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RESEARCH ARTICLE

DESIGN AND DEVELOPMENT OF EUDRAGIT COATED CHITOSAN MICROSPHERES FOR COLON TARGETED DELIVERY

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ABSTRACT

Oral route is the most convenient and preferred route but other routes for CDDS may be used. Rectal administration offers the shortest route for targeting drugs to the colon. However, reaching the proximal part of colon via rectal administration is difficult. Rectal administration can also be uncomfortable for patients and compliance may be less than optimal. Administration of glucocorticoids namely dexamethasone by oral route produce systemic side effects including adenosuppression, immunosuppression, cushinoid symptoms, and bone resorption. Thus selective delivery of drugs to the colon could not only lower the required dose but also reduce the systemic side effects caused by high doses. The aim of the present investigation was to prepare colon targeted microspheres of Dexamethasone using a combination of time and pH dependent polymer that offer protection to the drug until it leaves the stomach which is provided by pH dependent polymethacrylate polymer, Eudragit S 100 and major drug release in small intestine is avoided by providing pH independent coating of Chtosan. For preparation of colon targeted microspheres the Microspheres of dexamethasone were first prepared with pH independent polymer Chitosan then with outer layer of a pH dependent polymer Eudragit L100.

Keywords: Chitosan, Eudragit S100, Colon Targeted, Dexamethasone

INTRODUCTION:

Targeted drug delivery into the colon is highly desirable for local treatment of a variety of bowel diseases such as ulcerative colitis, Crohn's disease, amebiosis, colonic cancer, local treatment of colonic pathologies, and systemic delivery of protein and peptide drugs. The colon specific drug delivery system (CDDS) should be capable of protecting the drug en route to the colon i.e. drug release and absorption should not occur in the stomach as well as the small intestine, and neither the bioactive agent should be degraded in either of the dissolution sites but only released and absorbed once the system reaches the colon. Dexamethasone is a synthetic corticosteroid exhibiting both anti-inflammatory and immune-suppressant properties. The anti-inflammatory property of dexamethasone is useful in the treatment of inflammatory bowel diseases (IBD). The purpose of the present study was to prepare and evaluate the potential of Eudragit L-100 coated chitosan microspheres of Dexamethasone for colon targeting. Chitosan microspheres were prepared by the emulsification method and cross-linking will be done with glutaraldehyde. These microspheres will be further coated with Eudragit S-100 by the solvent evaporation technique so as to prevent drug release in the stomach and small intestine. Physiochemical characterization of the microspheres like shape, size, size distribution, surface morphology, incorporation efficiency and in-vitro drug release studies were performed.[1-5]

MATERIALS AND METHODS:

MATERIALS:

Dexamethasone as a gift sample from torent pharmaceutical, India, chitosan was obtained from HiMedia Laboratories Ltd, Mumbai, India.Eudragit S-100 (ES) was obtained from Ranbaxy Laboratory Ltd (Haryana, India). span 80,glutaraldehyde,toluene,acetone, hexane, and light liquid paraffin were purchased from Central Drug House Pvt Ltd, Mumbai, India. All other chemicals used were of analytical reagent grade and were used as received.

METHODS:

Preformulation Studies:

A) Organoleptic evaluation:

It refers to the evaluation by sensory characters-taste, appearance, odor, feel of the drug, etc.

B) Solubility (at room temp):

The spontaneous interaction of two or more substances to form a homogenous molecular dispersion is called as

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solubility in that solvent**.** For the qualitative or crude solubility study, a known amount of drug (10 mg) was suspended in a series of different solvents (10 mL) at room temperature in tightly closed test tubes and shaken on wrist action shaker for 24 hrs. The crude solubility was observed only by visual inspection.

C) Identification Test:

FTIR Spectroscopy:

Infra- red spectrum is an important record which gives sufficient information about the structure of a compound. This technique provides a spectrum containing a large number of absorption band from which a wealth of information can be derived about the structure of an organic compound.

D) Loss on drying:

Loss on drying directly measuring by IR moisture balance. Firstly calibrate the instrument by knob then take 5.000 gm sample (powder) and set the temp at 100°C to 105°C for 5 minutes and constant reading set the knob and check % moisture.

E) Determination of pH (1% w/v solution in water):

1gm of the Powder was taken and dissolved in 100ml of distilled water with sonication and filtered, pH of the filtrate was checked with standard glass electrode.

F) Melting point:

It is one of the parameters to judge the purity of drugs. A small quantity of powder was placed into a fusion tube. That tube is placed in the melting point determining apparatus containing castor oil. The temperature of the castor oil was gradual increased automatically and read the temperature at which powder started to melt and the temperature when all the powder gets melted.

G) Bulk properties:

Bulk density largely depends on particle shape, as the particles become more spherical in shape, bulk density increases. In addition as granules size increase, bulk density decrease. Bulk density was determined by measuring the volume of a known mass of powder sample that has been passed through a screen into a graduated cylinder or through a volumetric measuring apparatus into a cup. A known quantity of powder was poured into the measuring cylinder carefully level the powder without compacting, if necessary and read the unsettled apparent volume, Vo, to the nearest graduated unit. The bulk density was calculated in gm per ml gm/cc, by the formula

Bulk density = Bulk Mass/ Bulk Volume

H) Compressibility index (Carr's index)

Compressibility index (C.I.) is an important measure that can be obtained from the bulk and tapped densities. Carr's index a material having values of less than 20% to 30% is defined as the free flowing material.

It can be calculated as per given formula:

$$C.I. = \frac{100\,(V_0 - V_f)}{V_0} \quad OR \quad C.I = \frac{\text{Tapped density- Bulk density}}{\text{Tapped density}} \times 100$$

I) Hausner ratio:

It indicates the flow properties of the powder and is measured by the ratio of tapped density to bulk density.

