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Research Article

FORMULATION AND EVALUATION OF CONTROLLED RELEASE MUCOADHESSIVE MATRIX TABLETS OF LEVAMISOLE: ASSEMENT OF PURIFIED FRUIT PULP POLYSACCHARIDE ISOLATED FROM *AEGLE MARMELOS* AS MUCOADHESIVE EXCIPIENT

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ABSTRACT

Objective: Mucoadhesive tablets have been developed for Levamisole using natural mucoadhesive material such as purified polysaccharide isolated from the ripe fruit pulp of Aegle marmelos. Lin (AMPS) and evaluate in-vitro, in-vivo mucoadhesive properties to assess its efficacy as mucoadhesive agent.

Method: The tablets were formulated with AMPS in different concentration employing wet granulation method. Pre-compression tests, tablet parametric tests, swelling index and In-vitro drug release kinetics were performed. Optimization of formulation was done on the basis of controlled and maximum amount of drug release with respect to time. In-vitro, ex-vivo evaluation of mucoadhesion was performed with all prepared formulation but in-vivo evaluation was done by X-ray with optimized formulation (F40) in rabbit. Stability studies were carried out according to ICH guide line with optimized formulation.

Results: The tensile strength, mucoadhesive strength and swelling index increase as the natural polymer concentration increase. The release Kinetics and mechanism of release were calculated by fitting in-vitro release data in various models demonstrating that release. In vitro drug release of the optimized batch (F40) was best fitted and explained by Korsemeyer–Peppas and zero order drug release; (Rp=0.9891) and (Ro=0.9842) showed highest linearity and n value 0.97>0.5<1. Hence, pattern of drug release by non-Fickian erosion controlled release. Studies of the optimized formulation demonstrate no significant change in the tensile strength, mucoadhesive strength and drug assay.

Conclusion: These research outcomes clearly specify the potential of the AMPS to be used as mucoadhesive, release retardent natural material in tablet formulation

Keywords: Polysaccharide, Acrylamidopropylmethane sulfonic acid, *Aegle marmelos*, Levamisole, Mucoadhessive.

INTRODUCTION

Bioadhesion can be defined as the binding of a natural or synthetic polymer to a biological substrate. When this substrate is a mucous layer, the term mucoadhesion is often used [1]. Mucoadhesive dosage forms designed to enable prolonged retention at the site of application, providing a controlled rate of drug release for improved therapeutic outcome. Application of dosage forms to mucosal surfaces may be of benefit to drug molecules not amenable to some specific route, such as those that undergo acid degradation or extensive first pass metabolism [2].

Polysaccharide hydrocolloids like mucilage, gums, and glucans abundant in nature and commonly found in many higher plants. These polysaccharides constitute a structurally diverse class of biological macromolecules with a broad range of physicochemical properties which are widely used for various applications in pharmacy and medicine. Recent trend toward the use of vegetable and nontoxic products demands the replacement of synthetic additives with natural one. Many natural polymeric materials have been successfully used in sustained-release tablets include guar gum, ispaghula husk, pectin, galactomannan from *Mimosa scabrella*, mucilage from the pods of *Hibiscus esculenta*, tamarind seed gum. Some newly explored gums *viz*. sesbenia gum, Tara gum are also reported for having sustained release properties. These have found application not only in sustaining the release of the drugs but are also proving useful for development of gastro retentive dosage form, bio adhesive system, microcapsules, etc. [3].

Aegle marmelos Corr. (Rutaceae) commonly called as "Bael" in Hindi language is indigenous to India and the fruits are official in The Ayurvedic Pharmacopoeia of India (2007). The fruit is edible and has been recommended for use as anti-amoebic, anti-diabetic, and antihistaminic [4].

The fruit pulp of *A. marmelos* contains carbohydrates, proteins, vitamin C, vitamin A, angelenine, marmeline, dictamine, O-methyl fordinol, and isopentyl halfordinol. The natural oligosaccharides were characterized a 3-0-beta-D-galactopyranosyl-L-arabinose, 5-0-(beta-D-galactopyranosyl-L-arabinose and 3-0-beta-D-galactopyranosyl-D-galactose and acidic oligosaccharides as 3-0-(beta-D-galactopyranosyluronic acid)-D-galactose and 3-0-(beta-D-galactopyranosyluronicacid)-3-O-beta-D-galactopyranosyl-D galactose [5].

Levamisole, marketed as the hydrochloride salt under the trade name Ergamisole (R12564), an anthelmintic and immunomodulator belonging to a class of synthetic imidazo-thiazole derivatives [6,7]. The low bioavailability (47%) and short biological half-life (4.4-5.6 hrs) of Levamisole following oral administration favors the development of sustained release formulation [8,9].

Gum isolated from the fruit pulp of *A. marmelos* used as tablet binder, mucoadhesive agent in formulating tablets and showing promising result. The use of DEAE-Sephadex-50 purified polysaccharide fraction of the fruit pulp as oral mucoadhessive tablet excipients is not reported till date. Therefore, the aim of this study was to formulate and evaluate mucoadhesive tablet by using the purified polysaccharide isolated from the fruit pulp of *A. marmelos* (acrylamidopropylmethane sulfonic acid [AMPS]) using Levamisole as a model drug by wet granulation method. The formulated tablets were evaluated for various physical characteristics, *in-vitro* dissolution and drug release kinetics study. Mucoadhesive property of the polysaccharide as well as the formulation made by AMPS was evaluate by *in-vitro* and *in*-vivo study in rabbit model.

METHODS

Levamisole was kindly gifted by Wockhardt Limited, Baddi, India. Polyvinyl pyrrolidine K-30 was obtained from Alembic, Vadodra, India. Other chemicals were purchased from SD Fine Chemicals Ltd., Mumbai, India. All other chemicals and reagents used were of analytical grade. AMPS was prepared in by purifying the crude polysaccharide isolated from the fruit pulp of *A. marmelos* with DEAE-Sephadex-50.

