Kedarnagalakshman M. et al., Vol.–08 (04) 2018 [226 – 232]

Research Article

Available Online at: www.ijpir.com

International Journal of Pharmacy and Industrial Research

ISSN	Print	2231 – 3648
	Online	2231 – 3656

Design, formulation and evaluation of abacavir microspheres

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Abstract

The objective of present study was to encapsulate the anti-retroviral drug in various polymers in order to provide the sustained release and to minimize or reduce the dose dependent side effects as well as to improve the patient compliance. Abacavir sulphate microspheres were prepared by w/o emulsification solvent evaporation method using different polymers viz. sodium alginate and gelatin. The prepared microspheres were characterized for drug entrapment efficiency, muco-adhesion test, particle size analysis, surface morphology and in-vitro drug release study. The in-vitro release studies were performed using pH 1.2 Hcl and pH 7.4 phosphate buffer and drug release is evaluated. Morphology of microspheres was characterized by using Scanning Electron Microscopy (SEM). The prepared microspheres were small, discrete, free flowing and spherical in shape. The mean diameter of microspheres was between 21.00±1.96 to32.43±2.19µm for different formulations. The drug loaded microspheres showed 40-65% and 45-50% of entrapment for sodium alginate and gelatin microspheres respectively. Fourier Transform- Infra Red (FT-IR) was performed to evaluate interaction between drug and polymer. The prepared microspheres exhibited prolonged drug release (10h) as the concentration of sodium alginate increased, the muco-adhesion is also increased as the sodium alginate concentration increases and the drug release rate was decreased at higher concentration of gelatin.

Keywords: Abacavir, Microspheres, Solvent evaporation method, SEM.

Introduction

Controlled drug delivery systems can include the maintenance of drug levels within a desired range, the need for fewer administrations, optimal use of the drug in question, and increased patient compliance. While these advantages can be significant, the potential disadvantages cannot be ignored like the possible toxicity or non-biocompatibility of the materials used, undesirable by-products of degradation, any surgery required to implant or remove the system, the chance of patient discomfort from the delivery device, and the higher cost of controlled-release systems compared with traditional pharmaceutical formulations. The ideal drug delivery system should be inert, biocompatible, mechanically strong, comfortable for the patient, capable of achieving high drug loading, safe from accidental release, simple to administer and remove, and easy to fabricate and sterilize. The goal of many of the original controlled-release systems was to achieve a delivery profile that would yield a high blood level of the drug over a long period of time. With traditional drug delivery systems, the drug level in the blood follows the in which the level rises after each administration of the drug and then decreases until the next administration. The key point with traditional drug administration is that the blood level of the agent should remain between a

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maximum value, which may represent a toxic level, and a minimum value, below which the drug is no longer effective.[1-9]

Microsphere is a term used for small spherical particles, with diameters in the micrometer range (typically 1µm to 1000µm (1mm). Microspheres are sometimes often referred to as microparticles (Microsphere, 2015). Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having a particle size less than 200 μm^3. [2]

Methodology

Preparation of Standard Graph

Different concentrations of abacavir sulphate (2- 20 µg/ml) are prepared using water as solvent and the absorbances were measured at 285 nm using water as blank. The standard graph for the drug was plotted by taking concentrations on x-axis and relative absorbances on y-axis.

Preparation of Hcl Buffer pH-1.2

Place 50ml of 0.2M Kcl (Dissolve 14.911gm of Kcl in water and dilute to 1000ml) in 200ml volumetric flask. Add 85ml 0.2M Hcl (Dissolve 7.292gm of Hcl in 1000ml) and make up the volume with water.

Preparation of Phosphate buffer pH-7.4

Take 50 ml of Potassium di hydrogen phosphate (27.218gm of Potassium di hydrogen phosphate in 1000 ml) and 39.1 ml of 0.2 M NaOH (8gm of NaOH in 1000ml) and makeup the volume to 200ml.

