Preparation And Evaluation Of Spherical Poly Electrolyte Coacervates Of Zidovudine Prepared By Emulsification Method

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***Abstract*: The objective of the present work is to prepare spherical polyelectrolyte microcapsules of Zidovudine by a means of complex coacervation method in an emulsion system. Chitosan was used as the cationic polymer and sodium alginate and acacia as the anionic polymers. The two polymers were made to interact at a concentration of 2% and the coacervation was achieved at pH6.8. Glutaraldehyde was used as the cross linking agent. Microspheres were prepared in drug to polymer ratios of 0.5:1, 0.75:1 and 1:1. Microsphere suspension in arachis oil was prepared in ratio of 0.5:1. The emulsification used for microparticle formation allows the preparation of particles with spherical shape and narrow size distribution. The suspension was better than the dry product with respect to shape, drug content and also order of release, which was zero order for suspension whereas first order for the dried product. The drug from the suspension formulation was found to release over a period of 10hrs. The oily suspension provides a means of parenteral administration of Zidovudine, provided the particle size can be further reduced. This sustained release parenteral formulation of Zidovudine would be beneficial in comparison with the current intermittent oral dosage regimen.**

***Keywords*: Acacia, Chitosan, Sodium alginate, Zidovudine.**

I. INTRODUCTION

Research, development and sales of drug delivery systems are increasing at a rapid pace throughout the world. This worldwide trend will intensify in the next decade, as cuts in public health expenses, demand lower costs and higher efficiency. To meet this demand, many efficient drugs currently in use will be reformulated within delivery systems that can be value added for optimal molecular activities[1].

Microencapsulation by polymer-polymer incompatibility is probably the most classical method to produce microcapsules by phase separation. The interaction of oppositely charged electrolytes can result in the formation of a complex having reduced solubility resulting in phase separation. Microcapsules or coacervates thus formed have irregular shapes and control over the rate of drug release is difficult.

Hence the objective of the present work is to formulate spherical polyelectrolyte microcapsules by combining the above process with emulsification. This is done in order to achieve a more predictable and controlled release of the drug over a prolonged period of time.

The drug selected for the study is Zidovudine (AZT). It is a anti retroviral drug. It has been shown to be effective in prolonging the survival of AIDS patients. AZT is typically administered orally as tablets or capsules. The half-life of the drug is approximately one hour, which requires the frequent administration of the drug. The development of a controlled release formulation of AZT would be beneficial in comparison with the current intermittent dosage regimen.

II. MATERIALS AND METHOD

Materials Used:

Zidovudine standard as gift sample was obtained from Cipla, India. Chitosan from C.I.F.T, Cochin, and Acacia from Qualigens Fine Chemicals, Mumbai, and Sodium Alginate from Glenmark Pharmaceuticals ltd were purchased. All the chemicals and reagents used were of analytical grade.

Preparation of microspheres.

Chitosan-acacia and Chitosan-Sodium alginate microspheres of the drug Zidovudine were prepared using complex coacervation technique following the method developed, which is based on the method followed Luzzi.L.A.et al. An additional emulsification step was introduced with the objective of obtaining spherical particles. Microspheres were prepared using optimum concentration of the polymers, volume of hardening agent and hardening time.

Procedure: 50ml of 2% Chitosan solution in 1% acetic acid was taken in a 250ml beaker. The required amount of drug i.e. Zidovudine was dissolved in this solution by stirring with a glass rod. This solution was emulsified into 250ml arachis oil containing 0.5% span 80. The stirring was continued for 5mins for the emulsion to form properly. To this emulsion, 50ml of 2% acacia solution was then added drop wise with continued stirring. It was further stirred for 5mins. The pH was then adjusted to 6.8 using 2% Naoh solution, while continuing the stirring. It was continued for 10 mins at a slower speed for the proper formation of

microspheres. The temperature was then reduced to 5 to 10□C using ice bath. While stirring at this stage 5ml of glutaraldehyde was added to harden the microspheres. Stirring was continued for 2hrs.

After hardening for 2 hrs, the oil was separated by centrifugation. The microspheres were washed repeatedly with petroleum ether until free from oil. Filtered, dried and stored in suitable containers for further evaluation.

Similarly microspheres of chitosan-Sodium alginate were prepared by replacing 50ml of acacia with 50ml of 2% sodium alginate. The rest of the procedure remaining the same.

Suspension of microspheres in arachis oil.

The microspheres obtained by the above method were not completely spherical. Also the drug content obtained was not satisfactory. Hence, In order to obtain a better control over the shape of the microspheres, the microspheres prepared by the above basic method were suspended in a definite volume of arachis oil. The oil used in the initial preparation was separated by centrifugation after the hardening step and the microspheres are then suspended in fresh oil to obtain the same initial volume. By avoiding the drying step the drug loss due to the loss of water could be minimized.

