FORMULATION AND EVALUATION OF CHITOSAN NANOSPHERES AS A CARRIER FOR THE TARGETED DELIVERY OF ARECOLINE TO THE BRAIN

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Abstract

The present study deals with the formulation and evaluation of surfactant coated and uncoated chitosan nanospheres containing Arecoline for the targeted delivery to the brain. Nanospheres were prepared by a modified spontaneous emulsification method and different process variables were optimized. 0.5 % of chitosan gel and 2.5ml of gluteraldehyde saturated toluene per 25mg of polymer were selected as the optimum concentrations of polymer and cross linking agent, which yielded nanospheres of discrete nature, with an average size range of 151.00- 185.01nm. There was a proportional relationship between the drug loading capacity and drug concentration up to a concentration of 20mg drug /50mg of polymer, thereafter, an inverse relationship was observed. The in-vitro release behavior from all the drug loaded batches was found to be biphasic with an initial burst release followed by a sustained release over a period of 24hr. Surfactant coated nanospheres were prepared by adsorption method, the in-vitro release study of which showed a delay in the initial burst release. Mechanism by which the drug is being released was found to be a non-Fickian analomous diffusion, and the drug release was followed by first order kinetics. The drug loaded batches showed good stability when stored at room temperature for 60 days. Hence the formulated Tween 80 coated nanospheres can be used as an alternative and cheaper carrier for the targeted delivery of Arecholin to the brain, especially for the treatment of Alzheimer's.

INTRODUCTION

The efficacy of many drugs is often limited by their potential to reach the site of therapeutic action. In most cases(conventional dosage forms), only a small amount of administered dose reaches the targeted site, while the majority of the drug distributes throughout the rest of the body in accordance with its physicochemical and biological properties. Therefore, developing a drug delivery system that optimizes the pharmacological action of a drug while reducing its toxic side effects *in-vivo* is a challenging task.[1]

Insufficient delivery of anti- Alzheimer drugs to the CNS is attributed to their lower permeability across the blood brain barrier (BBB). Therefore, developing novel approaches that are targeted at enhancing the CNS delivery of anti- Alzheimer drugs are required.

The colloidal systems allow access across the BBB of non- transportable drugs by masking their physicochemical characteristics through their encapsulation in these systems. Among the particulate carriers, nanospheres are being increasingly investigated for targeted delivery of anti- Alzheimer drug. Their encapsulation into such systems may provide improved efficacy, decreased drug resistance, the reduction in dosage, a decrease in systemic toxicity and side effects, and an improved patient compliance. These successfully cross the barrier when coated with polyethylene glycol (PEG), polysorbate, or other polymer or co-surfactant[2].

Amyloid beta (Aβ or Abeta) is a peptide of 36–43 amino acids that is processed from the Amyloid precursor protein. While best known as a component of amyloid plaques in association with Alzheimer's disease, evidence has been found that Aβ is a highly multifunctional peptide with significant non-pathological activity. Aβ is the main component of deposits found in the brains of patients with Alzheimer's disease.

Aracoline have affinity to precipitated Amyloid protein & optimal dose for memory enhancement in patients with Alzheimer's disease.[3]

MATERIALS AND METHODS

Formulation of chitosan nanospheres

Plain chitosan nanospheres were prepared by modified spontaneous emulsification

method. 250 mg of chitosan and 1 gm of sodium chloride was dispersed in 50 ml of 3% glacial acetic acid and stirred continuously for 2 hours to obtain a 0.5 % chitosan gel solution, which was then stabilized overnight to obtain a clear gel.

To prepare a single batch of nanospheres, 5 ml of chitosan gel (0.5 % w/w) was taken in a 100 ml beaker and mixed with 5 ml of acetone to obtain a clear gel. The polymer gel was added into 10 ml of linseed oil and allowed to emulsify under magnetic stirring at room temperature by covering the beaker with a suitable lid. The system was stirred for 30 minutes and then the beaker was kept opened and stirring continued for another 1 hour, which resulted in the precipitation of polymer due to the evaporation of acetone with subsequent formation of smaller spheres suspended in oil phase. To solidify and stabilize the spheres, 2.5 ml of gluteraldehyde saturated toluene containing 10 % Span 80 was selected as a cross linking agent, which was slowly added with a micropipette to the system and stirring was continued for 2 hours.

