RESEARCH ARTICLE

Preparation and *in vitro* characterization of mucoadhesive Norethisterone - egg albumin microspheres for nasal administration

B. K. Jain

*Research Scholar, J.N.T.U. Hyderabad, Lecturer Pharmacy, K.N.Polytechnic, College, Jabalpur.

M. P. INDIA

ABSTRACT

In this study suitable, smooth, spherical, cross-linked egg albumin microspheres in the size range of 15-40 um loaded with norethisterone were prepared by multiple emulsion (o/w/o) method by the glutarldehyde cross-linking and thermal denaturation technique. Microspheres were prepared by using different drug/polymer ratios. Egg albumin was used as a mucoadhesive polymer in the formulation to increase the residence time of the microspheres on the nasal mucosa. The albumin microspheres were characterized and 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. The *in vitro* diffusion of NE from the prepared microspheres exhibited the extent of drug release decreased from 95 –60%. The release of the drug has been controlled by swelling control release mechanism. No initial burst release has been recorded except for NE microspheres stabilized using 25% w/v glutarldehyde as cross-linking agent. The drug release from NE microspheres obeys Higuchi diffusion controlled model.

Keywords: Mucoadhesive microspheres; nasal drug delivery, multiple emulsion (o/w/o), norethisterone(NE), egg albumin,polysorbate, olive oil, glutarldehyde, glutarldehyde cross-linking.

1. INTRODUCTION

Most of the microencapsulated techniques have been employed for lipophilic drugs. Various natural polymers have received attention in the development of suitable sustained drug delivery system(s). Albumin microparticles have been used as carrier for a wide range of drug – Anticancer (Doxorubicin, Methotrexate, etc.) antihistamines, antiferitiliy, anticancer, antituberculosis, etc. (1-4). Egg albumin microspheres have been widely accepted for drug delivery, fabrication of biosmers as well as delivery of both hydrophilic and lipophilic drugs.

The development of biodegradable polymeric delivery systems for antifertility steroids has received considerable attention in past 20 years. 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. (5-8). 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/ leucine co-polymers in the forms of tubes and solid rods were prepared by Sidman et al to provide controlled release of progesterone(9). Norethiserone was covalently bounded to poly (Hydroxyl-alkyl) –L- glutamine and the release of drug was examined by Peterson et al (10). Lee et al incorporated progesterone into glutarldehyde cross-

*Corresponding author: B. K. Jain | K.N.Polytechnic, College, Jabalpur.M. P. INDIA| Email: jainbhanukumar@yahoo.in

linked serum albumin microspheres and showed that an extended release of 1-2 ng/hr/ml of serum was possible for about 20 days (11). Albumin microcapsules and microspheres cross linked with glutarldehyde and 2, 3 butanedione were investigated for progesterone delivery by Oienti and Zecchi (12). Jameela S. R. et al studied in laboratory animals that glutarldehyde cross- linked chitosan microspheres are long acting biodegradable carriers suitable for controlled delivery of many drug (13). Roberto R. et al formulated norethisterone contraceptive microspheres for fertility regulation by using biodegradable polymers. The NE microspheres are administered by I.M. route. No important side effect was present. The method was effective, safe and well accepted (14).

The potentiality of nasal route for the administration of antifertility has been also explored. Nasal route is the alternative to the parenteral route for systemic drug delivery. Nasal cavity has certain obvious advantages of large surface area and highly vascularized epithelial layer. Nasal route avoid first pass metabolism. 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. (15-18)

Illum etal. (19) 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.(20-21). Therefore the present study aims at the development of an effective delivery system for norethisterone via nasal route. (22-23) Norethisterone is poorly water soluble drug thereby complicating absorption. Hence polysorbate has been selected as solubilizer and absorption enhancer. (24-28)

2 MATERIAL AND METHODS
2.1. Materials
Norethisterone was procured as a gift sample from to Famy Care, Pharmaceuticals, Navi Mumbai and Liquid paraffin, glutarldehyde, olive oil, ether, polysorbate were purchased from Loba Chemical, Mumbai. All reagents used were of analytical reagent grade

2.2. Preparation of Egg Albumin Microspheres of Norethisterone. (12)

1. Albumin microspheres were prepared by a multiple emulsion method. Norethisterone (30 mg) was dispersed in 0.5 ml olive oil. The dispersion was mixed with 1 ml of an aqueous solution containing 24%w/v egg albumin solution and 0.5 ml of 1% polysorbate solution. The mixture was stirred for 10 minutes to produce o/w emulsion. The emulsion was added to 3 ml of olive oil and the mixture was stirred again for 2 minutes by using magnetic stirrer to obtain the corresponding o/w/o multiple emulsions.

2. From the dropping funnel, this mixture was added drop wise to olive oil with continuous stirring at 1200 rpm for 15 minutes.

3. The microspheres were stabilized by adding 0.1 ml of 25% w/v glutarldehyde solution with continuous stirring for 15 minutes or by adding emulsion system to the preheated olive oil (100 ml) at 120º C drop wise with continuous stirring.

