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Research/Review

Formulation And Evaluation Of Cytarabine Microspheres For Sustained Drug Delivery

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	Abstract
Published on: 25 Apr 2024	The Drug Cytarabine has short half life and hence requires frequent administration. Therefore the possible way for formulating a sustained release formulation of mucoadhesive microspheres. These formulations are prepared by solvent evaporation technique by using polymers HPMC15cps+Carbopol 934p and HPMC15000cps+Carbopol 934p. Various evaluation parameters assessed, with a view to obtain sustained release of Cytarabine. In the present study six formulations are formulated by using Sodium Alginate and HPMC15cps+Carbopol 934p and HPMC15000cps + Carbopol 934p various proportions. The prepared Cytarabine microspheres are then subjected to IR, SEM, particle size, % yield, Swelling Index, Micrometric, % Drug entrapment efficiency, <i>In-vitro</i> mucoadhesion test and <i>in vitro</i> dissolution studies. The IR Spectra revealed that, there is no interaction between the polymer and Cytarabine. Cytarabine microspheres are spherical in nature, which was confirmed by SEM. The Optimized formulation C3 was found to release the drug for 12 h (99.13%) and follows peppas drug release kinetics model in dissolution studies.
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	Keywords: Cytarabine, HPMC15cps +Carbopol 934p, HPMC15000cps+Carbopol 934p and solvent evaporation technique.

INTRODUCTION

One of the most challenging areas of research in pharmaceuticals is the development of novel delivery systems for the controlled release of drugs and their delivery at the targeted site in the body to minimize the side effects and enhance the therapeutic efficacy of drugs. The basic principle behind the controlled drug delivery system is to optimize the biopharmaceutic, pharmacokinetic and pharmacodynamics properties of drug in such a way that its efficacy is maximized by reducing side effects, dose frequency and cure the disease in short time by using low amount of drug administered with the most suitable route¹.

The objective of controlled release drug delivery includes two important aspects namely spatial placement and temporal delivery of drug. Spatial placement relates to targeting a drug to a specific organ or tissue, while Temporal delivery refers to controlling the rate of drug delivery to the target tissue².

Oral controlled release dosage forms have been developed over the past three decades due to their considerable therapeutic advantages such as ease of administration, patient compliance and flexibility in formulation. However, this approach is beset with several physiological difficulties such as inability to restrain and locate the controlled drug delivery system within the desired region of the gastrointestinal tract (GIT) due to variable motility and relatively brief gastric emptying time (GET) in humans which normally averages 2-3 h through the major absorption zone, i.e., stomach and upper part of the intestine can result in incomplete drug release from the drug delivery system leading to reduced efficacy of the administered dose.^{10,11}

The objective in designing a controlled release system is to deliver the drug at a rate necessary to achieve and maintain a constant drug blood level. This rate should be similar to that achieved by continuous intravenous infusion where a drug is provided to the patient at a rate just equal to its rate of elimination. This implies that the rate of delivery must be independent of the amount of drug remaining in the dosage form and constant over time, i.e. release from the dosage form should follow zero-order kinetics.³

Microsphere carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery systems^{4,5}. Microspheres have varied applications and are prepared using assorted polymers. However; the success of these microspheres is limited owing to their short residence time at the site of absorption. So, various attempts have been made to increase the bioavailability as well as prolong the gastric residence time of dosage form in the stomach resulted in development of bio adhesive drug delivery system which will provide an intimate contact of the drug delivery system with the absorbing membranes⁶. This can be achieved by coupling mucoadhesion characteristics to microspheres and developing mucoadhesive microspheres. Mucoadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site⁷. Gastric mucoadhesive drug delivery offers a number of applications for drugs having poor bioavailability because of narrow absorption window in the upper part of gastrointestinal tract. It retains the dosage form at the site of absorption and thus enhances the bioavailability.⁸⁻¹⁰