Table 1: Amount of drug, polymers and glutaraldehyde used in different formulations

Drug to polymer ratio	Wt of drug in grams	Total wt of polymers in grams	Hardening time in hrs	Volume of glutaraldehyde (25% v/v)
0.5:1	1	2	2	5
0.75:1	1.5	2	2	5
1:1	2.0	2	2	5

Evaluation of microspheres

1. **UV scanning** was done for the pure drug and the microspheres from 200 to 400nmn and the spectra were compared to confirm the presence of unchanged drug in the microcapsules.
2. **Percentage yield**: the percentage yield was found out for all the products based on the dry weight of the drug and the polymers taken.
3. **Preparation of standard graph**.
   100mg of pure drug Zidovudine was accurately weighed and transferred into 100ml volumetric flask. The volume was made to using phosphate buffer pH7.4. From this 10ml was diluted to 100ml using phosphate buffer pH7.4. This is standard stock solution. From the standard stock solution a series of dilutions were made to get 5, 10, 15, 20, 25 and 30□g/ml solutions using phosphate buffer pH 7.4. The absorbance of these solutions was measured against a blank pH7.4 phosphate buffer in UV visible spectrometer at 266nm. A standard graph was drawn using the average values of six trials and it is shown in Fig.
4. **Drug content in the microspheres**:
   Microspheres equivalent to 50mg drug were taken for evaluation. The amount of Zidovudine entrapped in the different microspheres was estimated by triturating the microspheres with aliquot amounts of pH 7.4 phosphate buffer repeatedly. The extracts were transferred to a 100ml volumetric flask and the volume made up using phosphate buffer pH7.4.The solution was filtered and the absorbance was measured after ten times dilution using Chemito 2500 UV visible Spectrophotometer at 266nm. The amount of drug entrapped in the microspheres was calculated.
5. **Size and shape of the microspheres**
   Size analysis was carried out using optical microscopic method. The size distribution data were obtained using calibrated eyepiece micrometer and the average diameter was calculated. The microspheres were dispersed in liquid paraffin and observed under an optical microscope. The photomicrographs are taken to study the shapes of the microspheres.
6. **In vitro release studies**:
   In vitro release studies were carried out for all the products and for the pure drug in dissolution test apparatus USP/BP/IP Type II. Medium used was 900ml of 7.4 pH phosphate buffer. 100mg of pure drug had been taken for the dissolution studies and microspheres equivalent to 100mg of the pure drug were used. The test was carried out in phosphate buffer pH 7.4 for 7hrs with 60 rpm at 37 ± 0.5°C. 5ml of the sample was withdrawn at 30min interval and filtered. The required dilutions (ten times) were made with phosphate buffer pH7.4 and the solution was analyzed for the drug content spectrophometrically at 266nm. 5ml of the dilution medium was replaced in the vessel after each withdrawal. Three trials were carried out for all the products and the average values were calculated. From this percentage drug released was calculated and this was plotted against function of time to find out the pattern of drug release.
7. **Diffusion study for the microsphere suspension.**
   The drug release from the oily suspension is determined using a diffusion cell using a cellophane membrane. Treated cellophane membrane is tied to one end of the diffusion cell. The cell is then placed in a beaker containing 200ml of phosphate buffer pH 7.4. Formulation equivalent to 20mg of the drug is taken inside the diffusion cell. The buffer in the beaker is stirred continuously using a magnetic stirrer. 5ml of the solution is withdrawn at 30min interval and analyzed for the drug content after two times dilution using UV spectrophotometer at 266nm. 5ml of fresh buffer is replaced after each withdrawal.
   From this the percentage drug released was calculated and this was plotted against the function of time to find out the pattern

of drug release.

III. Results

The microspheres were prepared based on the basic method proposed by Luzzi and Gerraughty, with the addition of an emulsification step in order to get spherical particles.

Several trials were carried out to find out the optimum concentration of the two colloids in the formulation. It was found that 2% chitosan produce the optimum viscosity in the formulation. Any higher concentration was highly viscous for the proper formation of an emulsion. Acacia was also taken as a 2% solution.

Arachis oil was used as the continuous phase because of lower viscosity compared to liquid paraffin. Also separation and washing of the microspheres was found to be easier with arachis oil. Volume of arachis oil to be taken also had to be optimized. 250 ml of arachis oil was found optimum because higher volume caused uneven stirring towards the sides of the beaker and lower volumes produced viscous emulsions, which were difficult to stir.

The drug to polymer ratio used in the present work was up to 1:1. Higher amount of drug i.e. 1:1.5 was tried and the drug content was found to be only up to 26% and also most of the drug remained as crystals and failed to get incorporated in the microspheres.

Air drying was preferred over drying in oven. Drying at higher temperatures caused loss of the spherical shape of the microspheres. Volume of glutaraldehyde was optimized as 5ml (25%v/v) after several trials and hardening time was fixed as 2hrs.

UV scanning

The UV absorption spectra for pure drug and microspheres in combination with both the polymers are shown in Fig.1 & Fig. 2 respectively.

