TENOFOVIR DISOPROXIL FUMARATE MUCOADHESIVE MICROSPHERE FORMULATION AND EVALUATION

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Abstract:
The objective of the research work to design, optimize, evaluate oral control release mucoadhesive microspheres of Tenofovir disoproxil fumarate. To design & formulate mucoadhesive microsohere of Tenofovir disoproxil fumarate by ionic gelation technique using few selected mucoadhesive polymer like sodium alginate, carbapol, chitosan & release retardant polymer for extended release like cmc and HPMC. Tenofovir prepared by using ionc gelation technique by using different ratios of polymer. One is cross linking polymer (Sodium alginate) and other is mucoadhesive polymers (HPMC, CMC, Carbapol). The microspheres were evaluated by different evaluation parameters like- drug entrapment efficiency, swelling index, micromeritics property, in vitro wash off test, in vitro drug release study and stability study. Drug content and entrapment efficiency of different formulation was found to be in the range of 82 to 95%. Swelling index of microspheres prepared as per experimental design were found to be satisfactory. In vitro wash off test showed that prepared microsphere exhibit for mucoadhesive properties. It was found that formulation with drug polymer ratio 1:2 released maximum amount of drug at 12hours. But the other formulation release the drug before 12 hours. Show that in comparison to all the 9 formulations F7 showing controlled release action.

Keywords: Mucoadhesion, Mucoadhesive microsphere, Tenofovir disoproxil fumarate, Invitro dissolution, Sodium alginate, Carbapol

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Introduction:
Mucoadhesion is a phenomenon in which two materials at one of which two materials at least one of which is biological in nature are held together by means of interfacial forces.[1] Mucoadhesion is a complex process and numerous theories have been proposed to explain the mechanisms involved.

Ionotropic Gelation method was developed by Lim F and Moss RD 22[4]. Using this method Microspheres are formed by dissolving the gel-type polymers, such as alginate, in an aqueous solution followed by suspending the active ingredient in the mixture and extruding the solution through needle to produce micro droplets which fall into a hardening solution containing calcium chloride under stirring at low speed. Divalent calcium ions present in the hardening solution crosslink the polymer, forming gelled microspheres.
Tenofovir belongs to class of Anti-retroviral drugs, known as Nucleotide Analogue Reverse Transcriptase Inhibitors (NRTs) with block Reverse transcriptase.
Tenofovir disoproxil fumarate is a prodrug of tenofovir. Upon oral administration, it is converted to tenofovir, an acyclic nucleoside phosphonate (nucleotide) analogue of adenosine 5'-monophosphate and blocks the reverse transcriptase enzyme that is important for HIV-viral synthesis.
Tenofovir disoproxil fumarate is given once a day as it remains in cells for longer period of time than other antiretroviral drug.

Material and Methods
Tenofovir was a gifted sample from Dr. Reddys Lab. Hyderabad. Sodium alginate, HPMC, CMC & carbapol were obtained from cipla phrma ltd. Goa. Calcium chloride was obtained from I.P.T. Lab.

Solubility analysis:
The solubility of Tenofovir was determined in distilled water, 0.1N HCL & phoshphate buffer 7.4. 1gm of Tenofovir was dissolved in 10ml. of distilled water, 0.1 N Hcl & phoshphate buffer pH 7.4. The solubility analysis of Tenofovir in different solvent is shown in Table 1

Table 1 Solubility analysis of Tenofovir in different solvent Melting point of drug was determined by two method.

Solvent	**Solubility(mg/ml)**
Distilled water	Slightly soluble(7.4mg/ml)
Methanol	Soluble(38.5mg/ml)
0.1N HCL	Soluble(47.2mg/ml)

Capillary method- In this method, a small quantity of drug is taken in a capillary tube & sealed the tip of one end with the help of bunsen burner & was placed in melting point apparatus & the temperature at which the drug was melted was observed.
Average of the triplet was noted.

