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Formulation Development and Evaluation of Wax Incorporated Floating Beads of Tenofovir Disoproxil Fumarate

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ABSTRACT:

Pectin, carnauba wax and olive oil were selected for the preparation of floating pectinate wax beads. The identity of Tenofovir was confirmed by physical characteristics, spectrophotometric analysis such as Ultra violet visible spectrophotometric, Fourier Transform – Infra red and differential thermal colorimetic studies by preparing the floating pectinate wax beads of Tenofovir, the effect of different variables on floating pectinate wax beads was studied. The prepared floating beads were evaluated for micromeritic properties, % drug contents, floating lag time, floating time, swelling index and % drug release in 0.1N Hydrochloric acid and its accelerated stability study.The floating pectinate wax beads containing Tenofovir were prepared. The effect of various process and formulation variables on Tenofovir floating beads were studied. The concentration of carnauba wax had significant effect on % drug release and floating lag time. However the drug release was greatly retarded as the concentration of carnauba wax increases and floating lag time was decreased.After evaluation parameter of floating pectinate wax beads, the best suited formulation (F2) was selected because of better floating lag time and sustained release of the drug. Formulation (F2) was evaluated for stability study, floating lag time, floating time and % drug release. The following conclusions can be drawn from present study.

Keywords: Tenofovir Disoproxil Fumarate, costing method, Floating Drug delivery system, Bioavailability enhancement, Floating beads

INTRODUCTION

Pharmaceutical dosage forms are drug delivery systems (DDS) by which drug molecules are delivered to sites of action within the body. Some common examples are tablets, capsules, suppositories, ointments, liquid, solutions, injections and transdermal patches. To achieve an optimum response from any dosage form, a drug should be delivered to its site of action at a rate and concentration that both minimize its side effects and maximize its therapeutic effects. The development of safe and effective drug dosage forms and delivery systems requires a thorough understanding of physicochemical properties that allow a drug to be formulated in to a pharmaceutical dosage form. Design of the appropriate dosage form or delivery system depends on the following factors:

- Physicochemical properties of the drug, such as solubility, oil-to- water partition coefficient (K o/w), pKa value and molecular weight.
- Dose of the drug.
- Route of administration.
- Type of DDS desired.
- Pathologic condition to be treated
- Desired therapeutic effect.
- Drug release from the delivery system.
- Bioavailability of the drug at the absorption site.

- Pharmacokinetics and pharmacodynamics of the drug. [1]

Various routes of administrations play a marked role in the bioavailability of the active drug in the body. Oral route of administration is one of the oldest and most extensively used routes for the administration of drug providing convenient method of effectively achieving both local and systemic effect. Various approaches are made in designing the formulations, which will overcome the disadvantages of conventional dosage forms, which include sustained/controlled release drug delivery system.[2]

1.5 Mechanism of Floating system [23]

Various attempts are made to obtain retention of dosage form in the stomach by increasing RT of stomach. These include introduction of different gastro retention dosage forms as floating systems (gas generating system and swelling and expanding system), muco-adhesive system, high density system, modified shape systems, gastric– emptying delaying devices and co administration of gastric empting delaying drugs. From this the floating drug delivery systems (FDDS) is most commonly used. FDDS have a bulk density less than gastric fluids and so remain buoyant in the stomach without affecting the gastric emptying rate for a prolong period of time. When the system floats on gastric contents the drug is released slowly at the desire rate from the system. After the drug is released, the residue is emptied from the stomach. This results in increasing the gastric empting time of stomach as well as controlling the fluctuations in PDC.

F = FBuoyancy–Fgravity = (Df- Ds) gv… (1) Where,

F = Total vertical force.

DF = Fluid density. DS = Object density. V = Volume.