4. The preparation was cooled to 25°C centrifuged at 600 rpm and supernatant was decanted. Microspheres thus obtained were washed with liquid paraffin and twice with ether to get a free flowing and discrete product. The microspheres were then suspended in anhydrous ether and stored at 4° C in an airtight container.

3. Characterization of microspheres
3.1. Particle Size Analysis
The particles were grossly separated into different fractions. Particle size analysis is carried out by using a compound microscope. Dried microspheres were first redispersed in distilled water and placed on a glass slide and the number of divisions of the calibrated eye piece was counted by a using a stage micrometer. The particle diameters of more than 300 microspheres were measured randomly. The average particle size was determined by using Edmundson's equation.

$D_{mean} = \sum nd/\sum n$, Where, n = Number of microspheres checked; d = Mean size.

Median size of the microspheres formulations ranged from 15 to 40 um were considered to be suitable for nasal administration

3.2. Determination of microsphere density
The density of dried microspheres was determined at 25º C using a specific gravity bottle and benzene (density 0.874 g/ml) as the medium in which practically no swelling of egg albumin microspheres was noted.

3.3. Encapsulation efficiency
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) .Microsphere were crushed and

Page 50

powdered by using pestle and mortar and accurately weighed amount of this powder was extracted into phosphate buffer pH 6.8 by stirring at 500 rpm for 2 hr .The solution was filtered, suitable dilutions made and estimated the drug content spectrophotometerically at 241nm. 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.

% Drug entrapment = Actual drug content/theoretical drug content ×100

3.4 Percentage yield

The prepared microspheres were collected and weighed. The yield was calculated for each batch. The percentage yield of microspheres was calculated as follows.

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

3.5 Morphology

The surface morphology of the microspheres was observed by means of scanning electron microscopy (JEOL – JSM - 840A, Japan).The samples were prepared by gently sprinkling the microspheres on a double adhesive tape. 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 using a 20 KV electron beam

3.6 Infrared spectroscopy

About 1mg of the microspheres was triturated with approximately 300mg of dry, finely powdered potassium bromide Infrared (IR), the mixture was grinded thoroughly, spreaded uniformly in the die and compressed under vacuum at a pressure of about 800Mpa. Mounted the resultant disc in a holder in the IR spectrophotometer and recorded the spectra in the IR region of 4000-625/cm^{-1}.The positions and the relative intensities of the absorption bands of the microspheres were compared obtained with that of the pure drug.

3.7 Compatibility studies

The pure drug and mixture of drug- albumin 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.

3.8 Mucoadhesion property

The in vitro mucoadhesion of microspheres was carried out by modifying the method described by Ranga Rao and Buri (29) using goat nasal mucosa. The dispersion (0.5 ml) of microspheres in water was placed on goat nasal mucosa after fixing to the polyethylene support. The mucosa was then placed in the desiccator 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 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 found by the following equation: Na = N/N $_0$ x100, 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. (30)

3.9 Swelling property

The swellability of microspheres in physiological media was determined by allowing the microspheres to swell in the phosphate buffered saline pH 6.8. 100 mg of accurately weighed microspheres were immersed in little excess of phosphate buffered saline of pH 6.8 for 24 hrs and washed thoroughly with deionised water (31). The degree of swelling was arrived at using the following formula- $\alpha = Ws - Wo / Wo$, where α is the degree of swelling, Wo is the weight of microspheres before swelling and Ws is the weight of microspheres after swelling.

3.10 Bioadhesive strength

The bioadhesive strength of all batches was determined using measuring modified pan balance device(32).Section of nasal mucosa was cut from the goat nasal cavity and instantly secured 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 the two vials

3.11 Effect of process variables on microsphere properties

NE microspheres were prepared with different drug to polymer ratio (1:2,1:3,1:4,1:5,) at temperature of 120º C and agitation speeds of 1200 rpm .The effect of process variables on the properties of the resulting microspheres is depicted in table (I-III)

3.12 *In Vitro* Drug Diffusion Studies

The experimental conditions of drug release experiments were similar to those encountered in the nasal cavity. The

in- vitro drug release test of the microspheres was carried out using Frenz diffusion cell (33-34). This apparatus was designed to imitate the nasal cavity and it comprised a donor and receptor compartments. Fresh goat nasal mucosa was collected from a nearby slaughter house. The nasal mucosa of goat was separated from sub layer bony tissues and stored in distilled water containing few drops of Gentamycin injection (with three openings each for sampling, thermometer and donor tube chamber). The receptor compartment with capacity of 60 ml was used in the study in which phosphate buffer pH 6.8 was taken. Within 80 min of removal, the nasal mucosa measuring an area of 3 cm was carefully cut with a scalpel and tied to the donor tube chamber and it was placed in contact with the diffusion medium in the recipient chamber. Microspheres equivalent to 5 mg of NE were spread on the goat nasal mucosa. At hourly intervals, 1 ml of the diffusion sample was withdrawn with the help of a hypodermic syringe, diluted to 10 ml and absorbance was read at 241 nm. Each time, the sample withdrawn was replaced with 1 ml of pre-warmed phosphate buffer (pH 6.8) to maintain a constant volume of the receptor compartment vehicle.

