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Chitosan Based Nasal Microspheres of Sumatriptan: Formulation and *In-Vitro* Evaluation

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ABSTRACT

The purpose of present work is to do preliminary research for optimized delivery of sumatriptan in the form of nasal mucoadhesive microspheres. This would avoid the first pass effect and thereby significantly improve the bioavailability of sumatriptan. Chitosan was selected as mucoadhesive polymer due to its non-toxic nature and potential for sustained release. Different formulations were prepared by varying the drug: polymer ratio. Uniform spherical microspheres were prepared by modified emulsion technique. The prepared microspheres were evaluated with respect to particle size, entrapment efficiency, swelling index, in-vitro drug release, drug permeation and stability studies. All the formulations showed good mucoadhesive properties and chitosan can be considered as a potential carrier for mucoadhesive microspheres. This study further suggests extensive preclinical and clinical studies on chitosan nasal microspheres.

Key words: Chitosan, Microspheres, Sumatriptan, Nasal delivery

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INTRODUCTION

Chitosan, a natural polysaccharide, is being widely used as a pharmaceutical excipient. It is obtained by the partial deacetylation of chitin, the second most abundant natural polymer. Chitosan comprises a series of polymers varying in their degree of deacetylation, molecular weight, viscosity, pKa etc. Chitosan has found wide applicability in conventional pharmaceutical devices as a potential formulation excipient, some of which include binding, disintegrating and tablet coating properties [1]. The polymer has also been investigated as a potential adjuvant for swellable controlled drug delivery systems. Use of chitosan in novel drug delivery as mucoadhesive, gene and peptide drug administration via the oral route as well as its absorption enhancing effects have been explored by a number of researchers [2].

Recently the use of chitosan in formulation development has increased many folds. Though chitosan exhibits excellent compatibility with organic compounds such as cationic dyes and surfactants, starches, quaternary ammonium salts and with most cationic and non-ionic polymers, multivalent anions easily crosslink with chitosan to form gels and precipitates. The cationic nature permits it to form complexes with oppositely charged drug(s) and excipient(s), thereby altering the physicochemical characteristics of the formulation [3]. Reacting chitosan with controlled amounts of multivalent anions, result in crosslinking between chitosan molecules. This may be achieved in acidic, neutral or basic environments depending on the method applied.

Miyazaki et al [4] observed the sustaining effect of chitosan on the release of indomethacin (water insoluble drug) from granules. A sustained plateau level of indomethacin was obtained for drug chitosan granules (1:2 mixture) when compared with a sharp peak of plasma concentration with conventional commercial capsules (in rabbits). Further, the applicability of chitosan (degree of deacetylation 85%) as a vehicle for sustained release (SR)-preparation of water soluble drug (propranolol HCl) was examined. Retardation in drug release was observed to be proportional to chitosan content and was attributed to the gel forming ability of chitosan in media of low pH [5].

Sumatriptan is the most commonly prescribed drug for migraine attacks. The usual clinical dose are 25mg, 50mg, 100mg oral tablets [6]. But the major problem with oral route is very low bioavailability (15%) due to pre-systemic metabolism and incomplete absorption [7]. Hence sumatriptan is selected as model drug for nasal delivery to overcome above problems.

As a drug delivery route, nasal cavity offers several possible advantages. The nasal epithelium is highly vascularized and offers a relatively large surface area for drug absorption. In addition porous endothelial basement membrane and direct transport of drugs into systemic circulation through nasal mucosa avoids the hepatic first-pass effect present in per oral administration [8].
In the present project mucoadhesive chitosan microspheres were being prepared for nasal delivery of sumatriptan. Chitosan is a widely used mucoadhesive polymer. It is a cationic

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hydrophilic polysaccharide comprising copolymers of glycosamine and N-acetyl glycosamine. Chitosan microspheres have been reported to provide controlled release of many drugs [9]. Therefore, it is aimed to exploit the mucoadhesive property of chitosan and improved permeability of drug in the nasal microspheres [10].

