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[Research article]

Formulation and *invitro* Characterisation of Mucoadhesive Microspheres of Oseltamivir by Ionic Gelation Method

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ABSTRACT

The objective of the present study was to prepare and evaluate the mucoadhesive microspheres of oseltamivir. Oseltamivir microspheres were prepared by ionotropic gelation method using polymers such as HPMC (K 100 M), carbopol 940P, sodium CMC, sodium alginate. Totally 10 different formulations of oseltamivir were prepared by using the above polymers. The microspheres were characterised for drug content, entrapment efficiency, swelling index, mucoadhesive property by *in vitro* wash-off test and *in-vitro* drug release. The formulation F8 was selected as an ideal formulation based on the *in vitro* release profile which showed a controlled drug release of 86.11% up to 12 hours in acidic buffer of pH 1.2. Surface morphology (SEM analysis) and drug-polymer interaction studies (FT-IR analysis) were performed only for all the formulations. The microspheres were smooth and elegant in appearance showed no visible cracks as confirmed by SEM and FT-IR studies indicated the lack of drug-polymer interactions in the ideal formulation F8. The *in vitro* release data of all microsphere formulations were plotted in various kinetic equations to understand the mechanisms and kinetics of drug release. The ideal formulation, F8 followed Higuchi model kinetics.

Key words*:* Mucoadhesion, Microspheres, Hydrophilic polymers, Oseltamivir, Ionotropic gelation method, Controlled release

INTRODUCTION

The oral route for drug delivery is the most popular,desirable, and most preferred method for administrating therapeutical agents for systemic effects because it is a natural, convenient, and cost effective to manufacturing process. Microspheres are small spherical particles,with diameters in the micrometer range (typically 1µm to 1000 µm). Microspheres are sometimes referred to as microparticles.Microspheres can be manufactured from various natural and synthetic materials.

MUCOADHESION SYSTEM [1]

Mucoadhesive drug delivery system are the systems which utilizes the property of bio adhesion of certain polymers which become adhesive on hydration and can be used for targeting a drug to a particular region of the body for extended periods of time. The term "mucoadhesion" was coined for the adhesion of the polymers with the surface of the mucosal layer. Bio adhesions are a phenomenon in which two materials at least one of which is biological and are held together by means of interfacial forces. In biological systems, bio adhesion can be classified into 3 types:

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1. Adhesion between two biological phases, for example, platelet aggregation and wound healing
2. Adhesion of a biological phase to an artificial substrate, for example, cell adhesion to culture dishes and bio film formation on prosthetic devices and inserts
3. Adhesion of an artificial material to a biological substrate, for example, adhesion of synthetic hydrogels to soft tissues and adhesion of sealants to dental enamel.

For drug delivery purposes, the term bio adhesion implies attachment of a drug carrier system to a specified biological location. The biological surface can be epithelial tissue or the mucus coat on the surface of a tissue. If adhesive attachment is to a mucus coat, the phenomenon is referred to as mucoadhesion / mucoadhesion as the interaction between a mucin surface and a synthetic or natural polymer. In bio adhesion, the polymer is attached to the biological membrane.

MECHANISM OF MUCOADHESION[1]

A complete understanding of how and why certain macromolecules attach to a mucus surface is not yet available, but a few steps involved in the process are generally accepted, at least for solid systems. Several theories have been proposed to explain the fundamental mechanism of adhesion. A general mechanism of mucoadhesion drug Delivery system is show in Figure