3.13 *In Vitro* Drug Release Kinetics

For understanding the mechanism of drug release and release rate kinetics of the drug from the dosage form, the data obtained was analyzed with software (PCP - Disso V2.08)[35] equipped with zero order, first order, Higuchi matrix and Krosmeyer – Peppas model kinetics. By analyzing the R_f values, the best fit model was arrived at Higuchi model.

3.14 Stability studies

The selected formulations were packed in amber colored glass containers and closed with air tight closures and stored for 90 days at 37º C ± 2 Cº. Samples were analyzed at the end of 30, 60 and 90 days and they were evaluated at variable for % Drug entrapment efficiency, in vitro mucoadhesion test and in vitro drug diffusion studies.

3.15 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.

Formulation code	Particle size(um)	Product Yield	Encapsulation efficiency (%)	Swell size (um)	Muco-adhesion (%)	Bio-adhesive strength (g)	Density (g/ml)	Stabilized chemically/ heat treatment
ALN-1	.40.00±1.25	68.50	75.21±1.65	42.53±1.57	85±1.87	8.13	1.29	glutaraldehyde
ALN-2	32.35 ±.89	75.50	80.21±1.55	32.53±1.57	79±1.89	8.56	1.39	At 120 º C,15 minutes
ALN-3	25.58±1.87	78.20	80.11±1.12	24.43±0.57	87±1.45	7.88	1.48	At 120 º C,30 minutes
ALN-4	24±2.12	80.20	82.31±1.19	21.53±1.57	83±1.67	8.99	1.58	At 120 º C,45 minutes
ALN-5	23.87±1.24	82.00	-83.21±1.15	19.53±1.57	80±.89	9.02	1.60	At 120 º C,60 minutes

Table I: Characteristion of Norethisterone Microspheres
In all the formulations D/P ratio of 1:4, agitation speed of 1200 rpm was kept constant,
*Data are expressed as mean ± SD., n = 3.

Formulation code	Drug to Polymer ratio	%yield	%Drug entrapment efficiency	Particle size(um)	Degree of swelling	% Mucoadhesion
ALN-6	1:2	63.56	65.18±1.18	15.56±1.86	0.832±.0412	79±1.41
ALN-7	1:3	67.56	75.26±1.19	20.48±1.82	0.932±.0412	81±1.43
ALN-8	1:4	78.20	80.21±1.15	25.53±1.57	1.032±.0412	87±1.44
ALN-9	1:5	84.20	79.25±1.12	35.28±1.17	1.142±.0412	89±1.45

Table II Influences of Polymer to Drug Ratio on % Yield, %Drug Entrapement Effiency, Particle Size, Degree of Swelling, and Mucoadhesion
In all the formulations, agitation speed of 1200 rpm, and temperature of120°C was kept constant,*Data are expressed as mean ± SD., n = 3.

Formulation code	Volume of processing medium	temperature (º C)	Agitation Speed (rpm)	Particle size(um)
ALN-10	100ml	120	1200	25.53±1.57
ALN-11	100ml	130	1200	22.33±1.27
ALN-12	100ml	140	1200	19.43±1.55
ALN-13	100ml	150	1200	15.13±1.17
ALN-14	100ml	120	1400	20.53±1.57
ALN-15	100ml	120	1600	15.54±1.57
ALN-16	100ml	120	1000	30.53±1.27
ALN-17	100ml	120	800	35.53±1.07
ALN-18	150 ml	120	1200	20.53±1.37
ALN-19	200 ml	120	1200	16.53±1.50

Table III: Effect of Volume of processing medium, Temperature, Agitation, Speed on Particle size of microspheres
In all the formulations D/P ratio of 1:4 was kept constant,*Data are expressed as mean ± SD., n = 3.

4. RESULTS AND DISCUSSION

4.1 Preparation of microspheres

Egg albumin microspheres of norethisterone were prepared by simple multiple emulsion technique and heat denaturizing process. . The microspheres obtained under these conditions were spherical, free flowing and without aggregation in the size range of 15-41 um, which are therefore suitable for nasal administration. In multiple emulsion systems involving aqueous albumin droplets in vegetable oils, the droplets may be relatively stable without the use of any stabilizer .Albumin molecules contain many hydrophobic residues. When aqueous albumin droplets rough in contact with a hydrophobic environment such as a vegetable oil, the polypeptide chains at the interface adopt conformations in which the hydrophobic side chains are positioned toward the surface of the droplets. This conformational change by the protein molecules leads to the formation of a hydrophobic layer around the droplets, and thus stabilizes the emulsion system. The initially formed albumin droplets to the corresponding particles are there result of the gradual hardening of the droplets by covalent crosslinking. This crosslinking process can be accomplished either thermally or by the addition of a chemical crosslinking agent.

