

Formulation and evaluation of lamivudine sustained release tablet using okra mucilage

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ARTICLE INFO

Article history:

Received on: 28/04/2016

Revised on: 19/05/2016

Accepted on: 12/06/2016

Available online: 26/09/2016

Key words:

Lamivudine, Okra mucilage, sustained release, matrix tablet.

ABSTRACT

The present study was to extract the mucilage from the Okra plant (*Abelmoschus esculentus*) and to study the effect of mucilage concentration on *in vitro* release of Lamivudine from its sustained release matrix tablets. Mucilage was extracted from the fruits of *Abelmoschus esculentus* using organic solvent Acetone. The extracted mucilage was subjected to various physiological properties for its suitability as an excipient in the preparation of tablet. Lamivudine sustained release tablets were prepared using different concentration of Okra mucilage as a sustained release matrix excipient. The formulated tablets were evaluated for post compression parameters such as weight variation, hardness, friability, wetting time, water absorption ratio, and *in vitro* drug release studies. Stability studies of optimized formulation were carried out for three months. The results of *in vitro* release revealed that the release rate decreased with increase in the concentration of mucilage. The release kinetics indicated that the nature of drug release from the matrix tablets was dependent on drug diffusion and polymer relaxation and therefore followed non-fickian or anomalous release. No incompatibility was observed between the drug and excipients used in the formulation of matrix tablets. The Okra mucilage showed promising results in terms of sustaining the release behavior of Lamivudine from the matrix. The developed sustained release tablets of Lamivudine, with extension of release up to 12 hours, can overcome all the disadvantages of conventional Lamivudine tablets.

INTRODUCTION

In recent years, plant gums and mucilages have evoked tremendous interest due to their diverse pharmaceutical applications such as diluents, binders, disintegrants in tablet, thickener in oral liquid, protective colloids in suspensions and gelling agent in gels (Singh *et al.*, 2009; Ameena *et al.*, 2010). Polysaccharides are obtained from plants that could be applied in various pharmaceutical products. Okra mucilage from the pods of *Abelmoschus esculentus* is currently being studied in the pharmaceutical industry as a hydrophilic polymer in pharmaceutical dosage forms. The Okra is a bulky annual plant cultivated throughout the tropical and subtropical areas of the

world, particularly in India. The fresh green pods are rich in mucilage. Okra is widely harvested and does not require toxicology studies. It has been investigated as a binding agent for tablets and has also been shown to produce tablets with good hardness, friability, and drug release profiles (Okoye *et al.*, 2011). Natural materials have advantages over synthetic materials because they are non toxic, less expensive and freely available. It has advantages over most commercial synthetic polymers as it is safe, chemically inert, non irritant, biodegradable, biocompatible, and eco-friendly (Malviya *et al.*, 2011). Mucilages serve as food reserve and membrane thickener and aid in water storage and seed germination. Okra mucilage contains polysaccharides such as galactose, galacturonic acid, rhamnose and when extracted in water these polysaccharides produce highly viscous solution. Therefore, the highly viscous property of Okra mucilage may be useful as a retarding polymer in the formulation of sustained release tablets (Zaharuddin *et al.*, 2014).

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In the present work, we have isolated and characterized Okra mucilage and evaluated its sustained-release properties employing Lamivudine as a model drug. Lamivudine has been used for the treatment of AIDS and chronic Hepatitis B. Lamivudine conventional formulations are administered multiple times a day (150 mg twice daily) because of its moderate half-life ($t_{1/2} = 5-7$ hours). Treatment of AIDS using Lamivudine conventional formulations is shown adverse side effects resulting from accumulation of drug in multidose therapy, poor patient compliance, and high cost. Therefore Lamivudine sustained release formulation can overcome some of these problems (Moyle *et al.*, 2007; Punna *et al.*, 2003). The matrix tablets of Lamivudine were formulated using wet granulation method and evaluated for weight variation, swelling behavior, hardness, friability and *in vitro* drug release.