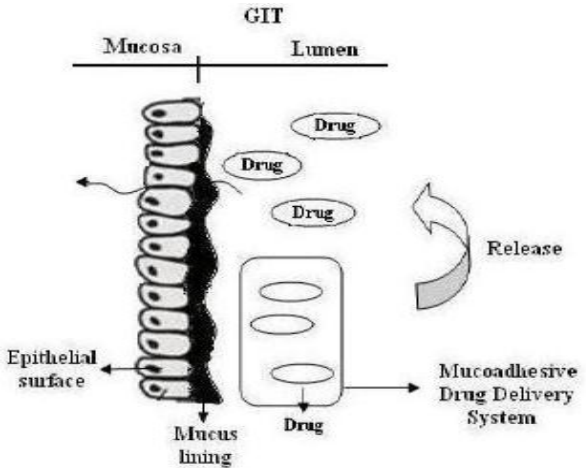


Fig no 1: Mechanism of Mucoadhesion

The drug Oseltamivir is a prodrug of oseltamivir carboxylate, an inhibitor of the enzyme neuraminidase (sialidase), which has a role in the infectivity and replication of influenza and B viruses. Its biological half life is 1 to 3 hours, hence frequent administration was necessary to maintain its therapeutic concentrations. This necessitates multiple daily dosing for maintenance of its plasma concentration of the drug with in therapeutic index , hence there was an impetus for developing controlled release dosage form that maintains improved bioavailability and therapeutic plasma drug concentration for long period compared to conventional dosage form.

MATERIALS AND METHODS:

Oseltamivir phosphate was obtained as a gift sample from Chandra labs, hyderabad. Carbopol 934, HPMC K4M was from BARIS Pharmaceuticals Pvt. Ltd, Sodium alginate was obtained from Sisco Research Laboratories Pvt. Ltd, Calcium chloride dehydrate, Hydrochloric acid was from Thermo Fisher Scientific India Pvt. Ltd. And methonal from SD fine-chem limited

UV-Visible spectrophotometer, Electronic weighing balance, Microbalance, Magnetic stirrer, Disintegration Apparatus, Dissolution apparatus, FT–IR Spectrometer, SEM

METHOD OF PREPARATION

The alginate microspheres were prepared by ionotropic external gelation technique. In this method, weighed quantity of the drug oseltamivir was dissolved in distilled water and stirred well and then polymer solution was prepared by dissolving sodium alginate, carbopol, hpmc and cmc in suitable solvent and stirred for 2 hours using magnetic stirrer at a speed of 1800rpm. For the

formation of microspheres, 50 ml of this solution was extruded drop wise from a needle of 18 G in diameter from a height of about 6 cm into 100 ml aqueous calcium chloride solution and stirred at 100 rpm. Then the solution containing the gel formed microspheres was filtered by using Whatmann filter paper no-1. The microspheres were allowed to dry at about 30-40°C and stored in well closed container for further use.

TABLE NO 1: FORMULATION TABLE

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Oseltamivir (mg)	100	100	100	100	100	100	100	100	100	100
Sodium Alginate (mg)	100	200	100	100	100	100	100	100	100	100
CMC (mg)	-	-	25	-	100	-	25	-	50	-
Carbopol (mg)	-	-	25	100	-	-	75	75	50	50
HPMC	-	-	50	-	-	100	-	25	-	50
Distilled water	Q.S									

EVALUATION TESTS:

Flow properties:

Angle of repose: [2]

The angle of repose of microspheres was determined by the fixed funnel and free standing cone method. The accurately weighed granules were taken in a funnel. The height of the funnel was adjusted in such a manner that the tip of the funnel just touched the apex of the heap of the granules.

$$\tan \theta = h/r$$

Where h = height of the powder heap

r = radius of the powder heap

θ = is the angle of repose.

Bulk Density and Tapped Density [2]:

Bulk density and tapped density were measured by using 50 ml of graduated cylinder. The sample poured in cylinder was tapped mechanically for 100 times and then tapped volume was noted down. Bulk density and tapped density were calculated.

Carr's Index [2]:

Compressibility index (Ci) or Carr's index value of microparticles was computed according to the following equation:

Carr's Compressibility Index (%) = [(TD-BD) X 100] / TD

Where,

TD = Tapped density and BD = bulk density

Hausner's Ratio: [8]

Hausner's Ratio indicates the flow properties of the powder and is measured by the ratio of tapped density to bulk density. It is the ratio of tapped density and bulk density..

Hausner's Ratio = Tapped density/Bulk Density

FOURIER TRANSFORM INFRARED S PECTROSCOPY (FT-IR):

In order to check the integrity (Compatibility) of drug in the formulation, FT IR spectra of the formulations along with the drug and other excipients were obtained and compared using Shimadzu FT IR 8400 spectrophotometer. In the present study, Potassium bromide (KBr) pellet method was employed. The samples were thoroughly blended with dry powdered potassium bromide crystals. The mixture was compressed to form a disc. The disc was placed in the spectrophotometer and the spectrum was recorded.The FT-IR spectra of the formulations were compared with the FT-IR spectra of the pure drug and the polymers.