Table-2- Measurement of melting point of drug (Tenofovir)

Sl.no.	**Melting point(^{0}c)**	**Average**
Trial 1	276^{o}c	
Trial 2	277^{o}c	277^{o}c
Trial 3	278^{o}c	

Tenoforvir stock solution: Stock solution is prepared taking 100mg of Tenofovir in 100ml of methanol .Then the stock solution is further diluted with methanol to get working solution of 4,6,8 µg/ml . The working solution scanned between 200nm to 400nm which shows the maximum absorbance at 259.5nm. The same λmax was used for further measurement of the drug.

Table-3:- Calibration curve of Tenofovir

Concentration (µg/ml)	Wavelength (nm)	Absorbance
4µg/ml	259.5nm	0.304
6µg/ml	259.5nm	0.390
8µg/ml	259.5nm	0.439
10µg/ml	259.5nm	0.481

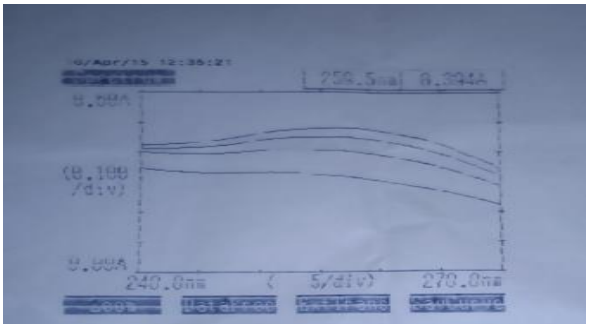

Figure- 1 UV Spectum of Tenofovir

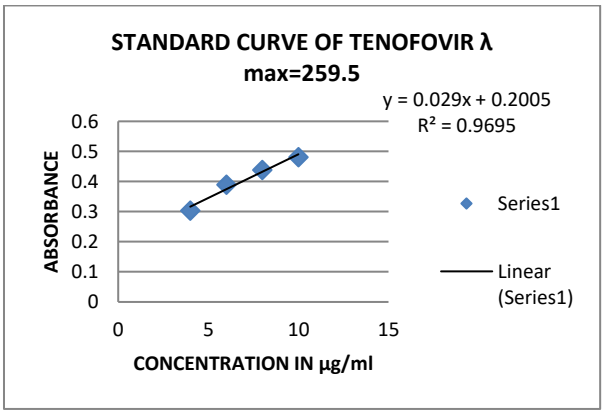

Figure-2: Standard curve of Tenofovir disoproxil fumarate

Formulation of mucoadhesive microsphehe of tenofovir disoproxil fumarate

Tenofovir mucoadhesive microsphere were prepared by ionic gelation technique. Microsphere containing tenofovir were prepared employing sodium alginate alone and in combination with HPMC, CMC and carbopol. The homogenous polymer solution was prepared in distilled water and stirred magnetically with gentle mix. The drug and cross-linking agent (sodium alginate) were added to the polymer solution and mixed thoroughly by stirring magnetically to form a viscous dispersion which was then extruded through a syringe with middle size no.18 into calcium chloride 5% solution kept under magnetic stirrer at 100rpm. The microsphere were retained in calcium chloride solution for 30mins to produce rigid discrete particles. They were collected by decantation and the product thus separated was washed with chloroform to remove the traces of calcium chloride. Then the microsphere were dried at 40% under vacuum for 12hrs, the composition of the microspheres are listed in table 1.