MATERIALS

Cytarabine-Procured from Million Health Pharmaceuticals. provided by SURA LABS, Dilsukhnagar, Hyderabad, HPMC15cps +Carbopol 934p (ratio)- Merk specialities Pvt Limited, Mumbai, HPMC15000cps + Carbopol 934p-Merk specialities Pvt Limited, Mumbai, Liquid paraffin (ml)-Merk specialities Pvt Limited, Mumbai, Span-80 (2%)-Merk specialities Pvt Limited, Mumbai

METHODOLOGY

PREFORMULATION STUDIES

SPECTROSCOPIC STUDIES

PREPARATION OF 0.1N HCl (pH 1.2)

Take 8.5ml of HCl in a 1000ml volumetric flask and make up the volume with distilled water

DETERMINATION OF λ_{MAX}

Weigh 10mg of Cytarabine and transferred into 10ml volumetric flask and dissolved in 10ml methanol (stock-I) to get concentration of 1000 $\mu\text{g/ml}$. From the stock-I take 1ml solution and make up 10ml with 0.1N HCL. From the second stock take 1ml solution and make up to 10ml with 0.1N HCL to get 10 $\mu\text{g/ml}$. Then scan from 200-400nm.

Preparation of Standard Calibration Curve of Cytarabine

1. 10 mg of Cytarabine was accurately weighed and dissolved in 10ml of methanol (Stock Solution – I) to get a concentration of 1000 $\mu\text{g/ml}$.
2. From the stock solution- I, 1ml of aliquots was taken and suitably diluted with 0.1N HCl (Stock Solution-II) to get concentrations of 100 $\mu\text{g/ml}$.
3. 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 5 to 25 $\mu\text{g/ml}$. The absorbance of these samples were analyzed by using UV-Visible Spectrophotometer at 272nm against reference solution 0.1N HCl (pH 1.2). The procedure repeated to pH 6.8 phosphate buffer and pH 7.4 phosphate buffer.

METHOD OF PREPARATION

Microspheres were prepared by emulsification solvent evaporation technique. Briefly, Cytarabine and polymers were mixed in 50ml distilled water. A different polymer ratio 1:1, 1:2 and 1:3 used to prepare the different formulations. Polymeric aqueous solution was made in which the drug was dispersed and then the

solution was added drop wise into 150 ml of light liquid paraffin containing 2% span-80 as an emulsifying agent. The aqueous phase was emulsified in oily phase by stirring the system in a 500ml beaker, Constant stirring at 500 rpm was carried out using magnetic stirrer at 80°C, stirring and heating were maintained for 4hrs, Until The aqueous phase was evaporated. The microspheres were washed 5 times with n-hexane, filtered through whattman's filter paper and dried in hot air oven at 50°C for 2 hours.

CHARACTERIZATION OF MICROSPHERES

Table 1: Prepared formulation of Microspheres

INGREDIENTS	FORMULATION CODES					
	C1	C2	C3	C4	C5	C6
Cytarabine	100	100	100	100	100	100
HPMC15cps + Carbopol 934p (ratio)	1:1	1:2	1:3	-	-	-
HPMC15000cps + Carbopol 934p	-	-	-	1:1	1:2	1:3
Liquid paraffin (ml)	150	150	150	150	150	150
Span-80 (2%)	2	2	2	2	2	2

RESULTS AND DISCUSSION

PREFORMULATION STUDIES

SPECTROSCOPIC STUDIES

Determination of $\lambda_{\max}$

A solution of 10 μ g/ml of Cytarabine was scanned in the range of 200 to 400nm. The drug exhibited a $\lambda_{\max}$ at 272 and 275nm in simulated gastric fluid pH 1.2 and pH 7.4 phosphate buffer respectively. Correlation between the concentration and absorbance was found to be near to 0.998, with a slope of 0.028 and intercept of 0.004.

Calibration curve of Cytarabine in simulated gastric fluid pH 1.2

Table 8.1 shows the calibration curve data of Cytarabine in simulated gastric fluid pH 1.2 at 272nm. Fig.8.1 shows the standard calibration curve with a regression value of 0.999, slope of 0.022 and intercept of 0.007 in simulated gastric fluid pH 1.2. The curve was found to be linear in the concentration range of 5-25 μ g/ml.