MATERIALS & METHODS

Materials

Chitosan (minimum 85% deacetylated) was obtained from Central institute of fisheries technology (CIFT), Cochin, India. Sumatriptan succinate was a kind gift received from Dr Reddys's Laboratory, Hyderabad, India. Span 80 and sodium taurocholate were procured from Rankem, New Delhi, India. Liquid paraffin (viscosity 90 cp at 30 c) was supplied by S.D Fine chemicals, Mumbai, India. All the other solvents and reagents were used of analytical grade.

Preparation of chitosan microspheres

Table 1: Formulation composition of chitosan microspheres

Formulation Code	Drug: Polymer ratio	Sumatriptan (mg)	Chitosan (mg)	Sodium Taurocholate (mg)
LP1	1:1.0	100	100	1
LP2	1:1.5	100	150	1
LP3	1:2.0	100	200	1
LP4	1:2.5	100	250	1
LP5	1:3.0	100	300	1
HP1	1:1.0	100	100	2
HP2	1:1.5	100	150	2
HP3	1:2.0	100	200	2
HP4	1:2.5	100	250	2
HP5	1:3.0	100	300	2

The Chitosan microspheres were prepared by modified emulsion technique [11]**.** Accurately weighed amount of drug, permeation enhancer and polymer are added step by step respectively in 5% acetic acid solution according to the formulation code in Table 1. This gives a viscous solution, which has to be mixed properly. This solution is added drop by drop to a beaker containing liquid paraffin which is kept under a remi propeller. The stirring should be done at 1000-4000 rpm. After 2 min a small quantity of cross-linking solution like glutaraldehyde is added. The stirring is continued for 4 hours and intermittently small amounts of span 80 are added to avoid clumping. After the stipulated time, the microspheres were centrifuged, washed several times with hexane, methanol and finally acetone. The microspheres were then dried at 50c and stored in desiccator for further use.

Characterization of chitosan microspheres

Particle size and shape analysis

The shape and surface morphology of chitosan microspheres were determined by scanning electron microscopy (JSM 6390, India). Briefly, samples were mounted on metal using double sided adhesive tapes and vacuum coated with gold film [12]. Further, particle size analysis was performed using laser diffraction method (Malvern sizer, UK). Effect of drug, crosslinking agent and permeation enhancer concentration on the particle size and shape are studied.

Entrapment efficiency

Entrapment efficiency is determined by placing accurately weighed (100 mg) in a mortar and crushed with pestle to form fine powder. Then 10 ml of PBS is added and shaken for 30 min on a magnetic stirrer. Then the solution is filtered and the filtrate is sent for quantitative estimation by HPLC. Entrapment efficiency can be calculated as, Total entrapment efficiency (%) = (weight of the drug recovered from microspheres / weight of the drug added during formulation) X 100

Swelling index

The swelling index is a property measured to know the behaviour of polymer in physiological solution. It is determined by keeping the microspheres in phosphate buffer saline (PBS) at pH 6.4. Accurately weighed amount of microspheres were immersed in PBS for 24 h and washed. The swelling index is calculated using formula, $\alpha = (W2-W1)/W1$, where α is swelling index, W1 is weight of microspheres before swelling and W2 is weight of microspheres after swelling [13].

***In-vitro* bioadhesion**

Goat intestine was collected freshly from slaughter house [14]. The intestine segment is everted and filled with phosphate buffer (pH 7.6). Both ends are tied. Further these sacs were inserted into tubes containing a suspension of accurately weighed microspheres (A1). These tubes are shaken for 30 min. The not attached microspheres are dried and weighed (A2). The bioadhesion (%) can be calculated as [(A1-A2) x 100].

***In-vitro* drug release studies**

The drug release from different formulations is studied using a Franz diffusion cell, which has lesser liquid capacity mimicking nasal compartment [15]. A treated cellophane membrane is used to separate the donor and receptor compartments. Accurately weighed drug loaded microspheres (100 mg) were placed on the cellophane membrane in the donor

compartment containing PBS (pH 6.4) maintained at 37±1 c. The samples are withdrawn at predetermined intervals and fresh PBS is replaced up to 24 h. Further to determine the concentration of sumatriptan, the samples were sent for HPLC studies.