Hausner ratio = Tapped density / Bulk Density

J) Flow properties

The angle of repose is a relatively simple technique for estimating the flowability of a powder through a funnel and fall freely onto a surface. The height and diameter of the resulting cone is measured and using the following equation, the angle of repose can be calculated.

Tan θ = h/r

Where h, r is the relatively height and radius of the powder cone.

For most pharmaceutical powders, the angle of repose values range form 25 to 45, with lower values indicating better flow characteristics. Values of angle of repose ≤ 30 usually indicate a free flowing material and angle ≥40 suggest a poorly flowing material.

L) MOISTURE CONTENT DETERMINATION

Principle: The titrimetric determination of water is based upon the quantitative reaction of water with an anhydrous solution of sulphur dioxide and iodine in the presence of a buffer that reacts with hydrogen ions.

In the original titrimetric solution, known as Karl Fisher Reagents, the sulfur dioxide and iodine was dissolved in pyridine and methanol. The test specimen may be titrated with the reagent directly, or the analysis may be carried out by a residual titration procedure. The titration of water is usually carried out with the use of anhydrous methanol as the solvent for the test specimen; however other suitable solvents may be used for special or unusual test specimens.

M) DRUG PARTITION STUDIES

Partition coefficient is defined as the ratio of unionized drug distributed between the organic and aqueous phase at equilibrium.

$$P_{O/W} = \frac{C_O}{C_W}$$

Where Co= Concentration of drug in n-octanol phase

C_w =Concentration of drug in water or PBS (7.4pH)

P.C. = Partition coefficient

Partition coefficient of Dexamethasone was determined in octanol: PBS (pH 7.4). Accurately weighed amount of drug (10mg) was taken in a glass stoppered test tube containing 10ml of n-octanol and 10 ml of PBS (pH 7.4).

The mixture was shaken on a wrist action shaker for 24hr. Both the phases were separated using separating funnel and the drug concentration in aqueous and octanol phase was determined by spectrophotometrically at 243.0 nm.

N) DETERMINATION OF λ_{max}

The absorption maximum of Dexamethasone was determined by running the spectrum of drug solution in double beam ultraviolet spectrophotometer.

Procedure: Accurately weighed 10 mg of drug was dissolved in 10 ml of 0.1 N HCl in 10 ml of volumetric flask and prepare suitable dilution to make it to a concentration of 10 µg/ml make adequate of sample with concentration range of 10-50 µg/ml. The spectrum of this solution was run in 200-400 nm range in U.V spectrophotometer (Labindia-3000+).

The spectrum peak point graph of Dexamethasone (absorbance versus wave length) was shown in figure no.

The Dexamethasone shows the absorbance maxima at 243.0 nm in 0.1 N HCl.

O) PREPARATION OF STANDARD CURVE OF DEXAMETHASONE IN 0.1 N HCl**:**

10 mg of Dexamethasone was accurately weighed and transferred to a 10 ml volumetric flask containing 10 ml of 0.1 N HCl and shaken to dissolve. The solution resulted is ≈1000 µg/ml. Then 0.1 ml of this solution is transferred to another 10ml volumetric flask to obtain solution of 10 µg/ml dilutions were done.

The absorbance was taken on double beam U.V. spectrophotometer using λmax at 243.0 nm. The absorbance values were plotted against concentration (µg/ml) to obtain the standard calibration curve.

Accurately weighed 10 mg of Dexamethasone was transferred into a 10 ml volumetric flask and dissolved in small amount of methanol and made up the volume with more of methanol to make the standard stock solution of 1000 µg/ml. The alliquots (0.1, 0.2, and 0.3….. upto 1.0 ml) of stock solution (1000 µg/ml) were transferred into 10 ml volumetric flask and volume was made up to 10 ml with PBS 7.4 pH Buffer to prepare concentration ranging from 10 to 50 µg/ml. The absorbance of these solutions was determined at 243.0 nm using Labindia UV spectrophotometer.

METHOD OF PREPRATION:

PRPRATION OF CHITOSAN MICROSPHERES[1,2,9,10]

Crosslinked Chitosan microspheres were prepared using emulsion method employing formaldehyde as crosslinker. Chitosan solution (2%w/v) was prepared in 2% aqueous acetic acid solution in which the drug was previously dissolved and dispersed in liquid paraffin (1:1 mixture of light and heavy) containing span 80 (1%w/v). The dispersion was stirred using a specially fabricated stainless steel half-moon paddle stirrer and formaldehyde saturated toluene solution (1ml to 3ml) was added with stirring. The stirring was continued further 4hr, then microspheres were centrifuged, washed two times with hexane and acetone and dried in vaccum dessicator for 48hrs.

Table 1: Preparation of Chitosan Microspheres

Batch No.	Drug: Polymer (w/w)	Emulsifier conc. (ml)
A1	1:2	0.75
A2	1:2	1.00
A3	1:2	1.25
A4	1:3	0.75
A5	1:3	1.00
A6	1:3	1.25
A7	1:4	0.75
A8	1:4	1.00
A9	1:4	1.25
A10	1:5	0.75
A11	1:5	1.00
A12	1:5	1.25

Coating of Chitosan Microsphres:

Chitosan microspheres were coated with ES100 using oil-in-oil solvent evaporation method. Chitosan microspheres (50 mg) were dispersed in 10 mL of coating solution prepared by dissolution of 500 mg of ES in ethanol:acetone (2:1) to give 5:1(coat:core ratio). This organic phase was then poured in 70 mL of light liquid paraffin containing 1% wt/vol Span 80. Thesystem was maintained under agitation speed of 1000 rpmat room

temperature for 3 hours to allow for the evaporationof solvent. Finally, the coated microspheres were filtered,washed with n-hexane, and dried in desicator.