Acute toxicity of purified polysaccharide

Healthy male and female Swiss albino mice (8-9 weeks) were used for the acute oral toxicity study, breed and reared at the animal house of the institution (Girijananda Chowdhury Institute of Pharmaceutical Science [GIPS]). The animals were housed in polypropylene cages and provided with bedding of clean paddy husk. The animals were acclimatized to laboratory conditions for 1-week prior to the experiment. The temperature in the animal house was maintained at 25±2°C with a relative humidity (RH) of 30-70% and illumination cycle set to 12 hrs light and 12 hrs dark. The mice were fed with standard laboratory pelleted feed. All the mice of both the sexes were fasted overnight before experimentation and were allowed to take food 1-hr after the experiment. AMPS was administered orally at a dose of 5, 50 300 2000 mg/kg body weight in distilled water. The animals were observed for any mortality and morbidity (convulsions, tremors, and grip strength and pupil dilatation) at an interval of 12 hrs for 14 days. This study was approved by the Animal Ethics Committee of GIPS (Regn. No.1372/C/10/CPCSEA), study approval no No-GIPS/IAEC/04 year 2011-13 [10,11].

Preparation of tablets

AMPS based mucoadhesive tablets of Levamisole were prepared by wet granulation method using different compositions as shown in Table 1. All the ingredients were screened through sieve no. 60 and then blended (except magnesium stearate and talc) for 15 minutes. A blend of all ingredients was granulated with 95% isopropyl alcohol. The wet masses were passed through sieve no. 12 and the resulting granules were dried at 40°C. The dried granules were again passed through sieve no. 22. Finally magnesium stearate and talc was added and mixed for 5 minutes. The micromeritic studies were carried out for all the granules. The results of angle of repose, Carr's index and Hausner ratio indicated that the granules possess good flow property and good packing ability. Tablets were compressed on a 8-station Mini Press-I rotary tablet compression machine (Shakti Pvt. Ltd) fitted with 8 mm flat-shaped punches using sufficient compression force to obtain a hardness of 4-5 kg/cm² containing 50 mg of Levamisole per tablet. No manufacturing defects were observed in tablets such as capping, lamination, and chipping. Tablets of batch F10-F50 contain single mucoadhesive polymer (AMPS) having concentration 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, and 50%, respectively [5].

Precompression study [5,12,13]

Dug excipient interaction study

Table 1: Formulae of Levamisole mucoadhesive tablet

Ingredients	Weight of ingredients mg/tablet								
	F10	F15	F20	F25	F30	F35	F40	F45	F50
Levamisole	50	50	50	50	50	50	50	50	50
AMPS	16	24	32	40	48	56	64	72	80
PVP (K30)	6	6	6	6	6	6	6	6	6
MCC	83.2	75.2	67.2	59.2	51.2	43.2	35.2	27.2	19.2
Magnesium stearate	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
Talc	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Total tablet weight	160	160	160	160	160	160	160	160	160

AMPS: Acrylamidopropylmethane sulfonic, MCC: Microcrystalline cellulose, PVP: Polyvinyl pyrollidone

The physicochemical interaction between Levamisole and was studied by Fourier transform infrared spectroscopy (FTIR) (spectrometer-430, Bruker Alpha FTIR) and differential scanning calorimetry (DSC) (JADE DSC, Perkin Elmer, USA).

Evaluation of the granule

The angle of repose was measured by using funnel method, which indicates the flow ability of the granules. Loose bulk density (LBD) and tapped bulk density (TBD) were measured using the formula: LBD = weight of the powder/volume of the packing. TBD = weight of the powder/tapped volume of the packing. Compressibility index of the granules was determined by using the formula: confidence interval (%) = ([TBD-LBD/TBD]) × 100.

Evaluation of the tablet

All prepared mucoadhesive tablets were evaluated for its uniformity of weight, hardness, friability and thickness according to official methods. The weight variation was determined by taking 20 tablets using an electronic balance. Tablet hardness was determined using a Monsanto tablet hardness tester; friability was determined by testing 10 tablets in a Roche friability tester for 4 minutes at 25 rpm.

Drug content

Five tablets were powdered in a mortar. An accurately weighed quantity of powdered tablets (100 mg) was extracted with 0.1 N HCl (pH 1.2 buffer) and the solution was filtered through 0.45 μ membranes. Each extract was suitably diluted and analyzed spectrophotometrically at 215 nm.

Swelling studies of the tablet [14-16]

Swelling study of individual batch was carried out using USP Type II dissolution apparatus (Basket type, Lab India, DISSO 2000). For each formulation batch, one tablet was weighed and placed in the stainless steel basket of the dissolution apparatus and weighed. The basket was then placed in the dissolution beaker containing 900 ml of 0.1 N HCl media and rotating speed of the basket is 100 rpm. The tablets were allowed to swell at 37±0.5°C; the basket was periodically weighed up to 12 hrs after removing the excess water on the surface with a filter paper. The swelling index was calculated using following formula.

$$\%\text{Swelling index} = \frac{W_t - W_0}{W_0} \times 100$$

Where, W_0 was the weight of the tablet before placing into the dissolution basket. W_t the difference of the ([basket and tablet weight after time t] – tablet weight at the initial time [W_0]).