G = Acceleration due to gravity

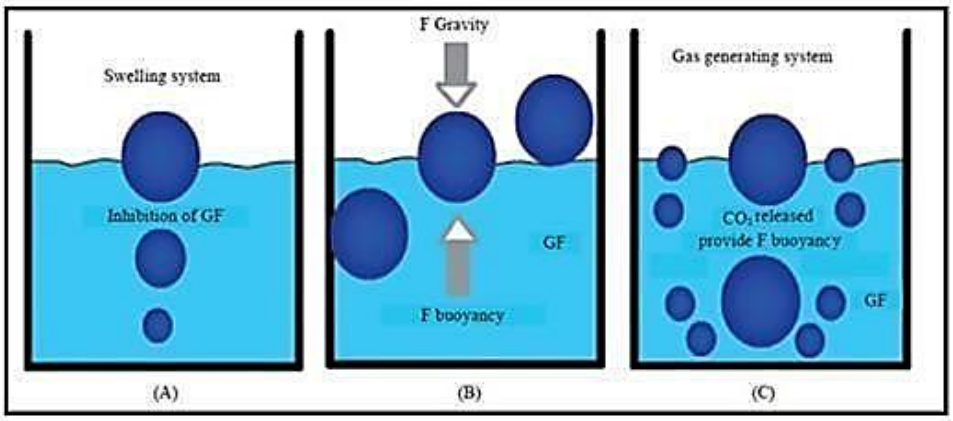

Fig. Mechanism of floating drug delivery

MATERIALS AND EQUIPMENTS:

Materials & Equipment used for experimental work are shown in the following table:-

Sr. No.	Materials	Manufacturer	Name of Equipment	Manufacturers
1	Tenofovir disoproxil fumarate	Hema Pharmaceutical,. Gujrat, india	Electronic analytical balance	AY220 Shimadzu corporation
2	Pectin	Research lab fine chem. Industries,	UV Visible	Shimadzu, Japan
3	Carnauba wax	Research lab fine chem. Industries,	Digital pH meter 335	Systronics, Ahmedabad
4	Olive oil	Research lab fine chem. Industries,	FTIR Spectrophotometer	Brucker, ECO- ATR
5	Calcium chloride	LOBA chemie Pvt. Ltd., Mumbai	Magnetic stirrer	DBK Instruments
6	Methanol AR	Research lab fine chem. Industries,	Sonicator	PCI Analytics
7	Potassium Dihydrogen Phosphate	Research lab fine chem. Industries, Mumbai	Water distillation unit	3361,Borocil-D.A.P.S.Controller
8	Disodium Hydrogen Phosphate	Research lab fine chem. Industries,	Dissolution apparatus	Electrolab Dissolution Tester USP
9	Hydrochloric acid	Research lab fine chem. Industries,	Stability chamber	Tempo Instruments Pvt. Ltd.
10	Sodium Hydroxide	Research lab fine chem. Industries,	DSC (Differential Scanning Colorometry)	DSC 60, Shimadzu, Japan
11	Distilled water	Research lab fine chem. Industries,	SEM (Scanning Electron Microscopy)	Carl Zeiss. Supra 5,Germany

Characterization of Excipients:

The description of all the additives were similar to those reported in the literature.

The excipients were evaluated for their appearance, colour. Pectin, Carnauba wax, Olive oil and Calcium chloride are the additives were used in the formulations.

- **Fourier Transform Infra-Red Spectroscopy (FTIR:**The FTIR spectrum of Tenofovir was recorded at wave number 4000 to 400 cm-1 using fourier transform spectrophotometer(Mode - FTIR, Bruker).

Method used for analysis was ATR. However, ATR method is able to measure powder sample directly. Attenuated total reflection (ATR) method involves pressing the sample against a high-refractive index prism and measuring theinfrared spectrum using infrared light that is totally internally reflected in the prism.A zinc selenide (ZnSc) or germanium (Ge) prism was used in the ATR accessory.

Preparation of floating beads:

Composition of formulation:

Formulation Code	Drug	Pectin	Olive Oil	Carnauba wax	Water Q.S
F1	300	4	30	4	100
F2	300	4	30	4	100
F3	300	4	30	4	100
F4	300	4	30	4	100

Tenofovir hydrochloride floating wax beads were prepared using polymers, viscosity enhancer, gelling agent & suspending agent.

