ISSN: 0975-766X
Research Article
Available Online through
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PREPARATION AND EVALUATION OF LAMIVUDINE MICROSPHERES USING VARIOUS CELLULOSE POLYMERS

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Received on 22-12-2010 *Accepted on 05-01-2011*

ABSTRACT: The present study was planed to prepare microspheres for sustained release of Lamivudine using various cellulose polymers such as Ethyl cellulose, Cellulose acetate phthalate, cellulose acetate by employing solvent evaporation technique. Microspheres were characterized for the particle size distribution, wall thickness by scanning electron microscopy (SEM), angle of repose, drug content, bulk density, entrapment efficiency and in vitro dissolution studies. Drug excepients compatibility was determined by FTIR and DTA. Accelerated stability studies were also carried out following ICH Guidelines. SEM shows that microspheres were found spherical in shape and free flowing. The entrapment efficiency and wall thickness was found in between 68.85%&42.88%, 120.28µ & 72.32µ respectively. The drug release was extended maximum up to 12 hr with ethyl cellulose .FTIR and DSC results showed Lamivudine was compatible with excepients .The curve fitting data shows that the drug release followed first order kinetics, higuchi's and peppa's plots stated non-fickian diffusion controlled.

Keywords: Microspheres, Lamivudine, Cellulose polymers, in-vitro dissolution studies, stability studies.

INTRODUCTION:

Lamivudine(3TC), a synthetic nucleoside analogue with activity against HIV-1 and HBV. The chemical name of lamivudine is (2R, cis)-4- amino-l-(2-hydroxymethyl-l,3-oxathiolan-5-yl)-(lH)-pyrimidin-2-one. Lamivudine is the (-) enantiomer of a dideoxy analogue of cytidine. Lamivudine has, also been referred to as (-) 2',3'dideoxy, 3'-thiacytidine. It has a molecular formula of $C_8H_{11}N_3O_3S$ and a molecular weight of 229.3. It has the following structural formula:

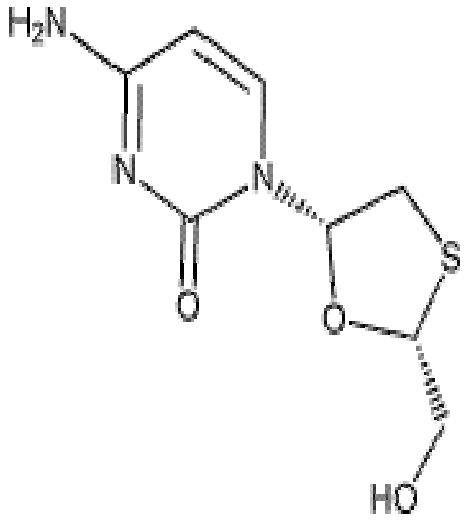


Fig-1

Lamivudine is a white to off-white crystalline solid with a solubility of approximately 70 mg/ml in water at 20°C. Lamivudine usually is given with other antiretroviral agents, such as ZDV or D4T.3TC at a dose of 600 mg/day reduced HIV cells by 75%, and in combination with ZDV (Zidovudine), the reduction in viral load was 94%.3CT is rapidly absorbed through the GI tract. Its bioavailability is approximately 86% after oral administration of 2mg/kg twice daily peak serum 3TC concentration is approximately 2mg/ml.3TC binding to human plasma is approximately 36%In vivo, it is converted to the trans sulfoxide metabolite, although a majority of the drug is eliminated unchanged in urine . The US FDA approved 3TC in combination with ZDV for the treatment of disease progression caused by HIV infection. The combination of 3TC with D4T (Stavudine) also are used for advanced HIV infections. Such combinations have the ability to delay resistance to ZDV and restore ZDV sensitivity in patients with AIDS. Recently, oral therapy with lower doses of 3TC has been approved by the US FDA for the treatment of chronic hepatitis B peripheral neuropathy and GI disturbance are the major side effects of 3TC.The minor side effects are nausea, vomiting, and diarrhea.

