ISSN: 2231-6078

INTERNATIONAL JOURNAL OF DRUG DISCOVERY AND HERBAL RESEARCH (IJDDHR)
06(1): April.-June.: (2016), Sppl 9-18

Research Lecturer

Preparation and *In-Vitro* Characterization of MUCOADHESIVE Progesterone -Chitosan Microspheres for Nasal Administration

B. K. Jain[1], and S.Kohli[2]

Pharmacy Department, K.N.Polytechnic College, Jabalpur-482002, M. P. INDIA[2]

Abstract

Chitosan microspheres have gained widespread acceptance in the fields of drug delivery, biosmer fabrication and delivery of hydrophilic and lipophilic drugs. Because it is biodegradable and biocompatible, chitosan, a polysaccride made of copolymers of glucosamine and N-acetyl glucosamine, is frequently used in the formulation of particulate drug delivery systems to achieve controlled drug delivery.

In this study suitable smooth, spherical, cross-linked chitosan microspheres in the size range of 20-60 um loaded with progesterone(PG) were prepared by glutaraldehydecross-linking of an aqueous acetic acid dispersion of chitosan containing PG in a nonaqueous 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. Shape and surface morphology were examined using scanning electron microscopy. The SEM of microspheres shows that a hollow spherical structure with a smooth surface.

Optimized process and formulation parameters resulted in spherical shape and rigid surface, homogenous population of microspheres in the size range of 33 to 65µm. The *in vitro* diffusion of PG from the prepared microspheres exhibited the extent of drug release decreased from 99–65%. The release of the drug has been controlled by swelling control release mechanism. Modeling drug release from polymeric controlled drug delivery systems systems has led to a wide spectrum of mathematical models. All the formulations followed first order kinetics. The drug release from progesterone microspheres obeys Krosmeyer-Peppas model and non-Fickian diffusion pattern.

Key Words: Mucoadhesive microspheres; Nasal drug delivery, progesterone, Chitosan, glutarldehyde, span60, .Liquid paraffin, glutarldehyde cross-linking.

Corresponding Author

*Corresponding Author-jainbhaanukumar@gmail.com

Introduction:

Over the past 20 years, there has been a lot of attention focused on the development of biodegradable polymeric delivery methods for antifertility steroids. Many synthetic biodegradable polymers have been used to prepare injectable microspheres, fibers, films, and rods for implants. The use of synthetic biodegradable polymeric carriers for antifertility steroids has been extensively studied in the literature; however, natural polymers like proteins and polysaccharides received far less attention.

A lipophilic drug progesterone is utilized in postmenopausal therapy, habitual abortion, suppression of estrous cycles, and reproductive function regulation. Progesterone has potential for fertility control since it

occurs in high concentration in the natural conditions and has no known adverse effects, unlike synthetic progestin. Progesterone's oral bioavailability is low because of its strong hepatic metabolism and short biological halflifeand its intolerance at higher dosages hinders its oral bioavailability. This significantly reduces progesterone's effectiveness when taken orally. Over the past 20 years, there has been a lot of attention focused on the development of biodegradable polymeric delivery methods for antifertility steroids.

The oral use of steroids is the primary method used in the pharmaceutical approach to fertility control. Even so, there has been some degree of clinical success with controlled release systems like Progestart and Norplants, which administer progesterone and levonorgestrel, respectively, from non-biodegradable polymer matrices. The oral route's daily ingesting need and the ensuing daily change in medication concentration are its drawbacks. Many synthetic biodegradable polymers have been used to prepare injectable microspheres, fibers, films, and rods for implants.

Injectable biodegradable drug reservoir from glutamic acid/leucine co-polymers in the forms of tubes and solid rods were prepared by Sidmanet al.[1] to provide controlled release of progesterone. Lee et al.[2] 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[3]. Jameela S. R. et al studied in laboratory animals that glutarldehyde cross- linked chitosan microsphers are long acting biodegradable carriers suitable for controlled delivery of many drug[4].