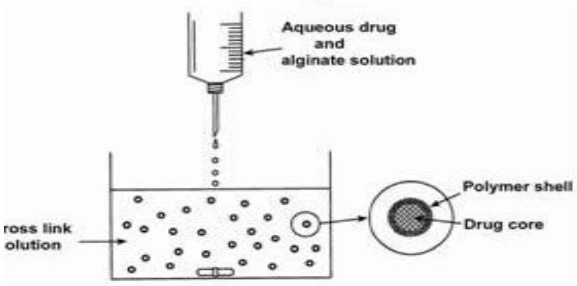

Figure -3 Procedure of microsphere preparation of ionic gelation method

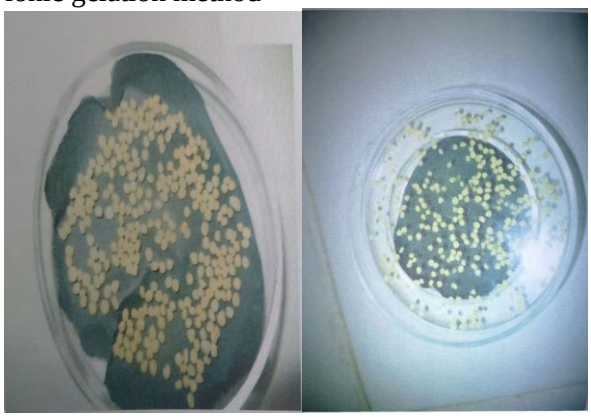

Figure-4 - Microsphere picture after drying

Table 4-Formulation of Tenofovir using different polymer

Formulation code	Drug(mg)	Sod. Alginate(mg)	HPMC(mg)	CMC(mg)	Carbopol (mg)	Calcium Chloride (mg)
F1	300mg	500mg	200mg	___	___	5%
F2	300mg	500mg	___	200mg	___	5%
F3	300mg	500mg	___	___	200mg	5%
F4	300mg	500mg	300mg	___	___	5%
F5	300mg	500mg	___	___	300mg	5%
F6	300mg	500mg	___	300mg	___	5%
F7	300mg	500mg	400mg	___	___	5%
F8	300mg	500mg	___	400mg	___	5%
F9	300mg	500mg	___	___	400mg	5%

Characterization of mucoad hesive microsphere:

Entrapment Efficiency: Initially the mucoadhesive microsphere were powdered by mortar & pestle. Then powder equivalent to 100mg was dissolved in 100ml of 0.1N Hcl. Then from the prepared solution 1ml of solution was taken and volume make up was done by 0.1N Hcl solution. The solution was filtered through the Whatman filter paper No.41 to obtain stock solution. The absorbance of resulting solution was taken at λmax 259.5nm by using UV-Spectrophotometer. The % of encapsulation efficiency can be estimated by following formula: %Entrapment= (Actual content/Theoretical content)×100

Swelling Index:

Swelling index illustrate the ability of mucoadhesive microspheres to get swelled at the absorbing surface by absorbing fluids available at the site of absorption, which is primary requirement for initiation of mucoadhesion.

Procedure:- The dynamic swelling property of microcapsules was determined by placing the microspheres in 100ml of distilled water for 24 hours. Further, the swollen microcapsules were dried by keeping on a filter paper and the weight was noted down. The percentage swelling was then calculated by using following formula:

$$\%Swelling=\{(D_T-D_O)/D_O\}\times100$$

Where, D_O=Weight of dried microsphere
D_T=Weight of swelled microsphere

% Yield Value of Microspheres:

The prepared microspheres were assessed for the yield value. The batch was weighed after total drying and the yield % was calculated using the formula given below. Each batch was formulated

in triplicate batches (n=3) to get a reproducible yield

$$\% \text{ Yield} = \frac{\text{The amount of microspheres practically obtained (g)}}{\text{The theoretical amount (g)}} \text{ X } 100$$

Percentage moisture loss:

The drug loaded microcapsules were evaluated for percentage moisture loss which gives idea about its hydrophilic nature. The microcapsules weighed 20 mg (W_1) were initially kept in desiccators containing calcium chloride at 37°C for 24 h. The final weight (W_2) was noted when no further change in weight of sample was observed. The percentage moisture loss was calculated by following formula:

%moisture loss={(W1-W2)/W2}×100

Fourier Transform Infrared Spectroscopy (FTIR):

FTIR Spectral measurement was performed using thermo electron FTIR spectrometer to confirm the presence of any interaction between the polymer and drug. The IR spectra of the free drug, physical mixture, formulation & empty microspheres were recorded. The identical peaks corresponding to the functional groups features confirm that neither the polymer nor the method of the preparation has affected the drug stability.

Differential Scanning Calorimetry(DSC):

DSC analysis was carried out to identify the compatibility between the drug and excipients. DSC analysis of pure drug 1:1 physical mixture of drug excipients were carried out. Sample (2-8mg) were accurately weighed and heated in sealed aluminium pans at a rate of 10°c/min between 0-30°c temperature ranges under nitrogen atmosphere.