This deoxycytidine analogue is phosphorylated intracellularly and inhibits HIV reverse transcriptase as well as hepatitis B virus (HBV) DNA polymerase .Its incorporation in to DNA result in chain termination. Most human DNA polymerases are not affected and systemic toxicity of 3TC is low. Point mutation in HIV-reverse transcriptase and HBV-DNA polymerase gives rise to rapid lamivudine resistance. Certain lamivudine –resistant mutants

become slowly growing. Oral bioavailability of 3TC is high and plasma $t_{1/2}$ longer (6-8 hours).Intracellular $t_{1/2}$ is still longer(>12hr).It is mainly excreted un changed in urine. Lamivudine is used in combination with other anti–HIV drugs, and appears to be as effective as AZT. It is also frequently used for chronic hepatitis B. HBV-DNA titre is markedly reduced and biochemical as well as histological indices of liver function improve. However, viral titres rise again after discontinuation. Even with continued medication HBV viraemia tends to return after 1 year due to emergence of resistant mutants. Dose for chronic hepatitis B -100mg OD for HIV infection-150mgBD

New drug delivery technologies are revolutionizing the drug discovery, development and creating R&D focused pharmaceutical industries to increase the momentum of global advancements. In this regard novel drug delivery systems (NDDS) have many benefits, which includes improved therapy by increasing the efficacy and duration of drug activity, increased patient compliance through decreased dosing frequency and convenient routes of administration and improved site specific delivery to reduce unwanted adverse effects (Amrita Bajaj et al, 2006). Lamivudine is an active antiretroviral drug belonging to non-nucleosides reverse transcriptase inhibitor. Lamivudine treatment has gained immense popularity in the AIDS treatment in the present era. Dosage and duration of Lamivudine therapy should be individualized according to requirement and response of the patient. The daily recommended dose is 150 mg b.i.d (J.H. Kao et al, 2000) (Indian Pharmacopeias, 1996). The oral administration of Lamivudine exhibits side effects in GIT as well as in CNS. Thrombocytopenia, parasthesias, anorexia, nausea, abdominal cramps, depressive disorders, cough and skin rashes etc have also been reported as possible adverse reactions (Caroline M. Perry et al, 1997). Controlled release (CR) preparations helps to achieve maximum therapeutic effect with simultaneous minimization of adverse effects. Micro particulate drug delivery posses many advantages such as high bioavailability, rapid kinetic of absorption as well as avoidance of hepatic first pass effect and improvement of patient compliance (Y.W. Chien, 1992). Absence of sufficient work in the direction of programmed delivery of Lamivudine as indicated by literature survey ignited the urge of this research

venture, which utilizes nine different formulation methods for preparation of Lamivudine microspheres and ultimately ascertain the most preferable method for industrial scale-up on the basis of physical characterization and *in vitro* drug release profile.

MATERIALS AND METHODS

Materials

Lamivudine was obtained as a gift sample from Dr.Reddy's (Hyderabad).Cellulose acetate phthalate and cellulose acetate was obtained from Nacto pharma (Hyderabad) ethyl cellulose was a gift sample matrix laboratories (Hyderabad) .All the solvents are procured of merck. All other chemicals and reagents used in the study were of analytical grade. XXI paddle type dissolution apparatus, FT-IR (Shimadzu IR spectrophotometer, Model 840, Japan) and UV-Visible spectrophotometer (UV-1700, Shimadzu, Japan) were the instruments employed in the current study.

