FORMULATION AND EVALUATION OF pH SENSITIVE MICROSPHERES OF N-SUCCINYL CHITOSAN FOR THE TREATMENT OF DIVERTICULITIS

ABHAY RAIZADAY, HEMANT K.S. YADAV, AMRUTHA JAYANTH, S. R. KAUSHI, MIDHUNA MATHEW and ACHOO BIBY ZACHARIAH

Department of Pharmaceutics, JSS College of Pharmacy, JSS University, S.S. Nagar, Mysore-570015, Karnataka, India
✉ *Corresponding author:AbhayRaizaday,abhayraizadaypharma@gmail.com*

The present study involves the preparation and *in vitro* evaluation of pH sensitive colon targeted microspheres containing ciprofloxacin for the treatment of diverticulitis. They were formulated using N-succinyl chitosan and ciprofloxacin was entrapped by the spray drying method. The prepared microspheres were characterized by FT-IR, DSC and SEM. The microspheres were also evaluated as to particle size, encapsulation efficiency, micromeritic properties, swelling and *in vitro* drug release. FTIR and DSC studies showed no chemical interaction between the drug and the polymers. SEM photographs showed that the microspheres were roughly spherical in shape and free from cracks. The particle size of optimized microspheres was found to range between 3.45-6.34µm. The formulation showed encapsulation of the drug in the range of 80-95.06%. The microspherespresented good flow properties. Swelling and *in vitro* drug release studies were carried out and the results found were between 125-298% and 73.52-99%, respectively. It was concluded that the prepared microspheres can be effectively used in the treatment of diverticulitis.

Keywords: ciprofloxacin HCl, N-succinyl chitosan, microparticle, spray drying

INTRODUCTION

Over many decades, oral drug delivery has been a widely accepted route of administration for therapeutic drugs, due to better patient compliance, convenience, and ease of administration,but the gastrointestinal tract presents several formidable barriers to drug delivery. Because of the dreadful nature of the gastrointestinal tract (GIT), many of the drugs get destroyed or become inactive before reaching the site of absorption.To overcome these problems and make drug delivery better,creating a pH sensitive drug delivery system (DDS) would bea promising strategy. Colonic drug delivery has gained increased importance not just for the delivery of drugs or for the treatment of local diseases associated with the colon, but also for its potential for the delivery of proteins and therapeutic peptides.[1,2,3] 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 etc.[4,5,6]

Diverticulitisis a commondigestive disease, which involves the formation of pouches (diverticula) within the bowel wall.[7]This process is known as diverticulosis, and typically occurs within the large intestine, or colon, although it can occasionally occur in thesmall intestineas well.[8]

Hence, to achieve a successful colonic delivery, a drug needs to be protected from absorption or from the environment of the upper gastrointestinal tract (GIT) and then be abruptly released into the proximal colon, which is considered the optimum site for colon targeted delivery of drugs.[1]For this, several natural polysaccharides, which suffer hydrolysis of theirglycosidic bonds in the colonand allow the encapsulated drug to release from the polymeric network, such as chitosan (CS), pectin, guar-gum, dextrans and chondroitin sulfate, are promising for colon targeting. Their use is limited by their high solubility in thegastrointestinal (GI) fluids, which prevents their integrity until the colonic region.[9,10] Therefore, chemical modification has been proposed to reduce polymer solubility in the upper GI tract.[11] N-succinyl-chitosan (NS-chitosan) is a pH-sensitive chitosan derivative that has been obtained by introducing succinyl groups into N-terminals of the chitosan glucosamine units.[12,13] NS-chitosan shows favorable properties as a drug colon carrier, such as poor solubility in acid environment and long-term retention in the

body, biocompatibility, and low toxicity. Moreover, the hydrophilic groups make the polymer mucoadhesive; the NS-chitosan adheres to the mucosal tissue via hydrogen bonding between its carboxyl groups and the hydroxyl groups of the glycoproteins of the intestinal mucosa.[14]

Hence, the present study involves the preparation of pH sensitive microspheres for delivering drugs to the colon. Chemically modified chitosan, i.e. N-succinyl chitosan, was used as polymer for delivering ciprofloxacin to the colon because of the irritability it produces in the upper GIT. The formulation of N-succinyl chitosan microspheres of ciprofloxacin will be useful for the treatment of diverticulitis.

EXPERIMENTAL

Materials

Ciprofloxacin was received as a gift sample from Strides Arcolab Ltd, Bangalore. Medium molecular weight chitosan (190,000-310,000 Da; 75-85% deacetylated) was purchased from Sigma Aldrich, Mumbai. Succinic anhydride, DMSO, and glacial acetic acid were purchased from Lobachemie, Mumbai. All the reagents used were of analytical grade.