Scanning Electron Microscopy (SEM):

The morphology of microspheres was examined by scanning electron microscopy(SEM). The outer surface of the microspheres was observed by SEM. By the SEM study the size, shape, outer structure of microspheres can be determined. A small amount of microspheres spread on gold stub. After that the stub containing microspheres placed in SEM. A scanning electron photomicrograph is taken at an acceleration of 5KV & chamber pressure of 0.6mmHg.

In vitro wash-off test:

A 4cm×4cm piece of goat stomach mucosa was tied onto a glass slide (3inch × 1inch) using a thread. Microspheres were spread onto the wet, rinsed, tissue specimen and the prepared slide was hung onto one of the groove of the USP tablet disintegrating test apparatus. The disintegrating apparatus was operated such that the tissue specimen was given regular up and down movements in a beaker containing the simulated gastric fluid. At the end of every time interval, the number of microspheres still adhering onto the tissue was counted and there adhesive strength was determined using the formula given below.

Mucoadhesive property = (No. of microsphere adhered/ no. of microsphere applied) ×100

***In–vitro* drug release study:**

Drug release studies were carried out in USP paddle type dissolution test apparatus. A quantity of microsphere equivalent to 100mg of drug was used for the test 0.1N Hcl was used as dissolution medium. The volume of the dissolution medium was 900ml & the bath temperature was maintained at 37±0.5°c. The microsphere were placed in the dissolution vessel & the vessel was covered, the apparatus was operated for 12hours at 100rpm . At definite time intervals 10ml of the dissolution fluid was withdrawn, filtered. 10ml of blank sample was replaced to the dissolution vessel, so as to maintain the volume. The samples withdrawn were analyzed spectrophotometrically at a ƛmax 259.5nm using UV- spectrophotometer.

Figure-5 Dissolution Test Apparatus

Stability study:

The microspheres were kept in a screw capped container. Then the accelerated stability study was carried out for the optimized formulation.

Results & Discussion

Table & Figures

Table No-5 Entrapment efficiency of all formulation:

Formulation Code	% Drug content
F1	60±0.2%
F2	65.55±0.1%
F3	69.23±0.2%
F4	70±0.1%
F5	61±0.2%
F6	63±0.1%
F7	92.3±0.2%
F8	73.3±0.1%
F9	82.12±0.2%

Table 6 Swelling Index

Formulation code	Percentage hydration (Swelling index)					
	1h	2h	4h	6h	8h	10h
F1	34.3±0.12	43.3±0.10	53.5±. 09	65.7±0.11	75.5±0.13	80.2±0.12
F2	36.2±0.11	44.5±0.13	55.2±0.13	63.5±013	72.5±0.11	77.5±0.12
F3	38.5±0.11	45.5±0.10	52.3±0.11	60.1±0.12	74.3±0.11	78.3±0.17
F4	40.1±0.12	50.1±0.12	56.3±0.12	63.4±0.12	70.2±0.13	77.8±0.12
F5	41.6±0.11	49.3±0.2	53.2±0.21	62.2±0.14	73.6±0.13	79.2±0.13
F6	43.8±0.12	47.4±0.2	53.15±0.12	65.2±0.13	74.7±0.14	80.1±0.14
F7	45.23±0.12	52.6±0.23	65.3±0.12	78.2±0.11	88.5±0.12	92.1±0.11
F8	44.2±0.13	50.2±0.13	54.2±0.12	68.4±0.13	75.3±0.12	82.5±0.10
F9	43.6±0.14	48.2±0.15	56.6±0.12	67.3±0.11	74.4±0.11	85.7±0.11

Table 7 Micromeritic Properties

Formulation Code	Weight of microsphere taken(gm)	Bulk volume	Tapped volume	Bulk Density	Tapped Density
F1	2g	1.3±0.1	1.3±0.2	1.53±0.2	1.58±0.1
F2	2g	1.5±0.1	1.4±0.1	1.33±0.1	1.42±0.2
F3	2g	1.4±0.1	1.2±0.1	1.42±0.2	1.66±0.2
F4	2g	1.6±0.1	1.4±0.1	1.25±0.1	1.42±0.1
F5	2g	1.7±0.1	1.3±0.1	1.17±0.1	1.5±0.1
F6	2g	1.8±0.1	1.4±0.1	1.1±0.1	1.6±0.2
F7	2g	1.8±0.1	1.6±0.2	1.111±0.1	1.25±0.3
F8	2g	1.5±0.3	1.2±0.4	1.333±0.2	1.66±0.4
F9	2g	1.4±0.1	1.3±0.1	1.428±0.2	1.538±0.2