MATERIALS AND METHODS

Materials

Okra fruits were obtained from the local market. Lamivudine was a gift sample from Aurobindo pharma, Hyderabad. Hydroxy propyl methyl cellulose (HPMC, E15 LV), Sodium carboxy methyl cellulose (Na CMC, Medium Viscosity grade), Microcrystalline cellulose, Starch were obtained from Loba chemie, Mumbai. Magnesium stearate was obtained from Sd fine chem, Mumbai. All chemicals are of analytical grade.

Isolation of okra mucilage

Okra mucilage was extracted from the fruits of *Abelmoschus esculentus* using organic solvent Acetone. The fruits of *Abelmoschus esculentus* were sliced into small pieces and soaked in 1000ml distilled water. It was boiled in a water bath for 1hr at 80°C. The mucilage was separated from *Abelmoschus esculentus* fruits after 1hr. Then the mucilage was precipitated from the filtrate by adding acetone. The precipitated mucilage was dried in oven at 45°C till it was completely dry. The mucilage was milled by using mortar and pestle. The dry powder was passed through 80 mesh sieve and stored in desiccator for further evaluation.

Characterization of okra mucilage

Solubility study

Solubility of the extracted mucilage was evaluated qualitatively by stirring 10mg of Okra powder in 10mL water, acetone, chloroform, and ethanol (1% dispersion). Solubility was determined by visual observation of the solute.

pH Determination

1% W/V okra mucilage dispersion of the sample in water was stirred consistently for 5 minutes and pH was determined using a pH meter.

Viscosity study

Viscosity of Okra gum at 1% W/V concentrations was performed using the Brookfield viscometer (Model DV-E, U.S.A) with helipath stand. Viscosity of the mucilage dispersion was studied at a rotational speed at 10 rpm using a S-64 spindle in triplicate.

Swelling ratio

One gram of mucilage was placed into a 25ml glass Stoppard measuring cylinder. 25 ml of water was added into the cylinder containing mucilage and mixture was shaken thoroughly at intervals of every 10 min for 1 h. The sample was allowed to stand for 3 h at room temperature and volume occupied by mucilage was measured. The mean value was calculated, related to 1 g of mucilage (Srinivas *et al.*, 2003).

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra was recorded of pure drug, polymer (Okra mucilage), and mixture of drug with polymer. The samples were analyzed by KBr pellet method using FTIR spectroscopy. The spectra were scanned over a frequency range 4000-400 cm^{-1} .

Formulation of Lamivudine tablets

The Lamivudine sustained release tablets were prepared using the wet granulation method. The granules were prepared using drug, excipients and okra mucilage powder in concentrations of 2.5%, 5%, 10%, 15%, 20 % (w/w). The moisten coherent mass was prepared and then passed through sieve no 16 and granules obtained were dried at 60°C for 30 min. Magnesium stearate and talc were mixed with dried granules. The uniformly mixed blend was compressed into 450 mg of tablets using rotary tablet compression machine. The tablets were stored in tightly closed containers. Same procedure was followed while preparing of Lamivudine sustained release tablets containing 15% w/w of HPMC and Na CMC as a retarding polymer (Table 1).

Pre compression Parameters

All formulations were evaluated for pre compression parameters such as angle of repose, bulk density, tapped density, Carr's consolidation index, and Hausner's ratio as per the official methods (Bi Y, 1996).

Table 1: Formulation of Lamivudine tablet containing Okra mucilage.

Ingredients	Formulation Code (FC)						
	F1 (2.5%)	F2 (5%)	F3 (10%)	F4 (15%)	F5 (20%)	F6(15%)	F7(15%)
Lamivudine (mg)	300	300	300	300	300	300	300
Okra mucilage (mg)	11.25	22.5	45	67.5	90	-	-
HPMC (mg)	-	-	-	-	-	67.5	-
Na CMC (mg)	-	-	-	-	-	-	67.5
Micro crystalline cellulose (mg)	118.75	107.5	85	62.5	40	62.5	62.5
Magnesium stearate (mg)	10	10	10	10	10	10	10
Talc (mg)	10	10	10	10	10	10	10

Total weight of each tablet was 450 mg; % was mentioned on the basis of concentration of polymer in tablet.