Method

Preparation of N-succinyl chitosan microspheres by spray drying technique

Preparation of N-succinyl chitosan as a pH sensitive polymer

Chitosan was dissolved in dimethyl sulfoxide with continuous stirring, to this solution succinic anhydride was added and this mixture was stirred for 3 hours using a magnetic stirrer. Glacial acetic acid was added in small quantities to increase the solubility of the chitosan polymer. This solution was kept standing for 24 hours. The pH of the above solution was adjusted to 5 using 5%w/v of NaOH solution and a precipitate was obtained. The precipitate obtained was filtered under suction and redispersed in distilled water. The pH of the above solution was increased to 10-12 using 5%w/v of NaOH. The formed solution was kept for filtration (membrane filtration) using presoaked cellophane sheets for 48hours. The obtained product was succinylated chitosan, which was characterized by using FTIR.The reaction is shown in Figure 1.

Preparation of drug loaded N-succinyl chitosan microspheres by spray drying technique

The polymer N-succinyl chitosan and the drug ciprofloxacin are freely soluble in water, so the drug and the polymer were dissolved in water; glacial acetic acid was used to prevent the precipitation of the polymer. The formed solution was spray dried under optimized conditions,i.e. input temperature: 150°C, output temperature: 110-115°C, feed rate: 2mL/min, vaccum pressure: -90mm, aspirator pressure:42 psi, nozzle diameter: 1mm.Microspheres were formed and collected in the receiver. The formulation chart is shown in Table 1.

Characterization of the prepared microspheres

Fourier transform infrared spectroscopy (FT-IR)

FT-IR studies were carried out using a Schimadzu FTIR 8400S. The pellets were prepared by pressing the sample (2mg) with KBr. The positions of the FT-IR bands of important functional groups of the drug and polymer were identified and were crosschecked withthe FT-IR spectra of the drug loaded formulation.

Differential scanning calorimeter (DSC)

DSC thermograms of the pure drug and of the prepared microspheres were obtained using a Schimadzu thermal analyzer DSC-60, Japan, at a scanning rate of 10ºC/min, over the temperature range 30-300ºC in liquid nitrogen environment (flow rate 10mL/min).

Scanning electron microscopy (SEM)

The morphology and surface characteristics of the prepared pH sensitive microspheres were determined using SEM (LEO, CFTRI Mysore). The photographs were observed for the morphological characteristics of the microspheres.

Evaluation of the prepared microspheres

Process yield[15]

The yield was determined by weighing the microspheres and then finding out the percentage yield with respect to the weight of the input materials, i.e. weight of the drug and polymers used. The percentage of yield was calculated using equation 1:

$$\% \text{ Yield} = \frac{\text{Weight of microspheres}}{\text{Weight of polymer} + \text{Weight of Drug}} * 100$$

Particle size analysis[16]

Particle size analysis was performed by optical microscopy using a compound microscope. A small amount of dry microspheres was suspended in liquid paraffin and was placed on the glass slide.The slide containing microspheres was mounted on the stage of the microscope, and the diameter of at least 300 microspheres was measured using a calibrated ocular micrometer. The process was repeated for each batch prepared.

Micromeritic properties of the prepared microspheres

Bulk density (ρ_b)

Accurately weighed quantities of granules were carefully placed into a graduated cylinder through a funnel and the bulk volume was noted.

Bulk densities were calculated from the following equation 2:

$$\rho b = \frac{M}{Vt} \quad (2)$$

Where **M** is the mass of the powder and $\mathbf{V_b}$ is the bulk volume of the powder.

Tapped density (δ_t)

An accurately weighed quantity of microspheres was carefully placed into the graduated cylinder through a funnel and the initial volume was noted; the graduated cylinder was tapped until there was no further reduction in volume and then the volume was noted. Tapped bulk densities were calculated from the following equation 3:

$$\rho t = \frac{M}{Vt} \quad (3)$$

Where **M** is the mass of the powder and $\mathbf{V_B}$ is the bulk volume of the powder.

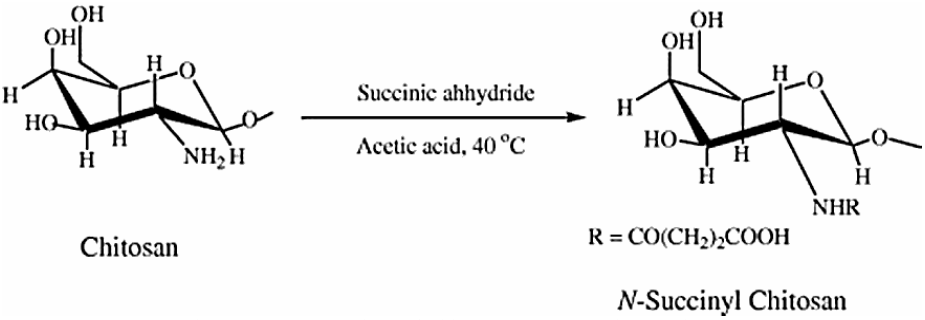


Figure 1: Conversion of chitosan to N-succinyl chitosan

Table 1
Formulation chart for preparation of microparticles

Formulation code	Drug:polymer ratio	Concentration of STPP
F1	1 : 0.5	0.0
F2	1 : 1.0	0.0
F3	1 : 1.5	0.0
F4	1 : 0.5	0.5
F5	1 : 1.0	0.5
F6	1 : 1.5	0.5
F7	1 : 0.5	1.0
F8	1 : 1.0	1.0
F9	1 : 1.5	1.0