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Formulation and Development:

- Preparation of floating beads :

Tenofovir hydrochloride floating wax beads were prepared using polymers, viscosity enhancer, gelling agent & suspending agent.

The following steps were carried for its preparation:

Step 1. Emulsion of pectin, olive oil and drug was prepared in the distilled water using the high speed homogeniser at 3000 RPM for 15 min.

Step 2. The pre-weighed amount of wax was melted in the porcelain dish on the heating water bath.

Step 3. The emulsion formed was heated to the temperature above the melting point of the wax.

Step 4. The molten wax was dispersed in the preheated emulsion using hot plate with magnetic stirrer.

Step 5. After stirring for 15 min the above solution was filled into the 22G syringe and air bubbles were removed.

Step 6. The solution was added drop wise into the calcium chloride solution.

Step 7. After addition of solution the beads are formed. The beaker was kept aside for 15 min.

Step 8. The beads were filtered from calcium chloride solution. The beads were rinsed thoroughly with distilled water and dried at room temperature.

Evaluation of Floating Beads:

5.1.1 Physical appearance

All the prepared floating beads formulations of Tenofovir was checked for their size, shape and colour.[61,62]

Micromeritic properties

All the prepared floating beads formulation of Tenofovir was checked for the Bulk density, Tapped density, Carr's index, Hausner's ratio.[64,71]

Bulk density: The bulk density was obtained by dividing the mass of powder by the bulk volume. The sample equivalent to 5 mg was accurately weighed and filled in a 100 ml graduated cylinder and the powder was leveled and unsettled volume (V0) was noted.

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Tapped density: The tapped density was determined by mechanically tapping the measuring cylinder or by using the digital bulk density tester (Meta Lab) USP Model no.I and the tapped volume were noted (USP, 2006).

Hausner's ratio: Hausner's ratio gives an idea regarding the flow of the blend to the apparent density.

Carr's index: The Carr's index measures of the propensity of the powder to be compressed. The packing ability of the drug was evaluated from change in the volume, which is due to rearrangement of packing occurring during tapping (USP, 2006).

Particle size determination: The particle size of beads was determined by the dry state using optical microscopy method. The stage micrometer and eyepiece micrometer were used for the measurement of the particle size. The size of the beads present in the $1cm^3$ area of the slide was counted.

Surface characterization: Surface characterization of beads were examined with a scanning Electron Microscopy (Diya labs, airoli, Mumbai) beads were mounted on metal grids using double-sided tape.

In- vitro drug release study: The release of Tenofovir from sustained release floating wax bead was determined using USP dissolution apparatus I at 50 rpm. The dissolution medium used 900ml of 0.1N HCl (pH1.2) and temperature was maintained at 37 0C. A sample (5ml) was withdrawn from the dissolution apparatus at 0 min., 1hr, 2hr, 4hr, 6hr, 8hr, 10hr, 12hr. The samples were filtered through Whatman filter paper and analysed using UV method. Cumulative % drug release was calculated and observed. The dissolution of the formulation was compared with the 250mg of the capsule containing 5mg of the drug.The data obtained from study of diffusion kinetics of the optimized formulation was studied to obtain the best fit model. The best fitted model is the one which gives the highest R2 value and least slope value.

Stability studies: Stability study of the formulation which gave maximum dissolution rate was carried out to point out any visual physical or chemical change made in the formulation after storing it at elevated temperature and humidity conditions. The optimized formulation was store in ambient colour bottle and stored at 40^0 C ± 2^0C and 75% ± 5% Relative humidity for three months. Floating wax beads was analysed for the drug content.

Results:

Organoleptic properties of Tenofovir:

S.no	**Properties**	**Observation**
1	Appearance	Crystalline powder
2	Colour	White
3	Odour	Odourless

Melting point determination:

Particle size determination

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Melting point determination:

The melting point of Tenofovir was given in table 9. The melting point of the drug matches with values found in literature.

Sr. No.	Drug	Melting point	
1.	Tenofovir	Literature	Observed
		278-282 ^{0}C	280.4^{0}C

Solubility:

Solubility of Tenofovir was determined in different solvent and given in table. The results were similar to those mentioned in literature.