4.2 SEM analysis

Surface morphology was using scanning electron microscopy (SEM). The examination of internal structure of microspheres shows that the interior of microspheres structure was solid in appearances with no pores or perforation. The microspheres were found to be spherical, with porous outer skins and quite smooth surfaces when viewed microscopically. (Fig 1 -2)
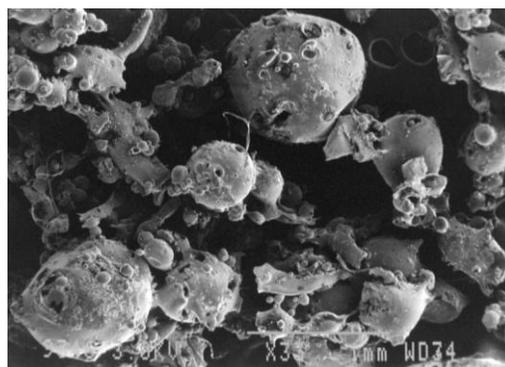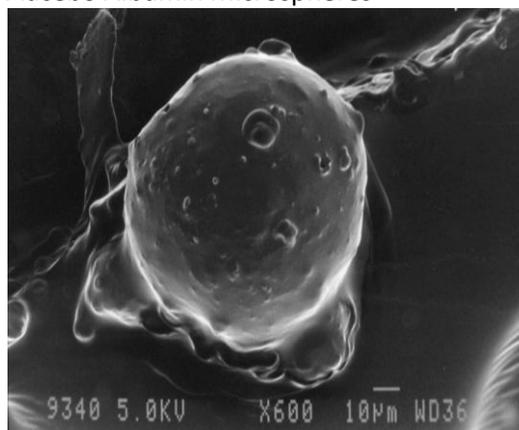

Fig 1   Placebo Albumin Microspheres

Fig 2   Norethisterone Albumin Microspheres

4.3 IR Spectroscopy

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-albumin 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 principle 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. The identical peaks corresponding to the functional groups and features confirm that neither the polymer nor the method of preparation has affected the drug stability. (Fig 3-6)
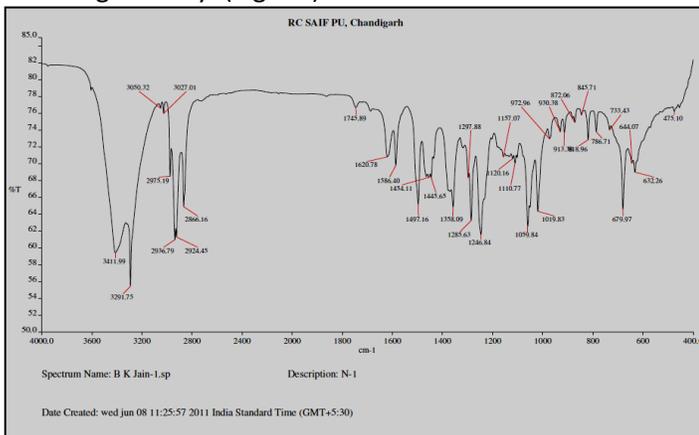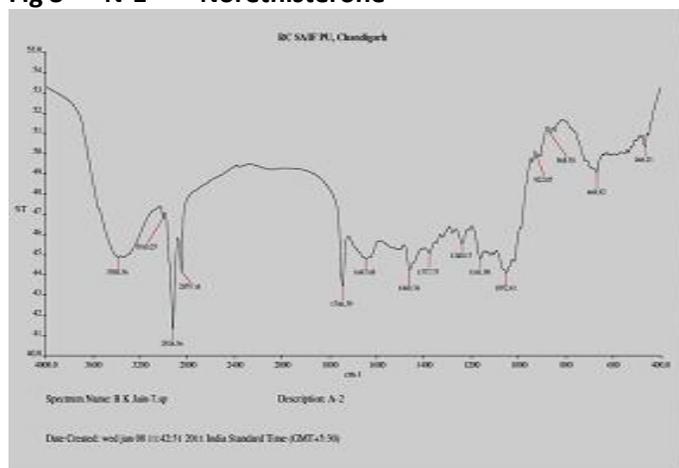

Fig 3   N-1 -   Norethisterone

Fig 4   A-2   Placebo Albumin Microspheres
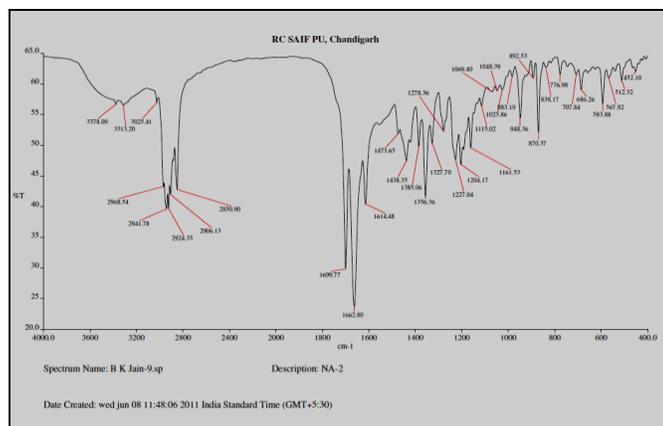