Compressibility index and Hausner ratio

The compressibility index and the Hausner ratio were determined by measuring both the bulk volume and tapped volume of the powder.

The compressibility index and Hausner ratio may be calculated by using the values measured for bulk density (δ_{bulk}) and tapped density (δ_{tapped}). The following equation was used to calculate the compressibility index and the Hausner ratio:

$$\%\ \text{Compressibility Index} = \frac{\rho tapped - \rho bulk}{\rho tapped} X\ 100 \quad (4)$$

Determination of drug loading and incorporation efficiency of pHsensitive microspheres[16]

The drug content of pH sensitive microspheres was estimated using a UV-Visible spectrophotometer, Schimadzu-1800. Accurately weighed quantities of microspheres (5 0mg) were taken and dissolved in 50mL distilled water and stirred for 2 hours. The microspheres were magnetically stirred to promote swelling and breakup of the crosslinked structure. This afforded the liberation and subsequent dissolution of ciprofloxacin. The resultant mixture was subjected to centrifugation for 10 min at 3000 rpm in a centrifuge; the supernatant liquid was carefully separated and analyzed for drug content at 277nm.

The drug content was calculated by using equation 5:

$$\text{Amount of Drug} = \frac{\text{Conc. from standard graph} * \text{Dilution factor}}{1000}$$

Percent drug loading and encapsulation efficiency were calculated usingequations 6 and 7:

$$\text{Amount of Drug} = \frac{\text{Weight of drug}}{\text{Weight of microspheres}} * 100 \quad (6)$$

$$\text{Encapsulation Efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} * 100 \quad (7)$$

Measurement of swelling ratio of prepared microspheres[17]

The swelling property of pH sensitive microspheres was studied by immersing the microspheres into aqueous solutions of pH 7.0 phosphate buffer for 12 hours. The microspheres were removed from the aqueous solution, after the removal of excess surface water with filter paper; the microspheres were weighed immediately using a digital balance. The degree of swelling (W_t) was calculated by equation 8:

$$S = \frac{(\text{Wt of swollen microparticles} - \text{Wt of microparticles})}{\text{Weight of swollen microparticles}} * 100 \quad (8)$$

Each swelling experiment was repeated three times and the average value was recorded.

***In vitro* release studies**[18]

In vitro studies on ciprofloxacin were carried out in triplicate at 37±0.1ºC in a USP XXII dissolution apparatus type II (paddle dissolution tester USP XXII, Electrolab, Mumbai) at paddle rotation of 50 rpm, in 900mL of pH 1.2 HCl buffer for the first 2 hours, and then in 900 mL of pH 7.0 phosphate buffer solution for 10 hours. An aliquot of the release medium (5mL) was withdrawn through a sampling syringe at predetermined time intervals and was replaced by an equivalent amount of fresh dissolution medium, which was prewarmed at 37ºC. The collected samples were then analyzed for ciprofloxacin content by measuring the absorbance at 277 nm after suitable dilution, using a Schimadzu UV-1700 double beam spectrophotometer.

RESULTS AND DISCUSSION

Synthesis and characterization of N-succinyl chitosan as pH sensitive polymer

From the chitosan spectrum in Figure 2, it can be noted that distinctive absorption bands appear at 1646 cm^{-1} (Amide I), 1603cm^{-1}(–NH_2 bending) and 1390 cm^{-1} (Amide III). The absorption bands at 1153cm^{-1} (asymmetric stretching of the C–O–C bridge), 1081 cm^{-1} (skeletal vibration involving the C–O stretching) are the characteristics of its saccharine structure.

Compared with the spectrum of chitosan, in that of N--succinyl chitosan, there appears an absorption band at 2922 cm^{-1} (stretching of –CH_2–), the peaks at 3409 and 1602 cm^{-1} (amino group characteristics) decrease greatly, and the peaks at 1659cm^{-1} (Amide I) and 1382 cm^{-1} (Amide III) increase, these results indicating that the succinyl derivation reaction took place at the N-position (Scheme 1) and –NH–CO– groups have been formed, which is shown in Figure 3.

