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Preparation and Evaluation Glutaraldehyde Cross Linked Chitosan Microspheres Containing Rivastigmine Tartrate

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ABSTRACT

Rivastigmine tartrate is an anticholine esterase inhibitor that used in the treatment of Alzheimer's disease. In this study an attempt was made to encapsulate the Rivastigmine tartrate in Chitosan microspheres for controlled delivery of it. Chitosan microspheres were prepared by the emulsion cross linking method were Glutaraldehyde saturated toluene (GST) was used as a cross linker. Total four batches were prepared with different amount of Glutaraldehyde. It was found that GST had no significant effect on % yield, also increase in amount of GST decrease the particle size and increases the entrapment. GST had shown significant effect on the in vitro drug release and found that increase in amount of GST prolongs the drug release from 36 to 96 hrs. Chitosan microspheres shown to be follow the Higuchi model.

KEYWORDS

Rivastigmine tartrate, Chitosan, microspheres, Glutaraldehyde

INTRODUCTION

Alzheimer's disease is a progressive, neurodegenerative disorder, preliminary affecting cholinergic neurons in the brain, which causes thinking and memory to become seriously impaired. It is the most common form of dementia. Dementia is a syndrome consisting of a number of symptoms that include loss of memory, judgment and reasoning, and changes in mood, behavior and communication abilities[1,2].

Rivastigmine is an anticholine esterase inhibitor that augments cholinergic transmission in the brain and reduces the neurodegradation[1, 2]. It has short half life approximately 1.5 hrs, this is due to its extensively metabolized primarily via cholinesterase-mediated hydrolysis to the decarbamylated metabolite NAP226-90, so it required frequent dose administration[3]. Therefore controlled release of Rivastigmine tartrate is an idle way.

Various types of controlled release dosage forms are formulated to overcome such difficulties. Controlled release drug delivery employs devices such as polymer based disks, rods, pellets, or microparticles, that encapsulate drug and release it at controlled rates for relatively long periods of time. Such systems offer several potential advantages over traditional methods of administration[4].

- Drug release rates can be tailored to the needs of a specific application; for example, providing a constant rate of delivery or pulsatile release.
- Controlled release systems provide protection of drugs, especially proteins that are otherwise rapidly destroyed by the body.

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- Controlled release systems can increase patient comfort and compliance by replacing frequent (e.g., daily) doses with infrequent (once per month or less) injection.

One such method is Microspheres as carriers of drug become an approach of controlled release dosage form in novel drug delivery system.

"Microspheres can be defined as a solid, approximately spherical particles ranging 1-1000 μm in size. They are made up of polymeric substances, in which the drug is dispersed throughout the microsphere matrix[5].

Chitin and chitosan are one of the most abundant polysaccharide and are biodegradable in nature. Many researches had been undertaken to evaluate the capability of chitosan as a controlled release polymer.

In this present work the microspheres of Rivastigmine Tartrate were prepared by using Chitosan as a polymer. In this experiment, our objectives were to study the effect of Glutaraldehyde on % Yield, % Entrapment efficiency, and Mean particle size and % drug release.

MATERIALS AND METHODS

MATERIALS

Rivastigmine tartrate was gifted by Alembic pharmaceuticals, Baroda. Chitosan was gifted by Central fisheries department, India. Light liquid paraffin, Span 80, n-Hexane, Potassium dihydrogen orthophosphate (KH_2Po_4), Sodium hydroxide (NaOH), Petroleum ether, Acetic acid, Toluene, were purchased from local Indian market.

METHOD

Drug- Polymer Compatibility Study

The DSC study was carried out using DSC-61000 (Seiko instrument, Japan).The samples were heated in sealed aluminum pans under air flow (30 ml/min) at a scanning rate of 20°C/min from 50 to 350°C. Empty aluminum pan was used as a reference. The heat flow as a function of temperature was measured for the samples.

Preparation of Chitosan Microspheres

Chitosan microspheres were prepared by simple emulsion Glutaraldehyde cross linking method previously described by the Jameela S.R.1995[6,7] with slight modification. 100 mg of Rivastigmine tartrate and 400 mg of chitosan was dissolved in 20 ml of 5% w/v acetic acid solution by stirring at 250 rpm for 30 min. on magnetic stirrer. The resultant solution was then added to the 100 ml of (60:40 ratio containing) mixture of light liquid paraffin and petroleum ether with 1% v/v span 80 by 22 gauge needle. The resultant emulsion was then stirred at 2000 rpm by propeller. Different amount of Glutaraldehyde saturated toluene (GST) as shown in table 1, was added drop wise in the emulsion at time interval of 5 min., 15min. and 30 min. and after 60 min. 0.8 ml of 25% v/v of aqueous solution was added drop wise and stirred continuously for total 1.5 hrs. Resultant microspheres then separated by centrifugation at 2500 rpm for 10min. and then washed thrice with n-Hexane, and with water, air dried and stored in refrigerator.