Table 8 Carr's index

Formulation Code	Carr's index	Flow property
F1	12.23±0.23	Good
F2	10.65±0.43	Excellent
F3	12.76±0.21	Good
F4	13.57±0.34	Good
F5	19.37±0.87	Passable
F6	16.22±0.76	Good
F7	7.64±0.67	Excellent
F8	11.46±0.45	Excellent
F9	12.54±0.34	Good

Table 9 Hausner's ratio

Formulation Code	Hausner's ratio	Flow property
F1	1.07±0.34	Free flowing
F2	1.16±0.23	Free flowing
F3	1.14±1.1	Free flowing
F4	1.30±0.45	Cohesive powder
F5	1.28±0.43	Cohesive powder
F6	1.25±0.56	Cohesive powder
F7	1.12±0.46	Free flowing
F8	1.07±0.34	Free flowing
F9	1.23±1.09	Cohesive powder

Table 10 Angle of repose

Formulation Code	Angle of repose	Flow property
F1	26±1	Good
F2	28±2	Good
F3	27±1	Good
F4	18±2	Excellent
F5	29±1	Good
F6	22±2	Excellent
F7	12±2	Excellent
F8	23±1	Excellent
F9	30±1	Good

Table 11 Percentage yield value

Formulation Code	% yield value
F1	70±1.12
F2	66.3±1.13
F3	75±1.23
F4	83±1.4
F5	79±1.5
F6	87±1.6
F7	95.23±1.5
F8	85±1.5
F9	92

Table-12 In vitro wash off test

Formulation code	**% Mucoahesion**						
	Time in hour						
	0.5h	1h	2h	3h	4h	5h	6h
F1	65±1	51±1	49±2	45±6	38±3	28±1	10±2
F2	63±1	52±2	47±1	43±5	32±3	27±3	7±4
F3	70±2	65±4	53±2	47±4	37±4	23±3	8±1
F4	72±2	63±2	59±2	45±5	32±3	30±1	12±4
F5	78±1	64±2	58±2	46±6	34±5	28±2	6±5
F6	73±1	62±3	53±1	43±5	38±1	20±2	7±5
F7	90±2	86±2	75±3	65±9	55±4	35±4	15±4
F8	82±1	76±1	65±5	59±6	48±3	39±3	11±4
F9	84±1	75±2	69±7	55±7	43±3	30±4	13±6

Table 13 *In-vitro* drug release

	% drug release				
	Time in hour				
Formulation code	2h	4h	6h	8h	12h
F1	25.23±0.21	38.57±0.13	55.65±0.11	82.22±0.11	90.27±0.45
F2	23.54±0.23	39.23±0.22	56.32±0.13	56.72±0.22	95.55±0.67
F3	30.72±0.12	38.54±0.13	69.52±0.23	58.23±0.23	90.23±0.56
F4	20.55±0.21	35.76±0.22	68.57±0.22	59.43±1.03	99.52±0.45
F5	15.75±0.22	30.63±0.22	69.84±0.34	58.95±0.56	97.12±0.58
F6	20.21±0.31	31.54±0.14	65.43±0.33	56.57±0.43	90.11±0.46
F7	15.18±0.41	21.92±0.14	32.72±0.34	49.44±0.87	91.23±0.47
F8	22.15±0.21	32.54±0.12	55.26±0.34	69.43±0.56	91.25±0.56
F9	20.13±0.22	38.15±0.15	56.92±0.22	58.79±0.45	95.15±0.47