SPECTROSCOPIC STUDIES:

Preparation of Standard Solution of 0.1N HCl (pH 1.2):

Take 8.32 ml of HCl in a 1000ml volumetric flask and dissolve it. Now make up the volume with distilled water up to the mark.

DETERMINATION OF λ MAX:

Stock solution (1000µg/ml) of Oseltamivir phosphate was prepared .This solution was appropriately diluted with 0.1N HCl (pH 1.2) to

obtain a concentration of 10µg/ ml. The resultant solution was scanned in the range of 200 nm to 400nm on UV-Visible spectrophotometer.The drug exhibited a λmax at 269 nm.

PREPARATION OF STANDARD CALIBRATION CURVE OF OSELTAMIVIR PHOSPHATE:

- 10mg of Oseltamivir phosphate was accurately weighed and dissolved in 10ml of distilled water (Stock Solution – I) to get a concentration of 1000 µg/ml.
- From the stock solution- I,1ml of aliquots was taken and suitably diluted with 0.1N HCl (Stock Solution II) to get concentrations of 100µg/ml.
- From the stock solution- II,aliquots were taken and suitably diluted with 0.1N HCl (pH 1.2) to get concentrations in the range of 2 to 18 µg/ml.The absorbance of these samples were analyzed by using UV-Visible Spectrophotometer at 269 nm against reference solution 0.1N HCl (pH 1.2).

Percentage yield [3]:

The percentage of production yield was calculated from the weight of dried microspheres recovered from each batch and the sum of the initial weight of starting materials. The percentage yield was calculated using the following formula:

$$\text{Percentage yield} = \frac{\text{Actual weight of product}}{\text{Total weight of drug and polymer}} \times 100$$

Drug entrapment efficiency: [4]

Microspheres equivalent to 15 mg of the drug Oseltamivir phosphate were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres. The powder was transferred to a 100 ml volumetric flask and dissolved in 10ml of methanol and the volume was made up using simulated gastric fluid pH 1.2 . After 24 hours the solution was filtered through Whatmann filter paper and the absorbance was measured after suitable dilution spectro photometrically at 269 nm. The amount of drug entrapped in the microspheres was calculated by the following formula.

$$\text{\% Drug entrapment} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \times 100$$

Particle size analysis: [6]

Samples of the microparticles were analyzed for particle size by optical microscope. The instrument was calibrated and found that 1unit of eyepiece micrometer was equal to 12.5µm. Nearly about 100 Microparticles sizes were calculated under 45x magnification.

The average particle size was determined by using t he Edmondson's equation:

$$D_{mean} = \frac{Nd}{n}$$

Swelling study:[4]

Swelling ratio of different dried microspheres were determined gravimetrically in simulated gastric fluid pH 1.2.The microspheres were removed periodically from the solution, blotted to remove excess surface liquid and weighed on balance. Swelling ratio (% w/v) was determined from the following relationship:

$$\text{Swelling ratio} = \frac{(Wt - W0)}{(W0)} \times 100$$

Where W0 &Wt are initial weight and Final weight of microspheres respectively.

Evaluation of mucoadhesive property:[5]

The mucoadhesive property of microspheres was evaluated by an in vitro adhesion testing method known as wash-off method. Freshly excised pieces of goat stomach mucous were mounted on to glass slides with cotton thread. About 20 microspheres were spread onto each prepared glass slide and immediately thereafter the slides were hung to USP II tablet disintegration test, when the test apparatus was operated, the sample is subjected to slow up and down movement in simulated gastric fluid pH 1.2 at 37^0C contained in a 1-litre vessel of the apparatus. At an interval of 1 hour up to 8 hours the machine is stopped and number of microspheres still adhering to mucosal surface was counted.

$$\text{\% Mucoadhesion} = \frac{\text{No of microspheres adhered}}{\text{No of microspheres applied}} \times 100$$

In vitro drug release study:[s]