The suspension obtained was centrifuged at 2500 rpm for 30 minutes, and washed three times each with toluene and acetone. After final washing, the product was suspended in acetone and poured in to a clean petri dish and dried at room temperature to obtain brown colored, free flowing, fine powder and was stored in a self sealed cover.[4]

Optimization of process variables

Study on effect of polymer concentration on particle formation

To study the relationship between the concentration of chitosan and particle formation, four different batches were prepared with various concentrations of chitosan gel. The morphology of the formed particles was observed through optical microscope and the percentage yield was calculated. (Table No.**1**)

Study on effect of volume of cross linking agent on particle solidification

In order to determine the effect of volume of cross linking agent on particle solidification, six different batches of nanospheres were prepared using increasing volumes of gluteraldehyde

saturated toluene. The formed particles were observed through optical microscope for their discreteness and uniformity of size.[5] (Table No. :**2**)

Fourier Transform Infra-Red spectroscopy analysis

The FT-IR spectra of pure Arecoline, chitosan,Arecoline- chitosan physical mixture and the drug loaded nanospheres were recorded to check drug polymer interaction and stability of drug.

Particle size analysis

The particles were observed through optical microscope to confirm the discreteness and uniformity in size. The particle size was analyzed by scanning electron microscopy. The SEM involves the use of vacuum dried nanospheres that are coated by conductive carbon gold layer for analysis.[6]

Formulation of the drug loaded nanospheres

5 ml of chitosan gel (0.5 % w/w) was taken in a 100 ml beaker and mixed with 5 ml of acetone to obtain a clear gel. 5 mg of the drug (Arecoline) was added into the gel and dispersed uniformly. The polymer gel containing drug was then added into 10 ml of linseed oil and allowed to emulsify under magnetic stirring at room temperature by covering the beaker with a suitable lid. The system was stirred for 30 minutes and then the beaker was kept opened and stirring continued for another 1 hour, which resulted in the precipitation of polymer due to the evaporation of acetone with subsequent formation of smaller spheres suspended in oil phase. To solidify and stabilize the spheres, 2.5 ml of gluteraldehyde saturated toluene containing 10 % Span 80 was selected as a cross linking agent, which was slowly added with a micropipette to the system and stirring was continued for 2 hours. The suspension obtained was centrifuged at 2500 rpm for 30 minutes, and washed three times each with toluene and acetone. After final washing, the product was suspended in acetone and poured in to a clean petri dish and dried at room temperature. Upon drying, brown colored, free flowing, fine powder was obtained and was stored in a self sealed cover.

Similarly, five other batches of drug loaded nanospheres were prepared with different

concentrations of drug as shown in the following table. The concentration of polymer was kept constant in all the batches.[6] (Table **3**)

Estimation of amount of Drug Incorporated to Chitosan Nanospheres

10 mg of drug loaded nanospheres from each batch was dissolved in 20 ml of 6 % acetic acid and stirred for 6 hours. It was then centrifuged at 2500 rpm for 30 minutes and the supernatant was analyzed by UV spectrophotometer at 254 nm. Plain nanosphere treated in the same manner was used as the blank. The percentage drug loading was calculated using the formula.[7]

%Drug Loading = Actual drug content/ Theoratical drug content × 100

In-vitro Drug Release of Nanospheres

A quantity of nanospheres, which is theoretically equivalent to 2 mg of drug, was calculated from each drug loaded batch and then it was added to 250ml beaker and to it 100ml of normal saline was added. Then the flask was kept in a magnetic stirrer, maintained at 37°C. 2ml of drug release medium was withdrawn at time intervals of 30 minutes, 1,2,3,4,5,6,7,8,9,10,11,12 and 24 hours while replacing it with fresh 2ml of normal saline (maintained at 37°C). The samples were centrifuged and filtered. From the filtered samples 1ml sample were withdrawn and diluted to 10 ml with normal saline and the drug content was analyzed by UV spectrophotometer at 254nm. The cumulative % drug release was calculated and a graph was plotted with cumulative % drug release Vs time.