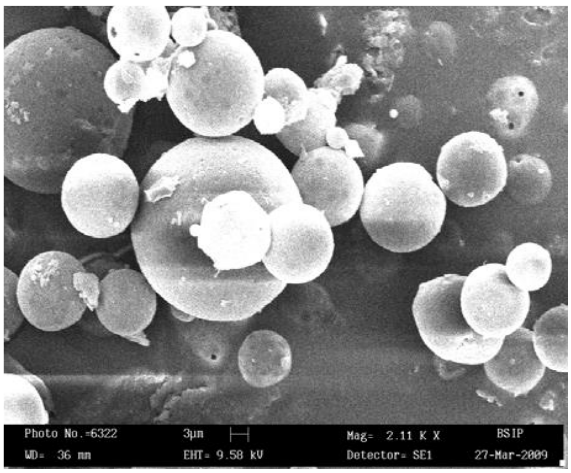

Figure-6 S.E.M of optimized formulation

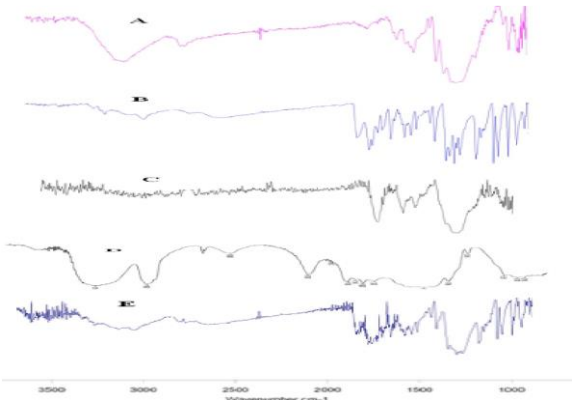

Figure 7 FTIR spectra of (A) Tenofovir, (B) Sodium alginate, (C) Sodium CMC, (D)HPMC K4M and (E) Optimized formulation