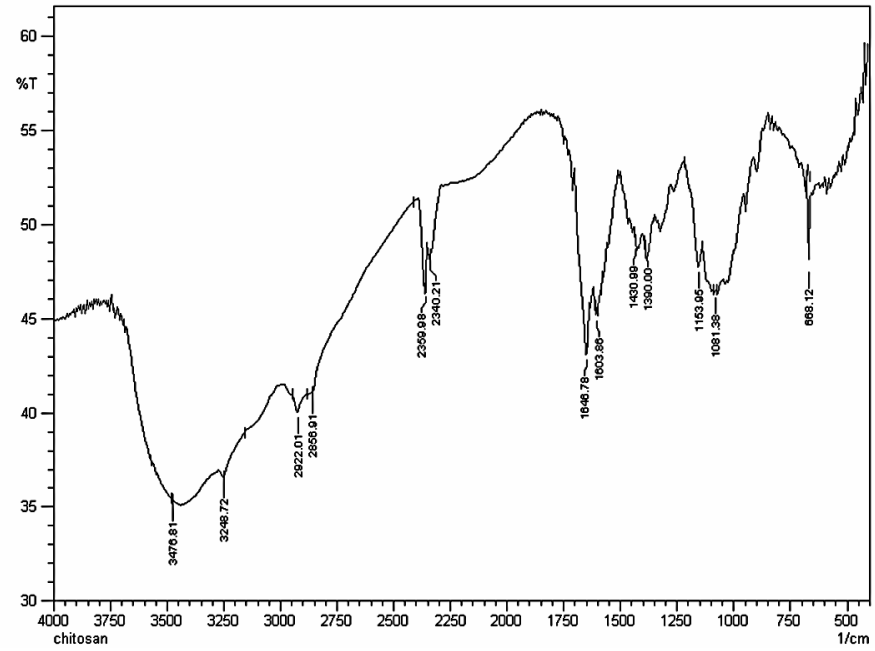


Figure 2:-FTIR of chitosan polymer

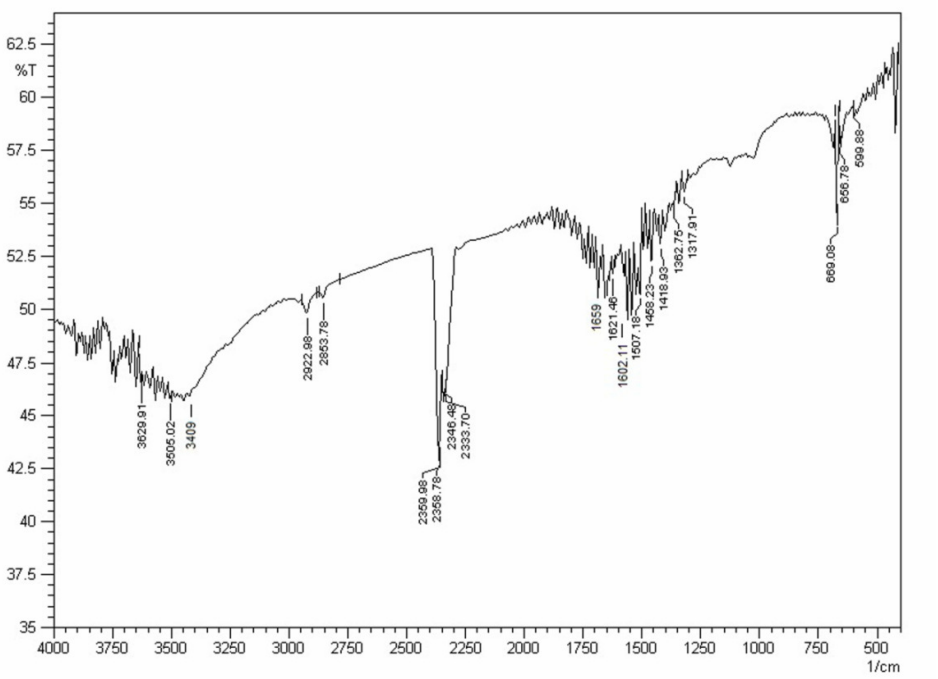


Figure 3: FTIR of N-succinyl chitosan polymer

Table 2
Different trials carried out to optimize the spray drying condition

Trial No	Inlet temp. (^{0}C)	Outlet temp. (^{0}C)	Aspiration (%)	Vacuum pressure (mm)	Compression pressure (Psi)	Feed rate (%)	%Yield
1	150	95-110	35	-75	25	6	12.1
2	150	95-110	35	-75	25	4	46.03
3	150	95-110	42	-95	25	2	66.6
4	150	95-110	40	-95	25	2	60.12
5	150	95-110	35	-95	25	2	45.09

Optimization of process parameters

Various process variables that could affect the preparation and properties of the microspheres were identified and optimized to get small discrete and spherical microspheres with a better production yield. The parameters to be optimized were: input temperature, output temperature, feed rate, vaccum pressure, aspirator pressure, nozzle diameter. The process giving the highest production yield was selected. Trial 3 gave the highest percentage yield because the feed rate was low and aspiration was 42%, allowing enough time for the liquid droplet to get dried, while trial 1 was gave the least percentage yield because the feed rate was high, leading to minimum drying of the feed material. The experimental conditions applied in different trials, i.e input temperature, output temperature, feed rate, vacuum pressure, aspirator pressure and nozzle diameter, are shown in Table 2.