In-vitro drug release study

Drug release was assessed by dissolution test under the following conditions: n=6 (in triplicate), USP Type II dissolution apparatus (Lab India, DISSO 2000) at 50 rpm in 900 ml of 0.1 N HCL maintained at 37±0.5°C. The tablet was allowed to sink to the bottom of the flask before stirring. Special precaution was taken not to form air pockets on the surface of the tablet. 5 ml of the sample was withdrawn by using a syringe filter at regular intervals and replaced with the same volume of pre warmed (37±0.5°C) fresh dissolution medium. The drug content in each sample was analyzed after suitable dilution using ultra violet spectrophotometer method at 215 nm.

In-vitro release kinetics

For the purpose to compare the dissolution profiles, several approaches can be followed such as Analysis of Variance (ANOVA)-based model-independent and model dependent approaches. In this work, model dependent approaches were used for comparison of dissolution profiles. ANOVA based is commonly used to detect significant differences between groups and thereby can be used to detect statistically significant differences between dissolution profiles.

In the model-dependent approaches, the order of drug release from matrix systems was described by using zero order or first order kinetics. The mechanism of drug release from matrix systems was studied by using Higuchi diffusion model and Hixon-Crowell erosion model. Korsemeyer–Peppas support the drug release mechanism for further judgment. The respective equations for these models are shown in Table 2. According to Korsmeyer–Peppas equation, the release exponent 'n' value is used to characterize different release mechanisms for a dosage form with cylindrical shape and summarized in Table 3 [17].

Optimization of formulation [11,18]

Optimization of formulation was done on the basis of sustained release pattern of drug release from the matrix tablet in different time interval. Amount of drug released at the end also considered as a criteria for optimization of the formulation.

***In-vitro* mucoadhesive test of the AMPS**

Shear stress method

The *in-vitro* bioadhesion was measured as a detachment force measurement, or the force required for detaching from the mucosal tissue. The reproducibility of the test system was initially examined. After each measurement, the tissue was replaced by fresh piece and finally detachment force was measured. The amount of shear in gram increases as the holding time increases. After 5, 10 and 15 minutes of holding time the detachment weight requirement was 14.78±0.26 g, 28.70±0.35, and 75.3±0.15, respectively. This result is comparable to the result as per synthetic polymer hydroxypropylmethylcellulose (HPMC) 3% and natural polymer chitosan 3% respectively as per literature [18]. This indicates that higher holding time requires the most maximum force in grams to break the bond between the polysaccharide and the mucosal surface. Further, the wetting time is more as the holding time increases and the carboxylic acid structure in polysaccharide gradually undergo hydrogen bonding.

Table 2: Mathematical model for comparision of dissolution data

Model	Equation
Zero order	$Q_t=Q_0+K_0t$
First order	$\text{Log } C=\log C_0-Kt/2.303$
Higuchi	$Q=K_H\times t^{1/2}$
Hixon-Crowell	$(W_0^{1/3}-W_t^{1/3})=\kappa t$
Korsmeyer-Peppas	$M_t/M_\infty=K.t^n$

Q_t: Amount of drug released in time t; Q_0: Initial amount of drug in the tablet; C_0: Initial amount of drug in the dosage form; W_t: Remaining amount of drug in the dosage form at time t; M_t: The amount of drug released at time t; M_∞: Amount released at time ∞; M_t/M_∞: Fraction of drug released at time t; K_0, K_t, K_H, κ, K: Rate constants

Table 3: Diffusion exponent and drug release mechanisms

Release exponent (n)	Drug release mechanism	Rate as function of time
0.5	Fickian diffusion	$t^{-0.5}$
0.45<n=0.89	Non-Fickian diffusion	t^{n-1}
0.89	Case II transport	Zero order release
Higher than 0.89	Super Case II transport	t^{n-1}

***In-vitro* evaluation by Falling sphere method**

In-vitro mucoadhesive property of the AMPS polysaccharide was evaluated by falling sphere method. For this purpose a clean burette (Fig. 5) was taken and filled with 10% mucus solution and fixed in a stainless steel tube. Mustard grains which retained on sieve size # 12 were taken and dipped in polysaccharide solutions (1%-5.0% w/v) and then each grain were slowly placed at the top of the mucus layer. Time taken by the grain to fall 50 divisions in the burette was noted. The result was compared with the result of the synthetic polymer in place of isolated polysaccharide as reported in literature [18-20].

***Ex-vivo* mucoadhesive time**

The *ex-vivo* mucoadhesion time was performed after application of the tablet on freshly goat stomach mucosa. The fresh goat stomach mucosa was tied on the glass slide with the help of double sided tape and the tablet was wetted with 1 drop of 0.1 M HCl (pH 1.2) and pasted to the goat stomach mucosa by applying a light force with a fingertip for 30 seconds. The glass slide was placed at the bottom of vessel paddle type USP Type-II (Lab India, DS 8000) apparatus. The test was performed with 900 ml of the 0.1 N HCl at 37±0.5°C. After 2 minutes, a 50 rpm stirring rate was applied to simulate the stomach environment, and tablet adhesion was monitored for 24 hrs. The time for the tablet to detach from the goat stomach mucosa was recorded as the mucoadhesion time [4,21].

Evaluation of *in-vivo* mucoadhesion of the optimized tablets

Evaluation of in-vivo mucoadhesion by X-ray imaging

In case of X-ray imaging technique of *in-vivo* evaluation of mucoadhesion, studies was carried out in healthy rabbit. Barium sulphate ($BaSO_4$=25 mg/80 mg tablet, 4 mm punch) was incorporated by replacing Levamisole in optimized mucoadhesive tablet formulation as an X-ray opaque material. The total study protocol was approved by animal ethics committee with CPCSEA Regn. No.1372/C/10 CPCSEA. Study approval No-GIPS/IAEC/04 year 2011-12. Optimized $BaSO_4$ tablet was prepared with polysaccharide (AMPS) by melt granulation method in the similar way as described in their preparation method. The prepared formulation was administered to the rabbit by oral feeding tube (tracheal tube no-4) with mild local anesthetic (xylocaine gel 2%). During the study, the rabbit was not allowed to eat any food, but water was available *ad libitum.* Images were recorded (Siemens, Polytron animal X-ray) at intervals of 1, 2, 4 and 8 hrs [22]. The study was performed under the supervision of Dr. Kushal Konwar Sarma, Head of the Department of Surgery and Radiology, College of veterinary Sciences, Khanapara, Guwati-22.