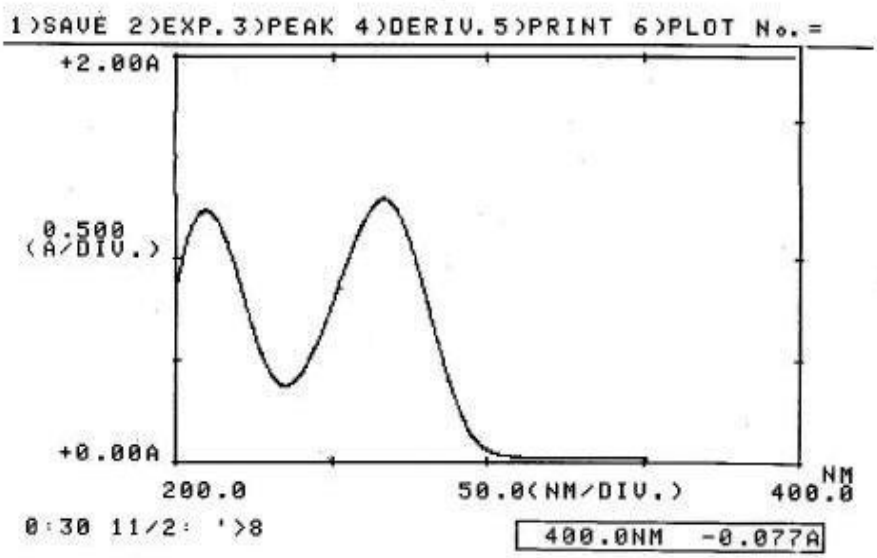


Fig 1: UV Spectra of Zidovudine

The UV absorption spectra for both the type of microspheres showed peak at 266 nm, which corresponds to the UV absorption peak of pure drug Zidovudine in the same solvent (phosphate buffer pH 7.4).The presence of peak at 266nm in the UV absorption spectrum of microspheres shows the presence of unchanged drug Zidovudine in the microspheres. This indicates no chemical interaction between the drug and the polymers.

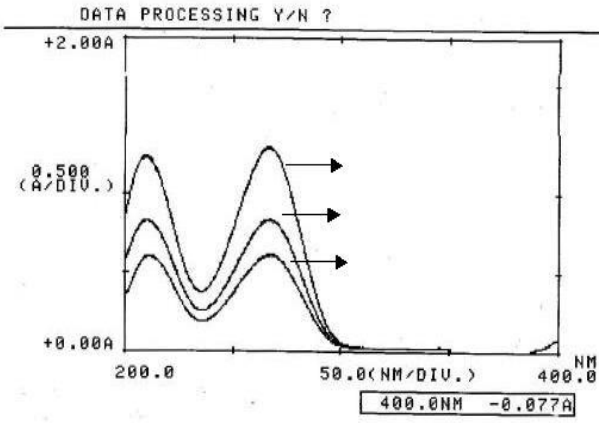


Fig 2: UV Spectra of Zidovudine & microspheres

Calibration curve for Zidovudine

The values of absorbance for the calibration curve of Zidovudine in phosphate buffer pH 7.4 are given in the following Table 2.

Table 2: Absorbance values for the calibration curve of Zidovudine

Concentration in µg/ml	Absorbance	Standard deviation
5	0.153	0.0051
10	0.304	0.0020
15	0.455	0.0015
20	0.615	0.0109
25	0.759	0.0043
30	0.909	0.0018

C = K x A + B
C = concentration of the sample
K = 32.940
A = Absorbance
B = -0.0334

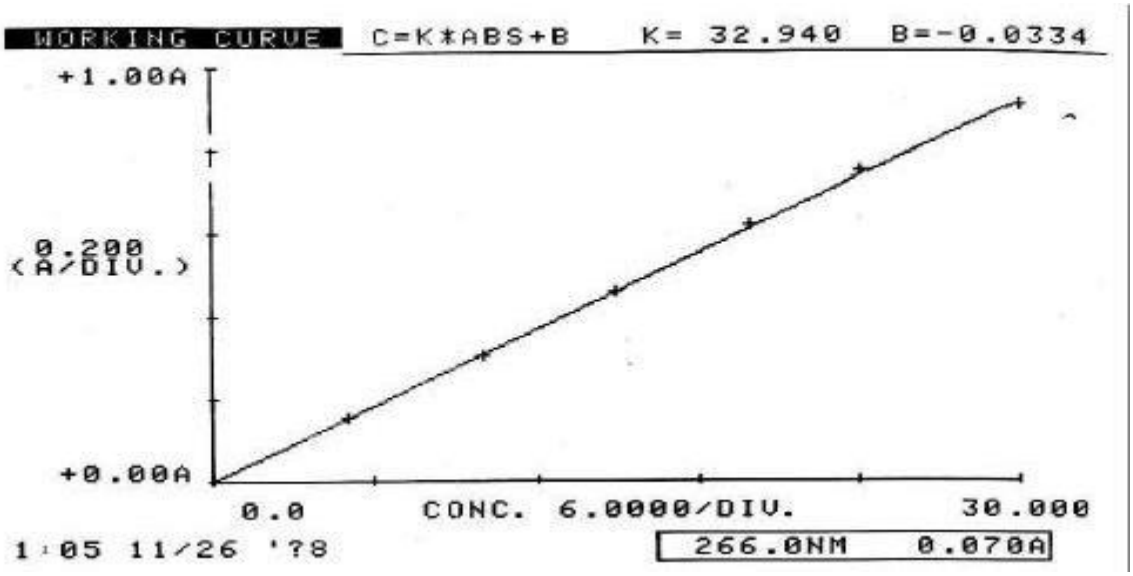


Fig 3: Calibration curve for Zidovudine in phosphate buffer pH 7.4 at266nm.

