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Research Article

Preparation and *In Vitro* Characterization of Mucoadhesive Chitosan Microspheres Containing Norethisterone for Nasal Administration

B. K. Jain *

***Research Scholar, J.N.T.U. Hyderabad,**
Pharmacy Department, K.N. Polytechnic College, Jabalpur, M.P. - India

Abstract

In this study suitable smooth, spherical, cross-linked chitosan microspheres in the size range of 20-60 um loaded with norethisterone were prepared by glutarldehyde cross-linking of an aqueous acetic acid dispersion of chitosan containing norethisrterone in a non-aqueous dispersion medium consisting of light and heavy liquid paraffin (60:40 ratio) stabilized using span 60. Microspheres were prepared by using different drug/polymer ratios in the microspheres formulations. Chitosan was used as a mucoadhesive polymer in the formulations to increase the residence time of the microspheres on the nasal mucosa. The in vitro characteristics of the microspheres were studied and were evaluated with respect to the particle size, production yield, encapsulation efficiency, shape and surface properties, drug polymer interaction, mucoadhesive property and suitability for nasal drug delivery.

Key Words: : Mucoadhesive microspheres; Nasal drug delivery, norethisterone, Chitosan, glutarldehyde, span-60, .Liquid paraffin, glutarldehyde cross-linking

Introduction

Chitosan microspheres have been widely accepted for drug delivery, fabrication of biosmers as well as delivery of both hydrophilic and lipophilic drugs. Chitosan a polysaccride comprising of copolymers of glucosamine and N- acetyl glucosamine, being biodegradable and biocompatible, is widely use in the formulation of particulate drug delivery system to achieve controlled drug delivery. (1-4)

The pharmacological approach to fertility control is mainly by oral administration of steroids. Although controlled release system such as Progestart and Norplants which deliver progesterone and levonorgestrel respectively from non- biodegradable polymer matrices, have met with a reasonable of clinical success. The disadvantage of oral route is the requirement of daily ingestion and the subsequent daily variation in drug concentrations.

The development of biodegradable polymeric delivery systems for antifertility steroids has received considerable attention in past 20 years. Implantable rods, fibres, films and injectable microspheres have been prepared from a number of synthetic biodegradable polymers. Although there is considerable literature on the use of synthetic biodegradable polymeric carrier for antifertility steroids: natural polymers such as proteins and polysaccharide have received much less attention. Injectable biodegradable drug reservoir from glutamic acid/ leucineco-polymers in the forms of tubes and solid rods were prepared by **Sidman et al** (5) to provide controlled release of progesterone. Norethiserone was covalently bounded to poly (hydroxyl-alkyl) –L-glutamine and the release of drug was examined by **Peterson et al**(6). **Lee et al** (7) incorporated progesterone into glutarldehyde cross- linked serum albumin microspheres and showed that an extended release of 1-2 ng/hr/ml of serum was possible for about 20 days. Albumin microcapsules and microspheres cross linked with glutarldehyde and 2,3 butanedione were investigated for progesterone delivery by **Oienti and Zecchi** (8). **Jameela S. R**(9). et al studied in laboratory animals that glutarldehyde cross- linked chitosan microsphers are long acting biodegradable carriers suitable for controlled delivery of many drug.

Roberto R. et al (10) formulated norethisterone contraceptive microspheres for fertility regulation by using biodegradable polymers. The NET microspheres are administered by I.M. route. No important side effect was present. The method was effective, safe and well accepted.

The present study was aimed at the development, characterization and evaluation of antifertility agent(s). Norethisterone undergoes extensive first pass hepatic metabolism. The contraceptive agents needs per day delivery of drug. And a missing of pill results in unwanted pregnancy, besides other complications to the user. The present study, therefore after realizing the effectiveness and need of norethisterone therapy via a convenient route is proposed.

It is proposed to evaluate the potentiality of nasal route for the administration of norethisterone considering the pharmacokinetic characteristics of drug it is realized that to avoid the rapid ciliary's clearance and to provide prolonged/ sustained action of drug a bioadhesive polymer based dosage form for nasal administration be developed and evaluated for *in vitro* and *in vivo* performance. Such a system could be exploited for single per day pulse systemic delivery of contraceptive agent.