Table no.1: Formulation Table

Formulation	Drug (mg)	Ethyl Cellulose(mg)	Cellulose acetate(mg)	Cellulose acetate phthalate(mg)	Drug:polymer
F1	300	300	-	-	1:1
F2	300	600	-	-	1:2
F3	300	900	-	-	1:3
F4	300	-	300	-	1:1
F5	300	-	600	-	1:2
F6	300	-	900	-	1:3
F7	300	-	-	300	1:1
F8	300	-	-	600	1:2
F9	300	-	-	900	1:3

Preparation of microspheres

Lamivudine Microspheres were prepared by the emulsion solvent evaporation method. The polymers(ethyl cellulose, cellulose acetate and cellulose acetate phthalate)[7-9] were dissolved in acetone by stirring the mixture at 800rpm the author dispersed the drug particles in liquid paraffin(50% heavy+50% light) containing 1% w/w

polysorbate [10-12].The polymer solution was added slowly to the drug dispersion by means of a burette. The mixture was agitated at room temperature (25^0c) until the acetone (polymer solvent) was evaporated[13-15].The rate of stirring was kept constant for all the batches and for all the methods and the ratio of drug to polymer was varied (Drug: Polymer as 1:1,1:2,1:3) and labeled as F1 to F9.The liquid paraffin was decanted and the microspheres were collected, washed with petroleum ether to remove any remaining oil phase & dried under reduced pressure for at least 12hrs.Table no.1

CHARACTERIZATION OF MICROSPHERES

Scanning electron microscopy (SEM) Morphological characterization of the microspheres was carried using scanning electron microscopy (SEM),For SEM the double sided sticking tape coated with gold film (thickness 200nm) was used under the reduced pressure.

Drug entrapment efficiency (DEE) (M.C. Gohel et al, 2005) the microspheres were evaluated for percentage yield and percent drug entrapment. The yield was calculated.

$$\text{Percentage yield} = \frac{\text{weight of microsphere recovered}}{\text{Weight (drug + polymer)}} \times 100 \quad \text{……..(1)}$$

Drug loaded microspheres (100 mg) were powdered and suspended in 100 ml methanol: water (1:99 v/v) solvent system. The resultant dispersion was kept for 20 min for complete mixing with continuous agitation and filtered through a 0.45 µm membrane filter. The drug content was determined spectrophotometrically (UV-Visible-1700, Shimadzu, Japan spectrophotometer) at 270 nm (3) using a regression equation derived from the standard graph (r^2 = 0.9978). The drug entrapment efficiency (DEE) was calculated by the equation

$$DEE = (Pc / Tc) \times 100 \quad \text{……(2)}$$

Pc is practical content, Tc is the theoretical content. All the formulations were analyzed in triplicate (n=3).

Particle size measurement (M.C. Gohel et al, 2005)The size of the prepared microspheres was measured by the optical microscopy method using a calibrated stage micrometer. Particle size was calculated by using equation

$$Xg = 10 \times [(ni \times \log Xi) / N] \text{…….(3)}$$

Xg is geometric mean diameter, ni is number of particle in range, xi is the mid point of range and N is the total number of particles. All the experimental units were analyzed in triplicate (n=3).

Encapsulation efficiency (EE) : Drug loaded microspheres were weighed and dissolved in phosphate buffer pH.7.4. and mixture was filtered. The percent entrapment was calculated using the Eq-4

Encapsulation efficiency= Actual drug content/Theoretical drug contentX100-----(4)

Wall thickness: The wall thickness of the prepared microspheres was calculated using Eq-5.

$$H=r(1-p)di/3(pd_2+(1-p)di\text{-----(5)}$$

Accelerated stability studies (G.T. Kulkarni et al, 2004) (L. Lachman, et al, 3rd ed., 1991) Stability studies were performed according to ICH guidelines. The formulations were stored in room temperature at 25 ± 1°, in hot air oven at 37 ± 1°, and at 60± 1° for a period of 14 weeks. The samples were analyzed for drug content every two weeks by spectrophotometer at 270 nm and compatibility of drug with excipients was determined by infrared spectroscopy using a Shimadzu FTIR-840 model IR spectrophotometer

Fourier Transforms infrared Spectroscopy (FTIR)

(D.R.Bhumkar et al, 2003)The FT-IR spectra acquired were taken from dried samples. A FT-IR (Shimadzu IR spectrophotometer, model 840, Japan) was used for the analysis in the frequency range between 4000 and 600 cm-1, an 8 cm-1 resolution and a 0.2 cm-1 rate. The results were the means of 16 determinations. A quantity equivalent to 2 mg of pure drug, empty microspheres of ethyl cellulose and drug loaded microspheres were selected separately.