Percentage yield of microspheres

The percentage yield of Zidovudine microspheres with different drug to polymer ratio by using different polymers is shown in the Table 3 and Table 4.

Table 3: Percentage yield of Zidovudine microspheres of chitosan-acacia

Drug to polymer ratio	Product code	Percentage yield
0.5:1	A1	80%
0.75:1	A2	74.3%
1:1	A3	73.75%

Table 4: Percentage yield of Zidovudine microspheres chitosan-Sodium Alginate

Drug to polymer ratio	Product code	Percentage yield
0.5:1	B1	76.66%
0.75:1	B2	71.43%
1:1	B3	72.50%

In both the formulations i.e. A and B, the yield was found to be highest in the case of lowest drug to polymer ratio. Increasing the drug incorporated, decreased the yield of the microspheres.

Estimation of drug content in the microspheres

The drug content was estimated in the microspheres and the drug content found is shown in Tables 5, 6 and 7.

Table 5: Drug Content in Chitosan-Acacia Microspheres

Product code	Ratio	Wt of microspheres taken in grams	Drug content		Percentage drug content
			Theoretical (mg)	Practical (mg)	
A1	0.5:1	0.15	50	21.97	43.94
A2	0.75:1	0.166	50	24.74	49.48
A3	1:1	0.1	50	20.36	40.72

Table 6:Drug Content in Chitosan-Sodium Alginate Microspheres

Product code	Ratio	Wt of microspheres taken in grams	Drug content		Percentage drug content
			Theoretical (mg)	Practical (mg)	
B1	0.5:1	0.15	50	24.67	49.34
B2	0.75:1	0.166	50	25.13	50.26
B3	1:1	0.1	50	22.46	44.92

In both the polymer combination, the maximum drug content was found to be in the drug to polymer ratio 0.75:1. Increase in the amount of drug causes loss of drug, probably during the separation and drying stages.

Table 7: Drug content in microsphere suspension

Product code	Ratio	Drug content		Percentage drug content
		Theoretical mg	Practical mg	
Chitosan + acacia SUSP1	0.5:1	20	18.64	93.2
Chitosan + Sodium alginate SUSP2	0.5	20	19.52	97.6

Drug content up to 50% could be obtained in the dry microspheres and upto 97% in the suspension. This difference shows hat the loss of drug in the dry product is during the drying stages due to the loss of water along with the drug.

In both the dry and suspension formulation, the drug content was greater in the microspheres prepared using chitosan and sodium alginate. Maximum drug content obtained in dry microspheres using chitosan and acacia was 49.48% whereas using chitosan and sodium alginate was 50.26%

Drug content in SUSP1 was 93.2% while that in SUSP2 was found to be 97.6%

Microscopic observation: Size and shape of the microspheres

Microscopic observation reveals that the microspheres are spherical in shape as shown in the photomicrographs Fig 4 and Fig 5.The shape was found to be better controlled in the case of suspension, which clearly shows that the loss of shape in the dry product was due to the loss of water during the drying stages.

Particle size distribution data for Zidovudine microspheres containing chitosan and acacia

Table 8: Particle size analysis for A1 (drug to polymer ratio 0.5:1)

Size range □m	Average diameter (d) □m	No of particles (n)	%n	%nd	Mean size □m
9.9-13.2	11.55	16	5.33	61.56	
13.2-16.5	14.85	21	7	103.95	
16.5-19.8	18.15	84	28	508.2	20.5237
19.8-23.1	21.45	102	34	729.3	
23.1-26.4	24.75	64	21.33	527.9	
26.4-29.7	28.05	13	4.33	121.46	

Table 9: Particle size analysis for A2 (drug to polymer ratio 0.75:1)

Size range □m	Average diameter (d) □m	No of particles (n)	%n	%nd	Mean size □m
9.9-13.2	11.55	2	0.66	7.62	
13.2-16.5	14.85	3	1	14.85	
16.5-19.8	18.15	41	13.66	247.93	21.1941

19.8-23.1	21.45	153	51	1093.95	
23.1-26.4	24.75	81	27	668.25	
26.4-29.7	28.05	20	6.66	186.81	

Table 10: Particle size analysis for A3 (drug to polymer ratio 1:1)

Size range □m	**Average diameter** (d) □m	**No of particles** (n)	**%n**	**%nd**	**Mean size** □m
9.9-13.2	11.55	4	1.33	15.36	
13.2-16.5	14.85	12	4	59.4	
16.5-19.8	18.15	61	20.33	368.99	21.5581
19.8-23.1	21.45	132	44	943.8	
23.1-26.4	24.75	75	25	618.75	
26.4-29.7	28.05	16	5.33	149.51	

∑%nd=2155.81

Particle size distribution data for Zidovudine microspheres containing chitosan and Sodium alginate.