***Corresponding Author**
E-mail: jainbhanukumar@yahoo.in

Nasal drug delivery is an interesting way and an alternative to the parenteral route for systemic drug delivery. The nasal cavity as a site for the systemic absorption of drugs has some advantages which include relatively large surface area, porous endothelial basement membrane, highly vascularized epithelial layer, high total blood flow per cm3, avoiding the first pass metabolism and easy access (11) However, there are some problems such as mucociliary clearance and low permeability of the nasal mucosa to some drugs that have a large influence on the efficiency of the nasal absorption of drugs (12)

Nasal mucociliary clearance is one of the most important limiting factors for nasal drug delivery. It severely limits the time allowed for drug absorption to occur and effectively rules out sustained nasal drug administration. However, mucoadhesive preparations have been developed to increase the contact time between the dosage form and mucosal layers of nasal cavities thus enhancing drug absorption (13, 14). Illum et al. (15) introduced mucoadhesive microsphere systems for nasal delivery and characterized them. The microspheres form a gel-like layer, which is cleared slowly from the nasal cavity, resulting in a prolonged residence time of the drug formulation. Another important limiting factor in nasal application is the low permeability of the nasal mucosa for the drugs with polar and high molecular size. It seems to be necessary to consider an absorption enhancement mechanism for co-administration of drugs with either mucoadhesive polymers or penetration enhancers or combination of the two approaches.

Material and Methods

2.1 Materials

Norethisterone was procured as a gift sample from to Famy Care, Pharmaceuticals, Navi Mumbai and chitosan from Central Institute of Fisher Technology Cochin. Liquid paraffin. span-60, glutarldehyde, acetic acid, ethanol, sodium hydroxide were purchased from Loba Chemical, Mumbai. All reagents used were of analytical reagent grade

2.2Preparation of Chitosan Norethisterone Microspheres

Four batches of the microspheres were prepared by taking different D/P ratio. The method reported by Thanoo et al (4) was adopted. A 2% (wt/vol) chitosan solution in aqueous acetic acid (2% v/v) was prepared. The drug (200 mg) was extruded through syringe in it and stirred at 1300 rpm for 2 hrs to get uniform dispersion. This dispersed phase was added to continuous phase (125 mL) consisting of light liquid paraffin and heavy liquid paraffin in the ratio of 1 .25:1 containing 0.5% (w/v) Span 20 to form w/o emulsion. After 20 min of stirring, 1 ml of glutaraldehyde (25% solution, as cross linking agent) was added (color was changed from white to yellow) and stirring was continued for 3 hrs.To this 1ml of 1N NaOH solution was added (1-2 drops every 5 minutes). Golden colour microspheres were obtained, filtered and washed several times with cyclohexane to remove oil, and finally washed with water to remove excess of glutaraldehyde. The volume of glutaraldehyde was varied to affect the cross-linking density. Washings were analyzed for drug contents. Microspheres were then finally air dried at room temperature. (Table 1)

2.3 Preparation of Placebo Chitosan Microspheres

Same procedure was adopted as above except omitting the drug norethisterone for the preparation of placebo chitosan Microspheres.

2.4 Particle Size Analysis

The particles were grossly separated. Particle Size Analysis is carried out by using a compound microscope. Dried microspheres were first redispersed in distilled water and placed in a glass slide and the number of divisions of the calibrated eye piece was counted by a micrometer using a stage micrometer. The average size of around 100 particles was determined.(Table 2)

2.5 Drug content determination

To calculate the entrapment efficiency of norethisterone into the microspheres, a weighed quantity of microspheres(10mg) was determined by extracting into phosphate buffer pH 6.8 .Microspheres were crushed and powdered by using pestle and mortar and accurately weighed amount of this powder was extracted into phosphate buffer Ph 6.8 by stirring at 1000 rpm for 2 hr .The solution was filtered ,suitable dilutions made and estimated the drug content spectrophotometerically at 241nm.