Research has also been done on the nasal route's potential for antifertility drug administration. The nasal cavity's broad surface area and highly vascularized epithelium are two of its clear advantages. Also avoiding first pass metabolism in the nasal route. It has been demonstrated that in rats, the nasal route of administration of steroidal drugs results in significantly higher bioavailability than the oral route.Nonetheless, several issues including mucociliary clearance and limited drug permeability of the nasal mucosa greatly impact how well drugs are absorbed through the nasal mucosa. Mucoadhesive microsphere systems for nasal administration were introduced and characterized by Illumet al[5]. The microspheres form a gel-like coating that gradually removes from the nasal canal, extending the drug's residence period.

The aim of this investigation was to develop nasal mucoadhesive microspheres that encapsulate progesterone as a model drug. Fertility can be regulated if steroids are administered in a sustained release manner. The current work focuses on the kinetics of in vitro drug release with the goal of developing an efficient nasal progesterone delivery system.

Materials and Methods[6-13]

Progesterone was procured as a gift sample from Famy Care, Pharmaceuticals, Navi Mumbai and liquid paraffin, glutarldehyde, olive oil, ether were purchased from Loba Chemical, Mumbai. All reagents used were of analytical reagent grade.

Chitosan microspheres were prepared by a modification of the methods used by Thanooet al[6]. The effect of process variables on preparation was studied and optimised. Five batches of the microspheres were prepared by taking different D/P ratio.A 2% (w/v) chitosan solution in aqueous acetic acid (2% v/v) was prepared. The drug (200 mg) was extruded through

syringe in chitosan solution and stirred at 1200 rpm for 2 hrs to get uniform dispersion. This dispersed phase was added to continuous phase (125 mL) comprising 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 25 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 number of times with cyclohexane to eliminate oil, and lastly washed with water to remove surplus of glutaraldehyde. The volume of glutaraldehyde was varied to affect the cross-linking density. Washings were tested for drug contents. Then microspheres were air dried at room temperature.

Characterization of microspheres [7-11]

Particle size analysis

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 200 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 37 µm was considered to be suitable for nasal administration.

Determination of microsphere density

The density of dried microspheres was determined at 25ºC 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.

Encapsulation efficiency

To calculate the entrapment efficiency of progesterone into the microspheres, a weighed quantity of microspheres (20 mg) 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 600 rpm for 1 hour. The solution was filtered; suitable dilutions were made and estimated the drug content spectrophotometrically at 241 nm. 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.

$$\% \text{ Drug entrapment} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

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.

Morphology

The surface morphology of the microspheres was observed by means of scanning electron microscopy. 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 Å) of gold, the microspheres were examined by SEM at different magnification using a

5-10 KV electron beam.

Infrared spectroscopy

About 1 mg of the microspheres was triturated with approximately 300 mg 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 800 Mpa. 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 obtained were compared with that of the pure drug.

Compatibility 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 IR spectrum of pure drug and excipients mixtures were compared to find any interaction between drug and excipients used for formulation.

Mucoadhesion property

The *in vitr o* mucoadhesion of microspheres was carried out by modifying the method described by Rao and Buri[12] 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 at 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. The mucosa was washed for 10min 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:

$$N_a = N/N_0 \times 100$$

Where N_a 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[28].

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[29]. The degree of swelling was arrived at using the following formula –

$$\alpha = W_s – W_o / W_o$$

Where α is the degree of swelling, W_o is the weight of microspheres before swelling and W_s is the weight of microspheres after swelling.

Bioadhesive strength

The bioadhesive strength of all batches was determined using modified pan balance device[13]. 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 37ºC 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.

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 of the microspheres was carried out using Frenz diffusion cell[14,15]. This apparatus was designed to imitate the nasal cavity and it comprises 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 progesterone 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

*In-vitro*drug release kinetics

The release data achieved were evaluated kinetically for zero order, first order and Higuchi model and Korsemeyer – Peppas to identify the mechanism of drug release from the formulations.The result of release kinetics for all the models is shown in Table 2.

Stability studies

The stability of the optimized formulations was evaluated as per ICH and WHO guidelines. Optimized formulations were filled in screw capped HDPE bottles and were filled at 40° C ± 2°C and 75 % RH ± 5 % RH for 6 months (Intermediate Storage Condition) using programmable environmental test chambers. The drug content and drug release rate were determined after storage for 6 months and they were evaluated for % Drug entrapment efficiency, *invitro* mucoadhesion test and *in vitro* drug diffusion studies.

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.