RESULTS

Acute toxicity of polysaccharide

A 14 day acute oral toxicity study was performed in swiss albino mice. It was observed that the animals fed with the AMPS were found to be healthy. No unusual changes in behavior or in locomotor activity, ataxia, and signs of toxicity were observed during the 14 days

Table4: Precompression parameters of granules

Formulation	Tab density	Bulk density	Hausner's ratio	Car's index	Angle of repose
F10	0.589±0.005	0.519±0.006	1.134±0.5	11.83±0.42	27.033±0.76
F15	0.58±0.01	0.48±0.01	1.166±0.02	14.19±1.65	27.910±0.52
F20	0.53±0.023	0.44±0.024	1.194±0.014	16.24±0.95	27.98±2.03
F25	0.585±0.025	0.487±0.018	1.20±0.02	16.68±0.95	28.15±0.58
F30	0.5767±0.008	0.5130±0.01	1.124±0.015	11.04±1.15	26.250±0.98
F35	0.580±0.004	0.495±0.012	1.172±0.020	14.65±1.47	27.1±1.645
F40	0.553±0.004	0.488±0.005	1.133±0.009	11.74±0.71	26.87±0.85
F45	0.587±0.018	0.489±0.005	1.20±0.024	16.615±1.6	27.057±0.97
F50	0.563±0.005	0.4613±0.004	1.220±0.018	18.05±1.20	28.973±1.27

(All the values are express as mean±SD, n=3), SD: Standard deviation, AMPS: Acrylamidopropylmethane sulfonic period. No differences were found in growth behavior between the control and treatment group in 14 days of study. The body weight of male and female swiss albino mice was found to be normal after treatment [10,11].

Drug polysaccharide interaction study

The FTIR spectra of pure drug Levamisole, AMPS and Levamisole-AMPS mixture are shown in Fig. 1 the FTIR spectrum of Levamisole showed peak at 2631.69 cm^{-1} due to C=N stretching, peaks at 1572.48, 1519.44 and 1435.14 cm^{-1} due to C=C (aromatic) stretching, 1201.33 cm^{-1} due to C-N stretching, 734.99 cm^{-1} due to C-S, 1964.92 cm^{-1} due to S $(=O)_2$ asymmetric stretching and 838.17 cm^{-1} due to C-Cl symmetric stretching confirming the drug structure. The IR spectrum purified AMPS showed peaks at 3246.85 cm^{-1} due to –OH stretching of primary alcohol. The absorption peaks at 2976 cm^{-1} and 2889 cm^{-1} are indicative of –CH stretching vibration of methyl group. The absence of significant aromatic stretches in the 1739 cm^{-1} region and the weakness of the stretches imply that there is modest amount of cross linking by peptides. The bands at 1607 cm^{-1} is characteristic of C=O of aldehyde. Peak at 1374 cm^{-1} is due to symmetrical deformation of $-CH_2$ and C-OH group. Weak bond at 769.38 cm^{-1} due to the r contribute to the ring stretching and ring deformation of α-D-(1-4) and α-D-(1-6) linkage.

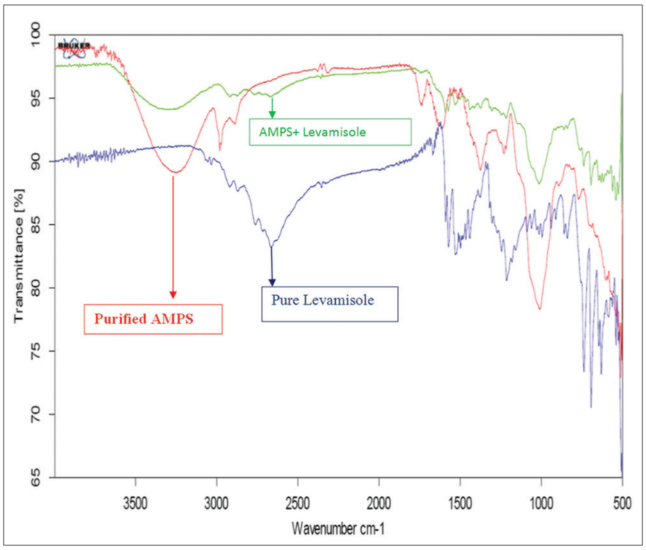

Fig. 1: Fourier transform infrared spectra of acrylamidopropylmethane sulfonic and Levamisole and mixture

The DSC curves of Levamisole, AMPS, Levamisole-AMPS mixtures, combination of three and DSC of the tablet are presented in Fig. 2 Levamisole exhibited a single melting endothermic peak corresponding to the melting of the drug. Onset of melting of Levamisole was observed at 234.24°C.

Per-compression parameter

Prepared granules of all the formulations are tested (Table 4) for their pre compression parameters like tap density, bulk density, Hausner's ratio, Car's index, and angle of repose for the evaluation of granules flow properties.

Evaluation of post compression parameters

The assessment results of thickness, hardness, friability and drug content are presented in Table 5. The tablet thickness was found to be in the range of 2.79±0.04 - 2.91±0.04. The hardness of all tablet were in the range of 4.0±0.26 - 6.27±0.1 kg/cm^2.