The dissolution studies were performed in a fully calibrated eight station dissolution test apparatus (37 ± 0.5^0C, 50 rpm) using the USP type – I rotating basket method in simulated gastric

fluid pH 1.2 (900ml). A quantity of accurately weighed microspheres equivalent to 15mg Oseltamivir phosphate each formulation was employed in all dissolution studies. Aliquots of sample were withdrawn at predetermined intervals of time and analyzed for drug release by measuring the absorbance at 269nm. At the same time the volume withdrawn at each time intervals were replenished immediately with the same volume of fresh pre-warmed simulated gastric fluid pH 1.2 maintaining sink conditions throughout the experiment

In-vitro drug release kinetics:

The release data obtained was fitted into various mathematical models.The parameters'n' and time component 'k',the release rate constant and 'R',the regression coefficient were determined by Korsmeyer Peppas equation to understand the release mechanism.

$$Mt / M\infty = Kt^n$$

Where, Mt / M∞ is the fractional release of drug, 't' denotes the releasetime,'K'represents a constant incorporating structural and geometrical characteristics of the device,'n' is the diffusional exponent and characterize the type of release mechanism during the release process.

Other equations to study the drug release kinetics from dosage forms

a. Zero Order

% R = kt

This modelrepresents an ideal release in order to ac hieve prolonged pharmacologicalaction.This is appl icable to dosage forms like transdermal systems, coated forms,osmoticsystems, as well as matrix tabl ets containing low soluble drugs.

b. First Order

log (fraction unreleased) = kt/2.303

The model is applicable to hydrolysis kinetics and to study the release profiles of pharmaceutical dosage forms such as those containing water soluble drugs in porous matrices.

c. Matrix (Higuchi Matrix)

$$\% R = kt^{0.5}$$

This model is applicable to systems withdrug disper sed in uniform swellable polymermatrix as in case o f matrix tablets with water soluble drug.

d. Peppas Korsmeyer Equation

$$\% R = kt^n$$

$$\log \% R = \log k + n \log t$$

This model is widely used when the release mechanism is well known or when more than one type of release phenomenon could be involved.

RESULTS AND DISCUSSION

Table no 2 : Data for mucoadhesive microspheres for micro particle analysis (F1-F10)

Formulation code	Bulk density (g/cc)	Tapped density (g/cc)	Carr's Index	Hausner Ratio	Angle of repose(θ)	Flow properties
F1	0.43±0.043	0.57± 0.07	14.80±0.2	1.13±0.02	27.11±0.22	Excellent
F2	0.42±0.042	0.51± 0.03	13.63±0.6	1.14±0.04	27.68±0.11	Excellent
F3	0.45±0.045	0.54± 0.08	14.58±0.8	1.15±0.08	27.44±0.16	Excellent
F4	0.46±0.046	0.58± 0.04	14.19±0.1	1.12±0.06	29.36±0.13	Excellent
F5	0.44±0.044	0.52 ±0.01	15.48±0.6	1.17±0.08	27.52±0.19	Excellent
F6	0.42±0.042	0.54± 0.06	13.48±0.8	1.19±0.09	28.32±0.19	Excellent
F7	0.41±0.041	0.59± 0.04	14.48±0.8	1.11±0.09	27.69±0.19	Excellent
F8	0.45±0.045	0.57± 0.04	15.19±0.1	1.19±0.05	28.36±0.23	Excellent
F9	0.44±0.044	0.50 ± 0.1	13.58±0.8	1.11±0.09	27.33±0.16	Excellent
F10	0.42±0.043	0.53± 0.02	13.55±0.6	1.17±0.09	25.59±0.13	Excellent

All the formulations were evaluated for bulk density, tapped density, % compressibility, Hausner's ratio and angle of repose. The results of % compressibility, Hausner's ratio and angle of repose were found to be <16, <1.25 and <30 respectively. These results show that the formulations have excellent flow properties

Drug Excipient Compatibility Studies:

Fig. 2: FTIR curve of oseltamivir

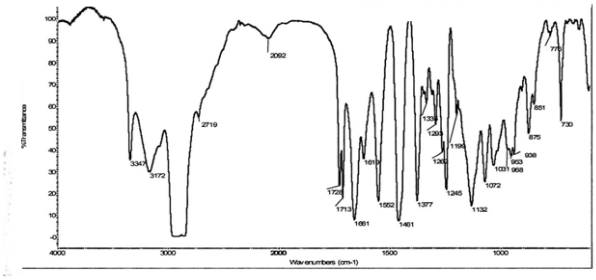


Fig. 3: FTIR curve of sodium alginate

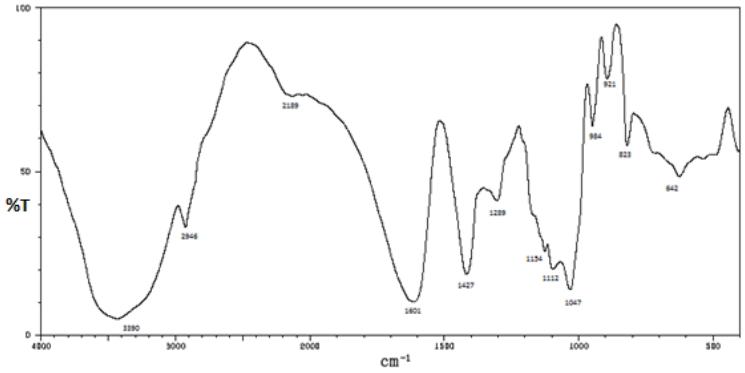


Fig.4: FTIR curve of cmc

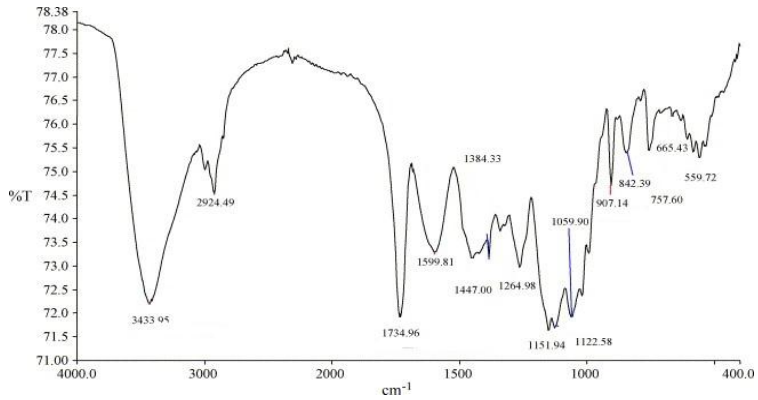


Fig .5: FTIR Curve of Carbopol

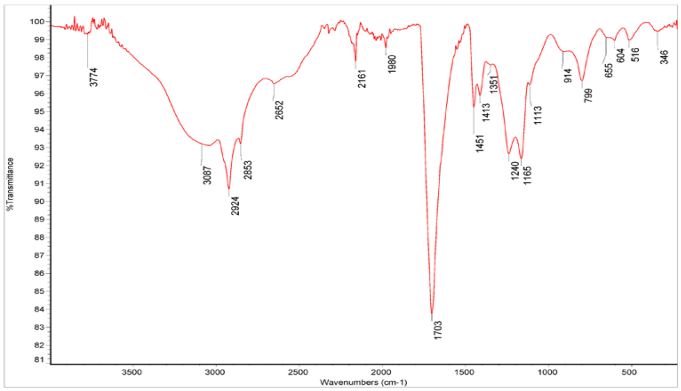
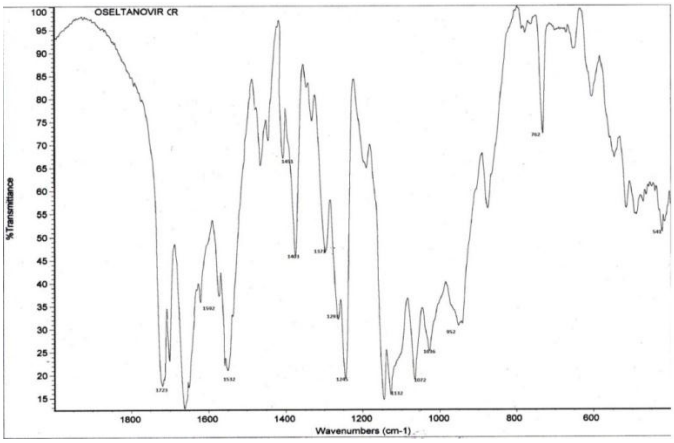