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Sr. No.	Solvent	Solubility
1	Methanol	Soluble
2	0.1 N Hydrochloric acid	Soluble
3	NaOH	Soluble
4	Water	Sparingly soluble
5	Phosphate buffer (pH 6.8)	Soluble

Ultraviolet –Visible spectroscopy study:

The 100 µg/ml stock solution of Tenofovir was prepared by dissolving 10 mg of drug in suitable volume of 0.1 N Hydrochloric acid with continuous shaking.

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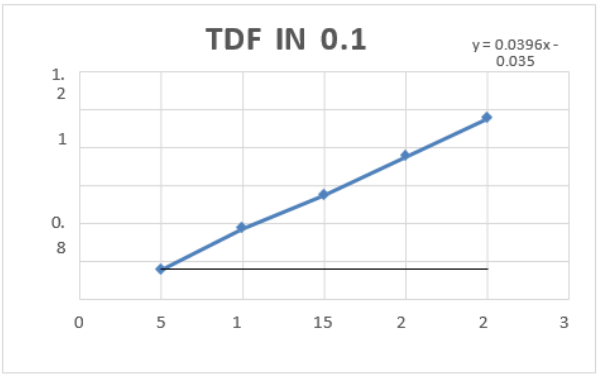
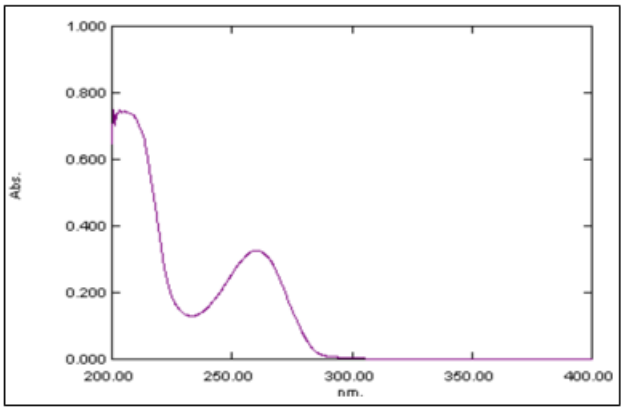
Determination of λmax of Tenofovir:

The UV spectrum of Tenofovir solution (10µg/ml) exhibited wavelength of absorbance maximum at 262nm. This is near to the reported value. However, Keeping in mind the probable concentrations likely to be encountered while carrying out the In-vitro release studies and considering the predicted theoretical λmax involved, the working λmax was decided as 262nm. The spectrum of Tenofovir is shown in fig.





Spectrum of Tenofovir in 0.1N HCl **Calibration curve of Tenofovir in 0.1N HCl**

Preparation of stock solution in phosphate buffer pH 6.8

The 100 µg/ml stock solution of Tenofovir was prepared by dissolving 10 mg of drug in suitable volume of phosphate buffer pH 6.8 with continuous shaking.