EVALUATION OF PREPARED MICROSPHERES

Table: 1 Different amount of GST to be added at different time interval

Batch No.	Total GST (ml)	Amount of GST at (ml)			Amount of aqueous glutaraldehyde at 60 min. (ml)
		5 min.	15 min.	30 min.	
C_1	3	1	1	1	0.8
C_2	6	2	2	2	0.8
C_3	9	3	3	3	0.8
C_4	12	4	4	4	0.8

Percentage Yield

The yield of microspheres was calculated from the amount of microspheres obtained divided by the total amount of all non-volatile components.

$$\% \text{ yield} = \frac{\text{Practical yield of microspheres after drying}}{\text{Total weight of Drug+Polymer}} * 100$$

Particle Size Determination

Size analysis was performed by the optical microscope. 100 particles of each batch were calculated by calibrated eye piece. The particle size range as well as the average particle size was calculated from a frequency distribution curve.

Shape and Surface Morphology

Scanning electron microscopy was used to study the morphology and surface characteristics of the microspheres.

% Entrapment Efficiency [8]

25 mg of microspheres were crushed in motor pastel and dispersed in 20 ml phosphate buffer pH 7.4 and then sonicate for 5 min by probe sonicator. Then this dispersion was allowed to rotate on magnetic stirrer for 24 hrs. Solution was then filtered and then absorption was measured at 264nm.

$$\% \text{ Entrapment Efficiency} = \frac{\textit{Practal drug loading}}{\textit{Theoritical drug loading}} * 100$$

In Vitro Drug Release

A modified dialysis method was used to evaluate in vitro release of drug. 200 mg of microspheres was weighed and suspended in the 2 ml of phosphate buffer pH7.4 in the dialysis bag (cellophane membrane, molecular weight cut off 10,000–12,000, Hi-Media, India) and bag was put in the iodine flask containing 50 ml of phosphate buffer pH 7.4. This flask was the put in the orbital shaker and rotated at 100 rpm at 37^0C. 3 ml sampling was done at predetermined time interval and released drug was analyzed by UV spectrometer at 264 nm.

Kinetic Model Fitting[9]

The in vitro drug release data were fitted to following model to evaluate the mechanism of drug release.

Table: 2 Model fitting equation for in vitro drug release data

Model	Equation	Graph
Zero order	$Q_0 - Q_t = K0_t$	cumulative amount of drug released *versus* time
Higuchi	$f_t = Q = \{A \sqrt{D(2C - Cs) Cs\ t}\}^{1/2}$	cumulative percentage drug release versus square root of time
First order	$\log C = \log C0 - Kt / 2.303$	log cumulative percentage of drug remaining *vs*. time which would yield a straight line with a slope of -K/2.303
Korsmeyer-Peppas	$Mt / M\infty = Ktn$	log cumulative percentage drug release versus log time.
Baker-Lonsdale	$f1 = 3/2 \{1 - (1- Mt / M\infty)^{2/3}\} Mt / M\infty = k_t$	[d (*Mt* / *M*∞)] / dt with respect to the root of time inverse.