Swelling study

The percentage of swelling Fig. 3 ranging from 16% to 19% in first hour, but increases over 200% in 10-12 hrs. Some tablet shows decrease amount of swelling between 10 and 12 hrs because of erosion and breaking occurred in 10-12 hrs due to swelling.

***In-vitro* dissolution profiles of Levamisole tablets prepared with AMPS**

The *in-vitro* drug release profiles of matrix AMPS tablets are shown in Fig. 4. The results indicated slow and controlled release of Levamisole

Table 5: Post compression parameters of the tablets of AMPS

Parameters	Formulation batches								
	F10	F15	F20	F25	F30	F35	F40	F45	F50
Thickness (mm)[a]	2.89±0.04	2.79±0.04	2.797±0.06	2.85±0.11	2.85±0.07	2.81±0.07	2.91±0.04	2.79±0.05	2.85±0.03
Hardness (Kg/cm^2)[a]	4.0±0.26	4.2±0.25	4.650±0.05	4.92±0.16	5.863±0.08	5.90±0.20	6.27±0.10	5.97±0.16	5.95±0.06
Friability (% w/w)[b]	0.54±0.02	0.48±0.01	0.445±0.01	0.46±0.02	0.628±0.01	0.64±0.01	0.58±0.01	0.51±0.02	0.49±0.04
Uniformity of weight (mg)[b]	157.8±1.01	161.20±2.99	163.57±1.20	158.53±1.06	160.9±1.44	159.67±0.81	160.80±0.70	158.73±0.67	160.03±1.65
Uniformity of content (% w/w)[b]	97.86±0.42	99.95±0.61	101.19±0.62	99.10±1.30	100.31±1.35	98.50±0.36	100.10±1.00	99.26±0.55	100.75±0.38

[a]: Mean±SD, n=6; [b]: Mean±SD, n=20, SD: Standard deviation, AMPS: Acrylamidopropylmethane sulfonic

Table 6: Release kinetics study of formulated baches of AMPS mucoadhesive matrix tablets

Model	Parameters	Formulation code								
		F10	F15	F20	F25	F30	F35	F40	F45	F50
Zero order	R^2	0.9843	0.9755	0.9886	0.9809	0.9593	0.9728	0.9842	0.9817	0.9790
	K_0	0.404	0.306	0.267	0.177	0.155	0.149	0.140	0.144	0.148
First order	R^2	0.8864	0.8768	0.9015	0.9436	0.9616	0.9584	0.9391	0.9559	0.9506
	K_1	0.007	0.005	0.004	0.003	0.003	0.003	0.002	0.002	0.003
Higuchi	R^2	0.8137	0.7854	0.8184	0.8759	0.9064	0.8993	0.8488	0.8877	0.8822
	KH	5.110	4.284	4.111	3.418	3.305	3.176	2.944	3.056	3.144
Hixon-crowel	R^2	0.9226	0.9109	0.9346	0.9703	0.9842	0.9811	0.9673	0.9785	0.9226
	KHC	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Korsemayer - Peppas	R^2	0.9927	0.9949	0.9957	0.9866	0.9840	0.9887	0.9891	0.9908	0.9876
	KKP	0.158	0.061	0.107	0.338	0.550	0.430	0.249	0.331	0.332
	n	1.179	1.294	1.162	0.893	0.796	0.830	0.907	0.867	0.871