Characterization of prepared pH sensitive microspheres

FT-IR

FT-IR studies were conducted to find out any possible interaction between the drugand polymer. It was concluded from the FT-IRstudies that the characteristic peaks of the drug, polymer and of the prepared formulation were not altered, indicating that there was no chemical interaction between drug and polymer,i.e.the N–H stretch of primaryamine was observed at 3530 cm^{-1}, primary amine (N–H bend) at 1611 cm^{-1}, carboxylic acid stretching (C=O stretch)at1486 cm^{-1} and C-H (out of plane band)at 804 cm^{-1}. The same peaksas those for the drug were observed for the formulation, with no significant shift in the peak

position. The FT-IR spectra of the pure drug and of the formulation are shown in Figures 4 and 5.

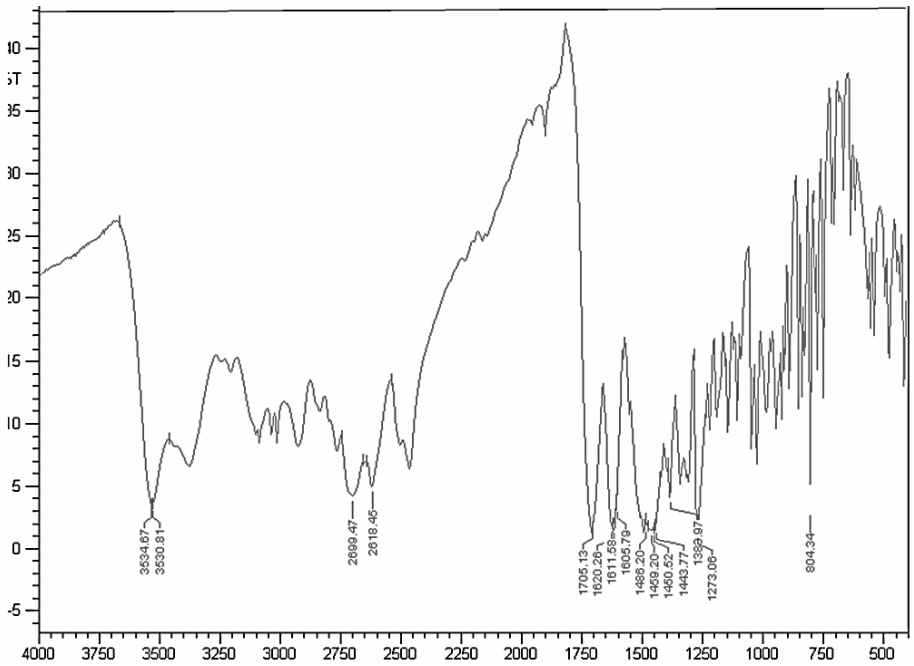


Figure 4: FTIR spectra of pure drug (Ciprofloxacin)