In-vitro drug permeation

The drug permeation study through biological mucosal membrane should be carried to ensure in-vivo drug absorption. This study is similar to in-vitro drug release study, except goat intestinal mucosa is used in the place of cellophane membrane. Further, the study is carried up to 24 h time to ensure complete permeation [16]. The samples withdrawn from receptor cell are sent for HPLC studies.

Stability studies

Stability studies are carried out on microspheres according to ICH guidelines to ensure their shelf life. Stability studies are carried out on the best two formulations based on in-vitro release [17]. The formulations are tested for stability in humidity chambers for intermediate term (30 c±2 c / 65 % RH ± 5% RH) for 6 months and accelerated stability (40 c± 2 c / 75% RH ± 5% RH) for 6 months. Sampling is done at 0 month, 3 month and 6 month and sent for quantification as described in the method of entrapment efficiency.

HPLC analysis of sumatriptan succinate

The concentration of Sumatriptan in the samples is analysed by HPLC using Shimadzu LC-2010c (Shimadzu Corporation, Japan). It consists of vacuum degasser, quaternary pump, auto sampler, column oven and UV detector. The chromatographic separation was carried out on a reverse phase C18 column (15X4.6 mm I.D, 4µm, Thermo, USA) maintained at 25 c. The wavelength of UV detector was set at 228 nm [18]. Mixture of ammonium phosphate monobasic (0.05M) – acetonitrile (84:16, v/v) was used as mobile phase at a flow rate of 1.0 ml/min. The sample injection volume was 50 µl.

RESULTS AND DISCUSSION

Particle size and shape analysis

Chitosan is a non-toxic polymer, which has good mucoadhesive properties. The microspheres are formed due to action of chemical crosslinking agent like glutaraldehyde. This is an instantaneous reaction where the aldehyde group form covalent imine bonds with amino group of chitosan. Hence the particles formed have a slightly roughed texture and uniform size. SEM photomicrographs show uniform spherical shaped microspheres (Fig 1). But, as the concentration of glutaraldehyde increased, the microspheres became more rough and irregular in shape, due to increased covalent bonds. Further the size analysis from Malvern sizer revealed that all formulations were in the size range of 11.01±0.45 to 26.31±0.73 µm (Table 2). There is a

significant increase in the size of microspheres, as the polymer ratio is increased. This may due to high availability of amino groups of chitosan, hence leads to higher crosslinking and larger size. Also it has been observed that as the speed of propeller increases, the size of microspheres is reduced. Further, the change in the concentration of penetration enhancer does not have any significant effect in particle size.

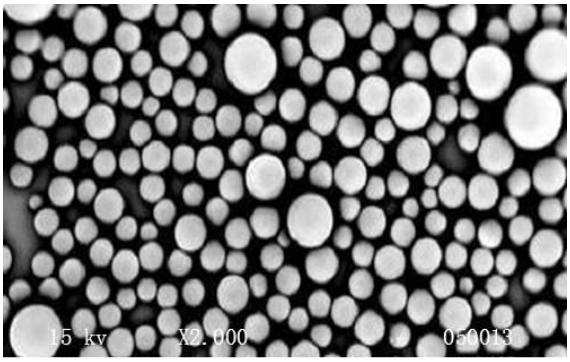

Figure 1: SEM photomicrograph of chitosan microspheres (LP1)

Table 2: Physical characteristics of chitosan microspheres

Formulation Code	Average particle size (µm)*	Entrapment efficiency (%)	Average swelling index #	Average bioadhesion (%)#
LP1	11.01±0.45	51	0.67±0.08	71.10±1.20
LP2	14.06±0.32	55	0.78±0.13	75.65±1.43
LP3	18.85±0.64	59	0.85±0.17	79.73±1.65
LP4	22.64±0.75	63	0.98±0.25	83.02±1.85
LP5	26.31±0.73	68	1.05±0.45	87.54±2.10
HP1	10.91±0.83	49	0.64±0.09	70.90±1.15
HP2	13.96±0.24	53	0.72±0.11	73.85±1.43
HP3	17.85±0.44	58	0.80±0.15	78.43±1.85
HP4	21.04±0.85	61	0.92±0.21	82.29±1.95
HP5	26.01±0.63	67	1.01±0.35	85.54±2.02

*values expressed as Mean±SD, n=100, #values expressed as Mean±SD, n=3

Entrapment efficiency

The overall entrapment efficiency was good, but a considerable amount of drug is lost, which remained in liquid paraffin solution. The entrapment efficiency improved with increase in polymer concentration, which may be due to more uptake of drug by polymer. The entrapment efficiency ranged from 51% to 68% (Table 2).