AMPS: Acrylamidopropylmethane sulfonic

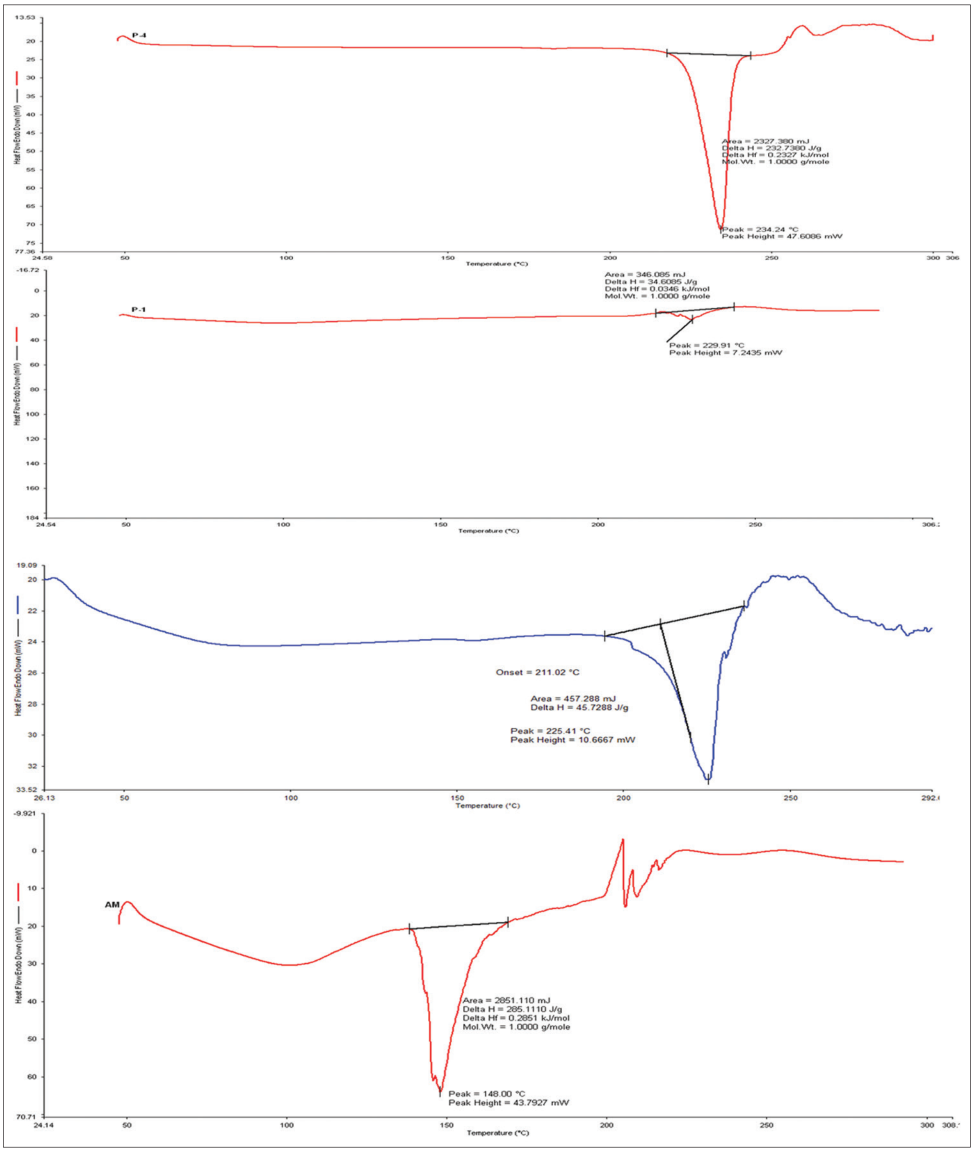

Fig. 2: Differential scanning calorimetry (DSC) thermogram of Levamisole, acrylamidopropylmethane sulfonic and Levamisole mixture, DSC of Levamisole tablet

from the matrix tablet. In the formulations, release of Levamisole in the first hour varied between 16.33±1.97 in F10 and 5.42±0.80 in F40. The release of drug extended from 10 hrs in F25 to more than 12 hrs in F40, F45, F50 in the matrix tablet.

Kinetics and mechanism of drug release

The release rate constant was calculated from the slope of the appropriate equations and the correlation coefficient (R) was determined for all formulations given in Table 6. In most of the formulated tablets, the R values were higher in zero order and Korsemayer–Peppas model than other model. Determination of correlation coefficient from various formulations, containing different proportions of AMPS indicates that zero order and Korsemeyer–Peppas equations seemed to be better fit than other equations.

Shear stress method

The *in-vitro* bio adhesion was measured as a detachment force measurement, or the force required for detaching from the mucosal tissue. The reproducibility of the test system was initially examined. After each measurement, the tissue was replaced by fresh piece and finally detachment force was measured. The amount of shear in gram increases as the holding time increases. After 5, 10, and 15 minutes of holding time the detachment weight requirement was 14.78±0.26 g, 28.70±0.35, and 75.3±0.15, respectively.

Falling sphere method

Time in seconds required to move the mustard grain from top to bottom in 5% w/v solution was found to be 57.04±0.08 seconds.

Ex-vivo evaluation of mucoadhesive time

The results of the mucoadhesive time of Levamisole tablet are presented in Table 7. The residence time of the formulations ranged between 66.2±2.7 minutes and 372±6 minutes.

Evaluation of *in-vivo* mucoadhesion by using X-ray study

For the purpose of confirming the mucoadhesive property of the optimized tablet, further *in-vivo* investigation was carried out by using X-ray study. X-ray images are shown in Fig. 5.

Extended stability study

The optimized mucoadhesive tablets (F40) was selected for stability study. The result of the stability study of the optimized formulation is given in Table 8. The tablets were kept in an environmental chamber for 3 months at 40±2°C and 75±5% RH.

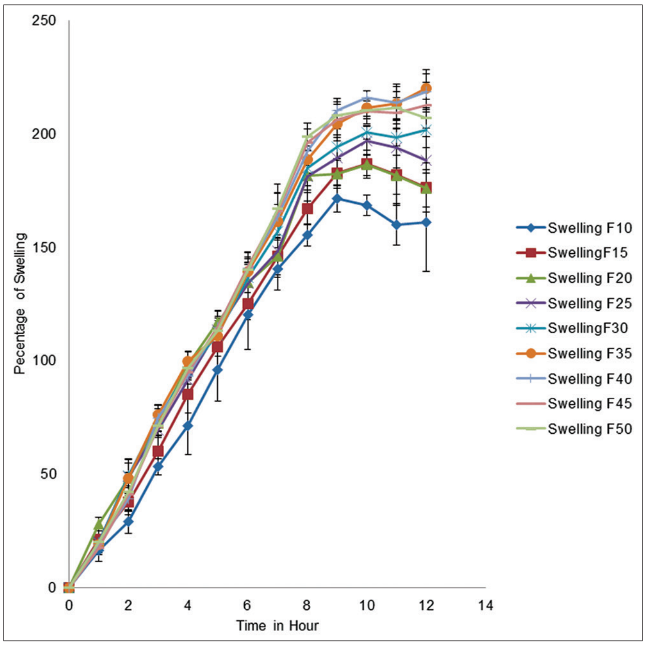

Fig. 3: Swelling index of the acrylamidopropylmethane sulfonic tablets

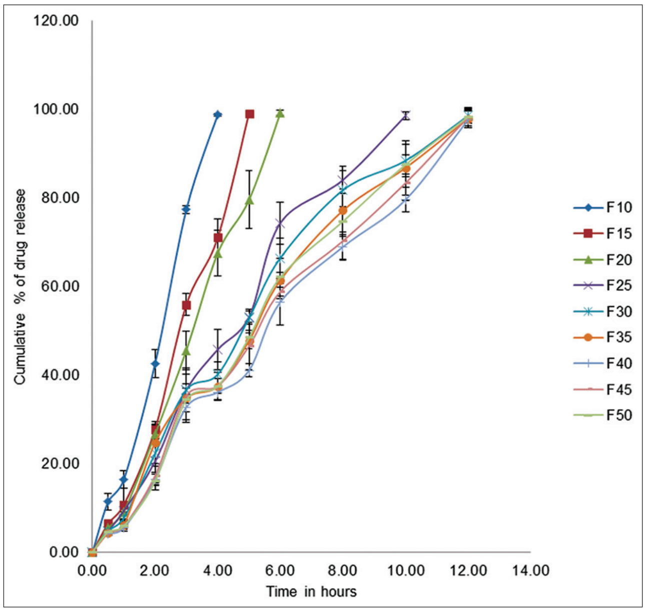

Fig. 4: *In-vitro* dissolution profiles of Levamisole tablets prepared with acrylamidopropylmethane sulfonic