RESULTS AND DISCUSSION

Drug Polymer Compatibility Study by DSC

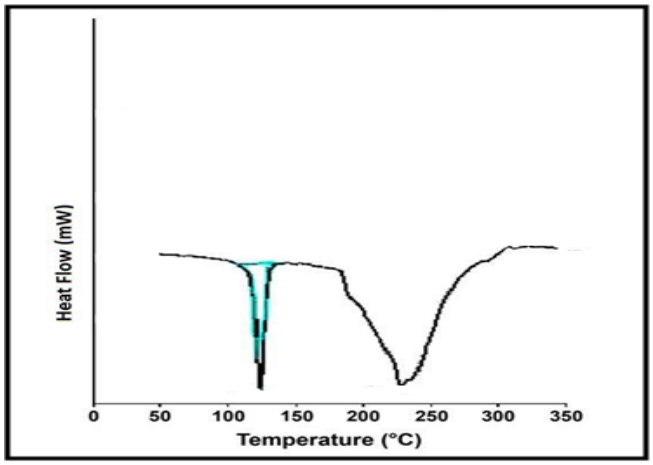


Figure: 1 (a) DSC of Rivastigmine tartrate

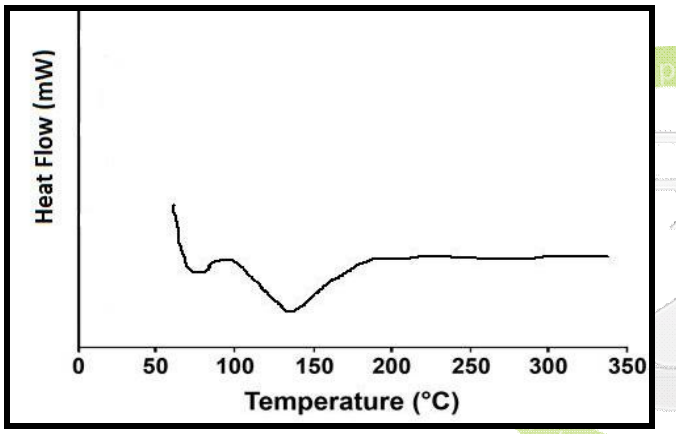


Figure: 1 (b) DSC of Chitosan

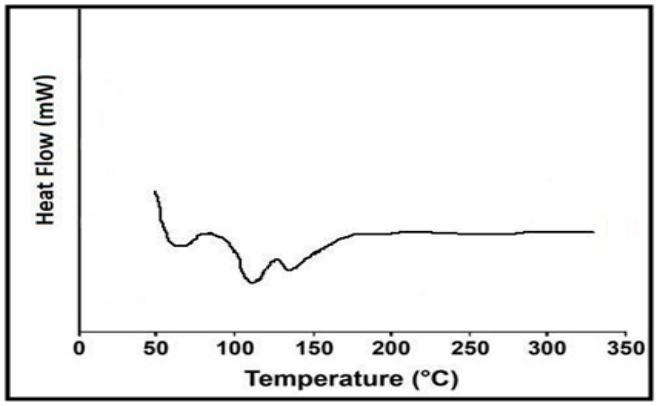


Figure: 1 (c) DSC of Physical mixture of Rivastigmine tartrate and Chitosan

As shown in figure 1 Rivastigmine tartrate shows endothermic peak around 125 ^{0}C, and Chitosan at around 135 ^{0}C. As shown in figure 1 (C) separate peaks of Rivastigmine tartrate and Chitosan was obtained so it was concluded that both were compatible.

Result of Rivastigmine Tartrate Microspheres:

Table: 3 Result of batches C_1 to C_4

Batch Code	% yield	Entrapment efficiency (%) ± S.D.	Mean Size (µm)
C_1	94.33	17.41 ± 3.31	19.22
C_2	95.11	22.5 ± 2.58	18.82
C_3	94.77	30.22 ± 2.35	17.64
C_4	96.54	31.75 ± 2.21	16.99

Percentage Yield

% yield of all batches are shown in table 3 and figure 2.

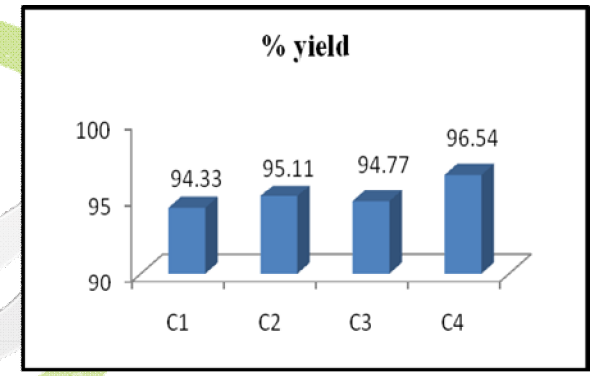


Figure: 2 % yield of batches C_1 to C_4

In all batches % yield was higher than 90%. There was no significant effect of Glutaraldehyde on % yield was found.

Entrapment Efficiency (%)

Results of entrapment efficiency are shown in table 3 and figure 3.

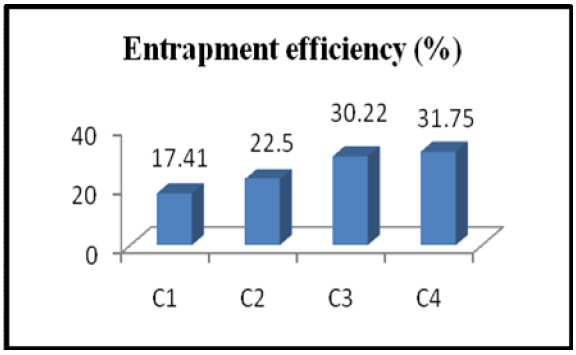


Figure: 3 entrapment efficiency of batches C_1 to C_4

As shown in figure 3 increases in amount in GST significantly increases the entrapment efficiency.