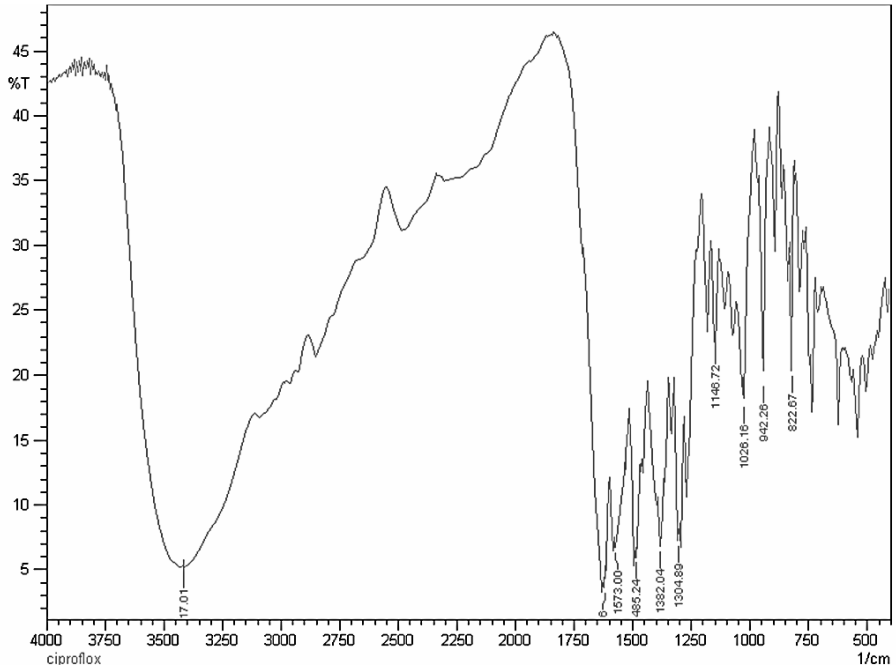


Figure 5: FTIR spectra of the formulation

Differential scanning calorimetry (DSC)

The thermogramsof the pure drug and of the physical mixture exhibit a characteristic, sharp endothermic peak at 320ºC, which is associated with the melting point of the drug and indicates the amorphous nature of the drug. The DSC of the pure drug and of the formulation are shown in Figures 6 and 7.

Scanning electron microscopy (SEM)

The shape and surface characteristics of the pH sensitive microspheres were observed by SEM. The SEM photograph indicated that the microspheres formed were roughly spherical. The SEM of the prepared microspheresis presented in Figure 8.

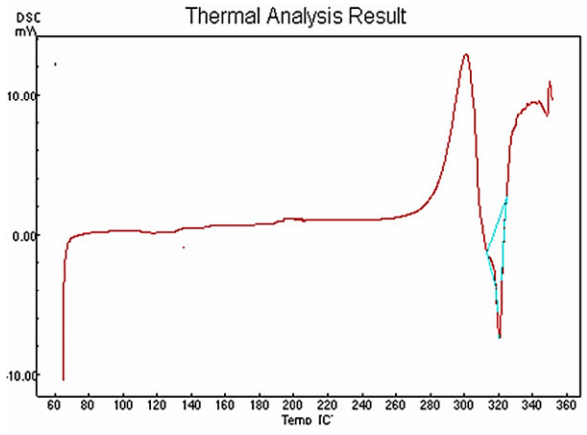


Figure 6: DSC of pure drug (Ciprofloxacin)

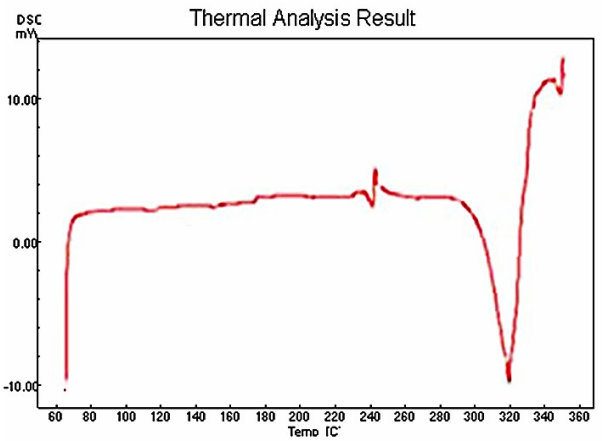


Figure 7:DSC of the formulation

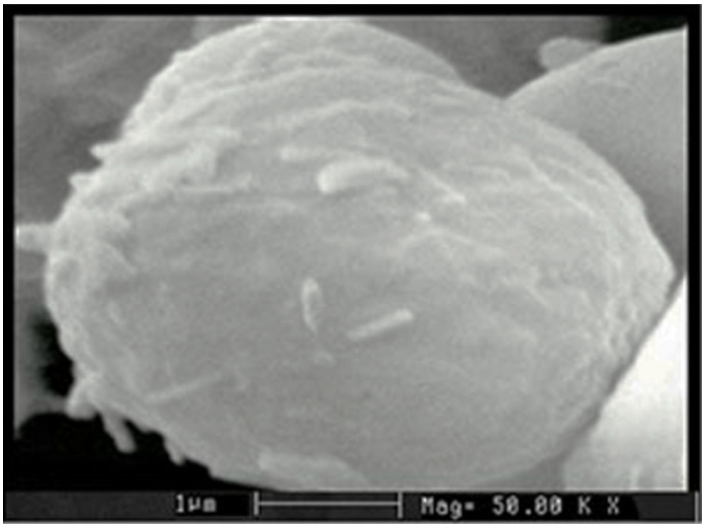


Figure 8: SEM image of n-succinyl drug loaded microparticles

Percentage yield of process

The ratio of polymer concentration and sodium tripolyphosphate(STPP) concentration showed a direct impact on the obtained percentage yield of microspheres. Graph 1shows the role of the STPP in the formation of the microspheres. As the concentration of the STPP increases with respect to the polymer the % yield also increases. This is due to the crosslinking of the polymer around the drug, leading to higher polymer coating onto the drug. The % yield of formulation F9 is greater compared to those of other formulations because the concentration of polymer is 1.5 %w/v and the concentration of STPP is 1.0 %w/v (crosslinking agent), causing the complete encapsulation of the drug within the polymer, leading to a greater production yield. The percentage yields of various formulations are shown in Graph 1.