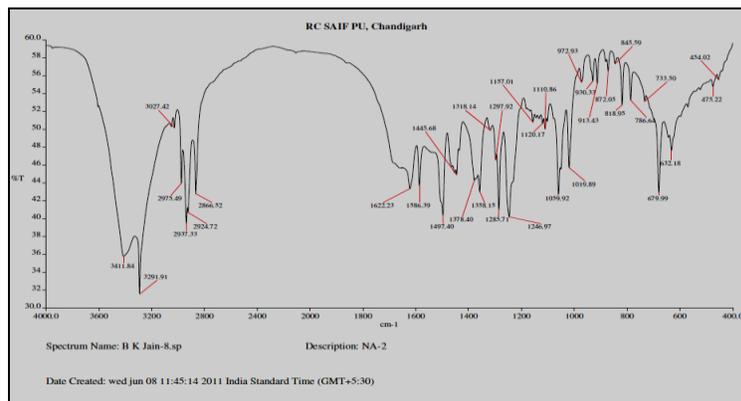**Fig 5 NA-2 - Loaded Norethisterone Albumin**

Fig 6 N+A-2   Physical Admixture (N+A) Microspheres

4.4. 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

4.5   Stability studies

It was observed that there was no significant change in the drug content of the microspheres which were stored at 37º C ± 2 Cº at the end of 30, 60 and 90 days The extent of mucoadhesion of the formulations did not show any significant change after the microspheres were subjected to stability studies. *In vitro* drug diffusion studies for all the four formulations were carried out at the end of 90 days and did not reveal any significant change in drug release from all the formulations. Thus, it can be conclude that the drug does not undergo degradation on storage

4.6   Mucoadhesion

Percentage mucoadhesion was found in the range from 79% to 90%. Bioadhesive strength was in range from 8.0 g to 9.02 g. The effect of heat treatment did not affect bioadhesion significantly. Thus norethisterone microspheres prepared were found to   have good mucoadhesive property.

4.7   Effect of experimental variables on particle size distribution

It was observed that with increase in egg   albumin concentration in the microspheres from batch ALN-6 to ALB-9(Table II) the particle size of microspheres increased, which may be due to the fact that increase in the concentration of polymer increases the cross linking, 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 which resulted in the formation of large droplets, thus increasing the particle size of microspheres

Increase in the temperature from 110º C to 120 º C led to decrease in the mean particle size. However, further increase in temperature above 120 º C did not produce any significant change in the mean particle size. Increase in temperature from 110 º C to 120 º C increases the degree of congealing or rigidization of the polymer, which ultimately results in shrinking of the particles, leading to a decrease in particle size. Hence for the final formulation design a temperature of 120 º C was optimized.

The results were in general agreement with the general theory of microspheres that the particle size of microspheres prepared at 1400 rpm were smaller than those prepared at 800,1000 and 1200 rpm. Since the microspheres obtained at 1200 rpm were in the size range of 15-40 μm suitable for nasal delivery, 1200 rpm was chosen to obtain microspheres. When the stirring speed was decreased from 1200 to 800 rpm, the mean particle size of the microspheres was increased and they were large and aggregated. When the speed was increased from 1200, 1400 to, 1600, rpm, the size of the microspheres was decreased in the size range.

 When the volume of the processing medium was increased from 50 to 100ml and to200ml, mean particle size of microspheres were decreased (Table II), because when the volume of processing medium was increased, the emulsion droplets can be moved freely in the medium and they had less chance to collide with each other there by yielding small and uniform microspheres. Conversely, when the volume is only 50 ml, the emulsion droplets had more opportunities to collide with each other and fuse together to form larger microspheres .This could be also be reason for higher drug extraction into processing medium resulting in lower entrapment efficiency.

4.8   Yield and entrapment efficiency.

The microspheres were analyzed for the drug content uniformity and the encapsulation efficiency.Norethisterone was found to be encapsulated 65–87% which shows that if there is an increase in the concentration of the polymer, the encapsulation efficiency also increases. The yield and entrapment efficiency of drug loaded microspheres of different polymer to-drug ratios are shown in (Table I-II). Encapsulation efficiency of the drug was dependent on its solubility in the solvents and processing medium and also depends on the physicochemical properties of the drug and polymer. The higher entrapment efficiency than expected may be attributed to the lower affinity of the oily inner phase of the o/w/o emulsion towards the external emulsion phase and to consequently higher stability of the double emulsion which inhibit drug migration. The decrease in entrapment efficiency with increase in drug concentration could be related to the increased extent of drug diffusion to the external phase

due to greater flux at higher drug content during the emulsification and microsphere formation process.