Solvent evaporation technique

Microspheres are prepared by 'emulsion solvent evaporation technique.' Abacavir is dissolved in aqueous polymer solution, the concentrations and amounts supplied are summarized in table-1, and the drug-polymer solution is thoroughly mixed by using magnetic stirrer. This solution was added drop wise to 100 ml of seesum oil containing 2% tween 80. The resultant emulsion was stirred at 800-1000rpm by using mechanical stirrer and heated to $60\text{-}70^0$ c to promote evaporation of water. Stirring is continued for 4 to 5 hrs, then the formed microspheres were subsequently separated from the oil by vaccum filtration, washed with n-hexane for 3-4 times and dried at room temperature for 24 hrs.[3]

Table no 1: Composition of Abacavir Microspheres Formulations

S.No	Composition	Formulation Code	Drug (gm)	Polymer(gm)		Water (ml)	Tween80 (ml)	Seesum oil (ml)	n-hexane (ml)
				Sodium alginate	gelatin				
1	Sodium alginate 3%	F1	1	3	-	100	2	100	50
2	Sodium alginate 4%	F2	1	4	-	100	2	100	50
3	Sodium alginate 5%	F3	1	5	-	100	2	100	50
4	Gelatin 5%	F4	1	-	1	10	2	100	50
5	Gelatin 10%	F5	1	-	1.5	10	2	100	50
6	Gelatin 15%	F6	1	-	2	10	2	100	50

Evaluation parameters

Particle Size Distribution[4]

The size distribution is carried out by optical microscopy and average of about 200 particles is carried out and the average particle size is determined.

Percentage Yield and Drug Entrapment Efficiency (DEE)

The microspheres were evaluated for percentage yield and percent drug entrapment. The yield was calculated as per equation:

Percentage yield = Weight of microsphere recovered / Weight (drug + polymer) X 100

Drug loaded microspheres (100 mg) were powdered and suspended in 100 ml water solvent system. The resultant dispersion was kept for 20 min for complete mixing with continuous agitation and filtered. The drug content was determined spectrophotometrically at 285 nm using a regression equation derived from the standard graph ($r2 = 0.9978$). The drug entrapment efficiency (DEE) was calculated by:

DEE = (Pc / Tc) X 100

Where,

Pc is practical content,

Tc is the theoretical content.

Scanning Electron Microscope[4]

A scanning electron microscope was used to characterize the surface topography of the microscope. The microspheres were placed on a metallic support with a thin adhesive tape and microspheres were coated with gold under vacuum. The surface was scanned and photographs were taken at 30kV accelerating voltage for the drug loaded microspheres.

In-vitro Drug Release[5]

Dissolution study was conducted in USP XXIII dissolution apparatus using basket containing 900 ml of pH 1.2 Hcl for 2 hrs and phosphate buffer of pH 7.4 for 8 hrs. The temperature was maintained at 37^0C, and the basket was rotated at 100 rpm, 5 ml of sample was taken periodically at 15, 30, 45 min, 1, 2, 3, 4, 5, 6, 7, 8, 9,and 10 hours time interval and estimated for abacavir, spectrophoto - metrically at 285 nm using buffer as a blank.

***In-vitro* Wash-off Test for Microspheres[6]**

The mucoadhesive properties of the microspheres were evaluated by *in-vitro* wash-off test. A 1-cm by 1-cm piece of rat stomach mucosa was tied onto a glass slide (3-inch by 1-inch) using thread. Microspheres were spread (∽50) onto the wet, rinsed, tissue specimen, and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in a beaker containing the simulated gastric fluid USP (pH 1.2). At the end of 30 minutes, 1 hour, and at hourly intervals up to 10 hours, the number of microspheres still adhering onto the tissue was counted. The results of *in-vitro* wash-off test of batches f1-f6 are tabulated.

FT-IR Study[7]

FT-IR spectra of pure drug and drug loaded microspheres were obtained at room temperature in KBr pellets by applying 6000 kg/cm2 pressure using Perkin Elmer FT-IR model 883, between the ranges of 400 to 4000 cm-1.

RESULTS AND DISCUSSION

SEM RESULTS

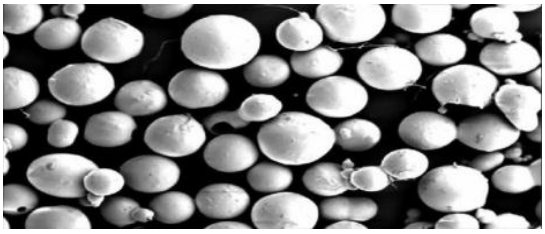

Figure no 1: Sodiumalginate Microspheres

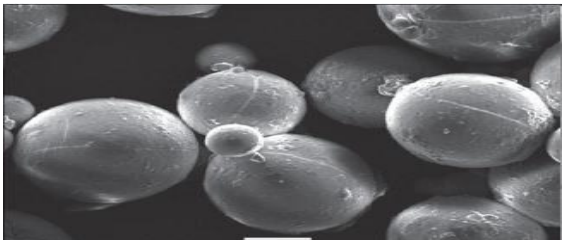

Figure no 2: Gelatin Microsheres

Table no 2: Particle size, % Yield, % Drug content, and Mucoadhesive Strength of Microspheres