CHARACTERIZATION OF MICROSPHERES:

Morphological Study of Microspheres[11,15]

The shape and surface morphology of chitosan microspheres were investigated by using scanning electron microscopy (SEM). The samples for SEM study were prepared by lightly sprinkling the formulation on a double adhesive tape stuck to an aluminum stub. The stubs were then coated with gold to a thickness of about 300 A^0 under an argon atmosphere using a gold sputter module is a high vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope.

Percentage Drug Entrapment[22,25]

10 milligram of microspheres were weighed and dissolved in 10 ml of 6.8 pH Phosphate Buffer. This solution was shaken with the help of wrist action shaking machine for 5 hrs and then kept for 24 hrs. Then it was filtered. The filtrate was assayed by UV spectrophotometer at 243.0 nm and percentage drug entrapment was determined using following formula.
% Drug entrapment= Practical content / Theoretical content*100

***In Vitro* Drug Release from Microspheres[30, 31, 32]**

In vitro drug release studies of both coated and uncoated microspheres were carried out in gastrointestinal fluid at different ph. extraction technique using USP dissolution test apparatus. The dissolution studies were carried out in 100 ml dissolution medium, which was stirred at 100rpm at 37±0.1°C (apparatus 2). The scheme of using the simulated fluids at different pH was as follows:

- **1st hour**: Simulated gastric fluid (SGF) of pH 1.2.
- **2nd and 3rd hour**: Mixture of simulated gastric and Intestinal fluid of pH 4.5.
- **4th and 5th hour**: Simulated intestinal fluid (SIF) of pH 6.8
- **6th hour:** SIF pH 7.5.Cross linked chitosan microspheres and Eudragit (S-100) coated chitosan Microspheres bearing drugs were suspended in dissolution media (100ml) at 37±0.1°C. Samples were withdrawn periodically and compensated with same amount of fresh dissolution media. The samples were analyzed for drug content by measuring absorbance using UV Spectrophotometer, Shimadzu 1700.

Stability Study Of Coated Crosslinked Chitosan Microspheres And Its Effect On Particle Size And Encapsulation Efficiency[27, 28, 33]

Stability of microspheres formulation on storage is of great concern as it is the major restraint in their development as marketed preparation. Eudragit (S 100) coated chitosan microspheres (Optimized formulation) was subjected to exhaustive stability testing during which they were stored in amber colored bottles at 4˚C, at Room Temperature and at 45±2˚C for 30 days period. Samples were withdrawn and observed for any change in particle size and encapsulation efficiency.

Partical Size[20,21,22]

Particle size analysis of microsphere was determined by using optical microscopy method. Approximately 500 microspheres were counted for particle size using a calibrated compound microscope.

Percent Entrapment Efficiency[25,26]

Percent Entrapment Efficiency of chitosan microspheres and Eudragit (S-100) coated chitosan microspheres was determined after removal of surface adsorbed drug. The surface adsorbed drug was removed by dispersing accurately weighed amount of microspheres in 10ml PBS pH 7.4 for 10min with occasional shaking. The suspension was then centrifuged at 3000rpm for 5min and the supernatant was kept aside. The sediment microspheres were retreated in the same manner and supernatant of this centrifuge was mixed with first supernatant and then these microspheres were then incubated for 48 hrs with PBS pH 7.4and the drug concentration was determined spectrophotometrically by UV at 230nm.
The Percent Entrapment Efficiency of Eudragit coated chitosan microspheres was determined in the same manner replacing PBS pH 7.4 with 0.1N HCl for removal of surface adsorbed.

RESULT AND DISCUSSION:

PHYSICO-CHEMICAL PROPERTIES OF DEXAMETHASONE
A.Organoleptic evaluation:

1. Description:

Table 2: Organoleptic property of Dexamethasone

Color	: White crystalline powder
Odor	: Characteristic

2. Solubility

Table 3: Solubility profile of Dexamethasone

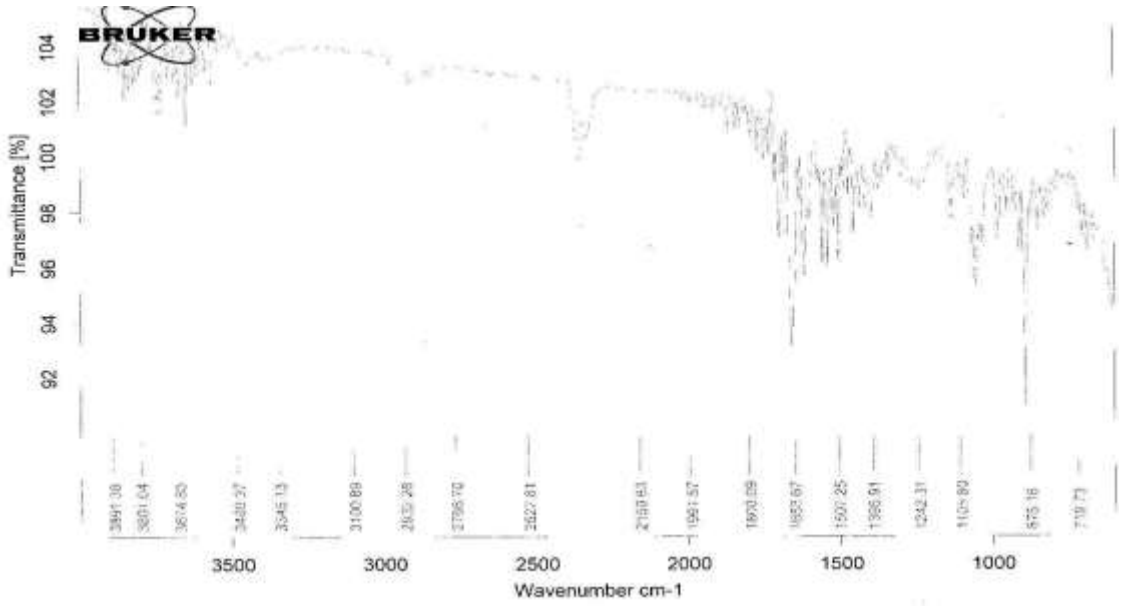
Sr. No.	Solvent	Solubility
		Deaxamethasone
1.	Water	+
2.	Methanol	++++
3.	Ethanol	++++
4.	Acetone	++++
5.	0.1 N HCl	+++
6.	PH 6.8 buffer	+++
7.	PH 7.4 buffer	+