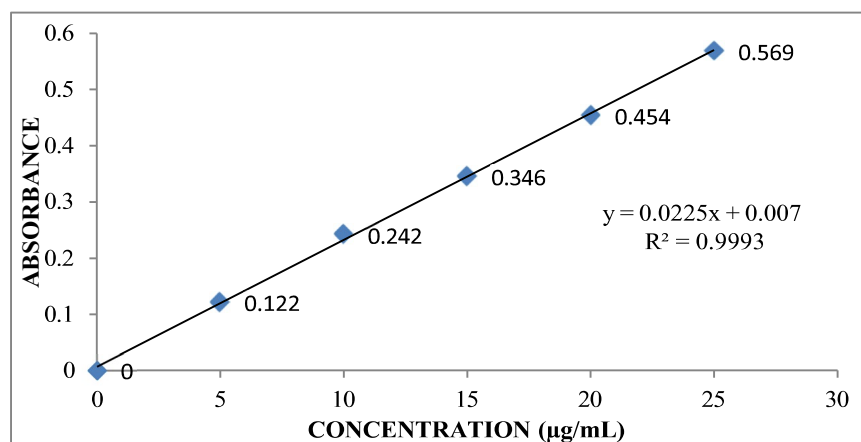


Fig 1 : Standard graph of Cytarabine in simulated gastric fluid pH 1.2

Calibration curve of Cytarabine in pH 7.4 phosphate buffer

Table 8.2 shows the calibration curve data of Cytarabine in pH 7.4 phosphate buffer at 275nm. Fig. 8.2 shows the standard calibration curve with a regression value of 0.999, slope of 0.027 and intercept of 0.007 in simulated gastric fluid pH 1.2. The curve was found to be linear in the concentration range of 5-25 μ g/ml.

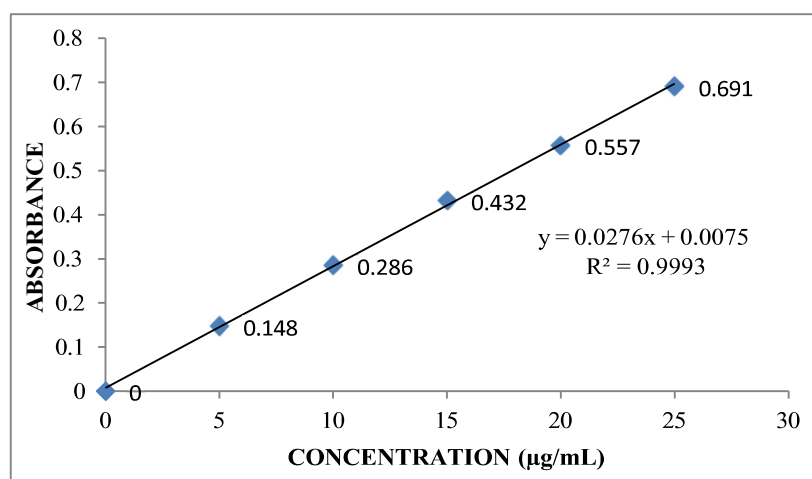


Fig 2: Standard graph of Cytarabine in pH 7.4 phosphate buffer

EVALUATION AND CHARACTERISATION OF MICROSPHERES

Micrometric Properties^{11,12}

The mean size increased with increasing polymer concentration which is due to a significant increase in the viscosity, thus leading to an increased droplet size and finally a higher microspheres size. Microspheres containing HPMC15cps + Carbopol 934p had a size range of 320.25 µm to 457.31 µm. Microspheres containing HPMC15000cps + Carbopol 934p as copolymer exhibited a size range between 305.45 µm to 385.19 µm.

The particle size data is presented in Tables 8.3. The effect of drug to polymer ratio on particle size is displayed in Figure. The particle size as well as % drug entrapment efficiency of the microspheres increased with increase in the polymer concentration.

The bulk density of formulation C1 to C6 containing HPMC15cps + Carbopol 934p and HPMC15000cps + Carbopol 934p formulation was in the range of 0.44 ± 0.03 to 0.55 ± 0.011 gm./cm³ (as shown in table 8.3), Tapped density 0.50 ± 0.061 to 0.62 ± 0.011 and hausners ratio 1.12 ± 0.015 to 1.16 ± 0.032 .