characteristics (F4 and F5), a quantity of nanospheres equivalent to 2 mg of Arecoline were dispersed in each of 20 ml of surfactant solution for six hours incubation at room temperature. After the incubation, nanospheres were collected by centrifugation at 2500 rpm for 30 minutes and dried to obtain coated nanospheres. Six different batches of coated nanospheres were prepared from three different concentrations of surfactants and they were checked for their stickiness after the coating has been established.[6]

Comparative study on the *in-vitro* release profile of coated nanospheres with that of uncoated nanospheres

All the six batches of surfactant coated nanospheres were subjected to *In-vitro* dissolution study. The dissolution conditions were maintained as same as that for uncoated nanospheres. The dissolution data obtained for surfactant coated nanospheres were compared with that of uncoated nanospheres.

Release kinetics study of drug loaded nanospheres

Determination of order of release of drug (Arecoline) from nanospheres by Graphical method

To determine the order of release of drug from nanospheres by graphical method from the dissolution data, a graph was plotted with % drug remaining Vs time to confirm Zero order release, and a graph was plotted with log % remaining Vs time to confirm first order release. If a straight line or linearity is observed, it can be confirmed that the drug release follows the corresponding order kinetics. The slope of the graph was found out and multiplied with 2.303 to obtain the zero/ first order rate constant. Regression co-efficient of the graph was found out to confirm the correlation between X and Y axis.

$$m_t/m_n = kt^n$$

Where, *Mt/mα* is the fraction release of the drug, '*t*' is the release time, '*k*' is the constant, which indicates the properties of the macromolecular polymeric system, and 'n' is the release exponent indicative of the mechanism of release. The '*n*' value was used for the analysis of drug release mechanism from drug loaded nanospheres. The '*n*' value was determined for all batches of drug loaded nanospheres by graphical method, which is explained below (table **4**).

Stability studies

The stability study was carried using the batch F4. The stability of drug loaded nanospheres was evaluated in terms of its drug content. A quantity of nanoparticle formulations was stored at room temperature for 60 days. 10 mg of samples were withdrawn at an interval of 15 days and drug content was analyzed by UV spectrophotometer at 254 nm. The change in drug content was determined at each time interval.[8](table **5**)

RESULTS AND DISCUSSION

IR studies ruled out the possibility of interaction between the selected polymer

chitosan and the drug Arecoline. The spectra obtained from IR studies at wavelength from 4000 cm-1 to 400 cm-1 showed that there are neither major shifts nor any loss of functional peaks between the spectra of drug, polymer, physical mixture of drug and polymer and also nanosphere form of drug [9 & 10](Figure **1**).

To determine the carrier capacity of chitosan with respect to Arecoline, various batches of drug-loaded nanospheres were prepared with varying concentrations of drug to a constant amount of polymer table shows the percentage drug loading of various drug-loaded batches. From Table No.**3**, it can be understood that there is a proportional increase in drug loading capacity with increase in concentration, thereafter a decrease in drug loading capacity with increase in concentration of the drug was noted. This indicates the saturation capacity of the polymer with respect to the selected drug due to saturation of the polymer matrix. From the above data, it has been understood that the saturation capacity of chitosan with respect to Arecoline occurs at a concentration of 20 mg/ 50 mg of polymer. To evaluate the release of drug from all the drug-loaded batches, an *in-vitro* release study was undertaken by centrifugal ultra filtration method. All the drug loaded batches showed a cumulative % release between 46.68 and 83.96. All formulations showed characteristic biphasic release with an initial burst release followed by a second phase with a much slower rate of drug release Figure No.**3**. The initial fast release phase can be due to immediate desorption of drug located in the surface of the nanospheres. The next slow phase could be due to slow desorption of drug located in the interior of the porous nanospheres and probably with erosion of the polymer matrix.

Among the six batches of nanospheres, the batches of nanospheres F4 and F5 (containing 20 mg and 30 mg of drug per 50 mg of polymer) showed maximum cumulative % drug release of 81.43 and 71.56 respectively. These batches were selected for the formulation of surfactant coated nanospheres.