Freely soluble + + + + (1-10 Parts of Solvents)
Soluble + ++ (10-30 parts of solvents)
Sparingly soluble + + (30-100 parts of solvents)
Slightly soluble + (100-1000 parts of solvents)
Insoluble - (More than 10,000 parts of solvents)

INFRARED SPECTRA OF DEXAMETHASONE

FTIR spectroscopy was performed using FT/IR Spectrometer and the curve found between the % transmittance and frequency (cm^{-1}) which is nearly similar with the reference spectrum of Dexamethasone. Results were shown in table

Figure 1: FTIR Spectra of Dexamethsone



Loss on Drying (LOD):

The percentage of loss on drying was found to be **0.215%w/w**.

Determination of pH (1 w/v solution in water):

The pH determination of Dexamethasone was found to be **3.8**

Melting Point: Melting point determine by Melting point apparatus and was found to be in the range **246-249°C**.

FLOW PROPERTY OF DEXAMETHASONE POWDER:

A. Bulk density:

Table 4: Bulk Density of Dexamethasone.

Sr. NO.	DENSITY	RESULT
1	Untapped Density	**0.344 g/cc**
2	Tapped Density (after 50 tapping)	**0.476 cc**

B. Compressibility Index (%): The compressibility index of Dexamethasone was found to be 18.75%

C. Hausner ratio: The Hausner ration of Dexamethasone was found to be **1.23.**

D. Angle of Repose

The Angle of repose of Dexamethasone was found to be **37.25** Degree.

Partical size pass through 40# is **100 (%w/w).**

E. Moisture by Karl-Fischer Apparatus (KF) : The Moisture content of Dexamethasone was found to be 0.285 **%.**

PARTITION COEFFICIENT

Partition coefficient is a measure of drug lipophilicity and an indication of its ability to cross biomembrane. For drug delivery, hydrophilic lipophilic balance (HLB) is an important factor. It is also useful in screening of some biologic properties .

Partition coefficient was found to be 1.82

DETERMINATION OF $\lambda_{max.}$

The dexamethasone shows the absorbance maxima at 243.0 nm in 0.1 N HCl and PBS Buffer pH-7.4.

PREPARATION OF STANDARD CURVE OF DEXAMETHASONE IN PBS BUFFER pH-7.4:

Standard Curve of Dexamethasone by Spectrophotometric Method

Table 5: Standard curve of Dexamethasone in 0.1 N HCL at 243.0 nm

Sr. No.	Concentration (µg/ml)	Absorbance	Equation of line
1.	10	0.234	y = 0.020x + 0.007 R^2 = 0.998
2.	20	0.425	
3.	30	0.615	
4.	40	0.815	
5.	50	1.063	