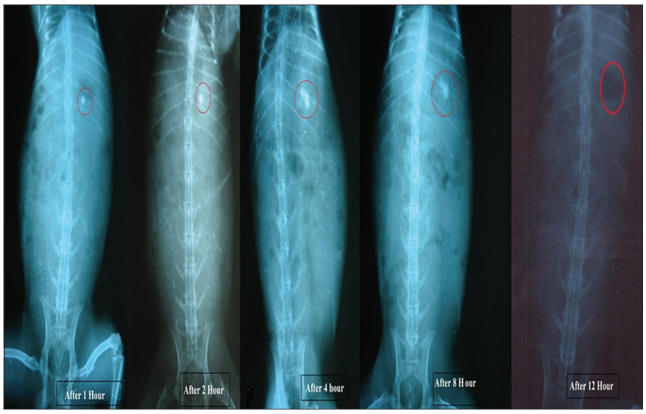

Fig. 5: X-Ray images of rabbit for mucoadhesive study of acrylamidopropylmethane sulfonic mucoadhesive tablet

Table 7: Detachment time in ex-vivo mucoadhession

Formulation	In 0.1 N HCl	
	Detachment time in hr	Detachment time in minutes
F10	1.10±0.05	66.2±2.7
F15	1.47±0.05	88±3
F20	2.43±0.03	145.8±1.6
F25	3.41±0.05	204.4±3.1
F30	4.41±0.10	264.6±5.8
F35	5.42±0.03	325±1.7
F40	6.14±0.56	384.6±5.8
F45	5.67±0.38	356±9.2
F50	6.2±0.1	372±6

The results are mean±SD (n=3), SD: Standard deviation

Table 8: Extended stability study of F40 tablets of AMPS

Parameters	Formulation F40		
	After 30 days	After 60 days	After 90 days
Hardness (Kg/cm^2)	3.2±0.35	3.8±0.2	3.85±0.15
Friability (% w/w)	1.55±0.12	1.40±0.15	1.95±0.15
Uniformity of weight (mg)	157.87±1.01	161.20±2.99	163.57±1.20
Uniformity of content (% w/w)	97.16±0.12	95.75±0.61	94.88±0.25
In-vitro drug release (%) 12 hrs	96.15±0.16	94.38±0.32	93.85±0.21
Mucoadhesive strength (N)	34.19±1.91	29.95±0.92	23.33±0.9

The results are mean±SD (n=3), SD: Standard deviation,
AMPS: Acrylamidopropylmethane sulfonic

DISCUSSIONS

The purpose of this study was to evaluate toxicity profile of the AMPS. The LD50 of all the AMPS was not further studied as they were found to be safe up to 2000 mg/kg on 24 hrs study basis [11].

The result showed polysaccharide structure that is neither starch nor cellulose, but has some peptide cross links and some amino sugars. The FTIR spectrum of mixture of the tablet showed all the characteristic peaks of Levamisole indicating the non-interaction between drug and polysaccharide in the formulation. All the peaks of AMPS remain unchanged. The results from FTIR spectroscopy showed that there was no significant change in the FTIR spectrum of Levamisole in mixture with AMPS [5,24,25].

The results from DSC study confirmed that there was no significant change in the melting endotherm of Levamisole in the mixture with AMPS as it moves to 225.41°C, which was not significant for considering as prominent drug excipient interaction. Any sign of interaction would be reflected by a change in melting endothermic of Levamisole. Hence, there was no well-defined chemical interaction between drug and polysaccharide. DSC of tablet also shows no interaction between Levamisole and other tablet excipients.

The angle of repose is a characteristic of internal friction or cohesion of the particle. So high value ø is indicating high cohesiveness and lower value signifies low cohesiveness among the particle. The angles of repose or ø values of all the formulation are within 25-29 indicating good flow properties of the granules. Compressibility index and Hausner's ratio of all formulation are within the range of 11-19 and below 1.22 satisfactory for compression. Hardness increased as the amount of concentration of AMPS increased. This indicates the binding potentiality of the polysaccharide. Since tablet hardness is not a perfect index to evaluate the strength of the tablets, friability percentage was also used to test the hardness of tablets. The friability values of all the prepared tablets were <1% which indicated that the test compiled with the official compendial tests for tablets as per IP. The tablets prepared in each batch were found to have within the limit of weight variation and content uniformity [25]. The content of each individual preparation been found to be within the limits of 85-115% of the average content indicating the content uniformity test compliance with the official compendial tests for tablets as per IP. All these results showed that AMPS produced good quality matrix tablets as per standard specified in pharmacopeia.

Appropriate swelling behavior of mucoadhesive tablet is very much essential for uniform prolong release of the drug and effective mucoadhesion [26]. It was observed that, as we the increased the concentration of polymer the amount of swelling also increased with time.