2.6 Encapsulation efficiency-

It was determined by taking weighed quantity of norethisterone microspheres (approximately 25 mg) in a 25 ml volumetric flask, sufficient quantity of methanol was added to make 25 ml. The suspension was shaken vigorously and then left for 24 h at room temperature with intermittent shaking. Supernatant was collected by centrifugation and drug content in supernatant was determined using UV spectrophotometer at 241 nm wave length. Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment (PDE) as per the formula given below. Corresponding drug concentrations in the samples were calculated from the calibration plot generated by regression of the data taken in triplicate. (Table 2)

Percent drug entrapment = Actual drug content/theoretical drug content ×100

2.7 Percentage yield-

The prepared microspheres were collected and weighed. The yield was calculated for each batch by dividing the measured weight by the total weight of non- volatile components. The percentage yield of microspheres was calculated as follows. .(Table 2)

% Yield= Weight of. Microspheres /Theoretical weight of drug and polymer x100

2.8Morphology

The surface morphology of the microspheres was investigated by scanning electron microscopy (SEM)). The microspheres were mounted in metal stubs using a double-sided adhesive tape. After being vacuum coated with a thin layer (100-150 A°) of gold, the microspheres were examined by SEM at different magnification. (Fig 1&2)

2.9 Infrared spectroscopy

Triturate about 1mg of the microspheres with approximately 300mg of dry, finely powdered potassium bromide Infrared (IR). Grind the mixture thoroughly, spread uniformly in the die, and compress under vacuum at a pressure of about 800Mpa. Mount the resultant disc in a holder in the IR spectrophotometer and record the spectra in the IR region of 4000-625/cm^{-1}. Compare the positions and the relative intensities of the absorption bands of the microspheres obtained with that of the pure drug. (Fig 3-6)

2.9.1Compatibility studies

The pure drug and mixture of drug- chitosan in the ratio of 1:1 were kept at room temperature for 30 days. Samples were subjected to FT-IR studies using KBr as a blank and the I R spectrum of pure drug and excipients mixtures were compared to find any interaction between drug and excipients used for formulation. (Fig 3-6)

2.9. 2 Mucoadhesion property

The in vitro mucoadhesion of microspheres was carried out by modifying the method described by Ranga Rao and Buri (13) using goat nasal mucosa. The dispersion (0.2 ml) of microspheres in water was placed on goat nasal mucosa after fixing to the polyethylene support. The mucosa was then placed in the desiccators to maintain at >80% relative humidity and room temperature for 30 min to allow the polymer to hydrate and interact with the glycoprotein and also to prevent drying of the mucus. The mucosa was then observed under microscope, and the number of particles attached to the particular area was counted. After 30 min, the polyethylene support was introduced into a plastic tube cut in circular manner and held in an inclined position at an angle of 45^0. Mucosa was washed for 5 min with phosphate buffer saline pH 6.8 at the rate of 22 ml/min using a peristaltic pump; tube carrying solution was placed 2-3 mm above the tissue so that the liquid flowed evenly over the mucosa. Tissue was again observed under microscope to see the number of microspheres remaining in the same field The adhesion number was determined by the following equation: $Na = N/N_0 \times 100$, where Na is adhesion number, N_0 is total number of particles in a particular area, and N is number of particles attached to the mucosa after washing area. (Table 2)

Table 1 Composition of the microspheres formulation

Formulation Code	D/P ratio	Norethisrterone (mg)	Chitosan (mg)
CHN-1	1:1	100	100
CHN-2	1:2	100	200
CHN-3	1:3	100	300
CHN-4	1:4	100	400
Placebo	-	-	100

Table 2 Physical Characteristics of Prepared Microspheres of Norethisterone

Formulation code	Particle size(um) (mean ±SD)	Product Yield(%) (mean ±SD)	Encapsulation efficiency (%)	Equilibrium swelling degree (ml/g) (mean ±SD)	Mucoadhesion.(%) (mean ±SD)	Bioadhesive strength(g) (mean ±SD)
CHN-1	35.12±1.27	75.50±2.37	15.0	1.69±0.089	75±1.87	6.23±1.25
CHN-2	45.35 ±.79	79.50±2.52	20.89	1.89±0.076	79±1.89	7.25±1.28
CHN-3	50.58±1.27	78.20±4.23	18.50	1.95±0.087	87±1.45	7.98±2.01
CHN-4	61±2.13	85.20±4.68	23.3	2.12±0.087	83±1.67	8.89±2.16
Placebo	34.12±1.29	80.50±5.23	-	1.79±0.089	85±1.87	8.23±2.22