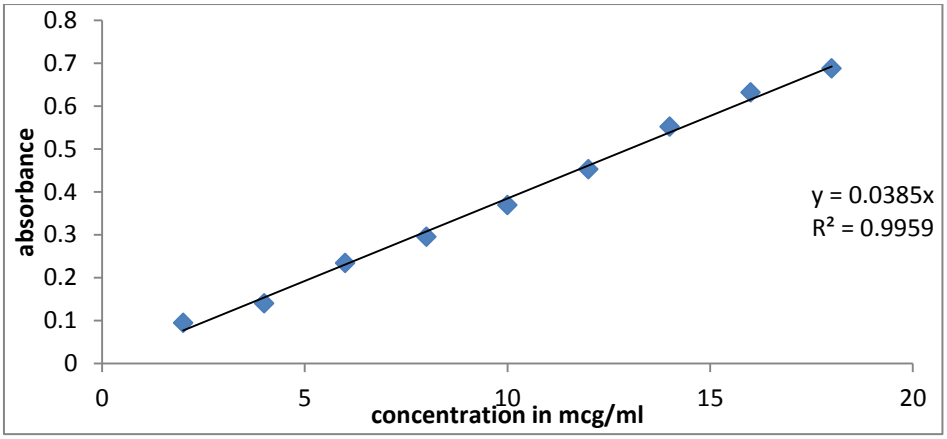
Fig 6: FTIR Curve of F8 Formulation



Drug - Excipient compatibility is confirmed by FTIR Spectroscopy for which, FTIR spectra of Oseltamivir, carbopol, Carboxy methyl cellulose, Sodium alginate alone were compared with FTIR spectrum of the physical mixture of Oseltamivir, Carbopol, Carboxy methyl Cellulose, Sodium alginate. And it shows no interaction between polymer and drug

Calibration Curve of Oseltamivir:

S.NO	Concentration(µg/ml)	Absorbance
1	0	0
2	2	0.095
3	4	0.140
4	6	0.234
5	8	0.295
6	10	0.369
7	12	0.453
8	14	0.552
9	16	0.632
10	18	0.668



Data for Calibration Curve of Oseltamivir in Acidic Buffer of pH-1.2

Different concentrations of oseltamivir from 2 to 18 µg/ml were prepared and the absorbance was taken at 269 nm against pH1.2 acidic buffer and graph was plotted between concentration and absorbance.

Data For Evalution Tests of microspheres

Formulation	Particle size (µm)	Drug entrapment (%)	Percentage yield (%)	Mucoadhesive strength (%)	Swelling index (%)
F1	510.5±1.32	58.6±1.64	64.2±1.40	40±1.3	24.3±0.89
F2	626.3±1.61	70.3±1.43	61.8±1.32	50±0.58	35±1.21
F3	715±2.22	74.1±1.48	59.3±0.99	45±0.69	49.3±1.20
F4	657.3±1.06	75.0±1.50	69.3±1.41	75±0.72	55.3±1.63
F5	757±1.11	49.8±1.82	59.2±1.08	45±0.81	64±0.79
F6	691.6±1.03	72.1±1.55	71.7±1.43	60±0.77	54.6±0.65
F7	707.3±2.04	59.6±1.68	65.3±0.40	60±0.93	42±0.74
F8	807.31±2.13	76.4±1.74	73.8±1.33	80±0.87	75.3±1.02
F9	791±2.37	55.3±1.84	71.6±0.41	65±0.96	72±0.36
F10	756±1.83	70.3±1.49	69.3±0.39	55±1.02	65.66±0.19

