

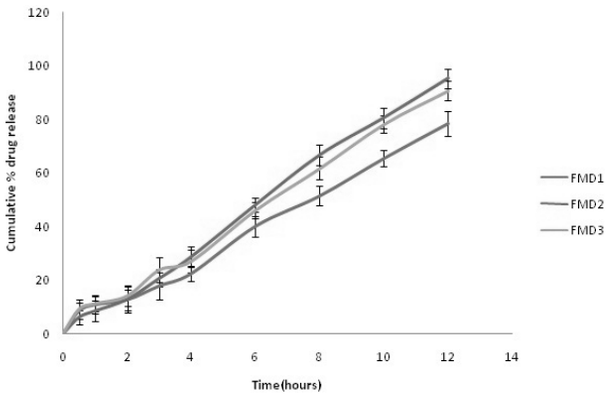

Fig. 3. Cumulative percentage drug release of formulations containing $HPMCK_4M$ and Chitosan (Mean ± S.D, n= 3).

All the physical parameters of the prepared formulations were good and complied to the pharmacopeial limits. In this study, the mucoadhesion of dosage form to gastric mucosal membrane is important so a new simple method was developed to determine the mucoadhesive properties. Earlier S.K Singh et.al and several others utilized a modified physical balance to determine the mucoadhesive strength, but our method of determination of mucoadhesive strength is more simple way to determine the mucoadhesive strength. All the prepared formulations showed good mucoadhesive strength. Formulation FMD2 containing chitosan and $HPMCK_4M$ in combination showed higher mucoadhesion than others and was considered for optimization.

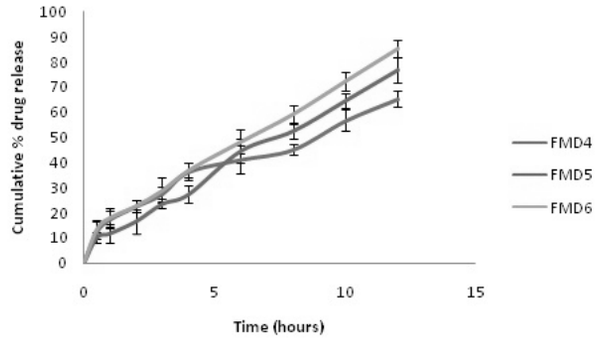

Fig. 4. Cumulative percentage drug release of formulations containing $HPMCK_4M$ and Isabgul husk (Mean ± S.D, n= 3).

The formulation FMD2, FMD3 and FMD8 have shown *in-vitro* drug release of more than 90%. The other formulations were unable to release the drug completely up to 12 hours which may be due to high concentration of polymer was utilized in order to achieve good mucoadhesion. The drug release from all the matrices followed zero order kinetics except FDM1 formulation, which followed first order kinetics. The r^2 values of Higuchi model which are nearer to one suggested diffusion controlled mechanism for all the formulations. The diffusional exponent (n) values for the formulations FMD2, FMD3, FMD5 and FMD8 were found to be higher than 0.45, suggesting a non-fickian diffusion (diffusion & erosion controlled) whereas other formulations followed fickian diffusion (diffusion controlled).

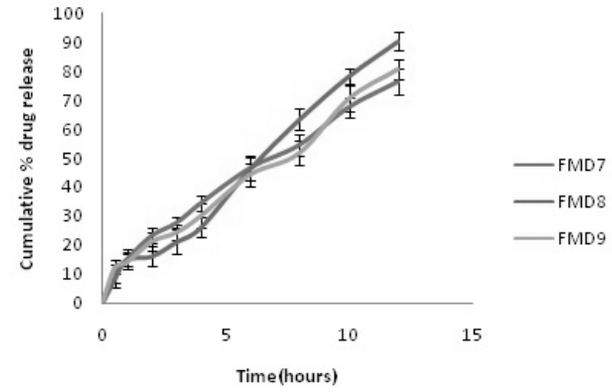

Fig. 5. Cumulative percentage drug release of formulations containing Chitosan, $HPMCK_4M$ and Isabgul husk (Mean ± S.D, n= 3).

Based on *in vitro* drug release and mucoadhesive properties, FMD2 formulation was selected as optimized formulation. It was subjected to swelling studies, stability studies and *in-vivo* radiographic studies and for the assessment of gastric residence time in human volunteers.