2.9.3 Swelling property

The swelling of microspheres was conducted in phosphate buffer pH 6.8 The equilibrium swelling degree (ESD) of norethisterone microspheres was determined by swelling a suitable volume of dried microspheres in 5 ml ethanol, overnight in a measuring cylinder. The ESD (ml/g) was expressed as the ratio of the swollen volume to the mass of dried microspheres.(Table 2)

2.9.4 Bioadhesive strength

The bioadhesive strength of all batches was determined using measuring device. Section of nasal mucosa was cut from the goat nasal cavity and instantly secure with mucosal side out on glass vial. The vial using nasal mucosa was stored at 37^0 for 5 min. Next, one vial with a section of mucosa was connected to the balance and the other vial was placed on a height-adjusted pan. Microspheres were placed in between the adjusted vial. The weight was increased until two vials were detached. Bioadhesive force was determined for the minimum weight that detached two vials.

2.9.5. Melting point

A small amount of the microspheres was taken and they were ground to remove the coating material and then subjected to melting point determination.

Results and Discussion

3.1 Particle Size.

The microspheres of norethisterone were prepared by the emulsification crosslinking method using glutaraladehyde as crosslinking agent. The microspheres obtained under these conditions were found to be spherical, free flowing and without aggregation, and median size ranged from 35 to 60 um .In previous studies Illum *et al* found that particle size was related to intranasal drug absorption. . Each step of microspheres preparation was keenly observed to understand the effect on particle size, total entrapment and release profile of the loaded microspheres suitable for nasal administration.

It was observed that with increase in chitosan concentration in the microspheres from batch CHN-1 to CHN-4 the particle size of microspheres increased, which may be due to the fact that increase in the concentration of polymer increases the crosslinking, and hence the matrix density of the microspheres increased, and that may result in the increase in the particle size of the microspheres

The increase in the particle size observed with increase in polymer and drug concentration was due to increase in viscosity of the droplet. Increase in polymer concentration attributed to increase in viscosity, which resulted in formation of large droplets, thus increasing the size of norethisterone microspheres.

3.2 Swelling Property.

Equilibrium swelling degree increases as the concentration of polymer increases while it decreases as concentration of drug increases as compared to plain microspheres. It can be concluded that incorporation of drug in microspheres decrease ESD.

3.3 Mucoadhesion

In vitro mucoadhesion of microspheres was the most important aspect of present investigation. It was found that, for batches CHN-1 to CHN-4, as the amount of polymer was increased, the % *in vitro* mucoadhesion also increased. This may be due to the fact that, as the amount of polymer increased, the amino groups available for binding with the sialic acid residues in mucus layer also increases, that results in the increase in the *in vitro* mucoadhesion of microspheres

Percentage mucoadhesion was found in the range from 75.0 % to 90.0%. Bioadhesive strength was in range from 6.23-8.23 g. Thus norethisterone microspheres prepared were found to be having good mucoadhesive property.

3.4 Morphology

The SEM of microspheres shows that a hollow spherical structure with a smooth surface morphology. Some of the microspheres show a dented surface structure. Outer surface of microspheres was smooth and dense, while internal surface was porous. Shell of the microspheres also showed some porous structure.