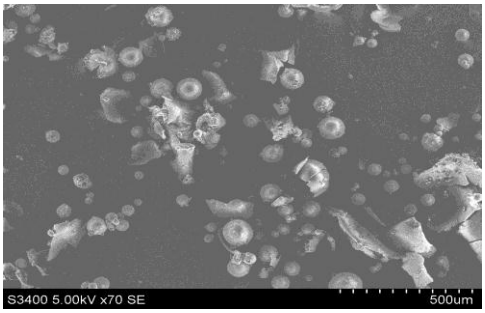
The particle size of the microsphere plays a vital role in the drug release so it is necessary to determine the particle size. The particle size of the microspheres obtained was analysed by laser difractrometry (which yielded the mean particle diameter of spheres. The diameter were calculated using volume distribution. The particle size obtained for the optimised formulation (f8) is found to be 807.31 The drug entrapment (DE) and percentage yield (PY) was analysed for 10 trails and results were noted in the above table the entrapment efficiency and percentage yield obtained for the optimised formulation (F8) was found to be 76.4% (DE)and 73.8%(PY) The mucoadhesive strength and swelling index was done for for all 10 formulations and the results

were noted. the mucoadhesive strength and swelling index for optimised formulation was found to be 80% and 75.3%

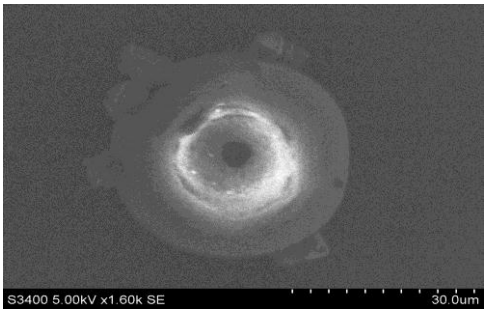
Scanning Electronic Microscopy:

Shape and surface characteristics of microspheres examine by Scanning Electron Microscopy analysis as shown in Fig. Surface morphology of F8 formulation examine at an different magnification 70X

SEM Analysis of F8 Formulation under 50X



SEM Analysis of F8 formulation under 70X



SEM Analysis of F8 Formulation

The microsphere surface appearance and shape were analysed by scanning electron microscope (SEM). SEM is the most commonly used method for characterising drug delivery system, due to simplicity in sample preparation and ease of operation. Scanning electron photomicrographs of all the ten formulations are taken under SEM. The average particle size of microsphere of oseltamivir was found for the optimised formulation 30.0 µm-500 µm. the particles which are obtained for F8 were smooth and spherical. while the other were slightly rough surface and irregular

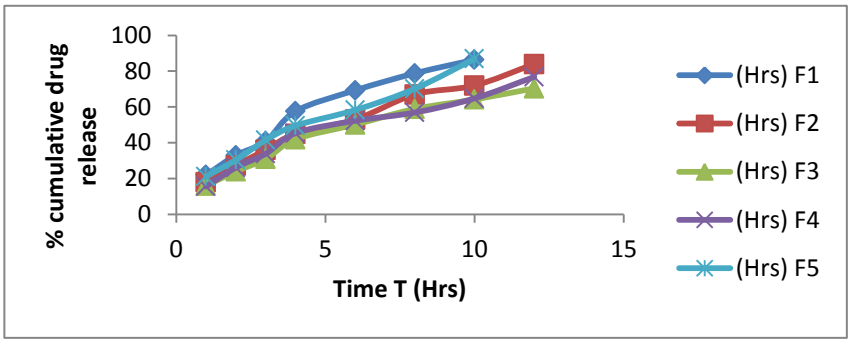
In vitro release studies:

The drug release data obtained for the formulations from F1 –F10 were tabulated in the table.

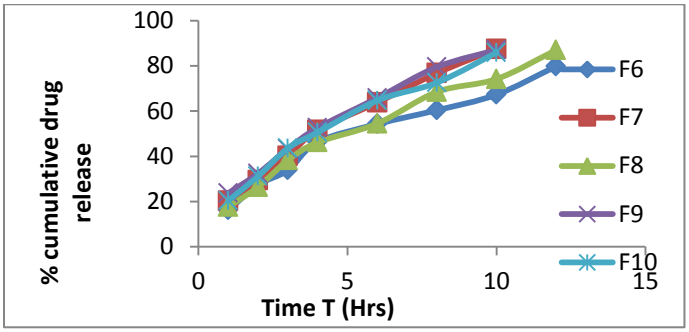
In vitro drug release studies of Oseltamivir