The Carr's index of formulation C1 to C6 containing different grades of HPMC15cps + Carbopol 934p and HPMC15000cps + Carbopol 934p 11.29 ± 0.35 to 14.28 ± 0.47 respectively. The angle of repose of formulation C1 to C6 containing HPMC15cps + Carbopol 934p and HPMC15000cps + Carbopol 934p formulation was in the range <28.53 respectively (as shown in table 8.3) The values of Carr's index and angle of repose indicate good flow properties.

Table 2: Micromeritic property of floating microspheres of Cytarabine

Formulation code	Mean particle size	Bulk density ((gm./cm ³))	Tapped density (gm./cm ³)	Hauseners ratio	Carr's index	Angle of repose
C1	320.25	0.44 ± 0.03	0.50 ± 0.061	1.13 ± 0.012	12 ± 0.58	26.12 ± 0.1
C2	338.15	0.48 ± 0.06	0.56 ± 0.08	1.16 ± 0.032	14.28 ± 0.47	28.53 ± 0.57
C3	457.31	0.55 ± 0.08	0.62 ± 0.011	1.12 ± 0.015	11.29 ± 0.57	25.46 ± 0.57
C4	305.45	0.53 ± 0.09	0.61 ± 0.071	1.15 ± 0.021	13.1 ± 0.15	27.61 ± 0.63
C5	351.12	0.49 ± 0.01	0.56 ± 0.08	1.14 ± 0.012	12.5 ± 0.21	25.15 ± 0.58
C6	385.19	0.55 ± 0.011	0.62 ± 0.06	1.12 ± 0.023	11.29 ± 0.35	26.08 ± 0.51

PERCENTAGE YIELD

It was observed that as the polymer ratio in the formulation increases, the product yield also increases. The low percentage yield in some formulations may be due to blocking of needle and wastage of the drug-polymer solution, adhesion of polymer solution to the magnetic bead and microspheres lost during the washing process. The percentage yield was found to be in the range of 86.15 to 98.17 % for microspheres containing HPMC15cps + Carbopol 934p, 82.24 to 95.12 % for microspheres containing HPMC15000cps + Carbopol 934p.

DRUG ENTRAPMENT EFFICIENCY

Percentage Drug entrapment efficiency of Cytarabine ranged from 76.29 to 98.38 % for microspheres containing HPMC15cps + Carbopol 934p, 80.77 to 93.54% for microspheres containing HPMC15000cps +

Carbopol 934p. The drug entrapment efficiency of the prepared microspheres increased progressively with an increase in proportion of the respective polymers. Increase in the polymer concentration increases the viscosity of the dispersed phase. The particle size increases exponentially with viscosity. The higher viscosity of the polymer solution at the highest polymer concentration would be expected to decrease the diffusion of the drug into the external phase which would result in higher entrapment efficiency. The % drug entrapment efficiency of the prepared microspheres is displayed in Table 3.

Table 3: Percentage yield and percentage drug entrapment efficiency of the prepared microspheres

S.No.	Formulation code	% yield	Drug Content (mg)	% Drug entrapment efficiency
1	C1	86.15	90.40	76.29
2	C2	90.60	92.83	85.14
3	C3	98.17	97.10	98.38
4	C4	82.24	83.91	80.77
5	C5	86.46	89.73	90.16
6	C6	95.12	92.50	93.54

Swelling studies

The swelling ratio is expressed as the percentage of water in the hydrogel at any instant during swelling. Swellability is an important characteristic as it affects mucoadhesion as well as drug release profiles of polymeric drug delivery systems. Swellability is an indicative parameter for rapid availability of drug solution for diffusion with greater flux. Swellability data revealed that amount of polymer plays an important role in solvent transfer. It can be concluded from the data shown in Table 8.5 that with an increase in polymer concentration, the percentage of swelling also increases.