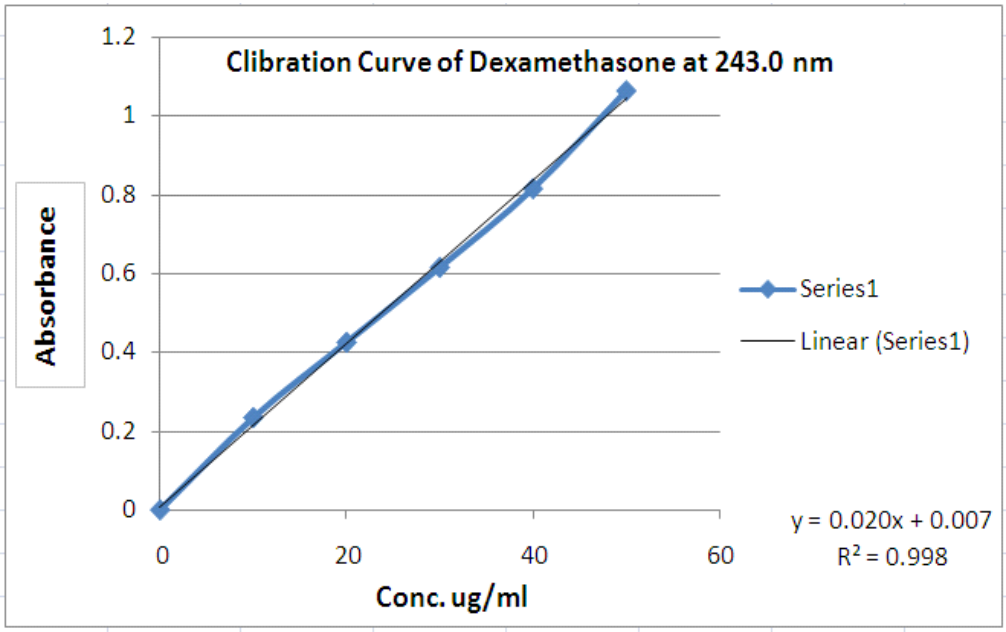


Figure 2: Preparation of calibration curve of Dexamethasone

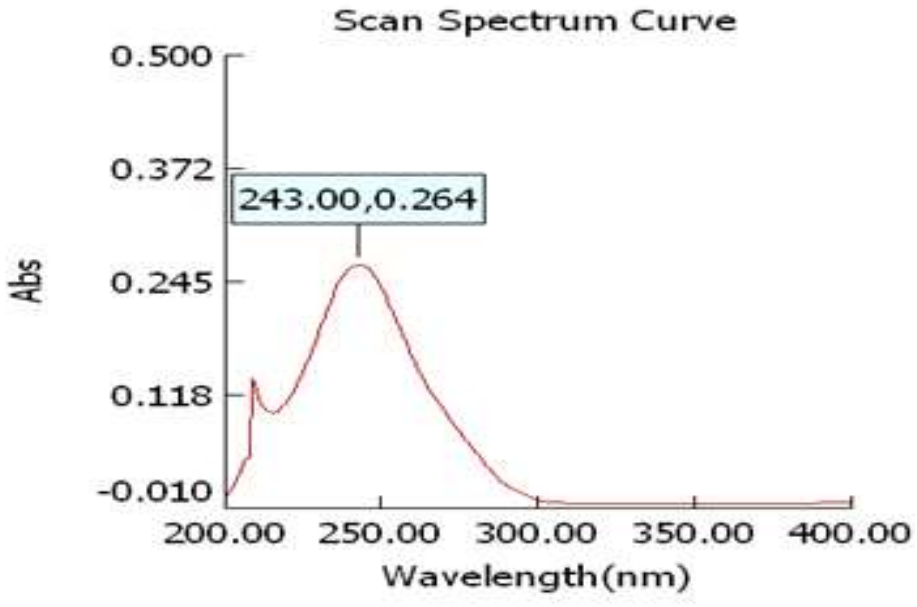


Figure 3: Standard curve of Dexamethasone in 0.1 N HCL at 243.0 nm

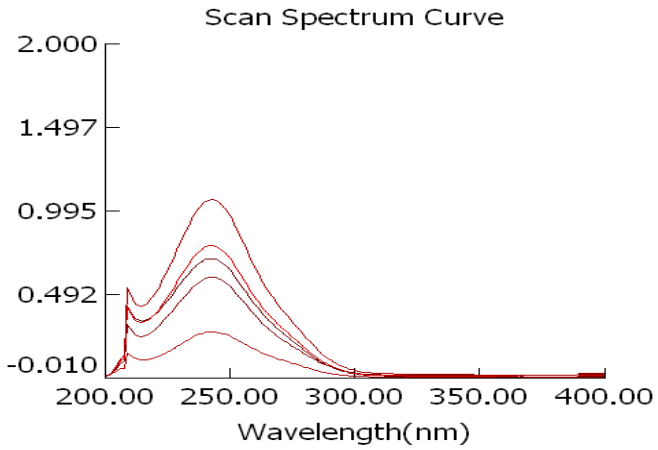


Figure 4: Calibration curve of Dexamethasone in PBS Buffer pH-7.4 at 243.0 nm

3D Spectra of Dexamethasone in PBS Buffer pH-7.4 at 243.0 nm

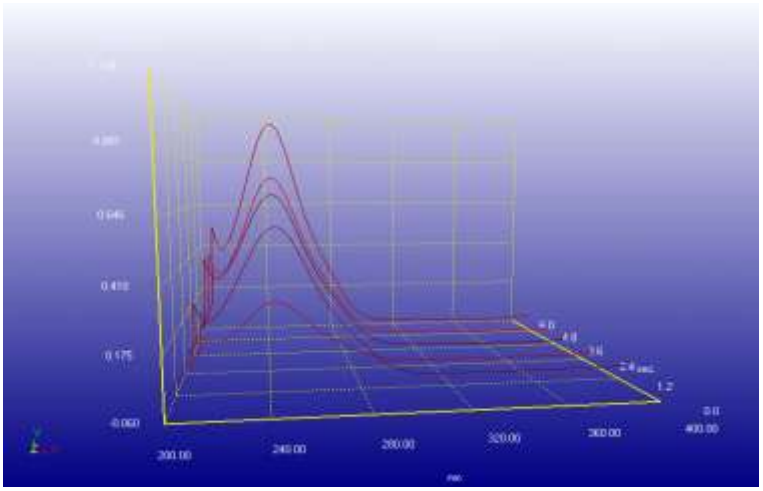


Figure 5: 3D Spectra of Dexamethasone in PBS Buffer pH-7.4 at 243.0 nm

RESULTS OF FORMULATION DEVELOPMENT:

Morphological Study of Microspheres:

Morphological Study of Microspheres of the chitosan microspheres were taken by scanning electron microscopy and were characterized in terms of sphericity and clumping of microspheres, as observed from the photomicrograph. Microspheres prepared from chitosan solution having concentration 4% were perfectly spherical, smaller clumping was found at 1000 rpm.