The swelling studies indicated that the chosen polymers were having marginally good swelling ability. The optimized formulation when studied for 24 hours, almost 3 times swelling was observed to initial diameter. The swelling index suggests that the dosage have good hydration and high water uptake property, which is essential for mucoadhesion.

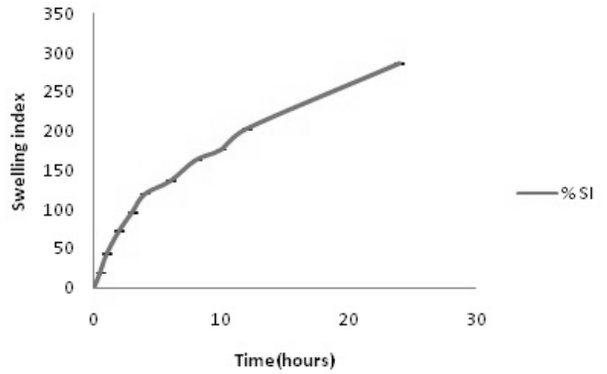

Fig. 6. Swelling index of optimized formulation FMD2 containing Chitosan and HPMC K_4M

The stability study was carried out for 6 months as per ICH and WHO guidelines. During the stability period there was no significant changes were observed for physicochemical parameters which was evident from the similarity factor (f_2) and dissimilarity factor (f_1). Hence, the prepared formulations were found to be stable.

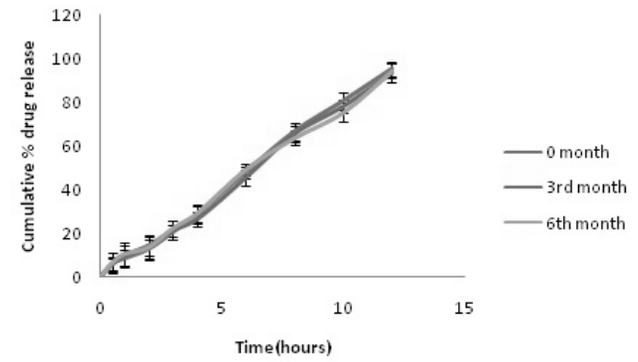

Fig. 7. Cumulative percentage drug release of optimized formulation FMD2 at 0, 3rd, 6th month (Mean ± S.D, n= 3).

Previously Doodipala et al; Katakam et al and several others reported the radiographic studies in human volunteers for floating GRDDS. Based on earlier reports we have conducted the *in-vivo* radiographic studies. The study proved that the optimized dosage form could be successfully retained in the stomach for more than 6 hours. The images of the dosage form when taken at different time intervals was seen at a particular location indicating that retention may be due to mucoadhesion.

The future scope of this study could be to establish the pharmacokinetic parameters of the drug in human volunteers by performing a bioavailability study in human volunteers.

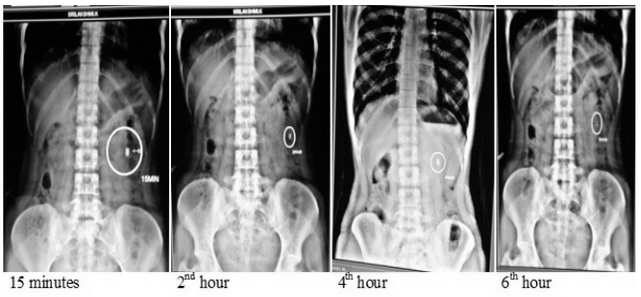

Fig. 8. X-ray images showing the presence of a barium sulfate loaded mucoadhesive tablet in the stomach at different time periods in fed condition (The tablet is indicated with an arrow). Images were taken after a 15 minutes, 2nd hour, 4th hour, and 6thhour.

Conclusions

Gastro-retentive mucoadhesive tablets of Dipyridamole could be successfully prepared by using polymers HPMC K4M and Chitosan. It was concluded that the prepared mucoadhesive tablets of Dipyridamole containing polymers Chitosan and HPMC K_4M formulation (FMD2)(2:1) is the optimized formulation among all other formulations. It showed *ex-vivo* mucoadhesive time of more than 12 hours with maximum drug release of 95.31% in 12 hours. *In-vivo* X-ray studies confirmed that the optimized formulation could be successfully retained in the stomach for more than 6 hours.

Acknowledgements

Authors thank to AET Laboratories for gift sample of Dipyridamole and Vaagdevi institute of pharmaceutical sciences for providing the facilities to carry out the research work.