In-vitro mucoadhesion test

As the polymer to drug ratio increased, microspheres containing HPMC15cps +Carbopol 934p exhibited % mucoadhesion ranging from 53.41 to 90.14 %, microspheres containing HPMC15000cps+Carbopol 934p exhibited % mucoadhesion ranging from 62.78 to 87.63%. The results of *in-vitro* mucoadhesion test are compiled in Table 4. Comparative depiction of % mucoadhesion is depicted.

Table 4: Percentage mucoadhesion of the prepared microspheres

S.No.	Formulation Code	No. Of Microspheres		Percentage Mucoadhesion
		Initial	Final	
1	C1	15	10.31	53.41
2	C2	15	13.47	71.51
3	C3	15	14.96	90.14
4	C4	15	11.85	62.78
5	C5	15	13.41	70.14
6	C6	15	14.28	87.63

IN-VITRO DRUG RELEASE STUDIES

Dissolution studies of all the formulations were carried out using dissolution apparatus USP type I. The dissolution studies were conducted by using dissolution media, pH 1.2. The results of the *in-vitro* dissolution studies of formulations C1 to C6 are shown in table. The plots of Cumulative percentage drug release Vs Time. Figure shows the comparison of % CDR for formulations C1 to C3, figure for formulations C4 to C6.

The formulations C1, C2 and C3 containing HPMC15cps+Carbopol 934p showed a maximum release of 96.14 % at 9 hours, 97.82 % after 10 hours and 99.13 % after 12 hours respectively.

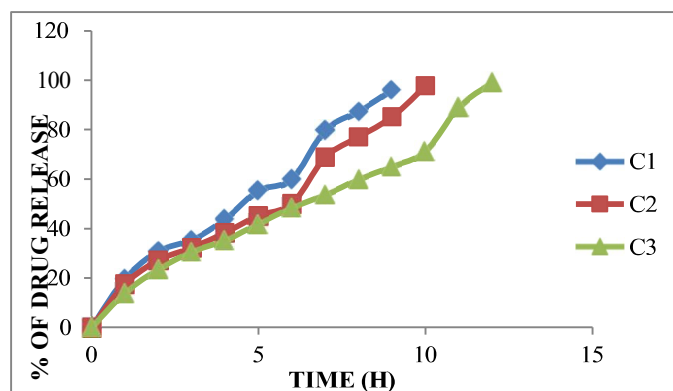
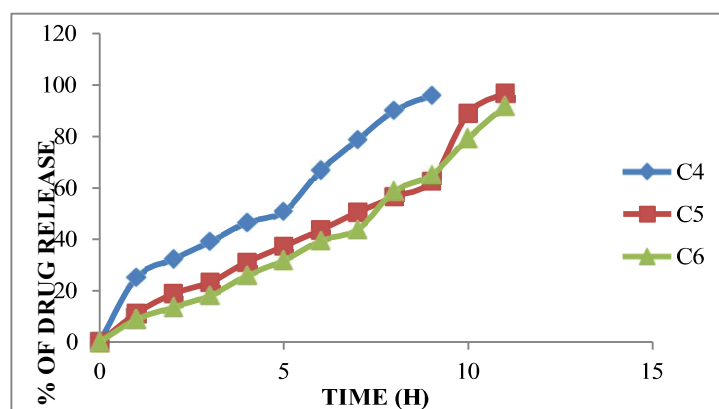
The formulations C4, C5 and C6 containing HPMC15000cps+Carbopol 934p showed a maximum release of 95.93% after 9 hours, 96.85% after 11 hours and 97.12% after 12 hours respectively.

This shows that more sustained release was observed with the increase in percentage of polymers.

As the polymer to drug ratio was increased the extent of drug release increased.