S.No	Formulation	Particle size (μm)	% yield	% drug entrapment	Percentage mucoadhesion
1	F1	31.8±1.41	81.56	35.73	68±1.51
2	F2	30.9±1.20	76.45	50.28	73±1.31
3	F3	29.8±1.08	70.52	65.01	80±1.20
4	F4	21.75±1.76	67.27	44.78	78.86±1.25
5	F5	21.00±1.96	67.25	50.48	76.67±2.19
6	F6	32.43±2.19	66.61	53.78	75.83±1.98

The particle size of microspheres was determined by optical microscopy and all the batches of microspheres show uniform size distribution. The average particle size was found to be in the range of 20-35µm.The formulation F1 has shown the highest % yield (81%) followed by other formulations. Sodium alginate microspheres have shown the highest % yield when compared to gelatin microspheres.

The percentage of encapsulation of six formulations was found to be in the range of 40 to 65 % and the drug entrapment efficiency increases with increase in concentration of polymers like sodium alginate and gelatin. The formulation F3 has shown the highest % of mucoadhesion (80%). The % mucoshesion increased with increasing concentrations of polymer in case of sodium alginate microspheres, but decreased in case of gelatin microspheres.

Dissolution Studies

Table no 3 :Cumulative % Drug Release of Sodium Alginate Formulations

Time (hrs)	F1			F2			F3		
	Abs.	Conc.	% drug release	Abs.	Conc.	% drug release	Abs.	Conc.	% drug release
0	0	0	0	0	0	0	0	0	0
0.25	0.04	1.81	8.14	0.12	3.16	14.22	0.09	2.66	11.97
0.5	0.07	2.32	10.44	0.16	3.84	17.28	0.15	3.67	16..51
0.75	0.10	2.83	12.73	0.19	4.35	19.57	0.18	4.18	18.81
1	0.13	3.33	14.98	0.25	5.37	24.16	0.21	4.69	21.10
1.5	0.20	4.52	20.34	0.29	6.05	27.22	0.28	5.88	26.46
2	0.26	5.54	24.93	0.34	6.89	31.05	0.36	7.23	32.53
3	0.38	7.57	34.06	0.50	9.61	43.24	0.51	9.77	43.96
4	0.45	8.76	39.42	0.57	10.79	48.55	0.60	11.30	50.85
5	0.50	9.61	43.24	0.62	11.64	52.29	0.63	11.81	53.14
6	0.54	10.28	46.26	0.66	12.32	55.44	0.65	12.15	54.67
7	0.63	11.81	53.14	0.73	13.50	60.75	0.71	13.16	59.22
8	0.72	13.33	59.98	0.81	14.86	66.87	0.79	14.52	65.34
9	0.87	15.88	71.46	0.91	16.55	74.47	0.89	16.22	72.99
10	0.98	17.74	79.83	1.04	18.76	84.42	1.03	18.54	83.65

The *in-vitro* dissolution studies showed that sodium alginate microspheres are found to be effective in sustaining drug release. Sodium alginate microspheres have shown 80-85% drug release in 10 hours.

Table no 4: Cumulative % Drug Release of Gelatin Formulations

Time (hrs)	F4			F5			F6		
	Abs.	Conc.	% drug release	Abs.	Conc.	% drug release	Abs.	Conc.	% drug release
0	0	0	0	0	0	0	0	0	0
0.25	0.37	7.40	66.60	0.34	6.89	62.01	0.19	4.35	39.15
0.5	0.39	7.74	69.66	0.35	7.06	63.54	0.22	4.86	43.74
0.75	0.40	7.91	71.19	0.36	7.23	65.07	0.24	5.20	46.80
1	0.41	8.08	72.72	0.37	7.40	66.60	0.26	5.54	49.86
1.5	0.43	8.42	75.78	0.39	7.74	69.66	0.27	5.71	51.39
2	0.45	8.76	78.84	0.44	8.59	77.31	0.29	6.05	54.45
3	0.47	9.10	81.90	0.46	8.93	80.37	0.31	6.38	57.42
4	0.50	9.61	86.49	0.49	9.44	84.96	0.34	6.89	62.01
5	0.52	10.11	90.99	0.52	10.11	90.99	0.37	7.40	66.60
6	0.54	10.28	92.52	0.55	10.45	94.05	0.43	8.42	75.78

Gelatin microspheres have shown 94% of drug release only within 6hrs, but the sustain release for gelatin microspheres can be increased by increasing the concentration of gelatin, because formulation F6 have shown better sustain release when compared to F4 and F5.