4.9   Swellability

 Swellability is an indicative parameter for rapid availability of drug solution for diffusion with greater flux swellability Equilibrium swelling degree increases as the concentration of polymer increases while it decreases as concentration of drug increases as compared to plain microspheres. In general, the higher the extent of crosslinking, the lower the swellability(hydration), and the lower the rate of particle biodegradation/drug release. The density of microspheres stabilized at 120ºC for 60 min was maximum whilst those stabilized for 120ºC for 15 min possessed minimum density (Table I). However, the swollen volume was remarkably different and was noted to be minimum in the case of microspheres treated at 120° C for 60 mm. Swellability could be attributed to the tortuosity of the microspheres whilst the latter could be accounted for by the degree of cross-linking or magnitude of denaturation that results in reorientation of albumin macromolecules. It can be concluded that incorporation of drug in microspheres decrease ESD .

4.10   Kinetic analysis of release

NE microspheres obtained by thermal crosslinking show higher drug content with respect to those obtained by chemical crosslinking. Thermal crosslinking probably reduces the time interval in which the drug can diffuse from the oily inner phase through the albumin aqueous solution due to its more rapid establishment compared with chemical crosslinking where the crosslinking agent has to diffuse from the external oily phase towards the aqueous albumin phase to start crosslinking. The differences observed between the different crosslinkers are probably due to their different partition rates between these two phases.

Microspheres (ALN-1---ALN-9) prepared at heat stabilization temperature of 120° C were studied for drug release rate. Measurable change in release rate was found. Release experiments with heat and chemically stabilized microspheres showed that with increasing density of microspheres due to heat treatment for varied time the release rate decreased. This is probably due to the closer trapping of solute molecules in the denatured albumin structure of high tortuosity. The decrease in release of drug from microspheres stabilized at 120ºC for 60 min was significant as compared to those which had not been heat-denatured but chemically stabilized.

Heat stabilization temperature of 120^oC was chosen on the basis of optimum drug release from microspheres. Slower drop rate of 20±10 also caused charring due to prolonged heating than microcapsules can be attributed.

 The in vitro diffusion of NE from the prepared microspheres exhibited the extent of drug release

decreased from 95 –60% (Fig7, 9). A significant decrease in the rate and extent of drug release is attributed to the increase in density of polymer matrix that results in increased diffusion path length which the drug molecules have to traverse. The release of the drug has been controlled by swelling control release mechanism. No initial burst release has been recorded except for NE microspheres stabilized using 25% w/v glutarldehyde as cross-linking agent. The findings are suggestive of effective washing of surfacial drug of microspheres after their stabilization. Additionally the larger particle size at higher polymer concentration also restricts the total surface area thus resulting in slower drug release over a span of 8 hr.

The release data obtained were evaluated kinetically by zero order, first order and Higuchi model. A linear graph was obtained by plotting the percentage of the drug released versus the square root of time. These profiles showed that drug release from NE microspheres obeys Higuchi diffusion controlled model (Fig8, 10). Further, the observed diffusion coefficient values are indicative of the fact that the drug release from the formulation follows non-Fickian transport mechanism The results obtain from the computer program were also supported by these profiles.

Fig.7: *In- vitro* diffusion profile of NE microspheres
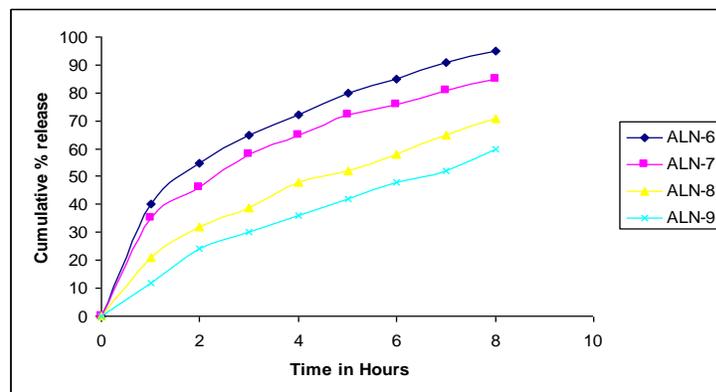

Fig. 8: Higuchi plot

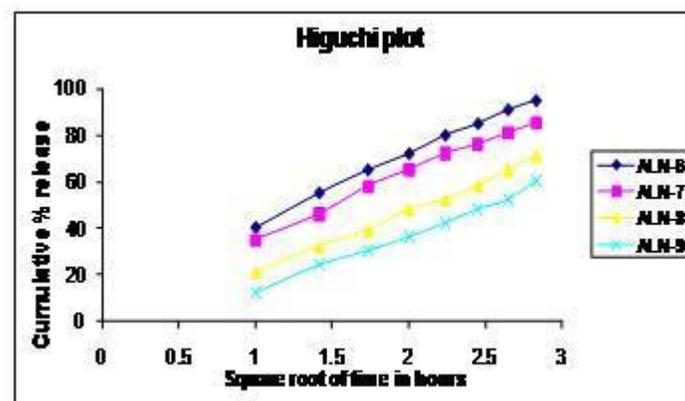Fig. 9: *In- vitro* diffusion profile of NE microspheres

Fig. 10: Higuchi plot

5. CONCLUSION

The multiple emulsification technique for obtaining microspheres has proved to be a useful tool in the preparation of microspheres for nasal drug delivery. By virtue of prolonged drug residence at the site of absorption, improved bioavailability can be achieved

Drug: polymer ratio, stirring speed and dispersing medium influenced the sphericity of the microspheres. The entrapments eiffcienciy, production yield, was high for all the formulations. It was suggested that mechanism of drug release from microspheres was diffusion and erosion controlled.