Tween 80 coated nanospheres were prepared by adsorption technique. Discrete, non-sticky and free flowing particles were obtained by this technique. The *In vitro* dissolution profiles of all surfactant coated

batches were observed for 24 hours at different time intervals. All the batches showed a cumulative percentage release between 63.26 and 86.265. All the surfactant coated batches showed a reduction in cumulative % release during the first two hours compared to the uncoated batches. There is a delay in the initial burst release; this could be due to the protective barrier effect of surfactant coating, and the time taken for the coating layer to dissolve in the dissolution medium, subsequently to diffuse out of the polymer matrix. (figure No.**4**)

In order to understand the mechanism of drug release, the result of *in-vitro* drug release study of nanospheres were fitted with various kinetic equation, Higuchi's model (cumulative % drug release vs. square root of time) and Korsmeyer-peppas model (log cumulative 5 drug release Vs log time). 'R2 'and 'n' values were calculated for the linear curve obtained by regression analysis of the above plots and it is given in (Table No.**4**). From the table, it is understood that the dissolution data is well fitted to Peppa's model. The 'n' value obtained for Peppa's model was in between 0.5 and 1 for all formulations so it was concluded that the mechanism by which drug is being released is a non-Fickian analomous diffusion mechanism, that is drug release is controlled by all diffusion, erosion and swelling mechanism.The *in-vitro* drug release of nanospheres was best explained by first order kinetics for all formulations.

The stability study data indicated that the drug loaded nanospheres shows good stability for 60 days, when it is stored in room temperature in terms of no change in percentage drug loading.

CONCLUSION

The formulated Arecoline nanospheres with chitosan as a carrier was found to be a suitable and potential natural carrier in terms of their particle size, drug loading capacity and *in vitro* release characteristics. Tween 80 coated nanospheres could be used as an alternative and cheaper carrier for the targeted delivery of Arecoline to the brain, especially for the treatment of Alzheimer. In turn, it may be beneficial in reducing the toxicity and side effects of the drug and also in reducing the total cost of the therapy, by reducing the total dose of the drug.

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Figure 1 Shows the IR spectra of A) Arecoline pure drug B) chitosan C) Arecoline loaded-nanospheres