ResultandDiscussion

Particle Size

The emulsification crosslinking technique was employed for the preparation of Progesterone microspheres by means of glutaraladehyde as crosslinking agent. These microspheres were discrete, spherical, free flowing and having particle size ranged from 33 to 65 um. It was observed that while increasing the polymers concentration from batch CHP-1 to CHP-5, the particle size of microspheres increased in proportion. This could be attributed to the fact that an increase in the polymer concentration leads to an increase in the degree of crosslinking.Subsequently the matrix density of the microspheres also increased that may result in the increase in the particle size of the microspheres. Increase in viscosity of the droplet resulted in formation of large droplets, consequently increasing the size of PG microspheres.

Swelling property

Equilibrium swelling degree (ESD)is largely governed by polymer and drug concentration.It was observed that on increasing the concentration of polymer ESD increases while it decreases as concentration of drug increases as compared to plain microspheres. It was suggested that incorporation of drug in microspheres decrease

ESD.Density of microspheres also increases while increasing the D/P ratio.

Mucoadhesion

The *In-vitro* mucoadhesionstudy of microspheres was the most significant feature of present study. It was found that, for batches CHP-1 to CHP-5 as the amount of polymer was increased, the % *in vitro* mucoadhesion also increased. This could be attributed 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 - 73.0 % to 83.0%. Bioadhesive strength was in range from 6.03-9.93 g. Therefore prepared microspheres were found to be having excellent mucoadhesive Property.

Stability studies

It was observed that there was no significant change in the drug content of the microspheres which were stored at 37ºC ± 2ºC 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.

SEM analysis

i.Progesterone Microspheres

The SEM of microspheres shows that a hollow spherical structure with a smooth surface. 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.

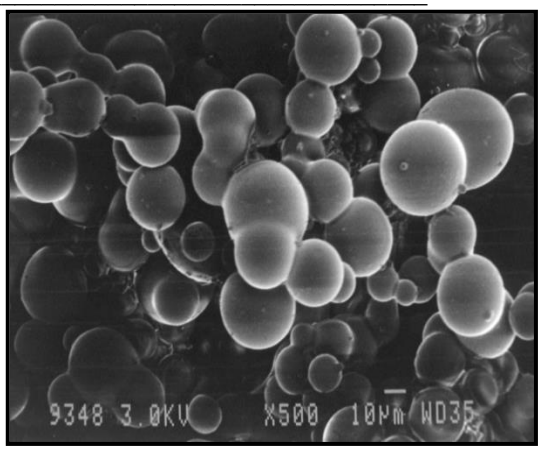

Fig.1: Placebo Chitosan Microspheres

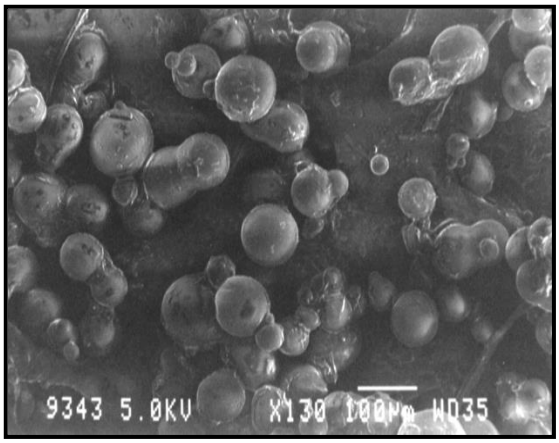

Fig. 2: Loaded Chitosan Progesterone Microspheres

MeltingPoint

The melting points of the Progesterone and the same in the microspheres were found to be the in the range of 127-131°C respectively signifying that there is no change in the nature of the entrapped drug because of the formulation process.

IR spectroscopy

IR spectra of the free drug, placebo microspheres and the drug loaded microspheres were documented and compared to find any variation in the frequency of functional group in microspheres with corresponding functional group of the drug.