All the microspheres were at a suitable size and had good mucoadhesive property for nasal administration. The hydrophilic polymer egg albumin and the microsphere system achieved to modify the in vitro release of NE. The stability data showed that there was no change in the appearance of the microspheres indicating that the formulations were stable at different conditions of storage

The result from the present study indicates that it is possible to achieve enhanced bioavailability of norethisterone by using egg albumin microspheres. It is concluded that NEcould be successfully steered via the nasal route Moreover, controlled drug release following
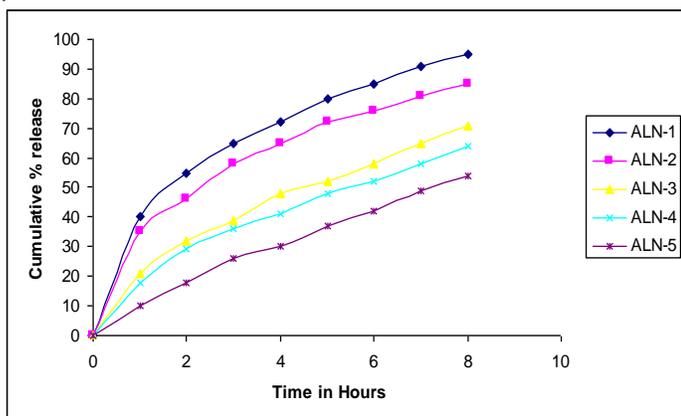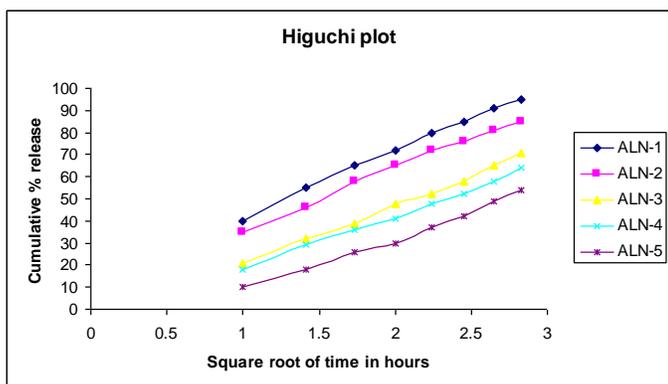
nasal administration of bioadhesive egg albumin microspheres resulted in sustained and controlled drug absorption.

7. Acknowledgement

1   Author is to Famy Care, Pharmaceuticals, Navi Mumbai, for providing contraceptive drugs as gift sample of Norethisterone.

2   Author is thankful to Punjab Technical University; Chandigarh for providing SEM & I.R. data of influences various characteristics of the system Last but not least I am indebted to my research guide Dr Seema Kohli H. O. D. Pharmacy,K. N. Polytechnic College, Jabalpur for their valuable contribution and guidance to fulfill this object.

8. REFERENCES

1   Lee,T. K. , Sokoloski,T. D., and Royer,G. P.,Progesterne release from glutarldehyde cross linking Caseinmicrospheres Science.pg 213, 233,(1981).

2   Longo,W. E. Iwata,H., Lindheimer,T.A. and Goldberg, Preparation of hydrophilic albumin microspheres, G. P. J. Pharm. Sci,.,,71,1323, (1982)

3   Tominson,E. and McVie,J. Methodology of manufacturing techniques ,G., Pharm. Int.,4,281,(1983),

4   Reza Arshady,Albumin microspheres and microcapsules ;Methodology of manufacturing techniques. J.Controlled Release 14, (1990) 111-131

5   Corbo. D. C. Transmucosal drug delivery and absorption: Ph. D. Dissertation, 1988, Rutgers University. N. J.

6   Corbo. D. C and Chien Y. W. Nasal Delivery of progestational steroids in ovariectomized rabbits Int. J,Pharm. , 46:133-140 (1988)

7   Chien, Y. W. Mucosal Drug Delivery: Potential Routes for Noninvasive Systemic Administration, J. Pharm., 1988, 46, 133

8   Hussain, A.A., Hirai, S. and Bawarshi, R., Nasal absorption of propranolol dosage forms by rats and dogs, J. Pharm. Sci. 1979, 68, 119

9   Sidman. K, et al, A biodegradable drug delivery system, in H.L. Gablelick(Ed.) Drug Delivery Systems, DHEW Publication Washington. C., 1993, pg120-130.