Table 5: *In-vitro* drug release data of Cytarabine microspheres

TIME (H)	CUMULATIVE PRECENT OF DRUG RELEASED					
	C1	C2	C3	C4	C5	C6
0	0	0	0	0	0	0
1	19.72	17.53	13.98	25.19	11.20	08.91
2	30.90	27.19	23.51	32.25	18.86	13.58
3	35.45	32.26	30.60	39.13	23.32	18.16
4	43.83	38.37	35.19	46.56	31.12	25.93
5	55.42	45.20	41.99	50.83	37.28	31.75
6	60.01	50.12	48.45	66.93	43.67	39.54
7	79.95	68.86	53.72	78.54	50.49	43.83
8	87.50	77.10	60.02	90.17	56.53	58.76
9	96.14	85.23	65.14	95.93	62.68	65.12
10		97.82	71.23		88.94	79.43
11			89.27		96.85	91.86
12			99.13			97.12

**Fig 3 : *In-Vitro* drug release profile of Cytarabine microspheres containing HPMC15cps + Carbopol 934p****Fig 4: *In-Vitro* drug release profile of Cytarabine microspheres containing HPMC15000cps + Carbopol 934p****IN-VITRO DRUG RELEASE KINETICS**

For understanding the mechanism of drug release and release rate kinetics of the drug from dosage form, the *in-vitro* drug dissolution data obtained was fitted to various mathematical models such as zero order, First order, Higuchi matrix, and Krosmeier-Peppas model. The values are compiled in Table 8.9. The coefficient of determination (R^2) was used as an indicator of the best fitting for each of the models considered. The kinetic data analysis of all the formulations reached higher coefficient of determination with the peppas drug release model whereas release exponent value (n) ranged from 0.983. From the coefficient of determination and release exponent

values, it can be suggested that the mechanism of drug release follows Korsmeyer-Peppas model along with non-Fickian diffusion mechanism which leading to the conclusion that a release mechanism of drug followed combination of diffusion and spheres erosion.

Table 6: Release kinetics studies of the optimized formulation (C3)

CUMULATIVE VE (%)	RELEASE Q TIME (T)	ROOT (T)	LOG(%) RELEASE	LOG (T)	LOG(%) REMAIN	RELEASE RATE (CUMULAT	1/CUM% RELEASE	PEPPAS log Q/100	% Drug Remaining	Q01/3	Qd1/3	Q01/3-Qd1/3
0	0	0			2.000				100	4.642	4.642	0.000
13.98	1	1.000	1.146	0.000	1.935	13.980	0.0715	-0.854	86.02	4.642	4.414	0.227
23.51	2	1.414	1.371	0.301	1.884	11.755	0.0425	-0.629	76.49	4.642	4.245	0.397
30.6	3	1.732	1.486	0.477	1.841	10.200	0.0327	-0.514	69.4	4.642	4.109	0.532
35.19	4	2.000	1.546	0.602	1.812	8.798	0.0284	-0.454	64.81	4.642	4.017	0.625
41.99	5	2.236	1.623	0.699	1.764	8.398	0.0238	-0.377	58.01	4.642	3.871	0.770
48.45	6	2.449	1.685	0.778	1.712	8.075	0.0206	-0.315	51.55	4.642	3.722	0.920
53.72	7	2.646	1.730	0.845	1.665	7.674	0.0186	-0.270	46.28	4.642	3.590	1.051
60.02	8	2.828	1.778	0.903	1.602	7.503	0.0167	-0.222	39.98	4.642	3.419	1.222
65.14	9	3.000	1.814	0.954	1.542	7.238	0.0154	-0.186	34.86	4.642	3.267	1.375
71.23	10	3.162	1.853	1.000	1.459	7.123	0.0140	-0.147	28.77	4.642	3.064	1.577
89.27	11	3.317	1.951	1.041	1.031	8.115	0.0112	-0.049	10.73	4.642	2.206	2.436
99.13	12	3.464	1.996	1.079	-0.060	8.261	0.0101	-0.004	0.87	4.642	0.955	3.687

Compatibility studies

Drug polymer compatibility studies were carried out using Fourier Transform Infra Red spectroscopy to establish any possible interaction of Drug with the polymers used in the formulation. The FT-IR spectra of the formulations were compared with the FTIR spectra of the pure drug.