Progesterone microspheres

Prominent peak of Progesterone are ketone (1707-1726 cm^{-1}), methyl ketone (1350-1369 cm^{-1}), and methyl (1375-1382 cm^{-1}). IR spectrums of the same exhibited the characteristic peaks at 1,661and 1698 cm^{-1}allocated to carbonyl stretching bands of C_3 and C_{20} in PG was also close in the IR spectrums of microsphere. Therefore said results indicate that neither the polymer nor the method of preparation has affected the drug stability.These IR spectral observations showed no alteration in the fingerprint of pure drug spectra, and prepared microspheres. Results are shown in Fig. (3-5)

Drug content and percentage of drug entrapped

The microspheres were assessed for the drug content uniformity and the encapsulation efficiency. Progesterone was found to be encapsulated 16.30-26.10% which indicates that if there is an increase in the concentration of the polymer, the encapsulation efficiency also increases.

Kinetic analysis of release

The release data obtained were evaluated kinetically. The entire formulations of CHP series exhibited good fit into first-order than zero order kinetics with the highest correlation coefficient (R^2 =0.9899-0.9961). The results of which were shown in table (2-4). It can be inferred that, all the formulations followed first order kinetics and also obeys Higuchi diffusion controlled model.The observed diffusion coefficient 'n' value in Peppa's model was between(0.5087-0.7872 implying that the mechanism of the drug release was diffusion and erosion controlled and follows Non-Fickian transport mechanism.

Conclusion

It has been demonstrated that the multiple-emulsification approach is a suitable technique to produce microspheres intended for nasal drug delivery. For nasal delivery, every microsphere had an appropriate size and strong mucoadhesive properties. For all formulations, there was a high manufacturing yield and entrapment efficiency. The drug-polymer ratio had an impact on the microspheres' size, sphericity, and other characteristics. Because of the contents of the stomach and the powerful first-pass metabolism, absorption of natural progesterone varies greatly. For this reason, progesterone administration by nasal route is beneficial. The in vitro release of progesterone was altered by the hydrophilic polymer chitosan and the microsphere system. It was proposed that diffusion and erosion determine the process of drug release from microspheres.

Enhancing the duration of the drug's residency at the site of absorption can lead to increased bioavailability. The present investigation demonstrates the potential of progesterone chitosan microspheres as a drug delivery matrix. In summary, based on the obtained results, it is possible to effectively steer the biocomptabile and cost-effective polymer chitosan to formulate an effective nasal micropartricles system for steroidal drugs that is appropriate for nasal administration. Additionally, controlled drug release after nasal administration leads to sustained controlled drug absorption.

Statistical analysis

Prism software and online statistical calculators were used to analyze the data that were collected. ANOVA and the Student t test were used to examine the differences in means between the two groups. The mean differences were considered statistically significant when the p-value was less than 0.05.