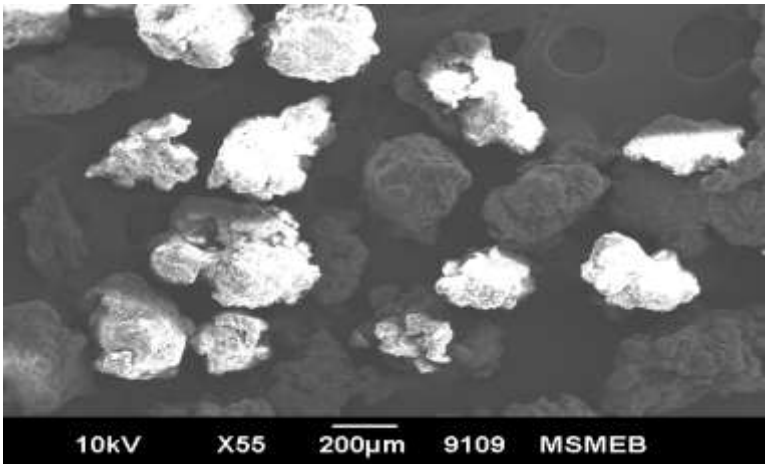


Figure 6: SEM of Microspheres

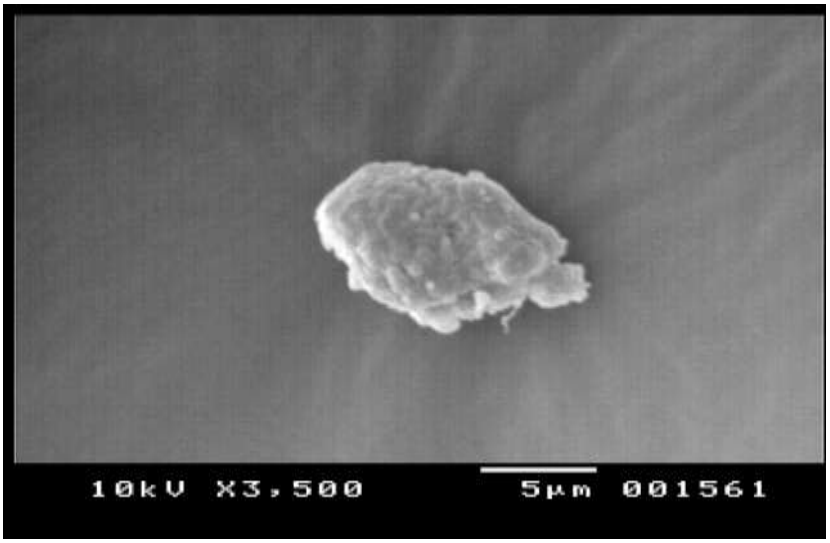


Figure 7: SEM of Microspheres (Magnified View)

In vitro drug release study:

Table No.6 In vitro drug release study

Sr. No.	Time (hrs)	*A1*	A2	A3	A4	B1	B2	B3	B4
1	1	*25.6*	22.1	20.4	19.5	0	0	0	0
2	2	*44.7*	40.2	38.5	36.9	0	0	0	0
3	3	*58.4*	54.5	52.2	51.5	0	0	0	0
4	4	*64.1*	60.4	57.8	59.9	6	4	2.8	0.8
5	5	*69.3*	65.0	63.6	62.1	17.8	11.3	8.5	4.5
6	6	*74.5*	70.9	67.5	66.7	29.5	22.2	19.5	13.5
7	7	*78.2*	75.1	73.3	71.7	39.8	36.2	31.3	27.3
8	8	*81.1*	78.8	76.4	75.2	49.0	43.2	38.5	33.5

%Drug release rate A1,A2,A3,A4 % drug release from chitosan microspheres(1:2,1:3,1:4,1:5) B1,B2,B3,B4 % drug release from eudragit coated chitosan microspheres (1:2,1:3,1:4,1:5)

RELEASE RATE FROM CHITOSAN: MICROSPHERES

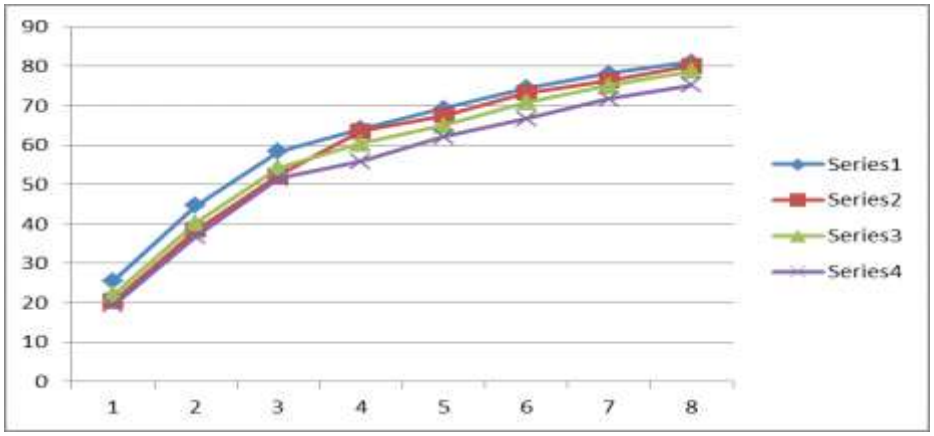


Figure 6: Graph of Release Rate from Chitosan Microspheres

RELEASE RATE FROM EUDRAGIT COATED CHITOSAN MICROSPHERES

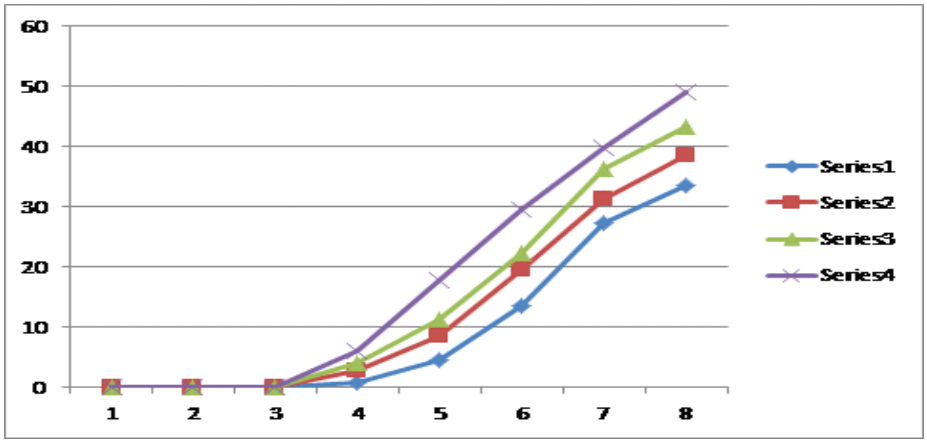
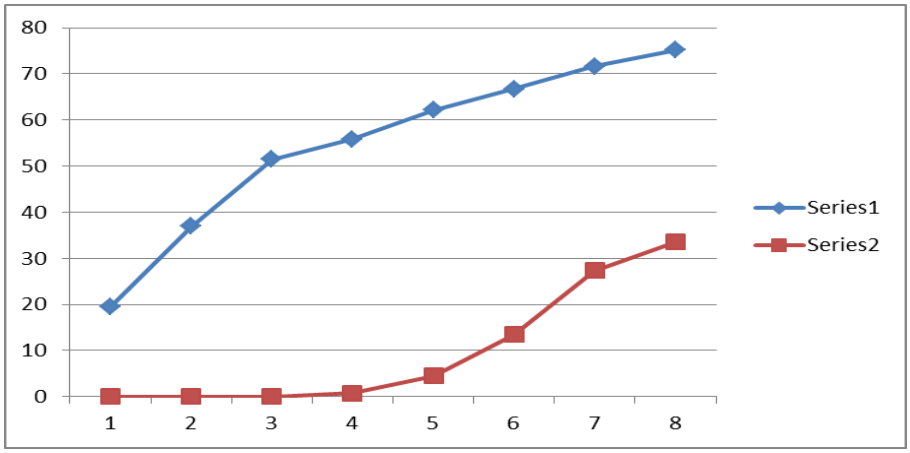