Post compression Parameters

Weight Variation

The weight variation test is carried out in order to ensure uniformity in the weight of the tablets in a batch. The total weight of 20 tablets from each formulation was determined and the average weight was calculated. The individual weight of the tablets was also determined accurately and the weight variation was calculated as specified in IP.

Thickness

Thickness of tablet was measured by using Vernier Calipers. Three tablets were selected at random from each batch and average measurement of three readings was taken.

Hardness

Hardness or crushing strength is the force required to break a tablet in a diametric compression which was measured using a Monsanto tablet hardness tester. It is expressed in kg/cm².

Friability Test

Friability test was carried out using Roche Friabilator. 20 tablets from each formulation were weighed and placed in Roche Friabilator rotated at 25rpm for 4 minutes. The tablets were dedusted and weighed again. The percentage of weight loss was calculated using the following formula (Bi YX *et al.*, 1996):

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

Where 'W1' is the weight of tablet before test and 'W2' is the weight of tablet after test.

Wetting Time

Five circular tissue papers of 10 cm diameter were placed in a Petri dish (10 cm diameter). Ten ml of water containing eosin (a water soluble dye) was added to the Petri dish. A tablet was placed on the surface of the tissue paper. The time required for water to reach the upper surface of the tablet was noted (Modasiya *et al.*, 2009).

Swelling index

Tablets were weighed individually and dispersed in 900 mL of the pH 6.8, phosphate buffer at 37± 0.5 °C and 50 rpm rotation. Tablets were withdrawn at 0.5 hour, 1 hour, 2 hour, 4 hour, 6 hour, and 8 hour intervals and soaked with filter paper to absorb excess buffer solution and then weighed again. Percentage swelling of tablets was expressed as the following (Ravindran *et al.*, 2012):

$$\text{Swelling index (Sw)} = \frac{[W_t - W_0]}{W_0} \times 100 \quad \dots (2)$$

Where 'Wt' is the weight of swollen tablet at time 't' and 'W0' is the initial weight of tablet.

In Vitro dissolution Study

In vitro dissolution studies of prepared tablets were performed using USP II dissolution apparatus (Electrolab TDT-08L) at 50 rpm in 900 ml of pH 6.8 phosphate buffers at 37 ± 0.5

°C. The aliquots were collected at specified time intervals (1,2,3,4,5,6,7,8,12 h) and analyzed at 280 nm by UV visible Spectrophotometer (Simadzu UV 1800, Japan). Sample volume used for analysis was replaced by equal volumes of fresh dissolution medium pre heated at 37 ± 0.5 °C to maintain the sink conditions. The cumulative drug release was then calculated. The study was performed in triplicate.

Drug release Kinetics

To determine the order and mechanism of Lamivudine release from matrix tablet, the release rate data were fitted to zero order, first-order, Higuchi square root equation and Korsmeyer Peppas equation.

Stability Study

The stability studies of selected tablet batches were carried out in stability chamber (Remi Instruments, India) kept at 40°C and 75% RH conditions for three months. The effects of temperature and time on the physical characteristics of the tablet were evaluated for assessing the stability of the prepared formulations.

RESULTS AND DISCUSSION

Extraction method was employed to produce a higher amount of mucilage from okra. Acetone was used as drying agent as it is able to precipitate out the mucilage from the filtrate. Characterization of the extracted Okra mucilage was performed to determine the physical and chemical attributes of the polymer.

Characterization of Okra mucilage

Characterization parameters such as solubility study, pH determination, viscosity study and swelling index of Okra mucilage were calculated and all characterization parameters results are shown in Table 2.