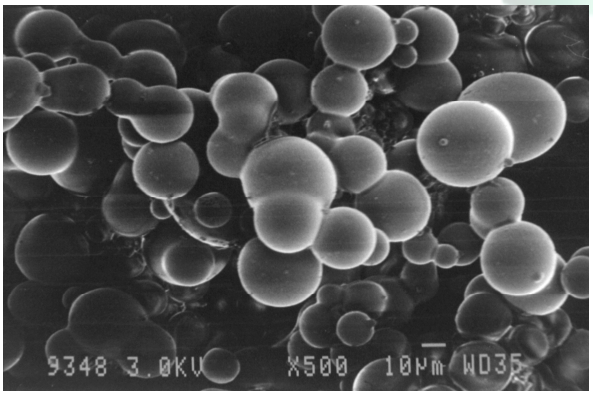

Fig 1 Placebo Chitosan Microspheres

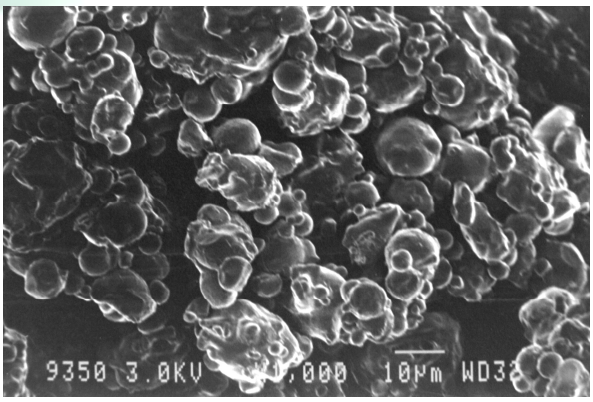

Fig2 NorethisteroneChitosa Microspheres

3.5 Melting Point

The melting points of the free drug and the drug in the microspheres were found to be the same 206°C indicating that there is no change in the nature of the entrapped drug due to the process of formulation of the microsphere.

3.6 IR Spectroscopic Studies

The FT- IR spectra of the free drug and the microspheres were recorded. The drug- excipients compatibility studies reveals that there is no physical changes observed in the drug and polymer mixtures. The IR spectrum of the drug, drug-chitosan mixture and microspheres formulation were compared to find any change in the frequency of functional group in microspheres with respective functional group of the drug. The spectral observations indicated that the prominent IR absorption peaks observed in the spectra of the drug were close to those in the spectra of the microspheres indicates that there is no interaction between the drug and the polymer. It indicates that that neither the polymer nor the method of preparation has affected the drug stability**.(**Fig3-6)

3.7 Drug Content and Percentage of Drug Entrapped

The microspheres were analyzed for the drug content uniformity and the encapsulation efficiency.Norethisterone was found to be encapsulated 15.0-23.3% which shows that if there is an increase in the concentration of the polymer, the encapsulation efficiency also increases.