Differential Thermal Analysis (DTA) :DTA of Lamivudine and drug loaded microspheres were performed by using Seiko(Japan)DTA.Samples were sealed in aluminum pans and the DTA thermo grams were reported at a heating rate of 10^0/min from 20^0c to 300^0c.

X ray diffractometer (XRDshimadzu7000): X ray diffractometry was used for diffraction studies. XRD studies were performed on the samples by exposing them to cupper (cu kα)radiation (40kv,30mA) and scanned from 2°C t0

80°C, 2 theta(θ) at a step size of 0.045° and step time of 0.5 sec. XRD analysis was performed on the pure drug and for the prepared formulation of microspheres with various polymers.

In vitro drug release studies

In vitro dissolution studies were performed using (USP type II dissolution apparatus). The rotating basket method specified in USP XXI at 75 rpm. The microspheres were weighed and tied in the muslin bag and placed in the basket. The dissolution medium (900ml) consisted of 0.1M hydrochloric acid for the first 2 hours and then changed to phosphate buffer pH 7.4 from the 3rd hour. The temperature was maintained at 37°C. An aliquot of (5ml) sample was withdrawn at specified time interval and replaced with an equivalent volume of dissolution fluid. Drug content was determined by UV-Visible spectrophotometer (Schimazdu UV 1700 E 23) at270nm. The release studies were conducted in triplicate.

***In vitro* drug release kinetics**

In order to study the exact mechanism of drug release from the microsphere, drug release data was analyzed according to Zero order (G.M. Khan, 2001), First order (D.M. Morkhade et al, 2006), Higuchi square root (T. Higuchi, 1963), Hixon-Crowell equation (J. Wang et al, 1999) .The criteria for selecting the most appropriate model was chosen on the basis of goodness of fit test.

Statistical Analysis

Statistical data analyses were performed using the ANOVA one way at 5 % level of significance $p < 0.05$ (Bolton S, 1997) and standard error mean.

RESULTS

The Lamivudine loaded with ethyl cellulose, cellulose acetate, cellulose acetate phthalate microspheres were prepared by solvent evaporation method as mentioned earlier. The microspheres obtained under these conditions were found to be spherical and without aggregation and mean particle size was found in a range of 27.89 to 41.28 µm (Table 2). The particle size distribution of all the formulations is displayed in Figure-2. The percentage yield of

all the formulations was found to be satisfactory and each formulation exhibited high drug entrapment efficiency (DEE), as summarized in Table 2. The Formulation F3 showed higher DEE among all the formulations.

The interaction study between the drug (lamivudine) and polymer (ethyl cellulose) in different formulations was evaluated using FTIR spectrophotometer. Four bands present in Lamivudine spectrum at 3445.91, 2930.92, 1736.51 and 1637.7 cm-1, due to the formation of N-H, O-H, C=O, C=N linkage respectively, was also detected and identified in the spectrum of the formulations, confirming no drug-polymer interaction as found. The accelerated stability studies were performed according to ICH guidelines for 14 Weeks and the results were found to be stable in varying temperature as shown in Table-3. The results were further verified with one way ANOVA method, the accelerated stability test data were found significant for F (3.395) at 5 % level of significance ($p< 0.05$).

The *in vitro* drug release profiles for all the batches were tabulated in Table-4. All the formulations showed constant release profile. To identify the kinetics of drug release from microspheres, release data was analyzed according to different kinetic models. Table-5 indicates that drug release from F1; F2 and F3 formulations obey Zero order kinetics, while the release data of F4, F5 and F6 seems to fit best in Higuchi square root model. The release mechanism was not significantly influenced by formulation variables and was predominately diffusion controlled. Statistical verification with one way ANOVA method attested the fact that the drug release data were found significant for F (27.3731) at 5 % level of significance ($p< 0.05$).