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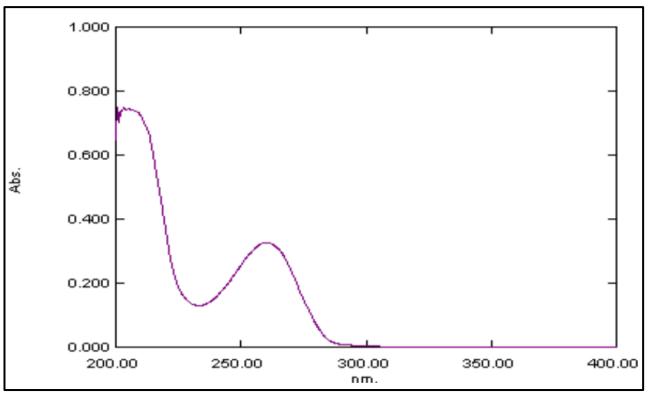
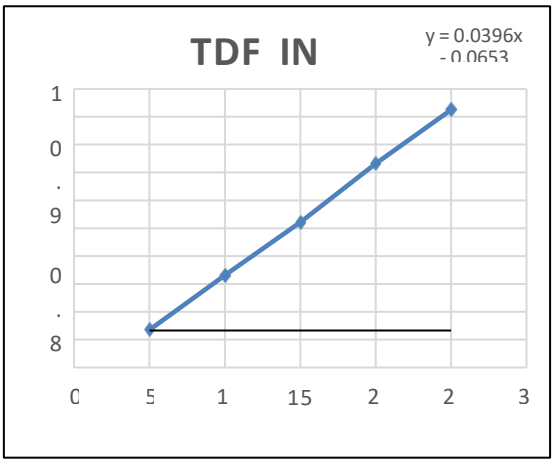
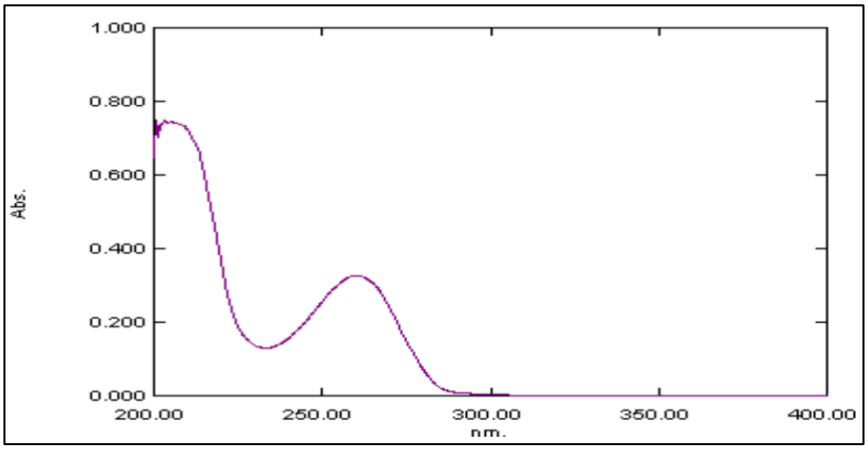




Calibration curve of Tenofovir in PBS pH 6.8 **Spectrum of Tenofovir in phosphate buffer pH 6.8**

Preparation of stock solution in methanol: The 100 µg/ml stock solution of Tenofovir was prepared by dissolving 10 mg of drug in suitable volume of methanol with continuous shaking.

Determination of λ max: of Tenofovir The UV spectrum of Tenofovir solution (10µg/ml) exhibited wavelength of absorbance maximum at 262nm. This is near to the reported value. However, Keeping in mind the probable concentrations likely to be encountered while carrying out the In-vitro release studies and considering the predicted theoretical λ max involved, the working λ max was decided as 262nm. The spectrum of Tenofovir.



Maximum wavelength of Tenofovir in 0.1N Hydrochloric acid (pH 1.2)

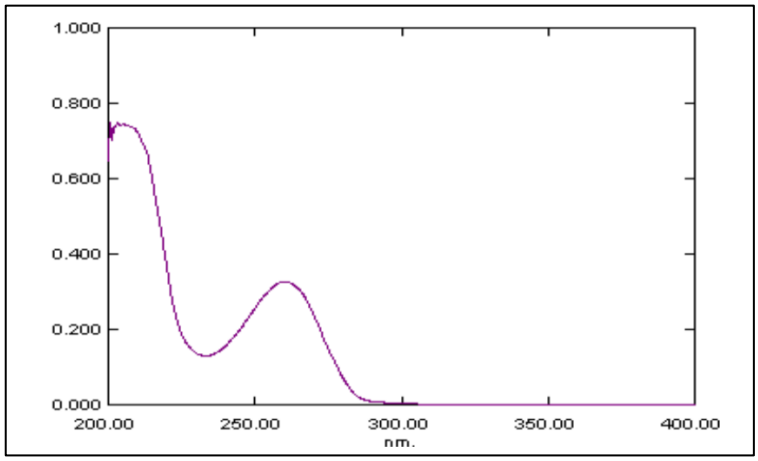
Solvent	Wavelength of maxima (nm)
0.1 N Hydrochloric acid (pH 1.2)	262 nm

Absorbance value of Tenofovir in 0.1N Hydrochloric acid (pH 1.2)

Sr, No.	Concentration	Absorbance
1	5	0.1564
2	10	0.3748
3	15	0.5517
4	20	0.7541
5	25	0.9561

Preparation of stock solution in phosphate buffer pH 6.8: The 100 µg/ml stock solution of Tenofovir was prepared by dissolving 10 mg of drug in suitable volume of phosphate buffer pH 6.8 with continuous shaking.