Particle size determination

The size of the particles depended on the concentration of the polymer and that of the crosslinker, and ranged from 3.45-6.34µm, as shown in Graph 2. It was observed that with the increase in polymer concentration, particle size increased, as noted for formulations F3, F6 and

F9; an opposite trend was noted with the increase in the amount of crosslinker. The increase in polymer concentration led to increased viscosity, which caused the formation of bigger droplets during atomization with a minimum change in particle size, whereas at low polymer concentration the bulk of the droplet consisted of solvent, which rapidly evaporated, resulting in shrinkage and accordingly small particle size, which decreased with an increasing concentration of STPP as crosslinker. The particle size of formulation F7 was 345nm, because during crosslinking, the polymeric network might have undergone rapid shrinking, leading to the formation of a smaller and rigid matrix at a higher crosslinking concentration.The particle size of various formulationsis shown in Graph 2.

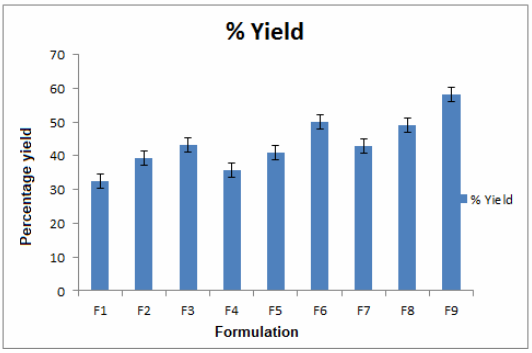
Determination of drug loading and encapsulation efficiency

Comparisons of the ciprofloxacin loading efficiency for different pH sensitive microspheres are shown in Graph 3. The encapsulation efficiency ranged between 80 to 95% in the prepared formulation. In formulations F1, F4 and F7, the encapsulation efficiency was 80.60, 83.42 and 85.50%, which was low when compared to F3 (88.49%), F6 (90.16%) and F9 (95.06%),and this may be attributed to the fact that the increase in the concentration of the polymer leads to higher encapsulation of the drug,which means an increase in thedrug encapsulation efficiency.

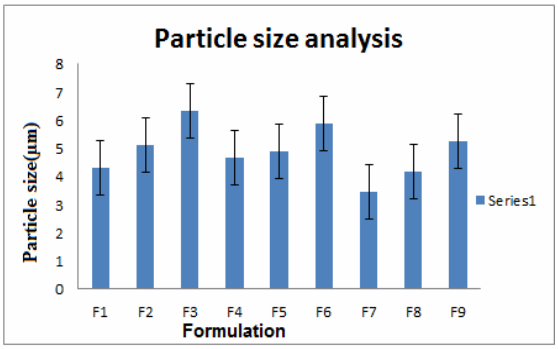
Formulations F4-F9 showed better encapsulation efficiency than formulations F1-F3, which can be attributed to the crosslinking (STPP) with the polymer, resulting in the formation of a more rigid network structure, which leads to greater encapsulation of the drug. Drug loading and encapsulation efficiency of various formulations are shown in Graphs 3 and 4.

Micrometric properties

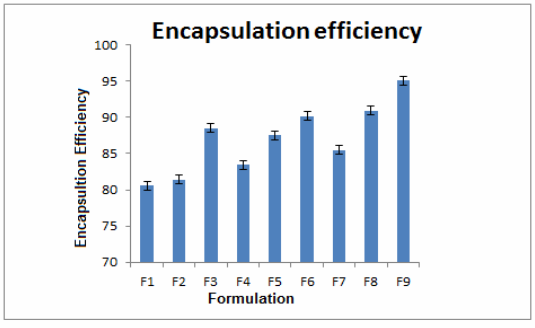
The flow property of the microspheres was studied by calculating the %compressibility index (CI). The obtained data along with optimum parameters are presented in Table 3. The result clearly showed that the prepared microspheres had reasonably good flow potential. The value of CI was found to be in the range of 8.7-13.12%. The values of tapped density ranged between 0.45-0.69g/cm^3.