Table 2: Characterization of Okra mucilage.

Parameters	Results
Solubility	Sparingly soluble in water and insoluble in acetone and ethanol.
pH	6.7±0.24
Viscosity	247.34±7.9 cp
Swelling ratio	3.6 ± 0.9

Data represented Mean ± S.D, n=3.

Solubility study

Okra powder was shown to be sparingly soluble in water and insoluble in acetone and ethanol. Okra powder was swelled and formed viscous dispersion when dispersed in water. The slightly soluble behavior of Okra gum is useful in this formulation as the swellable and viscous dispersion represents a strong matrix polymeric system that is able to control the release of Lamivudine.

pH determination

The pH of Okra gum was found to be 6.7. Okra gum is known to have maximum viscosity at a neutral pH range, which

helps in the retarding effect for the development of sustained release tablet.

Neutral pH also causes minimum irritation to the gastrointestinal tract and is suitable for uncoated tablets (Malviya *et al.*, 2011). Moreover, the neutral pH of Okra gum will not alter the pH of Okra tablet that is formulated with Lamivudine.

Viscosity study

Viscosity of Okra mucilage 1% solution was found to be 247.34 cp. Due to more viscous properties of Okra mucilage, it produces more sticky mass, which helps to slowdown the drug release from tablets and provides better tensile strength (Kalu *et al.*, 2007). The mucilage with a higher degree of stickiness creates a more dense material with heavier cross linkage of molecules, therefore it is able to hold the ingredients in a tablet more efficiently and produce tablets with better retarding effects.

Swelling ratio

The swelling ratio of mucilage, determined in distilled water, was observed to be 3.6 ± 0.9 . There was a significant change in swelling by the end of the study, which indicated that the mucilage had excellent swelling properties.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of pure drug, Okra mucilage, and mixture of both are shown in fig.1, fig.2, and fig.3 respectively. The prominent IR absorption peaks of Lamivudine showed at the characteristic peak of the carbonyl group present in the cystidine nucleus at 1650.0 cm^{-1} , a band of peaks at 3328.4 and 3200.2 cm^{-1} owing to amino and hydroxyl groups. Peaks at 1286.2 and 1160.4 cm^{-1} owing to asymmetrical and symmetrical stretching of the C-O-C system present in the oxathiolane ring. The presence of all the characteristic peaks of drug in the IR spectra of drug polymer mixture indicates no interaction between drug and carrier.

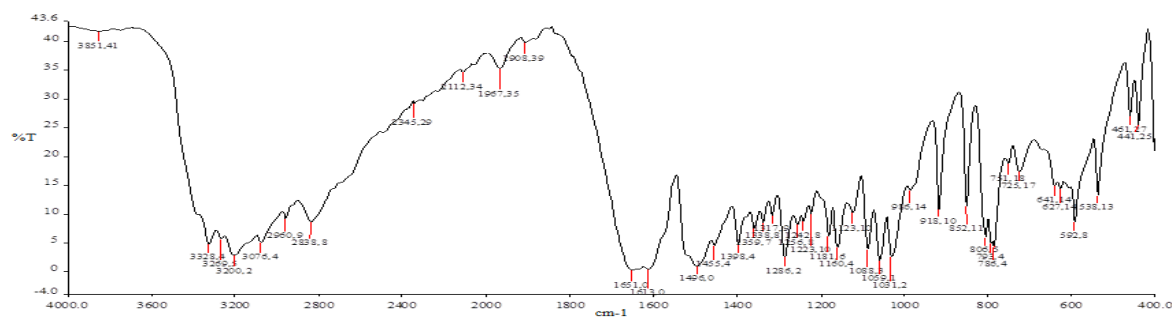


Fig. 1: FTIR spectra of Pure drug (Lamivudine).