Figure 7: Release Rate from Eudragit Coated Chitosan Microspheres

Table 6 In vitro drug release from chitosan microspheres and Eudragit Coated Chitosan Microspheres

Sr. No.	Time (hrs.)	Percent Drug Release	
		Chitosan Microspheres	**Eudragit Coated Chitosan Microspheres**
1	1	19.5%	0
2	2	36.9%	0
3	3	51.5%	0
4	4	59.9%	0.8%
5	5	62.1%	4.5%
6	6	66.7%	13.5%
7	7	71.7%	27.3%
8	8	75.2%	33.5%

Figure 8: Drug release from chitosan microspheres and Eudragit Coated Chitosan Microspheres



In vitro dissolution study was conducted to understand in-vitro drug release profile of uncoated and coated microspheres. The purpose of this formulation was to avoid release of drug in gastric and upper intestinal region but to release the drug slowly in the lower part of the intestine maximizing drug concentration in the colon. Accordingly, the in-vitro drug release study was conducted in pH change method as per USP protocol.

STABILITY STUDIES:

Table 7: Effect of Storage on Particle Size and Percent Entrapment Efficiency of Chitosan Microspheres.

Parameters	Initial Observation	After 30 days		
		At 4ºC	**At RT**	**At 45±2 ºC**
Particle Size (µm)	105.5	104.8	105.4	107.3
Percent Entrapment Efficiency	80.4%	79.8%	80.1%	76.2%

Stability studies were carried out with optimized formulation which was stored for a period of 45 days at 4±1°C, RT and 40±1°C. The particle size of formulation was determined by optical microscopy using a calibrated ocular micrometer. The particle size of the microspheres was found to increase at RT, which may be attributed to the aggregation of microspheres at higher temperature. At 452°C the microspheres aggregate i.e. these microspheres were unstable at higher temperature like 452°C .Percent efficiency of microspheres also decrease at higher temperatureLike 452°C.

PARTICAL SIZE AND ENTRAPMENT EFFICIENCY:

Several batches of chitosan microspheres prepared using different drug: polymer ratios yielded microspheres in the size range between 61 and 110 µm. The percentage entrapment varied between 54 to 82 % depending on drug: polymer ratio and the emulsifier concentration. With increase in drug: polymer ratio, an increase in the entrapment efficiency and particle size . D:P ratio of 1:2 and 1:3 showed marginal change in the entrapment efficiency (54-66%) and particle size (61 – 80) while, D:P ratio of 1:4 and 1:5 recorded entrapment efficiencies in the range between 71-82 % and particle size between 90 – 110 microns. These figures demonstrate the requirement of D:P ratio in this range for satisfactory entrapment efficiency. Hence, D: P ratio of 1:5 and emulsifier concentration of 1 ml was optimized.

CONCLUSION:

After various searches on literature it is found that no work has been done on Dexamethasone for colon delivery. Dexamethasone is a glucocorticoid wirh proven anti-inflammatory & immunosuppressive action and found to be more effective than other glucocorticoid.

Microspheres are small spherical particles, with diameters in the micrometer range (typically 1 µm to 1000 µm (1 mm)). Microspheres are sometimes referred

to as microparticles. Dexamethasone microsphere offers site specific delivery directly in colon without any degradation in stomach. One useful discovery made from the research of microspheres is a way to fight cancer on a molecular level. Cancer microsphere technology is the latest trend in cancer therapy. It helps the pharmacist to formulate the product with maximum therapeutic value and minimum or negligible range side effects. A major disadvantage of anticancer drugs is their lack of selectivity for tumor tissue alone, which causes severe side effects and results in low cure rates. Thus, it is very difficult to target abnormal cells by the conventional method of the drug delivery system. Microsphere technology is probably the only method that can be used for site-specific action, without causing significant side effects on normal cells.

Controlled release drug delivery employs drug-encapsulating devices from which therapeutic agents may be released at controlled rates for long periods of time, ranging from days to months. Such systems offer numerous advantages over traditional methods of drug delivery, including tailoring of drug release rates, protection of fragile drugs and increased patient comfort and compliance. Polymeric microspheres are ideal vehicles for many controlled delivery applications due to their ability to encapsulate a variety of drugs, biocompatibility, high bioavailability and sustained drug release characteristics.

Research discussed in this thesis is focused on improving large-scale manufacturing, maintaining drug stability and enhancing control of drug release rate.