DISCUSSION

Lamivudine loaded Ethyl cellulose microsphere were prepared and evaluated with different reported methods. No significant differences in particle size were found for the microspheres prepared by all formulations. Small variations may be attributed to different conditions like stirring speed and stirring time or fluctuations in temperatures. The smaller particle size can be due to application of wide range (5-800) of temperature change with constant stirring at 800 rpm. The reduced particle size helps in achieving our goal by enhancing the controlled delivery of Lamivudine. The F3 showed higher DEE among all the formulations. This can be justified on the basis of minimum process

parameters, minimum drug solubility in the external phase and smaller particle size of the thermal change method in comparison with the other methods, leading to minimum drug loss. The minimal use of process parameters during the formulation of Lamivudine microsphere confirms the high yield of F3.

The FTIR study attests the safety profile of the microspheres due to avoidance of drug polymer interaction. The accelerated stability study indicate the broader horizon of storage conditions complying with the ICH guidelines. The efficiency of release of Lamivudine from ethyl cellulose matrix of prepared microspheres is the key factor in the successful optimization of a method. The present study demonstrated that F3, among all other formulations, have a significantly slower release pattern in terms of their total drug load. The drug release rate was following zero order kinetic which complies with the controlled delivery of Lamivudine over 10 hours. However the other formulations F1, F2, F4, F5 and F6, had shown insignificant difference in drug entrapment efficiency but found to be significantly (S.E.M< 0.010) distinguished in particle size and percentage of yield. Except F1, F2, F3, the other formulations followed Higuchi square root kinetic model indicating the diffusion controlled drug release, which creates a restriction in optimizing the methods, as zero order model is the most desirable.

Table-2: Evaluation parameters of various formulations

Formulation code	Yield (%)	Particle size (µm) (X ± S.D)	Drug Entrapment efficiency (X± S.D)
F1	93.00±0.017	36.22±0.015	97.01±0.120
F2	96.00±0.014	29.55±0.021	95.36±0.110
F3	99.10±0.019	27.89±0.026	99.66±0.220
F4	94.56±0.027	28.69±0.019	99.01±0.190
F5	97.55±0.026	38.88±0.028	98.45±0.170
F6	98.80±0.023	41.28±0.026	96.21±0.230

All the results are mean ± standard deviation (n=3)
(Standard Error mean SEM<0.01)
F1=Drug: Ethyl cellulose (1:1)
F2= Drug: Ethyl cellulose(1:2)
F3= Drug: Ethyl cellulose(1:3)
F4= Drug: Cellulose acetate(1:1)
F5= Drug: Cellulose acetate(1:2)
F6= Drug: Cellulose acetate(1:3)

F7= Drug: Cellulose acetate phthalate(1:1)
F8= Drug: Cellulose acetate phthalate (1:2)
F9= Drug: Cellulose acetate phthalate (1:3)

Table-3: Stability profile of various formulations in different temperature.