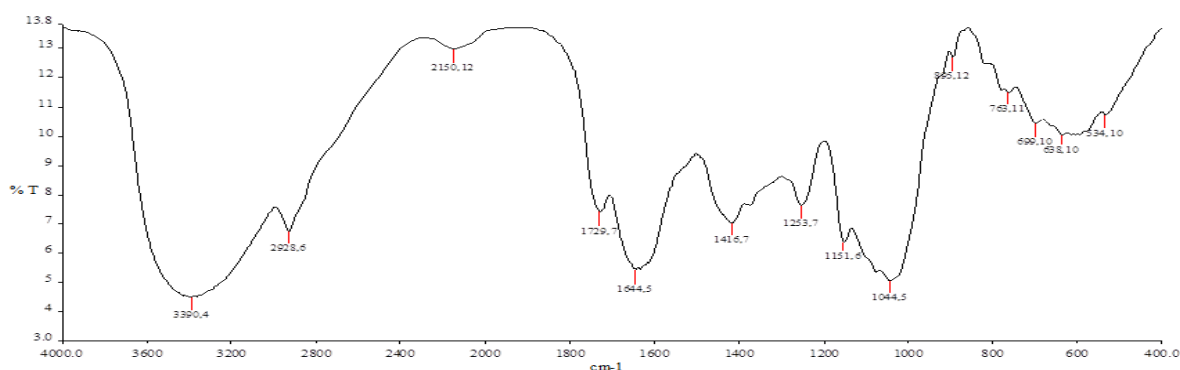


Fig. 2: FTIR spectra of Okra mucilage.

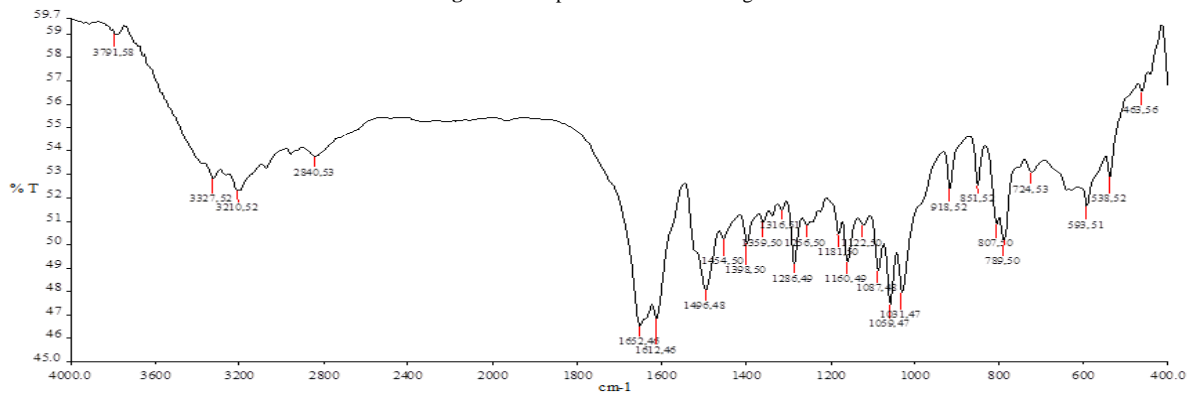


Fig 3: FTIR spectra of mixture of drug and mucilage.

Table 3: Evaluation of Precompression parameters for mixed blends.

Formulation code	Bulk density(gm/cc)	Tapped density(gm/cc)	Angle of repose(°)	Carr's index(%)	Hausner's ratio
F1	0.48±0.09	0.55±0.08	27.6±0.61	12.72±0.91	1.14±0.37
F2	0.47±0.06	0.56±0.05	28.6±0.54	16.07±0.83	1.19±0.72
F3	0.45±0.06	0.53±0.06	27.9±0.69	15.05±1.10	1.26±0.36
F4	0.48±0.08	0.56±0.05	27.9±0.47	14.28±0.96	1.16±0.49
F5	0.46±0.06	0.55±0.05	26.1±0.55	16.36±1.09	1.19±0.43
F6	0.43±0.08	0.51±0.05	25.67 ±0.85	15.68±0.89	1.18±0.54
F7	0.46±0.08	0.54±0.05	27.33±0.45	14.81±1.12	1.17±0.38

Data represented Mean±S.D, n=3

Table 4: Evaluation of Post compression parameters of Lamivudine tablet.