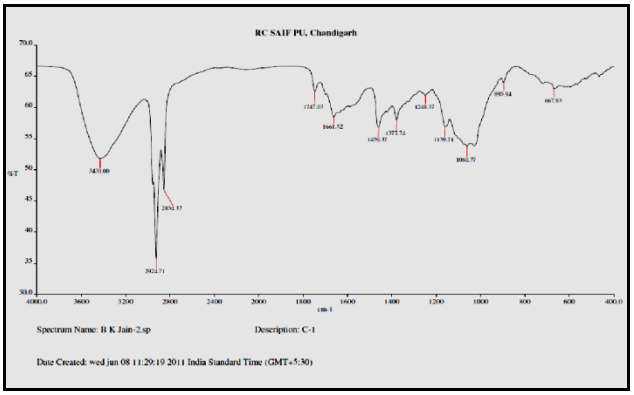

Fig.3 : FTIR spectra ofPlacebo ChitosanMicrospheres

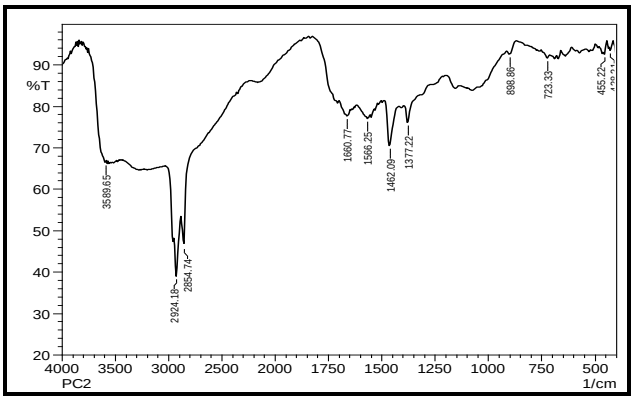

Fig.4:FTIR spectra of Loaded Chitosan Progesterone

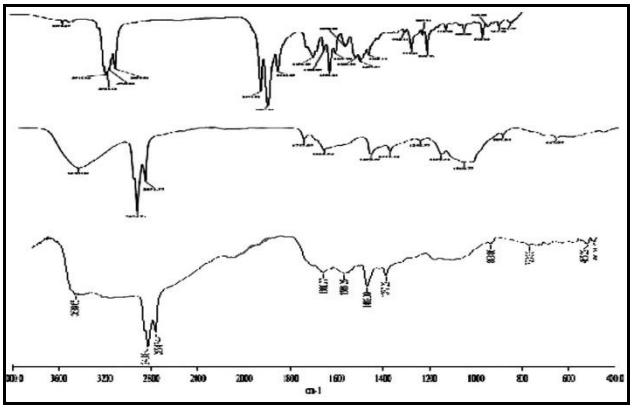

Fig.5: Comparison of FTIR spectrum of pure Progesterone (a), Placebo Chitosan Microspheres(b) and Loaded Progesterone Microspheres(c)

Drug content and percentage of drug entrapped

The microspheres were assessed for the drug content uniformity and the encapsulation efficiency. Progesterone was found to be encapsulated 16.30-26.10% which indicates that if there is an increase in the concentration of the polymer, the encapsulation efficiency also increases.

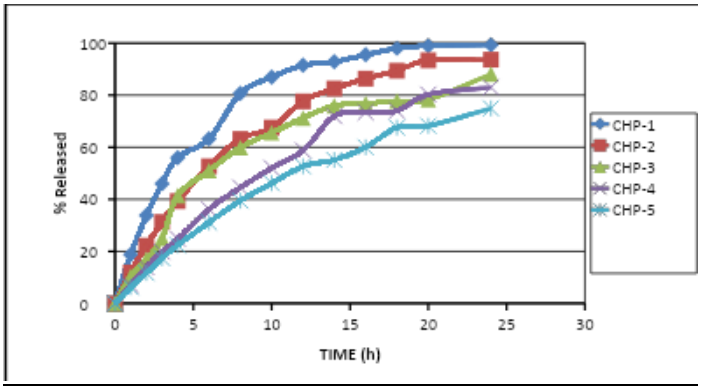

Fig.6: *In-vitro* drug Release profiles of Chitosan PG microspheres formulations

Table 2: Correlation coefficient (R^2) values in the analysis of release data as per various kinetic models

Formulation Code	Zero order	First order	Higuchi's	Peppa's
CHP-1	0.7855	0.9899	0.9361	0.9390
CHP-2	0.8867	0.9899	0.9766	0.9719
CHP-3	0.8523	0.9637	0.9516	0.9453
CHP-4	0.9426	0.9870	0.9746	0.9882
CHP-5	0.9569	0.9961	0.9812	0.9912

Table 3: First order linear regression equations, correlation coefficient (r^2) values in the analysis of drug release from Chitosan Progesterone Microspheres formulations

Formulation code	Linear regression equation	Correlation coefficient (R^2)
CHP-1	Y = -0.0922*X + 2.029	0.9899
CHP-2	Y = -0.0535*X + 1.998	0.9899
CHP-3	Y = -0.0360*X + 1.949	0.9637
CHP-4	Y = -0.0340*X + 2.003	0.9870
CHP-5	Y = -0.0251*X +	0.9961

Abbr.CHP-Chitosan Progesterone Microspheres

Table 4: Release characteristics of Chitosan Progesterone Microspheres formulations

Formulation code	T_{50}	T_{90}	K_1 (h^{-1})	n in Peppa's equation
CHP-1	3.2642	10.8530	0.2123	0.5087
CHP-2	5.6250	18.7021	0.1232	0.6478
CHP-3	8.3493	27.7602	0.0830	0.6694
CHP-4	8.8392	29.3890	0.0784	0.7872
CHP-5	11.9896	39.8633	0.0578	0.7827