It can be explained by the higher degree of cross linking occurred by higher concentration of GST. Here from batch C1 to C4 increased amount of GST was used. Increase in amount of GST produces much denser matrix due to increased cross linking with chitosan that reduces the out flow of drug during stirring and increases the entrapment efficiency which was comply with the early findings of Kotadiya R. et.al. 2011[10].

Size of Microspheres

Size of microspheres were shown in table 3 and figure 4.

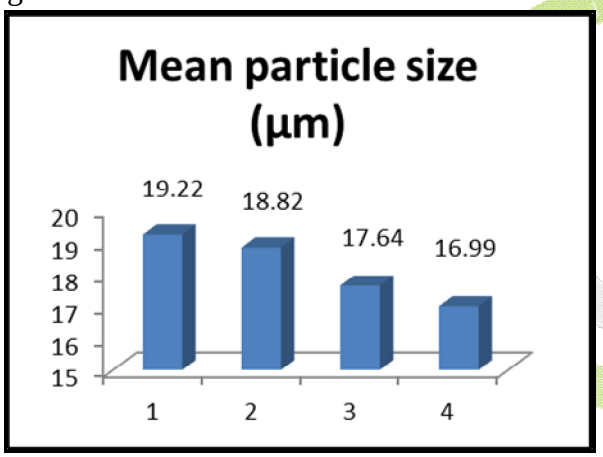


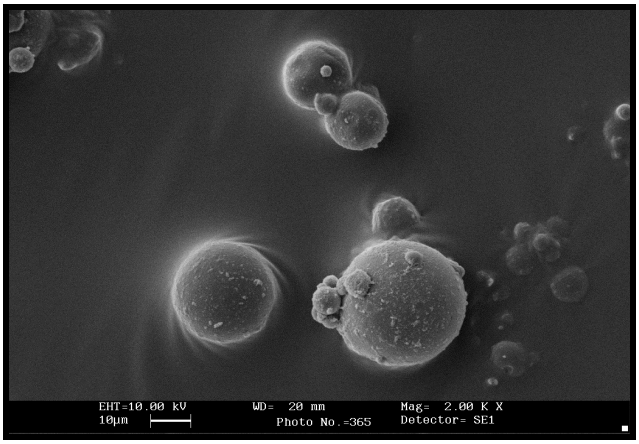
Figure: 4 Size of batches C_1 to C_4

From the figure 4 it can be seen that increase in amount of GST decreases the particle size. Here negligible decrease in size was achieved up to batch C_3 further increase in amount of GST showed almost no reduction in particle size.

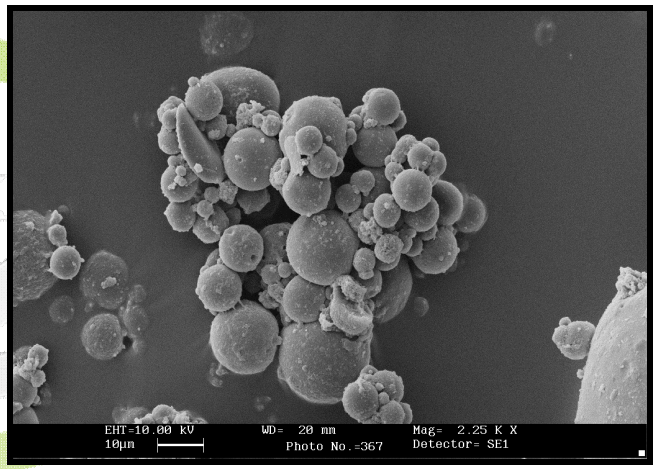
It was probably explained by the higher cross linking density with increase in GST that produces the denser and stiff structure that reduces the particle size. Which was complies with the early findings of Khalandar K.S. et.al. 2011[11], Eroğlu H. et.al. 2008[12], Genta I.et. al. 1997[13]. Also it can be seen that increased in GST, reduces the particle size distribution.