Table 11: Particle size analysis for B1 (drug to polymer ratio of 0.5:1)

Size range □m	**Average diameter** (d) □m	**No of particles** (n)	**%n**	**%nd**	**Mean size** □m
9.9-13.2	11.55	2	0.66	7.62	
13.2-16.5	14.85	12	4	59.4	
16.5-19.8	18.15	47	15.66	204.23	
19.8-23.1	21.45	128	42.66	915.06	21.634
23.1-26.4	24.75	71	23.66	585.59	
26.4-29.7	28.05	24	8	224.4	
29.7-33	31.35	16	5.33	167.1	

∑%nd=2163.4

Table 12: Particle size analysis for B2 (drug to polymer ratio 0.75:1)

Size range □m	Average diameter (d) □m	No of particles (n)	%n	%nd	Mean size □m
13.2-16.5	14.85	3	1	14.85	
16.5-19.8	18.15	31	10.33	187.49	
19.8-23.1	21.45	74	24.66	528.96	23.3854
23.1-26.4	24.75	113	37.66	932.09	
26.4-29.7	28.05	63	21	508.05	
29.7-33	31.35	16	5.33	167.1	

□%nd=2338.54

Table 13: Particle size analysis for B3 (drug to polymer ratio 1:1)

Size range □m	Average diameter (d) □m	No of particles (n)	%n	%nd	Mean size □m
13.2-16.5	14.85	7	2.33	34.60	
16.5-19.8	18.15	26	8.66	157.18	
19.8-23.1	21.45	71	23.66	507.51	24.1051
23.1-26.4	24.75	121	40.33	998.17	
26.4-29.7	28.05	64	21.33	598.31	
29.7-33	31.35	11	3.66	114.74	

□%nd=2410.51

Particle size analysis for the suspensions (drug to polymer ratio 0.5:1)

Table 14: Particle size analysis for SUSP1 (chitosan-acacia)

Size range □m	Average diameter (d) □m	No of particles (n)	%n	%nd	Mean size □m
6.6-9.9	8.28	42	13	107.25	
9.9-13.2	11.55	67	22.33	257.57	
13.2-16.5	14.85	99	33	490.05	

16.5-19.8	18.15	43	14.33	260.09	14.991
19.8-23.1	21.45	25	8.33	178.68	
23.1-26.4	24.75	17	5.66	140.09	
26.4-29.7	28.05	7	2.33	65.36	

%nd=1499.1

Table 15: Particle size analysis for SUSP2 (chitosan-sodium alginate)

Size range m	**Average diameter** (d) m	**No of particles** (n)	**%n**	**%nd**	**Mean size** m
6.6-9.9	8.28	38	12.66	104.82	
9.9-13.2	11.55	49	16.33	188.61	
13.2-16.5	14.85	58	19.33	287.05	
16.5-19.8	18.15	94	31.33	568.64	16.193
19.8-23.1	21.45	33	11	235.95	
23.1-26.4	24.75	25	8.33	206.17	
26.4-29.7	28.05	3	1	28.05	

%nd = 1619.31

Table 16: Consolidated particle size

Product code	**Drug to polymer ratio**	**Particle size** m
Chitosan + acacia		
1A	0.5:1	20.5237
1B	0.75:1	21.1941
1C	1:1	21.5581
Chitosan + sodium alginate		
2A	0.5:1	21.634
2B	0.75:1	23.3854
2C	1:1	24.1051
SUSP1	0.5:1	14.911
SUSP2	0.5:1	16.193

The particle size of the suspended microspheres was found to be smaller than the dry microspheres. This may be due to the perfectly spherical shape of the suspended microspheres.

Fig 4: Photomicrograph of Microsphere Suspension Containing Chitosan-Acacia (SUSP1)