Determination of λmax of Tenofovir in phosphate buffer pH 6.8: The UV spectrum of Tenofovir solution (10µg/ml) exhibited wavelength of absorbance maximum at 262nm. This is near to the reported value. However, Keeping in mind the probable concentrations likely to be encountered while carrying out the In-vitro release studies and considering the predicted theoretical λmax involved, the working λmax was decided as 262nm. The spectrum of Tenofovir is shown in fig.



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Spectrum of Tenofovir in phosphate buffer pH 6.8

Calibration curve of Tenofovir in phosphate buffer pH 6.8 The calibration curve was found to linear in the concentration range of 20 – 100 µg/ml having coefficient of regression value R^2 = 0.9998 and slope y = 0.0396x – 0.0653

Sr. No	Concentration	Absorbance
1	5	0.1348
2	10	0.3315
3	15	0.5211
4	20	0.7319
5	25	0.9248

Absorbance value of Tenofovir in PBS pH 6.8

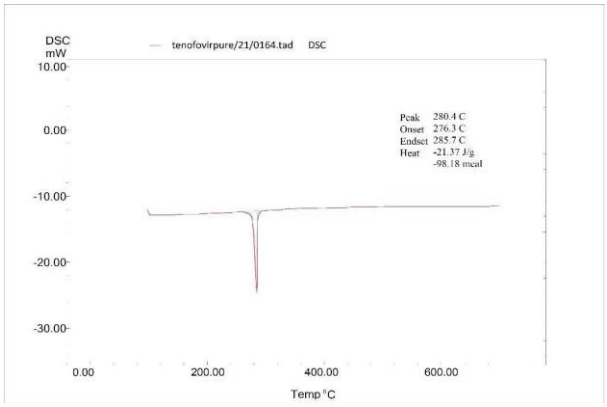
Preparation of stock solution in methanol ; The 100 µg/ml stock solution of Tenofovir was prepared by dissolving 10 mg of drug in suitable volume of methanol with continuous shaking.

Determination of λmax of Tenofovir in methanol; The UV spectrum of Tenofovir solution (10µg/ml) exhibited wavelength of absorbance maximum at 262nm. This is near to the reported value. However, Keeping in mind the probable concentrations likely to be encountered while carrying out the In-vitro release studies and considering the predicted theoretical λmax involved, the working λmax was decided as 262 nm. The spectrum of Tenofovir is shown in fig.

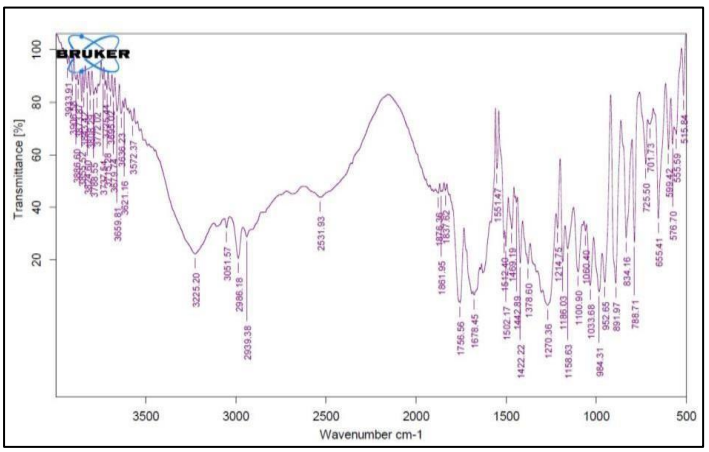
Sr, No.	Concentration	Absorbance (nm)
1	5	0.1497
2	10	0.3516
3	15	0.5414
4	20	0.7513
5	25	0.9661