Shape and Surface Morphology

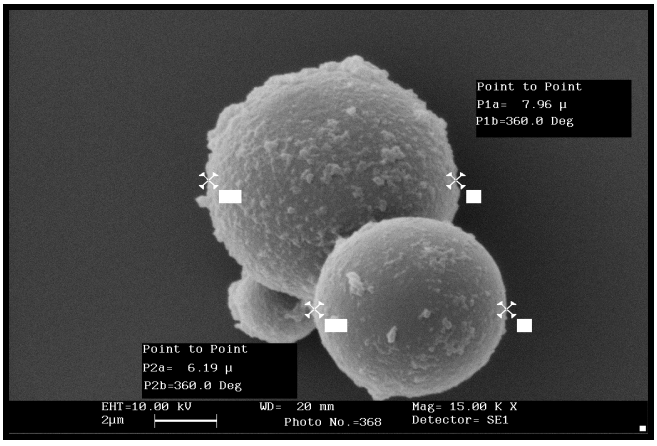
SEM photographs of chitosan microspheres are shown in figure 5.



(a)



(b)



(c)

Figure: 5 (a), (b), and (c) SEM photograph of chitosan microspheres

Shape of microspheres seems to be spherical with fairly smooth surface.

In Vitro Drug Release

Results of in vitro drug release data are shown in table 4 and figure 6.

in GST. It can be explained by the reduction in size of microspheres with increase in GST. Reduction in size increases the effective surface area in contact with dissolution medium and also the increase surface area result in increased surface bound drugs so burst release was increased.

Table: 4 *In vitro* drug release of Chitosan Microspheres

Time (hrs)	% Cumulative drug Release			
	C_1	C_2	C_3	C_4
0	0	0	0	0
2	22.11	23.56	24.71	25.09
4	24.12	24.98	25.98	25.11
6	46.12	35.16	28.12	26.51
8	59.71	41.58	31.44	29.12
10	67.13	47.13	36.55	32.11
12	71.55	52.57	41.48	33.57
16	81.25	62.16	49.99	39.18
24	90.18	72.56	60.13	46.15
36	98.51	84.12	69.22	51.58
48		91.18	78.16	60.11
60		96.15	89.13	70.95
72			99.16	79.11
84				88.65
96				98.13

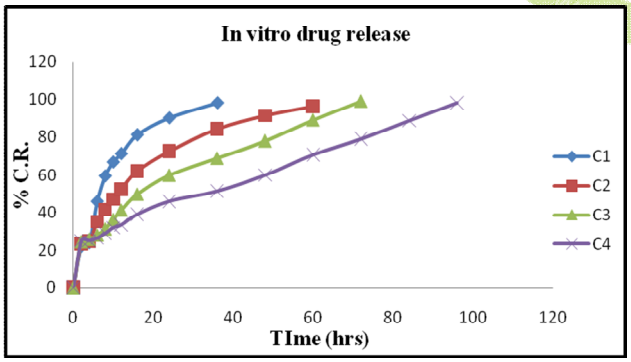


Figure 6**:** *In vitro* drug release of Chitosan microspheres.

From the above table 4 and figure 6 it can be seen that chitosan microspheres follows bi-release pattern first burst release within 2 hrs and then sustain release period.

Initial burst release was due to the surface associated drug crystals. Also there was slight increase in burst release observed with increase

There are significant reduction in release rate was found with increase in amount of GST. It can be explained by the increased cross liking density of chitosan with increased amount of GST. During the microspheres formation reaction take place between -NH_2 group of chitosan and –COO group of Glutaraldehyde that forms new bonds and matrix structure form. Drug release from chitosan matrix occur only after swelling of the matrix, but increase in cross linking density increases the hydrophobicity of chitosan matrix that increase the time for hydration and drug release decreases which was comply with the early findings of Jameela S.R.al. 1995[6] and Jameela S.R.al. 1998[7].

Kinetics of *in vitro* drug release data

In vitro drug release of C_4 batch was fitted to various mathematical model by Microsoft excel

to find the kinetics of release data. Results are shown in table 5.

Table: 5 Model fitting of batch C_4

Values	Model				
	Zero order	**First order**	**Higuchi**	**Coresmann and Pepaas**	**Baker and Londsen**
R^2	0.949	0.785	**0.978**	0.687	0.893
Slope	0.165	0.016	**8.92**	0.662	1.027
Intercept	4.128	0.668	**3.97**	0.752	0.205

Here from table 5 it could be seen that Higuchi shows highest R^2 value so, drug release follows diffusion mechanism.

CONCLUSION

Chitosan microspheres were prepared by the simple emulsion cross linking method to study the effect of Glutaraldehyde on properties of microspheres. Microspheres with fairly spherical shape and with rough surface were obtained. It was found that increase in amount of GST resulted in decreased particle size, increased entrapment while sustained drug release from 36 to 96 hrs.

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