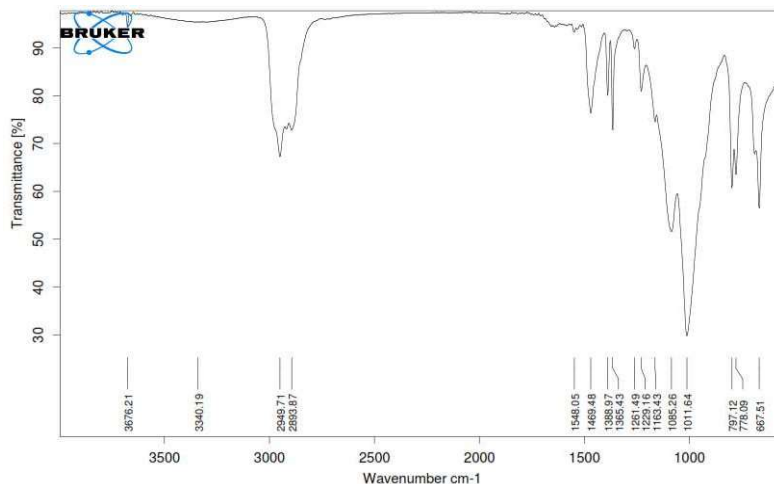


Fig 5: FT-IR spectra of Pure drug

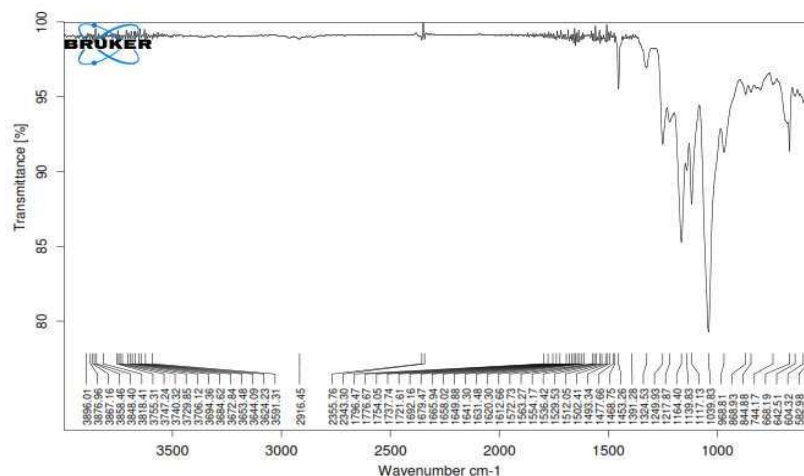


Fig 6: FT-IR spectra of Optimised formulation

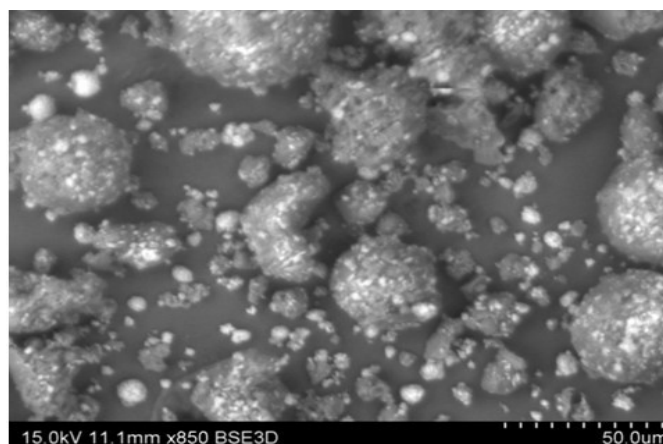


Fig 7: SEM of Optimised formulation

CONCLUSION

Cytarabine loaded microspheres were prepared solvent evaporation technique. Six batches of formulations, of drug: polymer ratio i.e. 1:1,1:2 and 1:3, were prepared and evaluated for particle size, % yield, drug content, drug entrapment efficiency, Swelling studies, *In-vitro* mucoadhesion test, *in vitro* drug release and Flow property had shown satisfactory results. The IR Spectra's revealed that, there is no interaction between polymer and Cytarabine. The polymer used is compatible with the Cytarabine. The basis of release data and graphical analysis formulation C3 showed a good sustained release profile with maximum entrapment efficiency because of high polymer concentration. Hence, from all the above obtained data it can be summarized that it is possible to formulate a promising sustained release mucoadhesive microspheres of Cytarabine by solvent evaporation technique using an ideal polymer like HPMC15cps +Carbopol 934p.

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