Week	Temp.(^{0}C)RT	F1	F2	F3	F4	F5	F6
Initial	Room Temp	99.24	99.42	99.56	99.88	101.25	99.23
	(RT)	99.20	99.01	99.66	99.78	99.44	99.84
	37±1	98.53	98.89	99.05	98.55	98.66	98.39
	60±1						
2	RT	98.23	98.88	99.00	96.36	99.02	98.81
	37±1	99.21	99.00	99.68	99.68	99.12	99.35
	60±1	98.65	98.87	98.97	98.58	98.25	98.01
4	RT	98.22	98.56	98.91	97.01	98.77	97.55
	37±1	98.92	98.97	98.99	98.90	98.88	98.95
	60±1	98.56	98.79	98.87	98.53	98.10	98.05
6	RT	98.12	98.50	98.82	97.21	98.62	97.49
	37±1	98.97	98.83	98.91	98.80	98.68	98.21
	60±1	98.62	98.69	98.78	98.41	97.92	97.98
8	RT	98.09	98.32	98.45	97.01	98.36	97.56
	37±1	98.89	98.45	98.85	98.78	98.59	98.19
	60±1	98.58	98.57	98.65	98.35	97.88	97.69
10	RT	97.98	98.29	98.39	97.01	98.26	98.18
	37±1	98.84	98.39	98.74	98.68	98.47	98.05
	60±1	98.52	98.48	98.60	98.31	97.79	97.58
12	RT	97.88	98.19	98.31	96.98	98.18	98.12
	37±1	98.71	98.31	98.69	98.57	98.41	98.00
	60±1	98.48	98.40	98.51	98.29	97.70	97.48
14	RT	97.69	98.08	98.29	96.92	98.10	97.95
	37±1	97.52	98.23	98.25	96.56	98.41	97.84
	60±1	97.33	98.11	98.32	96.35	98.36	97.65

The results of the present study suggest that method F3 is the most suited one to develop Lamivudine microspheres, keeping in consideration, the zero order

F1, F2 was rejected on the basis of particle size, yield factor, entrapment efficacy and prolongation of drug release in comparison with F3. release profile, high DEE (99.66 %), small particle size (27.89 µm) and high yield of the microspheres of this method.

In context to the intense world wide research to combat AIDS, it can be envisaged that future workers would indulge in optimization of the various process parameters of the selected method (F3), to promote its commercial scale up, leading to Lamivudine (Caroline M. Perry et al, 1997) loaded ethyl cellulose microspheres for effective management of AIDS.

Table values represents correlation coefficient (**r**) for linearity according to different kinetic equations used for describing the drug release from various formulations Verifying with one way ANOVA significant at 5% level of significance(F=3.395)

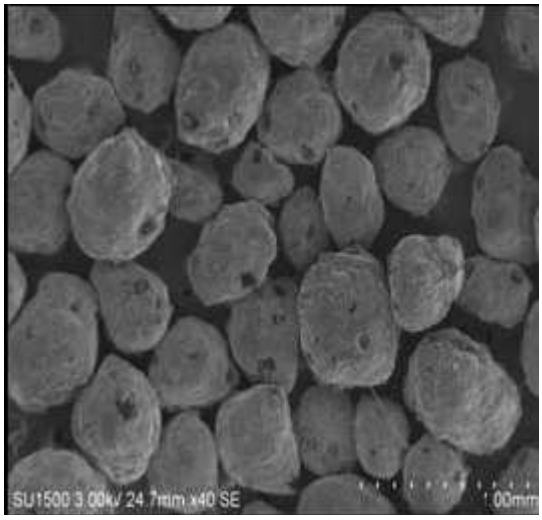
Table 4: *In vitro* drug release profile of formulations.

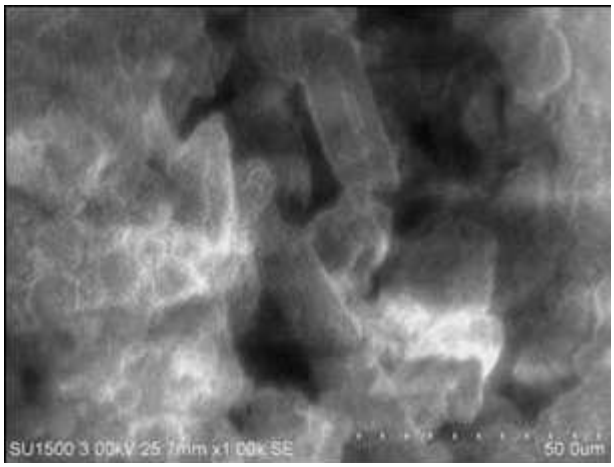
Time(hr)	F1	F2	F3	F4	F5	F6
1	19.36	60.22	25.35	23.47	27.62	24.69
2	15.73	70.47	21.30	22.63	30.91	23.34
3	20.03	74.91	19.57	15.59	21.77	19.79
4	17.94	72.09	21.27	21.31	19.66	22.05
5	19.38	74.80	21.28	10.37	18.78	20.83
6	24.02	72.09	18.41	10.89	17.87	23.99
7	22.60	76.29	19.31	15.01	28.61	17.76
8	24.71	72.93	21.06	18.83	28.33	28.47
9	17.38	71.16	21.41	23.12	29.22	32.69