Table no 5: Log % Undissolved Drug in Different Formulations

S. No	Time (hrs)	F1	F2	F3	F4	F5	F6
1	0	2	2	2	2	2	2
2	0.25	1.963	1.933	1.944	1.523	1.579	1.784
3	0.5	1.952	1.917	1.921	1.482	1.561	1.750
4	0.75	1.940	1.905	1.909	1.459	1.543	1.725
5	1	1.929	1.879	1.897	1.435	1.523	1.700
6	1.5	1.901	1.862	1.866	1.384	1.482	1.686
7	2	1.875	1.838	1.829	1.325	1.355	1.658
8	3	1.819	1.754	1.748	1.257	1.292	1.629
9	4	1.782	1.711	1.691	1.130	1.177	1.579
10	5	1.754	1.678	1.670	0.954	0.954	1.523
11	6	1.730	1.648	1.656	0.873	0.774	1.384
12	7	1.670	1.593	1.610	-	-	-
13	8	1.602	1.520	1.539	-	-	-
14	9	1.455	1.407	1.431	-	-	-
15	10	1.304	1.192	1.213	-	-	-

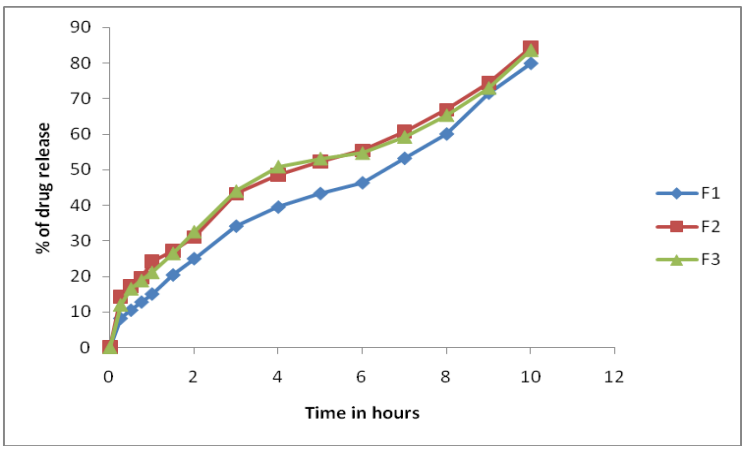

Figure no 3: Drug Release Patterns of Sodium Alginate Microspheres

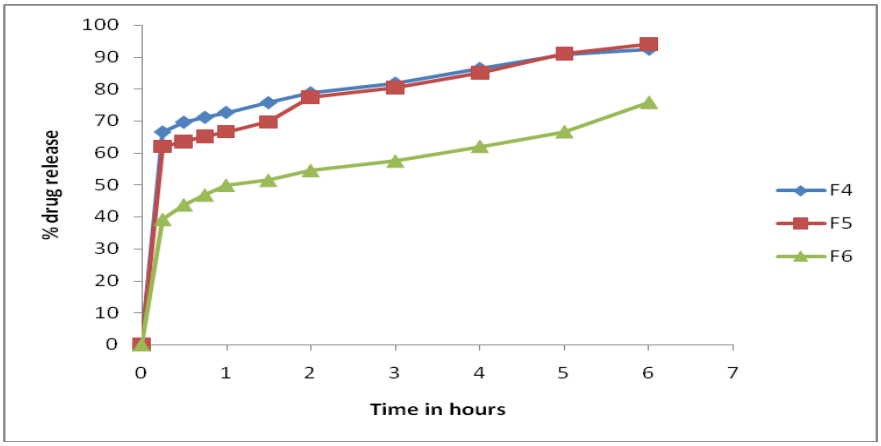

Figure no 4: Drug Release Patterns of Gelatin Microspheres

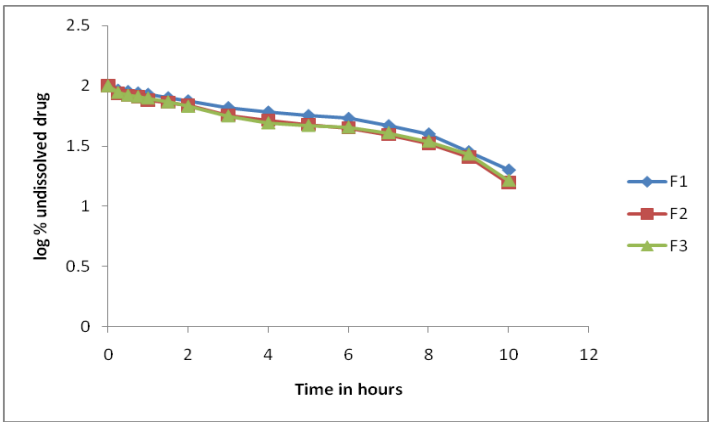

Figure no 5: Log % Undissolved Time Curve of Sodium Alginate Microspheres

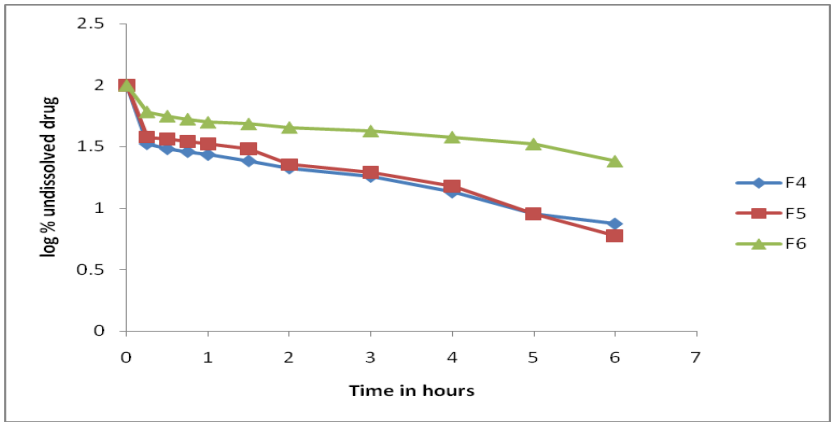

Figure no 6: Log % Undissolved Time Curve of Gelatin Microsheres

CONCLUSION

Microspheres are small spherical particles, with diameters in the micrometer range (typically 1µm to 1000µm (1mm)). Microspheres are prepared to obtain prolonged or controlled drug delivery, to improve bio availability or stability and to target drug to specific sites. Abacavir sulphate is anucleoside analogue reverse transcriptase inhibitor (NRTI), widely used to treat HIV and AIDS effectively. The drug has a relatively short half life (1.54 ± 0.63 h) in oral administration and it favors the development of a sustain release formulation. Abacavir sulphate microspheres were prepared by 'w/o emulsification solvent evaporation method' using different polymers viz. sodium alginate and gelatin. The prepared microspheres were characterized for particle size analysis, % yield, drug entrapment efficiency, muco-adhesion test, surface morphology and in-vitro drug release study.