Abbr.CHP-Chitosan Progesterone Microsphere

Table 1: characterization of Chitosan Progesterone Microspheres

Formulation code	D/P Ratio	Particle size(um) (mean ±SD)	Product Yield (%) (mean ±SD)	Entrapement efficiency (%)	Equilibrium swelling degree (ml/g) (mean ±SD)	Mucoadhesion (%) (mean ±SD)	Bioadhesive strength (g) (mean ±SD)	Density (g/ml)	Melting point
CHP-1	1:1	33.12±1.17	73.40±2.27	16.30	1.79±0.089	73±1.85	6.03±1.25	1.29	131^0C
CHP-2	1:2	44.34 ±.79	77.40±2.62	21.09	1.85±0.076	79±1.69	7.15±1.28	1.32	131^0C
CHP-3	1:3	50.98±1.17	81.20±4.23	19.50	1.90±0.085	87±1.45	8.28±2.01	1.46	129^0C
CHP-4	1:4	58.01±2.13	84.10±4.58	24.50	2.22±0.087	89±1.57	8.99±2.16	1.60	129^0C
CHP-5	1:5	61.12±1.26	89.60±5.23	26.10	2.39±0.057	86±1.27	9.93±2.56	1.68	130^0C

ACKNOWLEDGEMENT

I am appreciative to Famy Care, Pharmaceuticals, Navi Mumbai, for donating a progesterone gift sample along with contraceptive drugs. I am also appreciative of the SEM and I.R. data provided by Diya Labs in Bombay and Punjab Technical University

Chandigarh. I owe the principal of K. N. Polytechnic College in Jabalpur for his assistance and collaboration in achieving this goal.

REFERENCES

1.K. Sidman, W. D. Steber and A. W. Burg, A Biodegradable Drug Delivery System, in H. L. Gablelick (Ed.) Drug Delivery Systems, DHEW Publication, Washington C. (**1993**) , 120-130.

2.T. K. Lee, T. D. Sokoloski and G. P. Royer, Progesterne Release from Glutarldehyde Cross Linking Casein Microspheres, Science (**1981**) pp. 213, 233.

3.V. Orienti and V. Zecchi, Progestrone Loaded Albumin Microparticles, J. Contol Rel., **27** (1993) pp. 233-235.

4.Jameela et al J.Contolled Releage,**52** (1998),pg 17-24.

5.L. Illum, H. Jorgensen, H. Bisgaard, O. Krogsgoard and N. Rossing, Bioadhesive Microspheres as a Potential Nasal Drug Delivery System, Int. J. Pharm., **39**, (1987). 189-199.

6.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***., 44,** (1992), 283-286.

7.RezaArshady, Albumin Microspheres and Microcapsules, Methodology of Manufacturing Techniques, J. Controlled Release, **14**, (1990).111-131.

8.S. Vyas and S. Bhatnagar, Preparation and Characterization of HAS-Propanolol Microspheres for Nasal Administration, J. Int. Pharma., **69,** (1991). 5-12 .

9.S. Rathode and S. G. Desshpande, Albumin Microspheres as an Ocular Delivery System for Pilocarpine Nitrate, Ind. J. Pharm. Science, **70(2),** (2008) 193-197.

10.C. Hascicek and N. Gonul, Mucoadhesive Microspheres Containing Gentamicin for Nasal Administration, Science Direct, II Framaco, **58,** (2003). 11-16.

11.K. R. Rao and P. Senapati, Formulation and Evaluation of Ethyl Cellulose Microspheres Containing Zidovudine, J. Microencapsulation, **22(8),** (2005).863-876.

12.K. R. Rao and P. Buri, Measurement of Mucoadhesive Strength, Int. J. Pharm., **52,** (1989) 265-270.

13Gupta, S. Garg and R. K. Khar, Modified Balance Method for Measurement of Mucoadhesive Strength, Indian Drugs, **30(14),** (1992). 152-155.

14.S. P. Vyas, N. Talwar, J. S. Karajgi and N. K. Jain, An Erythrocyte Based Bioadhesive System for Nasal Delivery of Propranolol, J. Controlled Release**, 23**, (1993) 231-237.

15.M. D. Abd El-Hameed and I. W. Kellaway, Preparation and *In Vitro*Characterisation of Mucoadhesive Polymeric Microspheres Intranasal Delivery Systems, Eur. J. Pharm. Biopharm., **44,** (1997). 53-60.