REFERENCES:

1. Aranaz et al "Functional Characterization of Chitin & Chitosan". Current Chemical Biology-2009.3, 203-230.
2. Sreenivas and S. A. Pai, K.V. "Synthesis of thiolated Chitosan : promising polymers for prolong mocoadhesive drug delivery". Int. J. Pharm-2009. 1 (3), 670-678.
3. Dodov, M.G. et al "Wheat germ agglutinin conjugated Chitosan-Ca-alginate microparticles for local colon delivery of 5–Fu": development and in vitro characterization. Int.J.Pharm-2009. 381 (2), 116-175.
4. Paharia, A. et al "Eudragit-coated pectin microspheres of 5-Fluorouracil for colon targeting". AAPS ParmsciTech-2007. 8 (1), E1-E7.
5. Asane, G.S. " Mucoadhesive gastrointestinal drug delivery system." Pharmainfo.net -2007, 5(6). 1-2.
6. Rahman, Z. et al, "Characterization of 5-FU microspheres for colon delivery". AAPS PharmSciTech-2006. 7 (2), 47-53.
7. Patel, J.K. et al. "Formulation and evaluation of mucoadhesive Glipzide microspheres". AAPS PharmsciTech-2005. 06 (1), E 49-E50.
8. Harsh and Mohan, "Textbook of Pathology" Jaypee Brothers medical Publishers (P) LTD-2005, 5th edition; New Delhi. 597-605.
9. Schnurch, A. B et al "Thiolated Chitosan: development and in vitro evaluation of a mucoadhesive, permeation enhancing oral drug delivery system". J. Control. Release-2004. 94, 117-186.
10. Dubey, R. R and Parikh, R. H. "Two stage optimization process for formulation of chitosan microspheres". AAPS PharmSciTech-2004. 7 (2), E1-E9.
11. Chowdary et al. "Mucoadhesive microspheres for controlled drug delivery". Biol. Pharm. Bull-2004. 27(11), 1717-1724.
12. Vasir, J.P., Tambwekar, K., Garg, S., "Bioadhesive microspheres as a controlled drug delivery system'. Int. J. Pharm 2003, 255, 13-32.
13. Anande, N. M et al. "Con-A conjugated mucoadhesive microspheres for the colonic delivery of diloxanide furoate". Int. J. Pharm-2002. 359(1-2), 182-189.
14. Vyas, S.P., Khar, R.K. , "Controlled drug delivery concepts and advances". Ist ed; CBS publishers and Distributors, 2002, New Delhi. 257-300.
15. Keisuke-Kurita. "Controlled functionalization of polysaccharide chitin". Polymer science-2002. 26, 1921-1971.
16. Vyas, S. P and Khar, R.K., "Targeted and controlled drug delivery". 1st edition. CBS Publishers and Distributors-2002, New Delhi. 417-450.
17. Rowe, R.C et al S. "Handbook of Pharmaceutical excipient".5th edition, Published by pharmaceutical press-2002.159-161.
18. Jain, N.K., "Advances in controlled and novel drug delivery", Published by CBS Publishers & Distributors, 1st edition-2001 New Delhi. 89-119.
19. Lorenzo-Lamosa A.L et al "Design of microencapsulated chitosan microspheres for colonic drug delivery". J. Control. Release-1998. 52, 109-118.
20. Lachman, L. et al "The theory and practice of Industrial Pharmacy", Varghese Publishing House-1991, Bombay,3rd edition,. 412-420.
21. V. Ravi, (2008) Novel Colon Targeted Drug Delivery System Using Natural Polymers70(1): 111–113.
22. Emeje M2009Preparation and evaluation of colon targeted drug delivery systems for albendazole using kneading, extrusion and compaction technology. 44(10):1152-1158
23. Sanjay J.KShirsagar, Mangesh R.Bhalekar ,Gajendra N.Shukla, Santosh K.Mohapatra2011Development

and Evaluation of MultiparticulateColon Targeted Drug Delivery System by CombineApproach of pH and Bacteria. Vol. 3, No.2, pp 1139-1149,

24. Deepak Prashar, Hemant Mittal & Rakesh Kumar(*2011)*Hydrocolloids Based Colon Specific Drug Delivery *Issue 3 (Vol. 1)*

25. http://en.wikipedia.org/wiki/Dexamethasone

26. http://www.nlm.nih.gov/medlineplus/druginfo/meds/a682792.html

27. Lorenzo-lamosa M.L., Design of microencapsulated chitosan microspheres for colonic drug delivery, Volume 52, Issues 1–2, 2 March 1998, Pages 109–118

28. Oosegi Tomago , Onishi Hiraku,Gastrointestinal distribution and absorption behavior of Eudragit-coated chitosan–prednisolone conjugate microspheres in rats with TNBS-induced colitis Volume 348, Issues 1–2, 4 February 2008, Pages 80–88

29. Pahariya Amol,Rai Gopal,Jain Sunil K.,Pancholi Shyam S., Eudragit-coated pectin microspheres of 5-fluorouracil for colon targeting, AAPS PharmSciTech ,March 2007, Volume 8, Issue 1, pp E87-E93

30. Thakral Naveen K, Ray Alok R., Majumdar Dipak K, Eudragit S-100 entrapped chitosan microspheres of valdecoxib for colon cancer, Journal of Materials Science: Materials in Medicine, September 2010, Volume 21, Issue 9, pp 2691-2699.

31. Ping He, Davis Stanley S, Chitosan microspheres prepared by spray drying, International Journal of Pharmaceutics , Volume 187, Issue 1, 30 September 1999, Pages 53–65.

32. Vaidya Ankur ,khare piyush,jain sanjay k, Metronidazole loaded pectin microspheres for colon targeting, Article first published online: 2 JUN 2009

33. DOI: 10.1002/jps.21742

34. R. DAVID, New oral delivery systems for treatment of inflammatory bowel disease, Advanced Drug Delivery Reviews, Volume 57, Issue 2, 6 January 2005, Pages 247–265