FC	Mucilage concentration (%)	Weight variation (mg), n=20	Hardness (kg/cm ²), n=10	Thickness(mm), n=10	Friability (%), n=20	Swelling index(%)after 8hr ,n=10	Wetting time(min) n=10
F1	2.5	450±2.6	4.9±0.92	3.7±0.1	0.82±0.11	40.16 ±1.1	7.5±0.5
F2	5	449±1.2	6.1±0.87	3.8±0.1	0.61±0.17	43.12±1.9	6.7±0.7
F3	10	452±0.8	6.8±0.92	4.1±0.1	0.21±0.15	44.56±2.8	5.0±0.9
F4	15	449±1.2	6.9±0.86	4.1±0.1	0.12±0.09	50.12±1.7	4.3±0.5
F5	20	447±1.2	7.2±0.58	4.1±0.1	0.13±0.07	58.61±2.1	3.9±0.5
F6	15	449±1.9	5.9±0.68	3.9±0.1	0.46±0.09	76.59±2.6	4.7±0.9
F7	15	452±2.8	6.1±0.89	4.0±0.1	0.73±0.04	82.45±4.7	5.1±0.8

Data represented Mean±S.D.

Precompression Parameters

The results of pre compression studies (bulk density, tapped density, angle of repose, Carr's index, etc.) of mixed blends (F1-F5) are given in Table 3. The results of bulk density and tapped density ranged from 0.45±0.06 to 0.48±0.08 and 0.53±0.06 to 0.56±0.05, respectively. The results of angle of repose (26.1±0.55 to 28.6 ±0.54) indicated good flow properties which were further supported by Carr's index (12.72 % to 16.36 %) and Hausner's ratio data (1.14 to 1.26).

Post compression parameters

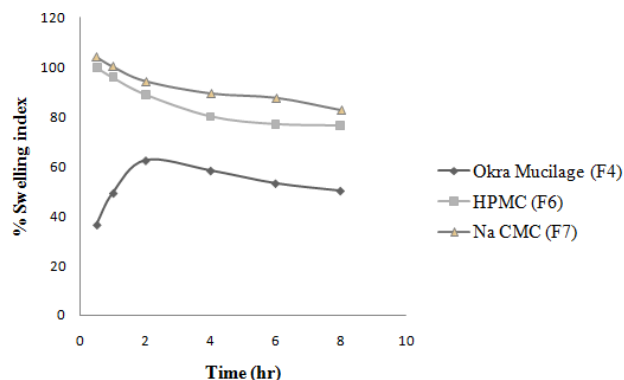
The results of post compression studies are shown in Table 4. All five formulations were uniform in dimensions and exhibited sufficient hardness in the range from 4.9±0.92 to 7.2±0.58. The friability data (<1%) indicated sufficient resistance to abrasion. All the formulation batches passed weight variation test.

The Okra mucilage has a significant impact on swelling and wetting characteristics as seen in batches F1-F5. This enhanced swelling characteristics may be due to the presence of mucilage (Kumar *et al.*, 2009 , Kaur *et al.*, 2014). Okra mucilage showed the highest hardness and lowest friability compared to HPMC and Sodium CMC. This indicates that Okra mucilage produces stronger tablets and is more capable of protecting tablets against capping and lamination (Okoye *et al.*, 2011). It is also a good plasticizer as it forms a smooth film that acts as a coating material for the tablets.

In vitro drug dissolution studies

In vitro dissolution studies are shown in fig 5. Dissolution studies were performed using pH 6.8 phosphate buffer. In order to investigate the effect of polymer concentration on drug release profile, different formulations containing various percentages of *Abelmoschus esculentus* mucilage were used.