The particle size of microspheres was determined by optical microscopy and all the batches of microspheres show uniform size distribution. The average particle size was found to be in the range of 20-35µm. The formulation F1 has shown the highest % yield (81%) followed by other formulations. Sodium alginate microspheres have shown the highest % yield when compared to gelatin microspheres. The percentage of encapsulation of six formulations was found to be in the range of 40 to 65 % and the drug entrapment efficiency increases with increase in concentration of polymers like sodium alginate and gelatin. Mucoadhesion of microspheres is estimated by performing *Invitro* wash-off test. The formulation F3 has shown the highest % of mucoadhesion (80%). The % mucoshesion increased with increasing concentrations of polymer in case of sodium alginate microspheres, but decreased in case of gelatin microspheres.

Morphology of microspheres was characterized by using Scanning Electron Microscopy (SEM). The prepared microspheres had good spherical geometry with smooth texture as evidenced by the SEM. Dissolution study for microspheres was conducted in USP XXIII dissolution apparatus using basket containing 900 ml of pH 1.2 Hcl for 2 hrs and phosphate buffer of pH 7.4 for 8 hrs.. The *in-vitro* dissolution studies showed that sodium alginate microspheres are found to be effective in sustaining drug release. Sodium alginate microspheres have shown 80-85% drug release in 10 hours, where as gelatin microspheres have shown 94% of drug release only within 6hrs. Fourier Transform- Infra Red (FT-IR) was performed to evaluate interaction between drug and polymer. From this study it was found that the drug and polymer are compatible with each other. Finally formulation F3 is found to be the best formulation because it has shown the maximum drug entrapment efficiency, highest % of mucodhesion and good sustain release of the drug.

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