Time	Percentage drug release									
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**
0	0	0	0	0	0	0	0	0	0	0
1	22	18	19.8	18.6	21.2	17.2	20.4	17.6	23.7	20.4
2	33	27.3	29	30.9	30.4	27.4	29.4	26.5	32.4	31.2
3	40.8	36	41.2	40.9	41.5	40.9	40.2	38.3	43.3	43.6
4	57.6	49	55.6	49	49.6	46.4	51.6	46.2	52.6	50.6
6	69.3	61.1	68.3	58.1	58.3	56.3	63.8	54.5	65.8	64.7
8	78.8	73.6	76.5	71.0	70.2	69.4	76.8	68.5	79.5	72.6
10	86.4	83.2	89.0	77.3	86.9	78.3	87.5	74.2	87	85.9
12	92.2	90.6	94.5	89.4	91.9	89.8	93.7	89.99	91.8	92.6

The percentage drug release for each of the formulation was found to be F1(92.2%), F2(90.6%), F3(94.5%), F4(89.4%), F5(91.9%), F6(89.8%), F7(93.7%), F8(86.9%), F9(91.8%), F10(92.6%). The F8 formulation shows 86.99% and having still drug release after 12 hrs and shows controlled release of drug.



% drug release of formulations F1 to F5



% drug release of formulations F6 to F10

Release kinetics:

Formulation	Zero order		First order		Higuchi model	Peppa's model
	R^2	K	R^2	K		
F1	0.950	3.0229	0.994	2.384	0.985	0.986
F2	0.978	2.877	0.975	2.363	0.994	0.996
F3	0.954	2.7899	0.992	2.343	0.991	0.990
F4	0.950	2.8066	0.968	2.343	0.967	0.981
F5	0.984	2.996	0.934	2.381	0.986	0.993
F6	0.986	2.8309	0.970	2.253	0.967	0.985
F7	0.982	3.0472	0.981	2.216	0.986	0.995
F8	0.975	2.9035	0.964	2.369	0.996	0.990
F9	0.979	3.0166	0.990	2.389	0.992	0.995
F10	0.972	2.994	0.976	2.381	0.980	0.995

Release kinetics for all 10 trails was done. The optimised formulation F8 follows Higuichi model and value was found to be 0.996. This shows than drug shows it follows controlled release

Summery and conclusion

Oseltamivir microspheres were prepared successfully using sodium alginate, Hydroxy propyl methyl cellulose, Carboxy methyl cellulose and Carbopol as a mucoadhesive polymer in different proportions by using ionic gelation method**.** Preformulation studies of oseltamivir was done initially and the results were directed for the further course of formulation Based on the Preformulation studies F1 to F10 batches were prepared using selected polymers

Prepared microspheres were evaluated for the percentage yield, drug content, drug entrapment efficiency, particle size determination, swelling index, *invitro* wash off test, *invitro* dissolution test.

The drug content and entrapment efficiency were good for all formulations. Among all formulations F8 shows better properties. So mucoadhesive property was better than other formulations. Dissolution was carried out at 1.2 P_H in 0.1 N Hcl at 269 nm.

All the formulations were evaluated different kinetic models like Zero order, First order, Higuchi matrix and Korsmeyer Peppas equation. The best formulation F8 contains polymers sodium alginate, carbopol and HPMC Which shows better mucoadhesive property and better drug release

The formulations were found to be linear in kinetic models and F8 was selected as optimised formulations and shows 86.99% of drug release after 12 hours.

For optimised formulation the drug entrapment efficiency was 76.4%, Percentage yield was 73.8%, Mucoadhesive strength was 80% and Swelling index was 75.3%

The polymer concentrations are major factor effecting the mucoadhesive strength of prepared microspheres.While control of drug release profile has been a major aim of pharmaceutical research and development of past decade, control of GI transmit profile could be the focus of next few decades and might results in the availability of new products with better therapeutic possibilities and substantial benefits for patients.Mucoadhesive microspheres would become promise candidate for delivery of various drugs in sustained release manner in future.Dosing frequency and loss of drug also reduced by the use of such type formulations and the bioavailability of drugs can also be increased.

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