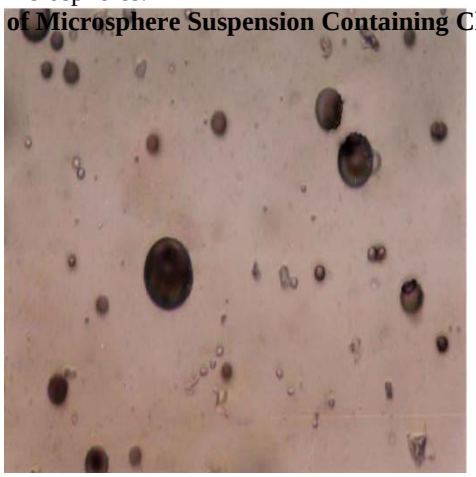
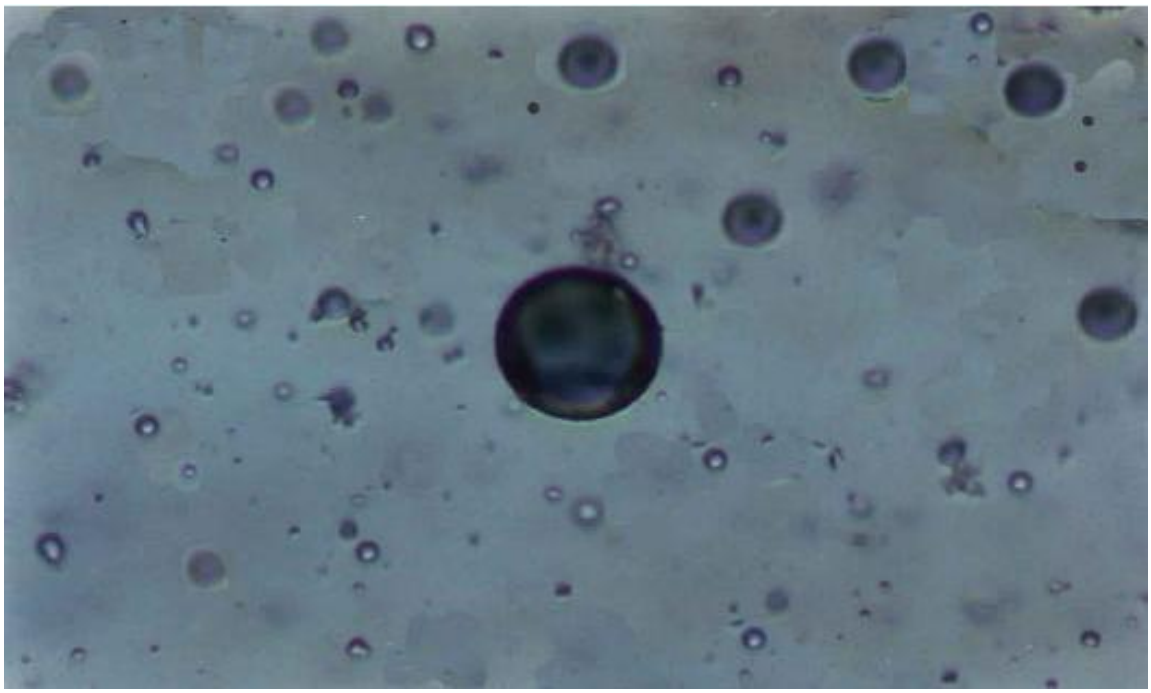


Fig 5: Photomicrograph of Microsphere Suspension Containing Chitosan-Sodium Alginate (SUSP2)



Dissolution studies:

The data regarding percent drug dissolved obtained from the dissolution tests and the corresponding dissolution profiles are shown in Tables 17 to 24 and Fig 6,7 and 8.

Dissolution data for microspheres of chitosan-acacia

Table 17: Dissolution data for 1A (drug to polymer ratio 0.5:1)

Time in (Hours)	**Absorbance*** □ SD	**Concentration** (C) (□g/ml)	**Amount of drug released** (mg) (C□900□D.F/1000)	**% Drug released**
0.5	0.080 □ 0.55	2.63	23.67	23.67
1.0	0.137 □ 0.092	4.48	40.32	40.32
1.5	0.175 □ 0.053	5.76	51.84	51.84
2.0	0.213 □ 0.061	7.01	63.11	63.11
2.5	0.242 □ 0.054	7.96	71.65	71.65
3.0	0.267 □ 0.083	8.78	79.05	79.05
3.5	0.293 □ 0.076	9.62	86.66	86.66
4.0	0.301 □ 0.091	9.90	89.11	89.11

4.5	0.311 □ 0.028	10.22	92.05	92.05
5.0	0.320 □ 0.039	10.52	94.72	94.72
5.5	0.323 □ 0.013	10.63	95.74	95.74
6.0	0.325 □ 0.021	10.68	96.17	96.17
6.5	0.333 □ 0.094	10.94	98.54	98.54
7.0	0.333 □ 0.064	10.94	98.54	98.54

*each value is an average of three determinations D.F = Dilution factor

Table 18: Dissolution data of 1B (drug to polymer ratio 0.75:1):

Time in (Hours)	**Absorbance*** □ SD	**Concentration** (C) (□g/ml)	**Amount of drug released** (mg) (C□900□D.F/1000)	**% Drug released**
0.5	0.092 □ 0.065	3.03	27.27	27.27
1.0	0.153 □ 0.021	5.01	45.09	45.09
1.5	0.186 □ 0.056	6.11	55.00	55.00
2.0	0.222 □ 0.058	7.28	65.52	65.52
2.5	0.256 □ 0.099	8.43	75.87	75.87
3.0	0.292 □ 0.064	9.59	86.31	86.31
3.5	0.317 □ 0.059	10.41	93.74	93.74
4.0	0.322 □ 0.099	10.60	95.40	95.40
4.5	0.326 □ 0.064	10.72	96.55	96.55
5.0	0.328 □ 0.059	10.80	97.23	97.23
5.5	0.331 □ 0.048	10.89	98.09	98.09
6.0	0.335 □ 0.091	11.01	99.11	99.11
6.5	0.335 □ 0.082	11.01	99.11	99.11
7.0	0.335 □ 0.062	11.01	99.11	99.11

*each value is an average of three determinations D.F = Dilution factor

Table 19: Dissolution data for 1C(drug to polymer ratio 1:1)