References

Agaiah Goud B, Kumara Swamy S and Praveen Kumar V (2011). Formulation and evaluation of mucoadhesive buccal tablets of Simvastatin. *JAPS* **1:** 23: 28.

Amish V panchal, Markand Mehta, Viral H. Shah and Umesh Upadhyay (2012). Formulation and *in-vitro* evaluation of mucoadhesive bilayered buccal tablets of Rosuvastatin Calcium. *IJPSR* **3:** 2733-2740.

B. R. Mathews (1999). Regulatory aspects of stability testing in Europe. *Drug Dev Ind Pharm* **25:** 831-856.

Bhupinder Singh, Sukhwinder Kaur chakkal and Naveen Ahuja (2006). Formulation and Optimization of Control release Mucoadhesive tablets of Atenolol using Response surface methodology. *AAPS Pharm Sci Tech* **7:** E19-E28.

Deshmukh VN, Harsulkar AA and Sakarkar (2009). Formulation and invitro evaluation of mucoadhesive tablets using hydrophilic gum blends. *Int J Pharm Res* **1:** 16-22.

Doodipala N, Palem CR, Reddy S and Madhusudan Rao. Y (2011). Pharmaceutical Development and Clinical Pharmacokinetic Evaluation of Gastroretentive floating matrix tablets of Levofloxacin. *Int J Pharm Sci Nanotech* **4:** 1461-1467.

Gupta A, Garg S and Khar RK (1993). Measurement of mucoadhesive strength of mucoadhesive buccal tablets: Design of an invitro assembly. *Indian Drugs* **30:** 152-155.

J. W. Moore and H. H. Flanner (1996). Mathematical comparison of curves with an emphasis on *in-vitro* dissolution profiles. *Pharm. Technol.* **20:** 64-74.

Katakam V.K, Reddy. S, Somagoni J.M, Panakanti P.K, Rallabandi RC and Rao Y.M (2012). Preparation and Evaluation of a Gas Formation-based Multiple-Unit Gastro-Retentive Floating Delivery System of Dipyridamole. *International Journal of Pharmaceutical Sciences and Nanotechnology* **5:** 1607-1616.

Kim SJ, Masaki T, Leypoldt JK, Kamerath CD, Mohammad SF and Cheung AK (2004). Arterial and venous smooth-muscle cells differ in their responses to antiproliferative drugs. *J Lab Clin Med* **144:** 156-62.

Kong SY, Lin R and Chen GM (2003). Preparation of Dipyridamole Floating Tablets in Stomach. *Chin J Pharm* **34:** 395-397.

Lingam. M, Bhasker. K, Krishna Mohan. C, Venkateswarlu. V and Madhussudan Rao Y (2008). Preparation of a matrix type multiple unit gastro retentive floating drug delivery system for captopril based on gas formation technique: *in vitro* evaluation. *AAPS Pharm Sci Tech* **9:** 612-619.

Moes AJ (1993). Gastroretentive dosage forms. *Crit Rev Ther Drug Carrier Syst* **10:** 143-195.

P. L. Ritger and NA. Peppas (1987). A simple equation for description of solute release. I. Fickian and non-Fickian release from nonswellable devices in the form of slabs, spheres, cylinders or discs. *J Contr Rel* **5:** 23-36.

Ranga Rao KV and Buri P (1989). A Novel in situ method to test polymers and coated microparticals for bioadhesion. *Int J Pharm* **52:** 256-270.

Singh JP, Rothfuss KJ, Wiernicki TR, Lacefield WB, Kurtz WL and Brown RF (1994). Dipyridamole directly inhibits vascular smooth muscle cell proliferation *in vitro* and *in vivo*: implications in the treatment of restenosis after angioplasty. *J Am Coll Cardiol* **23:** 665-671.

Singh S.K, Bothara S. B, Singh. S, Patel. R and Dodia. R (2010). Formulation and Evaluation of Mucoadhesive Tablet: Influence of some Hydrophilic Polymers on the Release Rate and *In vitro* Evaluation. *Int J Pharm Sci Nanotech* **3:** 1111-1121.

Address correspondence to: Mr. Kiran Kumar, *Department of Pharmaceutics, Vaagdevi Institute of Pharmaceutical Sciences Bollikunta, Warangal 506005, Telangana State. India.*

Mob: +91-9603799038; E-mail: kiranrips@gmail.com