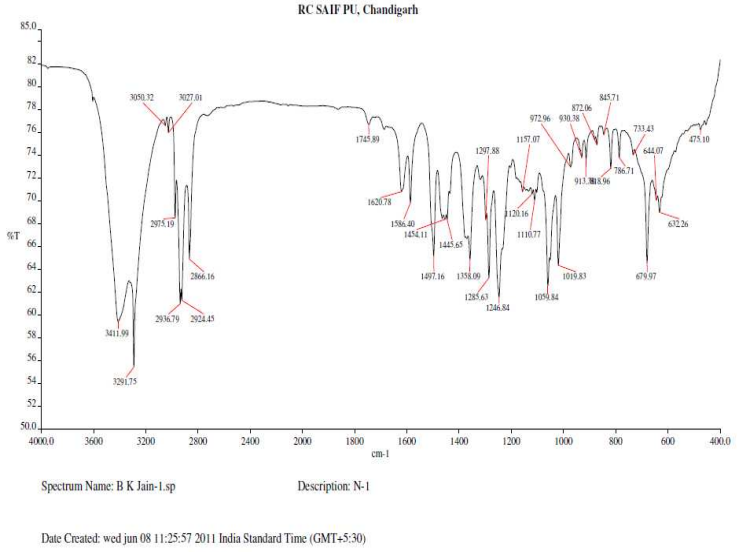

Fig 3 N-1 - Norethisterone

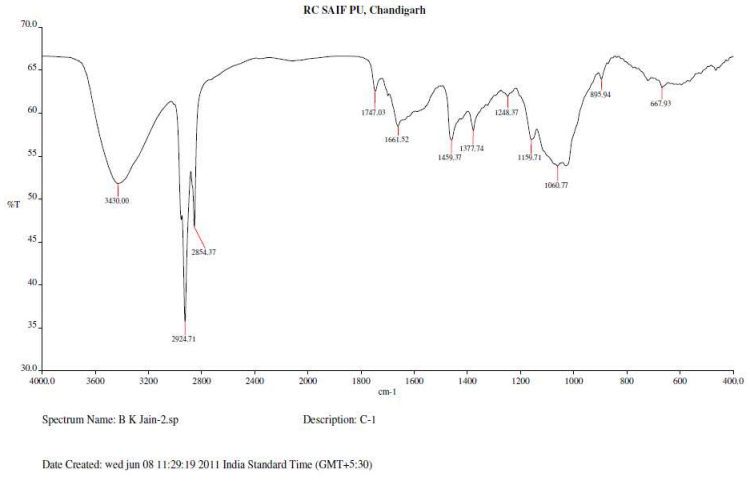

Fig 4 C-1 - Placebo Chitosan Microspheres

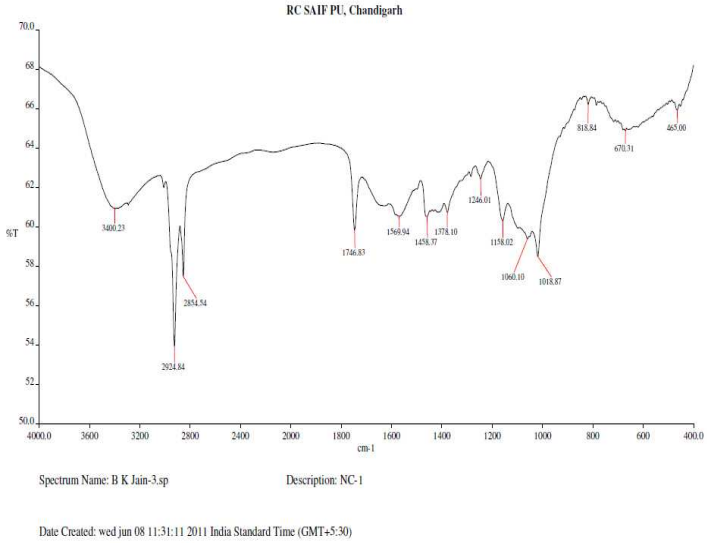

Fig 5 NC-1 - Loaded Norethisterone Chitosan Microspheres

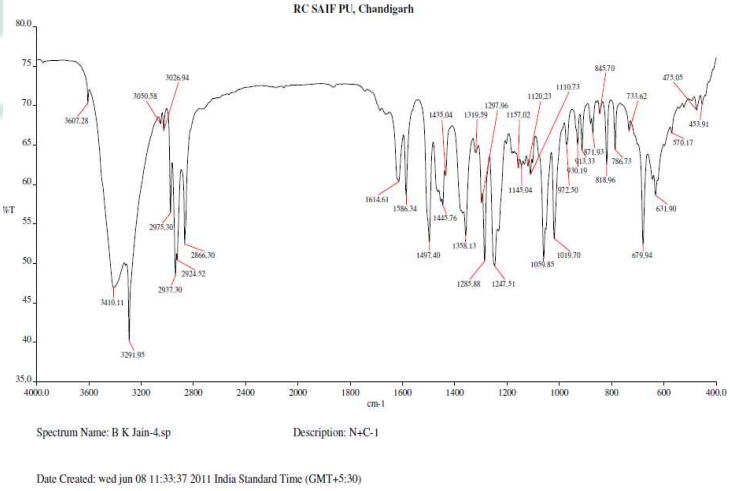

Fig 6 N+C-1 - Physical Admixture (N+A)

Conclusion

Chemical cross-linking is a rapid and simple technique for producing norethisterone chitosan loaded microspheres. They were produced with sufficient production yield, average drug encapsulation efficiency and reproducible from all batches. All the microspheres were of a suitable size and had good mucoadhesive property for nasal administration.The mucoadhesive properties of the microspheres could be altered by varying the proportion and the type of polymer. Thus the present investigation show promising results of chitosan microspheres as a matrix for drug delivery and also warrant for in vivo study for scale up the technology. From the results It can be concluded that the biocomptabile and cost effective polymer chitosan can be used to formulate an efficient nasal micropartricles system suitable with respect to the in vitro characteristics for in vivo studies.

Further studies are required to optimize release profile of the chitosan loaded microspheres delivering therapeutically desirable concentration of norethisterone to produce contraceptive action over prolonged periods (1 day).

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3. I am also grateful to Central Institute of Fisher Technology, Cochin for providing gift sample of chitosan.

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