Absorbance value of Tenofovir in methanol

Fourier Transform Infra- Red Spectroscopy (FTIR);Infrared spectrum of Tenofovir is shown in .The major peaks observed and corresponding functional groups are given in Table 17. Infra-red spectrum shows peak characteristics of structure of Tenofovir. Infrared spectrum of Tenofovir is shown in fig.15. The major peaks observed and corresponding functional groups are given in Table 17. Infra-red spectrum shows peak characteristics of structure of Tenofovir.



FTIR Spectrum of Tenofovir Disoproxil Fumarate



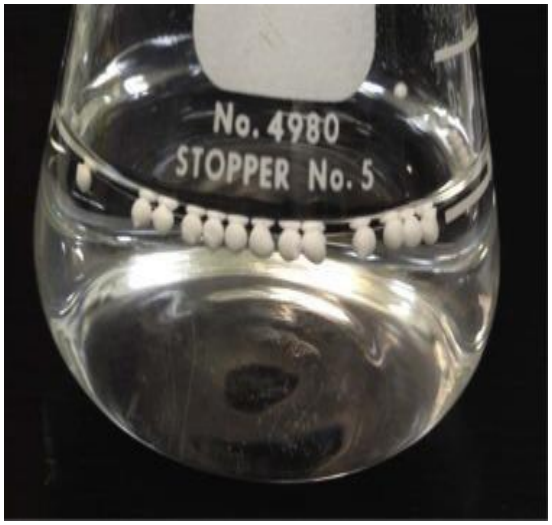
DSC thermogram of Tenofovir

Differential scanning colorimetric studies (DSC):DSC thermogram of Tenofovir is shown in fig. The DSC analysis of Tenofovir against reported values are given . The DSC thermogram peak value matches with value found in literature.

Observed value	**280.4 ^{0}C**
Peak	280.4 ^{0}C
Onset	276.3^{0}C
End	285.7^{0}C
Heat	-21.37 J/g

Evaluation of floating wax beads;

Physical Appearance; To developed formulation, dissolve all the pre-requisite to become a floating wax beads system and floated instantaneously at pH condition of the stomach.



Physical appearance of the formulated beads

Micromeritic properties; The micromeritic properties (Bulk density, Tapped density, Carr's index, Hausner's ratio) of all the formulated batches was measured.

Micromeritic properties of the formulation

Batch Code	**Bulk density (gm/ml) ± SD**	**Tapped density (gm/ml) ± SD**	**Carr's index ± SD**	**Hausner's ratio ± SD**
F1	0.3920 ± 0.0013	0.4693 ± 0.0021	11.70 ± 0.0578	1.1904 ± 0.0085
F2	0.4804 ± 0.0045	0.5563 ± 0.0049	6.76 ± 0.0357	1.1277 ± 0.0010
F3	0.4312 ± 0.0020	0.5182 ± 0.0016	9.22 ± 0.0441	1.1834 ± 0.0058
F4	0.3604 ± 0.0011	0.4569 ± 0.0025	8.10 ± 0.0482	1.2658 ± 0.0011