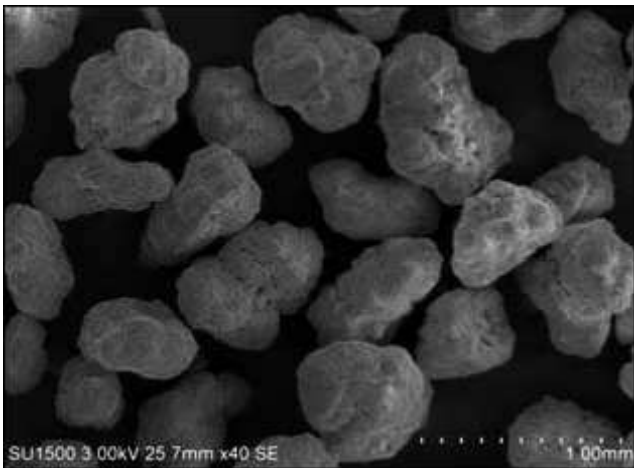
Table-5: Correlation coefficients according to different kinetic equations.

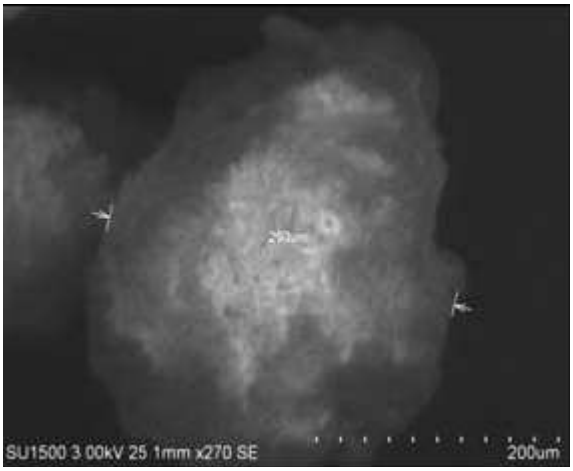
Kinetic Models	F1	F2	F3	F4	F5	F6
Zero order	0.9992	0.9952	0.9891	0.9841	0.9968	0.9871
First order	0.7397	0.8282	0.8021	0.8724	0.8641	0.8559
Hixon-crowell model	0.8927	0.9443	0.9803	0.9727	0.9849	0.9853
Higuchi square root	0.9564	0.9931	0.9864	0.9932	0.9985	0.9877

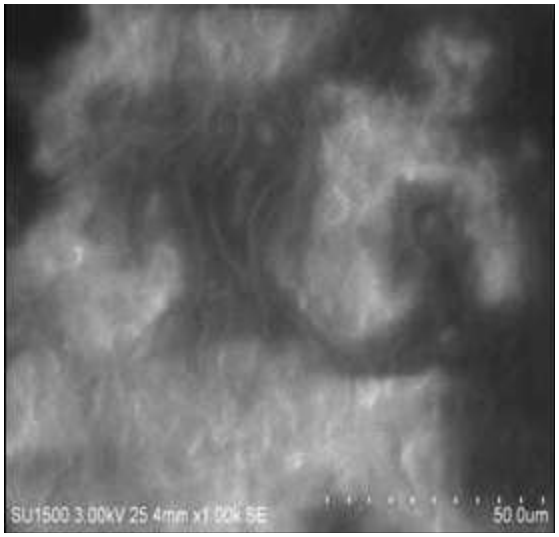
Figures of different microspheres:











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