Swelling index

The swelling index is an indicative parameter showing the ability of polymer to absorb water and produces pores for rapid availability of drug entrapped inside the microspheres. It was observed that swelling index increases with increasing concentration of chitosan (Table 2).

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The swelling index values varied from 0.67±0.05 to 1.05±0.15. Also, the concentration of penetration enhancer has not shown any change in swelling index.

In-vitro bioadhesion

This is one of the major parameter required by nasal microspheres, as they have to show significant mucoadhesive property to extend a sustained release action and also to avoid drug loss due to sneezing. The study shows that chitosan microspheres are having good bioadhesive property ranging from 71.10±1.20 to 87.54±2.10. Further, it was observed that as the polymer concentration increases, the mucoadhesive property has increased (Table 2). The penetration enhancer however showed a slight decrease in mucoadhesive property may be due to interaction and disruption of biological membranes.

In-vitro drug release studies

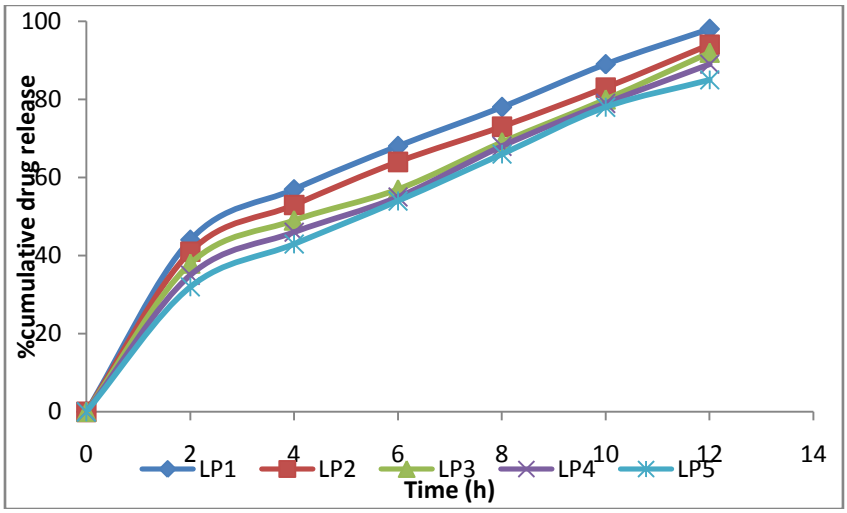

Figure 2: % cumulative drug release of chitosan microspheres (LP1 –LP5)

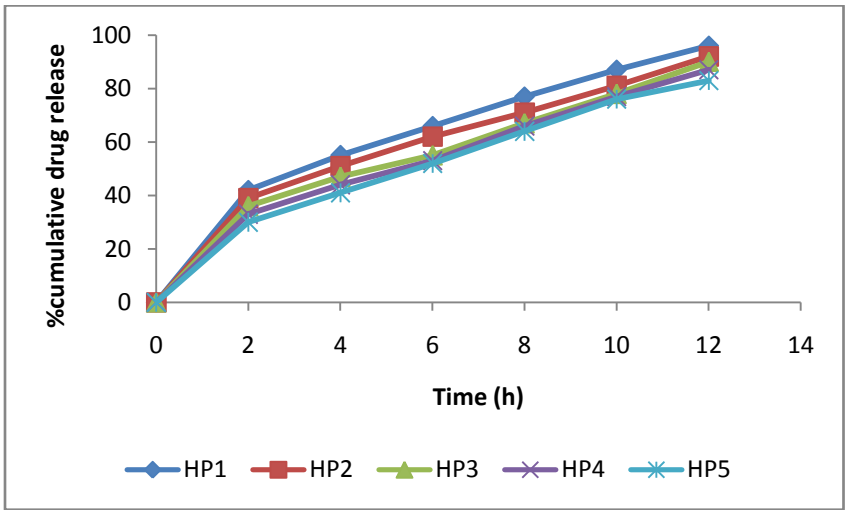

Figure 3: % cumulative drug release of chitosan microspheres (HP1-HP5)