Time in (Hours)	**Absorbance*** □ SD	**Concentration** (C) (□g/ml)	**Amount of drug released** (mg) (C□900□D.F/1000)	**% Drug released**
0.50	0.112 □ 0.041	3.66	32.94	32.94
1.00	0.167 □ 0.052	5.49	49.47	49.47
1.50	0.213 □ 0.027	7.00	63.00	63.00
2.00	0.247 □ 0.062	8.11	73.00	73.00
2.50	0.284 □ 0.081	9.33	84.00	84.00
3.00	0.310 □ 0.026	10.19	91.74	91.74
3.50	0.334 □ 0.033	11.00	99.01	99.01
4.00	0.335 □ 0.051	11.01	99.16	99.16
4.50	0.337 □ 0.062	11.08	99.76	99.76
5.00	0.338 □ 0.052	11.10	99.96	99.96
5.50	0.338 □ 0.051	11.10	99.96	99.96
6.00	0.338 □ 0.092	11.10	99.96	99.96
6.50	0.338 □ 0.049	11.10	99.96	99.96
7.00	0.338 □ 0.057	11.10	99.96	99.96

*each value is an average of three determinations D.F = Dilution factor

Dissolution data for mirospheres of chitosan-sodium alginate

Table 20: Dissolution data of 2A (drug to polymer ratio 0.5:1)

Time in (Hours)	**Absorbance*** □ SD	**Concentration** (C) (□g/ml)	**Amount of drug released** (mg) (C□900□D.F/1000)	**% Drug released**
0.50	0.052 □ 0.065	1.71	15.39	15.39
1.00	0.093□ 0.024	3.05	27.46	27.46
1.50	0.134□ 0.028	4.40	39.62	39.62
2.00	0.153□ 0.035	5.03	45.31	45.31
2.50	0.200□ 0.062	6.55	59.01	59.01
3.00	0.242 □ 0.023	7.97	71.73	71.73
3.50	0.270 □ 0.035	8.88	79.99	79.99
4.00	0.283 □ 0.057	9.29	83.63	83.63
4.50	0.297 □ 0.026	9.76	87.92	87.92
5.00	0.304 □ 0.039	10.00	90.05	90.05
5.50	0.313 □ 0.095	10.30	92.75	92.75
6.00	0.320 □ 0.091	10.53	94.83	94.83
6.50	0.324 □ 0.072	10.65	95.92	95.92
7.00	0.328 □ 0.061	10.79	97.11	97.11

*each value is an average of three determinations D.F = Dilution factor

Table 21: Dissolution data of 2B (drug to polymer ratio 0.75:1)

Time in (Hours)	**Absorbance*** □ SD	**Concentration** (C) (□g/ml)	**Amount of drug released** (mg) (C□900□D.F/1000)	**% Drug released**
0.50	0.073 □□0.025	2.39	21.51	21.51
1.00	0.127 □□0.051	4.15	37.37	37.37
1.50	0.154 □□0.078	5.04	45.41	45.41
2.00	0.192 □ 0.061	6.31	56.8	56.8
2.50	0.222 □ 0.055	7.30	65.77	65.77
3.00	0.259 □ 0.062	8.50	76.51	76.51
3.50	0.277 □ 0.081	9.10	81.94	81.94
4.00	0.293 □ 0.044	9.63	86.73	86.73
4.50	0.300 □ 0.058	9.88	88.92	88.92
5.00	0.313 □ 0.039	10.28	92.54	92.54
5.50	0.324□□0.051	10.65	95.86	95.86
6.00	0.325 □ 0.064	10.67	96.1	96.1
6.50	0.329 □ 0.037	10.82	97.42	97.42
7.00	0.331□ 0.064	10.89	98.03	98.03

*each value is an average of three determinations D.F = Dilution factor

Table 22: Dissolution data of 2C (drug to polymer ratio 1:1)

Time in (Hours)	**Absorbance*** □ SD	**Concentration** (C) (□g/ml)	**Amount of drug released** (mg) (C□900□D.F/1000)	**% Drug released**
0.50	0.096 □ 0.062	3.15	28.43	28.43
1.00	0.140 □ 0.071	4.58	41.22	41.22
1.50	0.186 □ 0.066	6.10	54.96	54.96
2.00	0.212 □ 0.085	6.97	62.74	62.74
2.50	0.243 □ 0.074	7.99	71.99	71.99
3.00	0.284 □□0.054	9.33	84.00	84.00
3.50	0.304 □ 0.025	9.99	89.94	89.94
4.00	0.306 □ 0.057	10.00	90.04	90.04
4.50	0.320 □ 0.068	10.52	94.76	94.76
5.00	0.322 □ 0.064	10.60	95.42	95.42
5.50	0.328 □ 0.071	10.78	97.09	97.09
6.00	0.333 □0.054	10.93	98.43	98.43
6.50	0.335 □0.064	11.01	99.17	99.17
7.00	0.335 □□0.052	11.01	99.17	99.17

*each value is an average of three determinations D.F = Dilution factor

Drug release from the suspension formulations

Table 23: Drug release data from microsphere suspension of chitosan-acacia (drug to polymer ratio 0.5:1)