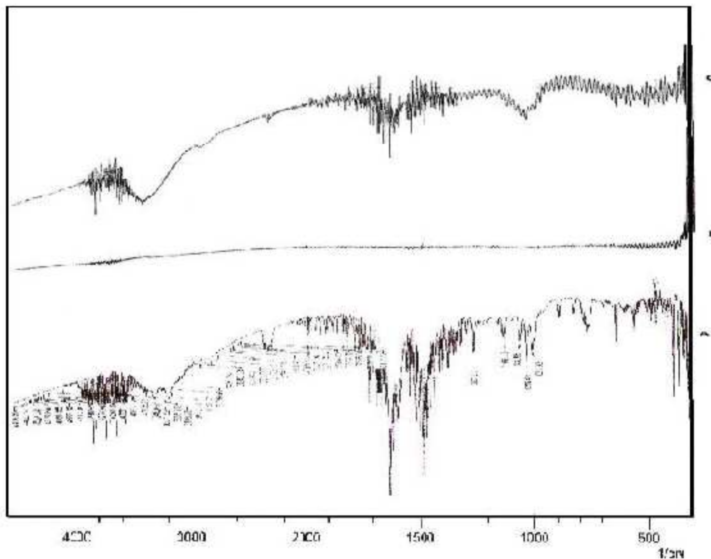

Figure 2 Scanning Electron Micrograph of Chitosan nanospheres

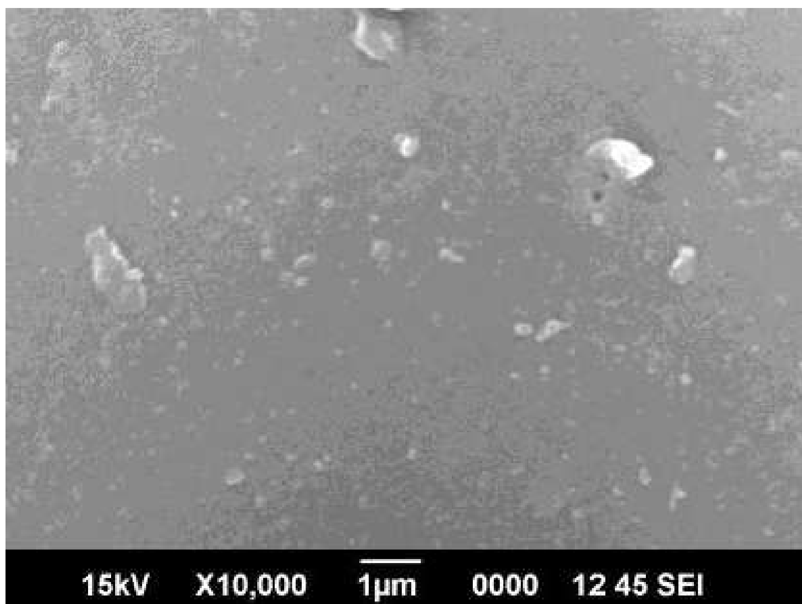

Figure 3 *In-vitro* release profile of drug loaded nanospheres

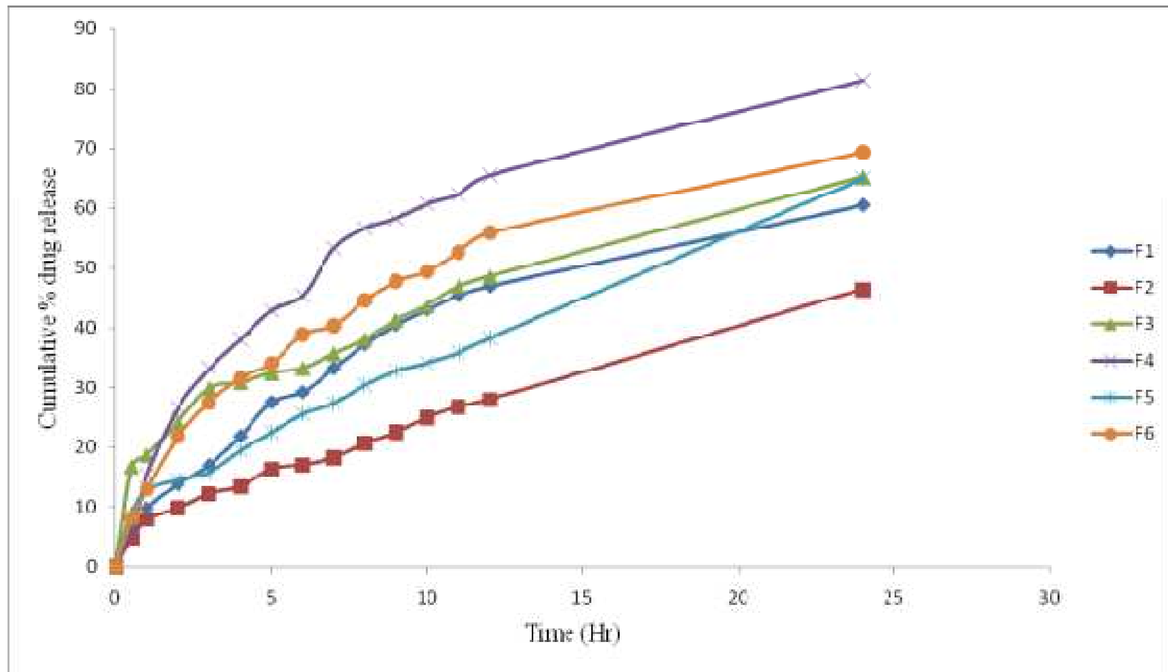

Figure 4 comparative *in-vitro* dissolution profiles of coated nanospheres with that of uncoated nanospheres