The drug release was found to retard more when the concentration of gum increases in the formulation. By increasing the polymer concentration a viscous gel layer is formed resisting to erosion and the diffusion of the drug is controlled primarily by the gel viscosity.

**Fig. 4:** Swelling index of tablets (F4,F6,F7).

The natural gum is hydrophilic, which is used as an excipient for retarding release of drug in controllable manners up to 12hrs. The formulations F3, F4, and F5 containing mucilage concentrations (10%, 15%, 20%) were able to sustain the drug release up to 12 hrs with percentage drug release 73.45%, 69.59% and 53.99%, respectively which indicate that as the concentration of mucilage increased, drug release was retarded due to increase in the gel strength and formation of gel layer with longer path of diffusion, resulting in reduction in diffusion coefficient of the drug (Zaharuddin *et al.*, 2014). The swelling of tablet occurred due to the formation of the matrix layer by the mucilage around the tablet, enabling it to sustain release of drugs. As the swelling continues, the swollen matrix retains more water until the shear forces in the dissolution medium disentangle the individual polymer chains from the matrix. The formulations F1 and F2

containing mucilage concentration 2.5%, 5% were not shown sustained release behavior satisfactorily due to decrease in the gel strength and reduced swelling behavior of the tablet.

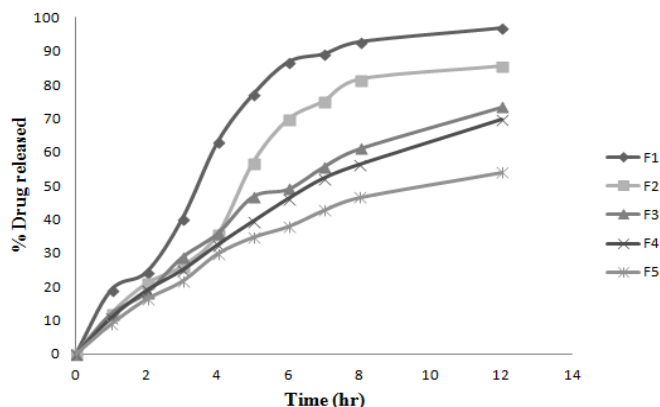


Fig. 5: *In vitro* drug release of Lamivudine (F1-F5) from tablet with various concentrations of Okra mucilage.

The dissolution profile showed that the drug was released in a moderate and consistent manner up to 12 hours. For the swelling index of tablets that were formulated with HPMC and Sodium CMC, the weight of tablets was seen to decrease at the first hour. This indicates that HPMC and Sodium CMC were not that successful in allowing the tablets to swell for the purpose of controlling the rate of release. This could also be seen from the rapid drug release of tablets formulated with these 2 polymers during the first 4 hours of dissolution studies (Fig. 6).

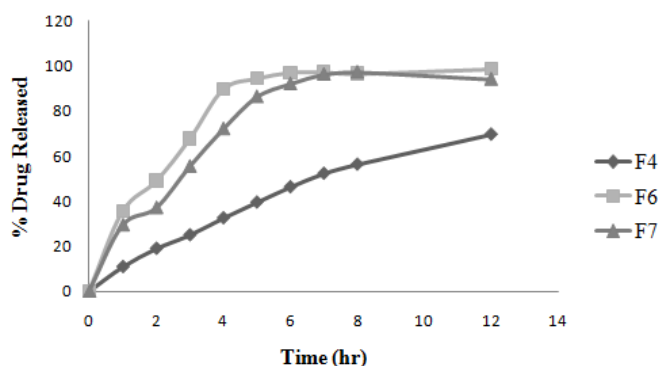


Fig. 6: *In vitro* drug release of Lamivudine (F4,F6,F7) from tablet with various polymers.