When matrices containing swellable polymers are exposed to dissolution medium, tablet surface becomes wet and hydrated to form a gel layer. The initial release of drug from these matrices occurs by the drug dissolution in the water penetrated into the matrix. The overall drug release from these matrices is governed by hydration, gel layer formation and drug diffusion into gel layer and to the dissolution media [27]. Polymer erosion also plays a major role in releasing drug from these matrices [28]. These considerations indicate that AMPS have the potential to sustain the release of the drug from matrix tablets. The results of the *in-vitro* drug release study (n=3) of all the batches of tablets is shown in Fig. 4. From the drug release profile of the batches from F10 to F20, it was seen that total amount of drug was released within 6-7 hrs (300-360 minutes), while from the batches F30 to F50 where amount of AMPS increase drug release showed a controlled release pattern. Batch F25 also had a controlled pattern of drug release but not so significant as compared to the other five bathes. Among the five batches (F30-F50) the F40 showed mean release of 16.46% drug in first 2 hrs, while within the first 6 hrs and 8 hrs mean release were 32.69% and 68.87%, reaming portion of drug was released during last 4 hrs of observation and yet 2.42% of drug remain in the formulation. In F40 though complete drug release did not occur within 12 hrs but 97.58% was not very significant for the considering low amount of drug release. On the other hand, F40 showed the best controlled release pattern when we compare with other. Hence, F40 was considered for further study [29-33].

Mughal *et al*. in their study reported that hydrophilic natural polymer has sustained the release of the drugs by first order kinetics which may be true for the present study as well [33]. The drug release mechanism from controlled release devices is very complex to explain and still not yet completely understood. Although some controlled release processes may be classified as either purely diffusional or purely erosion controlled and many others can only be interpreted as being governed by both the mechanisms. To evaluate the *in-vitro* drug release profile, the data at various time points were fitted into the Korsemeyer–Peppas equation. In this equation KKP is the release rate constant and n is the characteristic for the mechanism of drug release. With an n value of 0.5, the equation becomes equal to the square root model described by Higuchi, which signifies that drug release from the matrix is governed by Fickian diffusion, for n value 0.5-1.0, anomalous non-Fickian drug diffusion occurs i.e. combination of both diffusion or swelling as well as erosion mechanism. For n>1, non-Fickian Case-II, erosion controlled or zero order release kinetics is followed. The R and n values of various batches of tablets are depicted in Table 6.

The R values of (0.9927, 0.9949, 0.9957, 0.9866, 0.9840, 0.9887, 0.9891, 0.9908, 0.9876) F10, F15, F20, F25, F30, F35, F40, F45 and F50 respectively which showed good linearity between log cumulative amount of drug release versus log time. The n values were found to be 1.179, 1.294, 1.162, 0.893, 0.796, 0.830, 0.907, 0.867, and 0.871 for the tablets F10, F15, F20, F25, F30, F35, F40, F45 and F50 respectively. Hence, the mechanism of drug release from the tablets was predicted from Korsemeyer–Peppas equations and from the obtained n values was a non-Fickian erosion controlled release.

In-vitro drug release of the optimized batch (F40) was best fitted and explained by Korsemeyer–Peppas and zero order drug release where (R_p=0.9891) and (R_o=0.9842) showed highest linearity and n value 0.97>0.5<1. Hence, pattern of drug release by non-Fickian erosion controlled release [33,34].

Optimization of formulation

Optimization of formulation was done on the basis of sustained release pattern of drug release from the matrix tablet in different time interval. Amount of drug released at the end also considered as a criteria for optimization of the formulation [11].

Evaluation of mucoadhesion

This result is comparable to the result as per synthetic polymer HPMC 3% and natural polymer Chitosan 3% respectively as per literature [18]. This indicates that higher holding time requires the most maximum force in grams to break the bond between the polysaccharide and the mucosal surface. Further, the wetting time is more as the holding time increases and the carboxylic acid structure in polysaccharide gradually undergo hydrogen bonding.

It was observed that as concentration of the polysaccharide was increased, resistance in terms of time to move of the mustard towards bottom side was also increased. The result was compared with the result of the synthetic polymer in place of isolated polysaccharide as reported in literature for synthetic polymer HPMC [16].

The results showed that the mucoadhesive time is concentration dependent. Increasing contact time may provide inter diffusion and chain entanglement between the polysaccharide and mucin chain in mucus membrane. This result supports the hypothesis of Leung and Robinson, those who demonstrated that mucoadhesion of carbomer was a time dependent process, supporting the proposed interpenetration as being a time dependent process [25]. An increase in the contact time resulted in an increase in formation of secondary bonds and diffusion path or depth of interpenetration between two macromolecules. Hence, an increase in contact time between the mucoadhesive polysaccharide and the mucus layer could therefore increase the mucoadhesive strength [27].

It was confirmed that tablet remained intact in its structural integrity and shape in stomach. The position of tablet at different time intervals in X-ray image clearly showed the evidence of bioadhesive nature of the tablet in rabbit's stomach. The unchanged position of tablet start from 2 hrs and is maintained throughout up to 8 hrs, indicating the bio adhesive property of the optimized formulation. After 12 hrs due to swelling the core and outer core get diminished, the integrity of size and shape of the tablet was broken and reduced.

Evaluations of various testing parameters such as friability percentage, hardness, amount of drug content, drug release as well as mucoadhesive strength were perfumed at an interval of 30, 60 and 90 days. There were no significant changes observed in the friability percentage, hardness, the amount of drug content, drug release as well as in mucoadhesive strength. Thus, tablets (formulation F40) were stable under these storage conditions for at least 3 months [5,34,35].

CONCLUSION

This study focused on the formulation of mucoadhesive tablets of Levamisole using AMPS as matrix forming hydrophobic polymer. It was summarized from the dissolution studies that the tablets containing less concentration of AMPS were disintegrated and the release of drug was not controlled less amount of gastric mucoadhessive property.

At higher concentration of AMPS the tablets released the drug in a controlled manner. The study of release mechanism exhibited anomalous non-fickian diffusion that involved both diffusion and erosion mechanisms.

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