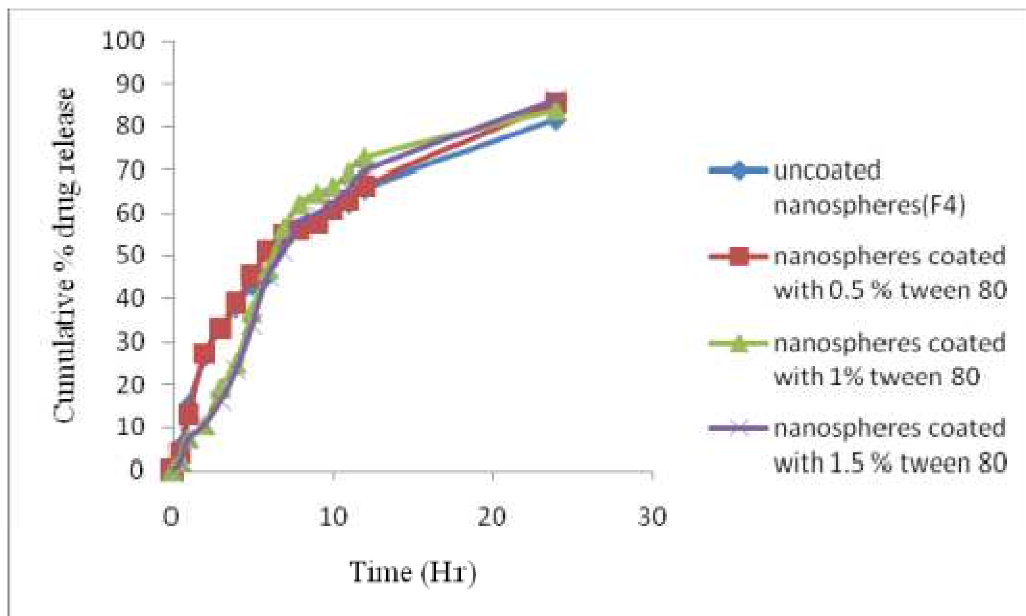

Table 1 Effect of polymer concentration on the properties of chitosan nanospheres

Batch code	Polymer concentration (%)	% yield	Description of formed particles(observed under optical microscope magnification 100X
C1	0.25	85	Clumpy masses formed, only few spheres observed
C2	0.50	92.33	Discrete spheres formed
C3	0.75	84.66	No discrete spheres and more larger clumpy particles formed
C4	1.00	83	Only large clumpy masses formed

Table 2 Effect of volume of cross linking agent on particle formation

Batch code	volume of cross linking agent (ml)	Nature of particles observed under optical microscope
G1	0.5	Number of discrete particles were few, clumpy masses observed
G2	1.0	Clumpy masses with few number of spherical particles were observed
G3	1.5	Few clumpy masses with different sizes of spherical particles observed
G4	2.0	More or less uniform sized discrete spherical particles were obtained
G5	2.5	More uniform sized discrete spheres were obtained
G6	3.0	Uniform sized spheres were obtained

Table 3 % yield and % drug loading of drug loaded nanospheres

Batch code	Amount of drug added per 50 mg of polymer (mg)	% yield	% Drug Loading
F1	5	85.05	8.33± 0.181
F2	10	86.76	11.72± 0.365
F3	15	88.75	18.20± 0.654
F4	20	89.50	27.44± 0.812
F5	30	87.30	22.28± 0.303
F6	40	68.55	15.15± 0.323

Table 4 In-vitro release kinetics data of chitosan nanospheres

Formulation code	Zero order regression (R2)	First order		Higuchi's		Peppa's	
		Regression (R2)	Rate constant, k1	Regression (R2)	Slope (n)	Regression (R2)	Slope (n)
F1	0.870	0.943	0.0392	0.981	13.56	0.989	0.641
F2	0.972	0.990	0.0230	0.962	8.808	0.980	0.548
F3	0.855	0.950	0.0368	0.979	12.11	0.970	0.347
F4	0.798	0.952	0.0668	0.973	18.21	0.939	0.650
F5	0.969	0.983	0.0368	0.959	12.01	0.956	0.485
F6	0.835	0.942	0.0461	0.987	15.01	0.986	0.562
T1	0.803	0.971	0.0736	0.972	18.99	0.914	0.729
T2	0.780	0.911	0.0829	0.917	21.80	0.954	0.986

T3	0.835	0.970	0.0852	0.938	21.34	0.964	0.976
T4	0.958	0.995	0.0392	0.978	12.61	0.984	0.584
T5	0.963	0.995	0.0392	0.968	12.77	0.975	0.582
T6	0.952	0.996	0.0415	0.976	13.50	0.987	0.653

Table 5 Stability of Arecoline nanospheres during storage at atmospheric temperature

Time (days)	Parameters observed	
	% drug loading	Change in morphology(by optical microscopy under magnification 100 X)
1	28.638	Discrete regular spheres
31	28.343	No significant change in morphology
45	28.932	No significant change in morphology
60	28.638	No significant change in morphology

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