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The in-vitro drug release studies are carried out using Franz diffusion cell. The release profile of Sumatriptan succinate up to 12 h shows constant release over a period of time (Fig 2& 3). Initial burst effect is very negligible, which indicates that the drug is uniformly mixed with polymer. The release profile shows that, as the polymer concentration is increased the release rate at the initial stage is same, but slowly decreases over a period of time. This may be due to larger size of microspheres and hence drug in the central part of microsphere has to travel a larger distance to get into the dissolution medium. Also, as the polymer concentration is increased, it shows much longer duration of release. The concentration of permeation enhancer does not have a significant effect on drug release.

***In-vitro* drug permeation**

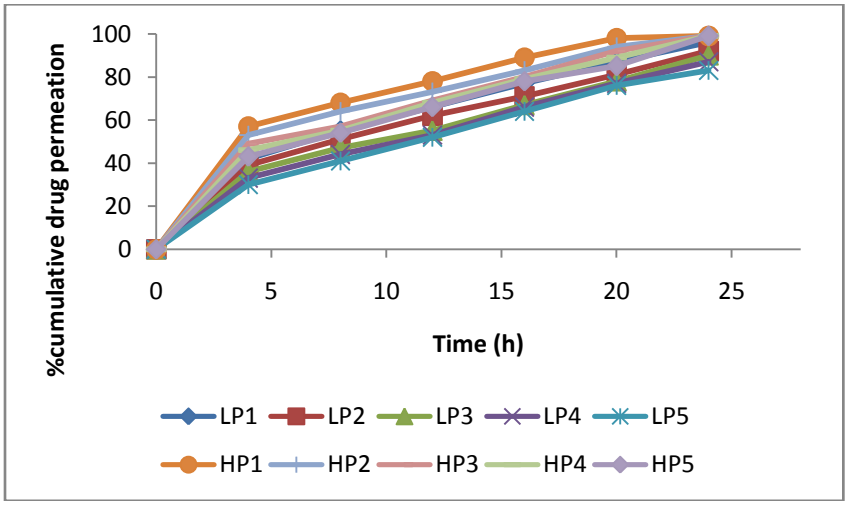

Figure 4: % cumulative drug permeation of chitosan microspheres

The drug permeation studies carried out on different formulation revealed much different drug permeation profile when compared to in-vitro drug release profile. The formulations containing higher concentration of permeation enhancer, sodium taurocholate showed 87% of drug permeation in 20 h whereas, the other formulations showed only 62% of dug permeation with less permeation enhancer (Fig 4). This is mainly due to membrane disrupting capability of permeation enhancer, which allows easy permeation of drug through biological membranes. But a note should be taken not exceed the prescribed limit of permeation enhancer, as they are toxic in nature.

Stability studies

At the end of stability studies, the microspheres were checked for any changes in physical stability, size, shape, drug content and release profile. The both formulations (LP1 & HP1) did not show any changes in physical stability, size, shape, drug content and release profile at intermediate conditions. But a slight loss of drug content at accelerated studies has

been observed (Table 3). This could be due to chemical changes in the drug due to elevated temperature.

Table 3: Stability studies of chitosan microspheres

Formulation code	Sampling time	Drug content (%)	
		Intermediate stability study	Accelerated stability study
LP1	0 months	99.5	99.5
	3months	99.0	96.1
	6months	98.0	93.7
HP1	0 months	99.4	99.4
	3 months	99.1	95.5
	6 months	98.5	92.8

CONCLUSION

The chitosan microspheres exhibited good bioadhesive properties and have potential for sustained release of different drugs. As sumatriptan has very low bioavailability, intranasal microspheres can be considered promising route for drug delivery. This study gives a significant lead for conducting extensive preclinical and clinical studies to improve bioavailability of sumatriptan in the form of nasal microspheres.

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