Time in (Hours)	**Absorbance*** □ SD	**Concentration** (C) (□g/ml)	**Amount of drug released** (mg) (C□200□D.F/1000)	**% Drug released**
0.50	0.094 □ 0.038	3.10	3.10	15.48
1.00	0.159 □ 0.022	5.21	5.21	26.06
1.50	0.198 □ 0.050	6.50	6.50	32.5
2.00	0.229 □ 0.045	7.50	7.50	37.5
2.50	0.257 □ 0.015	8.44	8.44	42.2
3.00	0.293 □ 0.029	9.60	9.60	48.0
3.50	0.319 □ 0.038	10.50	10.50	52.3
4.00	0.348 □ 0.019	11.40	11.40	57.0
4.50	0.370 □ 0.027	12.10	12.10	60.61
5.00	0.400 □ 0.036	13.10	13.10	65.55
5.50	0.427 □0.071	14.00	14.00	70.0
6.00	0.462 □0.036	15.20	15.20	75.8
6.50	0.500 □ 0.009	16.40	16.40	82.02
7.00	0.526 □ 0.026	17.30	17.30	86.3
7.50	0.546 □ 0.018	17.90	17.90	89.5
8.00	0.561 □ 0.034	18.40	18.40	92.07
8.50	0.570 □ 0.074	18.70	18.70	93.5
9.00	0.574 □ 0.059	18.80	18.80	94.20
9.50	0.575 □ 0.017	18.90	18.90	94.37
10.0	0.575 □ 0.030	18.90	18.90	94.37

*each value is an average of three determinations D.F = Dilution factor

Table 24: Drug release data from microsphere suspension of chitosan-sodium alginate (drug to polymer ratio 0.5:1)

Time in (Hours)	Absorbance* □ SD	Concentration (C) (□g/ml)	Amount of drug released (mg) (C□200□D.F/1000)	% Drug released
0.50	0.073 □ 0.053	2.40	2.40	12.00
1.00	0.123 □ 0.009	4.04	4.04	20.20
1.50	0.159 □ 0.021	5.20	5.20	26.00
2.00	0.194 □ 0.067	6.36	6.36	31.80
2.50	0.219 □ 0.086	7.18	7.18	35.90
3.00	0.249 □ 0.095	8.16	8.16	40.80
3.50	0.275 □ 0.074	9.02	9.02	45.10
4.00	0.304 □ 0.080	9.96	9.96	49.80
4.50	0.328 □ 0.061	10.80	10.80	53.80
5.00	0.353 □ 0.071	11.60	11.60	57.97
5.50	0.387 □ 0.037	12.70	12.70	63.50
6.00	0.427 □ 0.066	14.00	14.00	70.00
6.50	0.460 □ 0.085	15.10	15.10	75.50
7.00	0.487 □ 0.074	16.00	16.00	79.87
7.50	0.517 □ 0.054	17.00	17.00	84.80
8.00	0.535 □ 0.058	17.50	17.50	87.70
8.50	0.544 □ 0.028	17.80	17.80	89.20
9.00	0.557 □ 0.015	18.30	18.30	91.28
9.50	0.561 □□0.059	18.40	18.40	92.00
10.0	0.564 □□0.062	18.50	18.50	92.50

*each value is an average of three determinations D.F = Dilution factor

Table 25: Comparison of 'r' values for different products

Product code	Coefficient of correlation (r)	
	Zero order	First order
1A	0.8453	0.9867
1B	0.7913	0.9805
1C	0.7252	0.9341
2A	0.8939	0.9928
2B	0.8824	0.9896
2C	0.8369	0.9838
SUSP1	0.9806	0.9602
SUSP2	0.9684	0.9571

The calculation of the first order release rate constants gave the following values.

Table 26: First order release rate constants for the dried microspheres

Product code	Release rate constants (hr-1)
1A	0.6064
1B	0.7280
1C	0.7668
2B	0.4659
2B	0.5320
2C	0.6908

The above data also shows a slower rate of release for the chitosan-sodium alginate microspheres (2A, 2B, 2C) compared to the chitosan-acacia (1A,1B,1C) microspheres. This may be due to a greater viscosity of sodium alginate compared to acacia.
Further the drug release from these microspheres followed matrix diffusion controlled process for most of the drug release i.e. upto 80% as shown by the 'r' values (Table 27). This may be possible because of the dispersion of the drug in the polymer matrix.

Table 27: 'r' values for the Higuchi plot

Product code	**Coefficient of correlation 'r'**
1A	0.9995
1B	0.9973
1C	0.9995
2A	0.9785
2B	0.9950
2C	0.9944

The respective rate constants were calculated and the values are as shown below.

Table 28: Comparison of zero order rate constants for the microsphere suspensions

Product code	**Zero order rate constants** Percent /Hour
SUSP1	9.7818
SUSP2	8.95

III CONCLUSION

The overall results presented in this study provided evidence that the emulsification used in the formulation of microspheres allows the preparation of particles with spherical shape, better drug content and good release kinetics.
The microspheres prepared using chitosan and sodium alginate were found to be better compared to chitosan and acacia with respect to duration of drug release, which was slower with chitosan and sodium alginate.
The suspension was better than the dry product with respect to shape, drug content and the release kinetics which was found to be zero order for the suspension whereas first order for the dried products.

The oily suspension provides a means of parenteral administration of Zidovudine, provided the particle size can be further reduced. This sustained release parenteral formulation of Zidovudine would be beneficial in comparison with the current intermittent oral dosage regimen.

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