10   Peterson. R. V. et al, Controlled release from progestins from poly(a aminoacid) carriers in R. Baker (Ed.) , Controlled release of Bioactive Materials. Academic, New York 1980, pg 45-60

11   Lee. T. K. et al Serum Albumin Beads. An injectable biodegradable systems for of sustained release of drugs, Science, 213,, 1981 pg213-215

12   Orienti V. and Zecchi V. Progestrone loaded albumin microparticles .J. Contol Rel, 27 (1993), pg 233-235

13   Jameela S. R.et al Progesterone loaded chitosan microspheres, J. Controlled Release, 52 (1998) pg17 -24

14   Roberto R. etal J. Norethisterone contraceptive microspheres,Steroid Biochem. V_27, No4 -6, pp-1003-7, 1987

15   A. Cornaz, P. Buri, Nasal mucosa as an absorption barrier, Eur.J. Pharm. Biopharm. 40 (1994) 261 – 270

16   L.Muggetti, M. Zurlo, A. Martini, P. Civaroli, R. De Ponti,Preformulation activities of intranasal dosage forms of temazepam, Int. J. Pharm. 136 (1996) 81 - 88.

17   P. Vidgren, M. Vidgren, P. Vainio, J. Nuutinen, P. Paronen,Double-labelling technique in the evaluation of nasal mucoadhesionof disodium cromoglycate microspheres, Int. J. Pharm. 73(1991) 131 - 136.

18   M.I. Ugwoke, G. Kaufmann, N. Verbeke, R. Kinget, Intranasal bioavability of apomorphine from carboxymethylcellulose-baseddrug delivery systems, Int. J. Pharm. 202 (2000) 125 – 131

19   L. Illum, H. Jorgensen, H. Bisgaard, O. Krogsgoard, N. RossingBioadhesive microspheres as a potential nasal drug delivery system, Int. J. Pharm. 39 (1987) 189 -199.

20   Hascicek C and Gonul N. mucoadhesive microspheres containing gentamicin for nasal administration, Science Direct, II Framaco58 (2003) 11-16

21   Patil S. B. and[1], Murthy R.S.R.[2,]"Preparation and in vitro evaluation of mucoadhesive chitosan microspheres of amlodipine besylate for nasal administration, I.J.P.S.Year : 2006 | Volume : 68, pg : 64-6722.   Rathode S. & DesshpandeS. G,Albumin Microspheres as an Ocular Delivery system for Pilocarpine Nitrate. Ind. J. Pharm.Science, Mar-Apr 2008, pg.193-197

23   Vyas S. and Bhatnagar S. Preparation and characterization of HAS- propanolol microspheres for nasal administration,J. INT. Pharma. 69(1991) 5-12

24   G.Rathnam,Narayan N,Liavarasan R., Preparation and in evaluation of carbopol based nasal gels for systemic delivery of progesterone.,IJPRD/2010/PUB/ ART/ VOV2/ISSUE1/MAR/OO5

25   Thanoo BC, Sunny MC, Jayakrishnan A. Cross-linked chitosan microspheres: preparation and evaluation   as a matrix for the controlled release of pharmaceuticals.   *J Pharm Pharmacol*. 1992; 44, pg 283-286

26   Rao K.R.and Senapati P.Formulation and evaluation of ethyl cellulose microspheres containing zidovudine. J. of Microencapsulation, Dec 2005, 22(8):863-876

27   Pande A. V. etal Ind. J. Pharm.Biomed Res2010, I(4) 122-128

28   K. Widder, G. Fluoret and A. Senyei, Magnetic microspheres,J. Pharm. Sci., 68 (1979) 79-82.

29   Rao K. R. and Buri P. (1989),Measerment of mucoadhesive strength, Int. J. Pharm 74:9.

30   Matazzovi S A and Smard JD. An in vitro method for assessing the duration of mucoadhesion.,J. Control Rel, 1994, 31, 207-12

31   Jain SK, Jain NK, Gupta Y, Jain A, Jain D, et al. Mucoadhesivechitosan microspheres for non-invasive and improved nasaldelivery of Insulin. Indian J Pharm Sci 2007; 69(4): 498-504

32   Gupta,. Garg S., and Khar R.K.,Modified balance method for measurement of mucoadhesive strength.Indian Drug.s, 30(14), 152 1992

33   .P. Vyas, N. Talwar, J.S. Karajgi, N.K. Jain, An erythrocytebased bioadhesive system for nasal delivery of propranolol, J.Controlled Release 23 (1993) 231_ 237

34   M.D. Abd El-Hameed, I.W. Kellaway, Preparation and in vitrocharacterisation of mucoadhesive polymeric microspheres as intranasaldelivery systems,, Eur. J. Pharm. Biopharm. 44 (1997)53_ 60

35   Swamy NGN, Rupa V, Abbas Z, Dasankoppa FS. Formulationand evaluation of Nanosuspensions for enhancing thedissolution of poorly soluble Mebendazole. Indian Drugs 2010;47(9): 47-54

36   Shahiwala A, Misra A , , Nasal delivery of levonorgestrel for contraception: an experimental study in rats. Fertility and Sterility, 81,, 2004,.,893-898.