The rate of drug release was observed to be the fastest in tablets formulated with HPMC where it reached 70% of release in the first 2 hours and released moderately until it reached maximum release at 4 hours. For Sodium CMC tablet, drug release was seen to be rapid for the first 3 hours and they experienced moderate release up to its maximum release at 5 hours. As for Okra tablets, the release was observed to be relatively consistent until it reached maximum release up to 12 hours.

Drug release kinetics

The drug release kinetics data are shown in Table 5. The *in vitro* release profiles of drug from all these formulations

could be best expressed by Higuchi's equation as the plots showed linearity ($R^2=0.901$ to 0.974). To confirm the diffusion mechanism, the data were fitted into Korsmeyer-Peppas equation. The formulations showed good linearity ($R^2=0.925$ to 0.990) and the 'n' values for all the formulations ranged from 0.75 to 0.83 indicating that the release mechanism was non-fickian or anomalous release ($0.45 < n < 0.89$). It can be inferred that the drug release was dependent on both drug diffusion and polymer relaxation.

For tablets with HPMC and Sodium CMC, the 'n' value of tablets was between 0.29 and 0.39 indicating fickian diffusion principle (Table 5).

The release kinetics studies clearly indicate that the drug release is controlled by diffusion principle. The tablets swell well if immersed in water and the swelling property may control the diffusion of drug from the tablets.

Table 5: Release kinetics of dissolution data of Lamivudine tablet.

FC	Zero order		First order		Higuchi		Korsmeyer-Peppas's	
	K	R ²	K	R ²	K	R ²	n	R ²
F1	8.889	0.829	-0.138	0.971	34.01	0.916	0.77	0.925
F2	8.143	0.889	-0.081	0.940	29.84	0.901	0.83	0.955
F3	6.274	0.943	-0.049	0.943	23.11	0.967	0.78	0.980
F4	5.904	0.964	-0.043	0.947	21.55	0.966	0.78	0.990
F5	4.602	0.926	-0.029	0.956	17.18	0.974	0.75	0.986
F6	6.951	0.634	-0.125	0.764	29.95	0.885	0.29	0.820
F7	6.329	0.524	-0.127	0.765	28.65	0.811	0.39	0.865

Stability Study

Stability studies were carried out using optimized batch F4 as per ICH guidelines for 90 days at accelerated stability condition ($40^{\circ}\text{C}/75\% \text{RH}$). No remarkable changes were observed in batch F4 (physicochemical properties as well as release profile) as shown in Table 6. This indicates good stability of the formulation even after stressed conditions.

Table 6: Stability study of Optimized formulation (F4) at $40^{\circ}\text{C}/75\% \text{RH}$.

Parameters	Time		
	1 month	2 months	3 months
Hardness(kg/cm ²)	6.9±0.86	6.9±0.72	6.6±0.77
Friability(%)	0.12±0.09	0.12±0.10	0.12±0.07
Swelling index (%)	50.12±1.7	51.87±2.1	50.45±1.9
Wetting time(Min)	4.3±0.5	4.5±0.38	4.3±0.65
% Drug released after 12 hours	69.59±3.9	67.15±4.2	66.03±4.6

Data represented Mean ± S.D, n=3.

CONCLUSIONS

Lamivudine sustained release tablets were prepared using Okra mucilage as a sustained release matrix excipient. The Lamivudine tablets were evaluated and the results indicate that as the concentration of mucilage increased, drug release was retarded due to increase in the gel strength and to the formation of the gel layer with longer path of diffusion, resulting in reduction in diffusion coefficient of the drug. It can therefore be concluded that okra mucilage, which is an effective sustained release matrix forming agent may be used for preparation of sustained release Lamivudine tablets.

Financial support and sponsorship: Nil.

Conflicts of interest: There are no conflicts of interest.

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How to cite this article:

Palei NN, Mamidi SK, Rajangam J. Formulation and evaluation of lamivudine sustained release tablet using okra mucilage. *J App Pharm Sci*, 2016; 6 (09): 069-075.