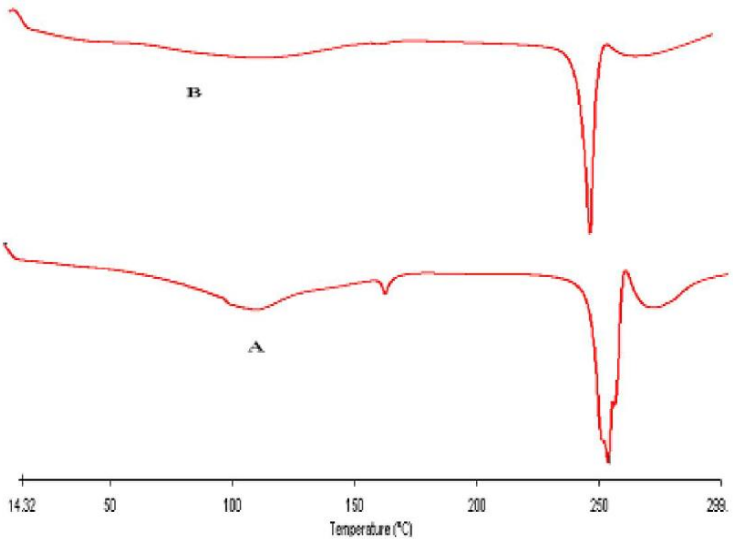

Figure 8 DSC thermogram of (A) Tenofovir and (B) Optimized formulation

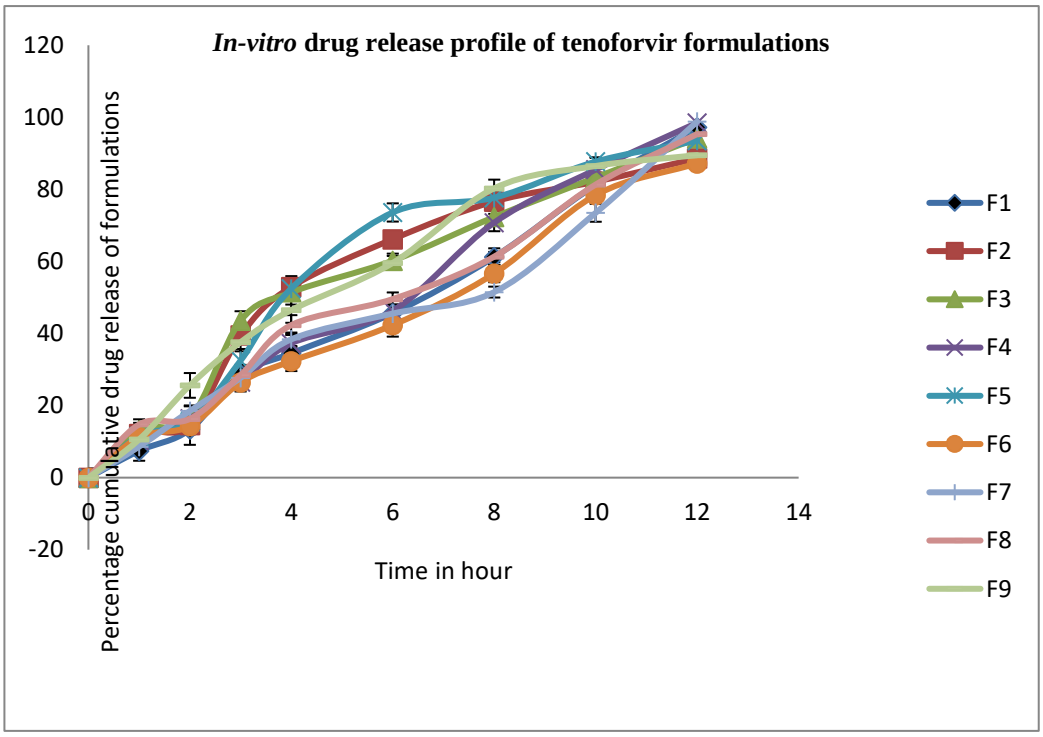

Figure 9 In-vitro drug release profile for tenofovir formulation

Discussion:
Tenofovir disoproxil fumarate mucoadhesive microsphere prepared by using ionic gelation technique by using different ratios of polymer. One is cross linking polymer (Sodium alginate) and other is mucoadhesive polymers (HPMC, CMC, Carbapol). The microspheres were evaluated by different evaluation parameters like- drug entrapment efficiency, swelling index, micromeritics property, in vitro wash off test, in vitro drug release study and stability study. And the results of evaluation study are discussed below.

Drug content and entrapment efficiency:
Drug content and entrapment efficiency of different formulation was found to be in the range of 82 to 95%. Formulation-7 containing HPMC based mucoadhesive microsphere showed maximum drug content and entrapment efficiency in comparison to other formulations.

Swelling index:
Swelling index of microspheres prepared as per experimental design were found to be satisfactory. Formulation F7, F8, F9 showed maximum swelling index.

Micromeritics property:
For the prepared formulation carr's index came in between 7.646 to 19.37%, hausner's ratio in between 1.07 to 1.30, angle of repose in between18

to 30°. This results confirms good flow property of microsphere.

FTIR:
It was observed from the spectra of pure drug and optimized formulation that there was neither remarkable shift in the wave number the peaks nor in the intensity of peaks; proved that there was no interaction between drug and selected polymers.

DSC:
It is cleared from the DSC that the characteristics peaks of the drug are also present in the formulation depicting no incompatibility between the drug and polymers in the formulation

In vitro wash off test:
In vitro wash off test showed that prepared microsphere exhibit for mucoadhesive properties. Formulation containing higher concentration of mucohesive polymer (HPMC), showed higher mucodhesive property and longer wash off test. Attributed due to electrostatic attraction between HPMC and mucin.

In vitro drug release study:
It was found that formulation with drug polymer ratio 1:2 released maximum amount of drug at 12hours. But the other formulation table 13 release the drug before 12 hours show that in comparison to all the 9 formulations F7 showing controlled release action.

Conclusion:
The present research was carried to develop mucoadhesive drug delivery system. Tenoforvir disoproxil fumarate loaded microspheres containing sodium alginate, HPMC polymers prepared by ionic gelation techniques. The influence of the formulation and dosage parameters in formulation of Tenofovir disoproxil fumarate microsphere was studied with respect to percentage yield value, entrapment efficiency, in vitro drug release, in vitro wash off test, stability study. In case of in vitro dissolution F7 release the drug in controlled manner up to 16hours; indicating promising potential of the tenofovir disoproxil fumarate over the conventional dosage form.

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