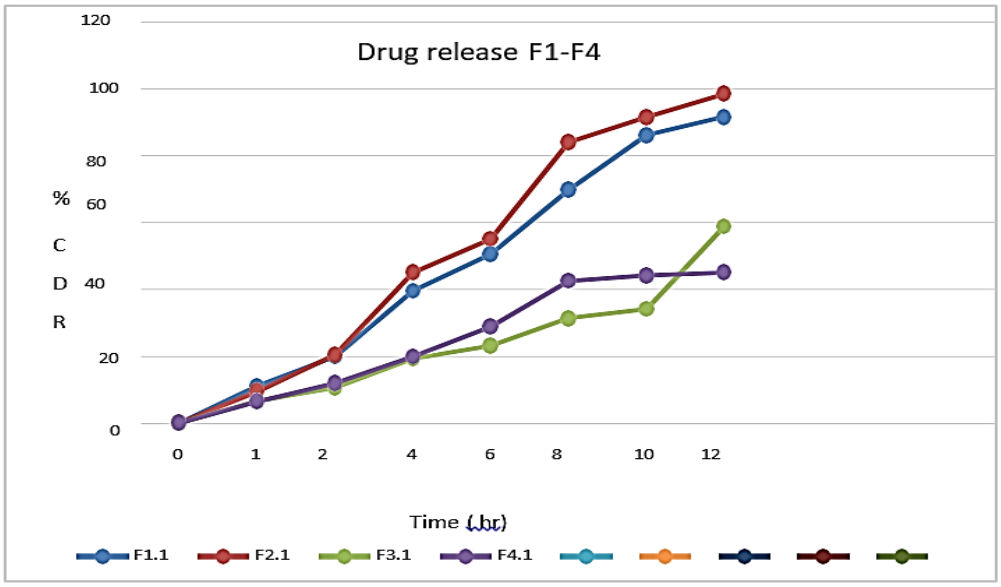
Percentage yield ; The percentage yield of floating beads of Tenofovir was measured.

Sr. No.	**Batch Code**	**Percentage Yield (%)**
1	F1	96.84
2	F2	97.14
3	F3	95.65
4	F4	95.20

Percentage yield of the formulations

All formulations F1 – F4 found percentage yield 97.14 – 95.20% which lied in the normal range in table.

In vitro drug release study;



Drug release profile of formulations F1-F4

Stability study; The sample were withdrawn after 1, 2 and 3 months and subjected to following a shown in Table.

Details of stability study for F2 batch

Test	Before	After		
	0 month	1 month	2 month	3 month
Drug release	93.64 ± 0.246	92.60 ± 0.236	91.07 ± 0.254	90.45 ± 0.251
Floating lag time	>12 hrs	>12hrs	>12hrs	>12hrs

The accelerated stability studies (carried for 3 months), at temperature of 40^0C± 2^0C and % RH 75% ± 5% RH indicated that the developed floating pectinate beads were unaffected after 03 months storage under accelerated condition as no change was observed in the appearance and colour of the formulation. On the basis of these results, it may be concluded that the optimized formulation developed is stable under accelerated condition of 03 months.

Conclusions;

a) Preformulation study of drug and polymers was done.

b) Compatibility study between drug and polymers was done by Fourier Transform Infrared spectrum analysis and it was found that there was no chemical interaction between drug and polymers. Conclusions.

c) Floating lag time of formulation was studied and it was found that as concentration of polymers increases the floating lag time also increases.

d) Surface characterization by Scanning Electron Microscopy of floating pectinate wax beads was studied and it shown the uneven surface with spherical shape.

The sustained release rate and evaluation of prepared edible oil entrapped floating pectinate wax beads was studied.

a) The beads of optimized batch shown the bulk density as 0.4804 ± 0.0045, tapped density of 0.5563 ± 0.0049, Carr's index as 6.76 ± 0.0357 and Hausner's ratio as 1.1277 ± 0.0010.

b) The beads of optimized batch shown the percentage yield as 97.14%, percentage drug content as 96.89 ± 0.8369 %, percentage drug entrapment efficiency as 92.21 ± 0.6864 %.

c) The optimized batch shown floating lag time of 1.21 min and floating time >12hr.

d) The F2 batch showed the maximum swelling index as 20.66 ± 0.05241 and average particle size was found to be 1.21 ± 0.0163.

e) The In vitro drug release study of different formulation was studied maximum drug release 98.51% was shown by optimized batch. After comparing the coefficient of regression (r2) values of different kinetic models, drug release kinetics for optimized floating beads best fitted in zero order kinetic release followed by Higuchi.

f) No significant change was observed in present drug release before and after stability studies carried out for 03 months of batch (F2).

g) The characterization of different excipients in prepared floating pectinate wax beads formulations was studied.

Thus it can be concluded that the floating wax beads can be a better approach for sustained release activity for drugs with short half life

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