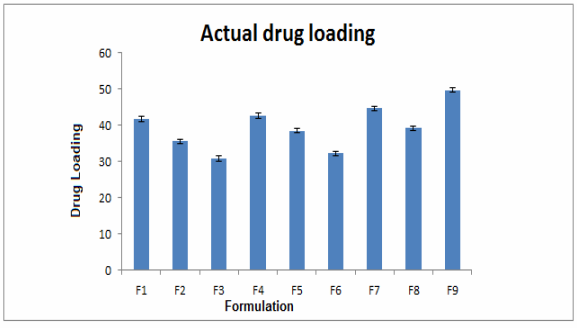
Graph 1: % Yield of pH sensitive microparticles



Graph 2: Particle size of pH sensitive microparticles



Graph 3: Actual drug loading of pH sensitive microparticles



Graph 4: Encapsulation efficiency of pH sensitive microparticles

Table 3
Micromeritic properties of microparticles

Formulation	Bulk density Mean ± SD	Tapped density Mean ± SD	Carr's Index Mean ± SD	Hausner's ratio Mean ± SD
F1	0.48 ± 0.23	0.621± 0.14	9.87 ± 0.11	1.104 ± 0.1O
F2	0.446 ± 0.11	0.516 ± 0.17	8.714 ± 0.41	1.128 ± 0.02
F3	0.412 ± 0.73	0.541 ± 0.61	10.01 ± 0.41	1.132 ± 0.05
F4	0.489 ± 0.12	0.612 ± 0.49	13.01± 0.13	1.135 ± 0.31
F5	0.506 ± 0.27	0.69 ± 0.17	12.864 ± 0.285	1.117 ± 0.29
F6	0.426 ± 0.18	0.545 ± 0.76	10.426 ± 0.31	1.136 ± 0.12
F7	0.489 ± 0.78	0.628 ± 0.61	13.12 ± 0.24	1.135 ± 0.22
F8	0.480 ± 0.91	0.613 ± 0.32	12.426 ± 0.25	1.110 ± 0.15
F9	0.442 ± 0.45	0.45 ± 0.75	8.529 ± 0.41	1.101 ± 0.21

*Standard deviation, n = 3

The difference in the density values of the formulations was negligible, the values being well within the limits, which indicated that the prepared microspheres were non-aggregated and spherical. Hausner's ratio was below 1.25, which indicates a good flowability of the prepared microspheres. Formulation F9 had better micrometric properties because the particles were of spherical shape and possessed good flowability behaviour. This was due to the fact that the ratio of polymer and crosslinking agent was at an optimized level. The micrometric properties of various formulations are shown in Table 3.

Swelling index

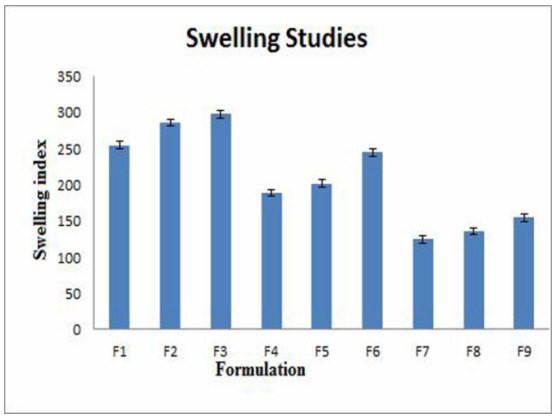
The *in vitro* swelling property of the microspheres was studied at pH 7.0. The swelling of the microspheres is due to the ionization of the acetyl group, leading to repulsion between similar charges along with an increase in osmotic pressure, and hence swelling occurs.[19] The swelling of the microspheresisillustrated in Graph 5, which indicates that with an increase in polymer concentration, the degree of swelling also increased. The formulations of uncrosslinked microspheres showed rapid swelling, followed by erosion, compared to the formulations of crosslinked microspheres. At the end of the study, the swelling was found to be greater in the formulation containing 0.5% STPP, compared to that with 1% STPP. This can be explained by the fact that the rate of swelling gradually increased without erosion.

The crosslinked microspheres using 1% STPP showed the least swelling, which can be attributed to the higher extent of crosslinking with enhanced matrix rigidity. These swelling results are very well supported by the *in vitro* drug release results. The swelling index of various formulationsis shown in Graph 5.

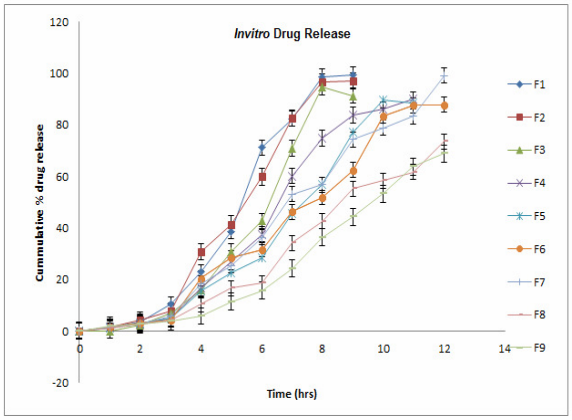
***In vitro* release studies**

The drug release for the first two hours was minimal, because the drug was encapsulated inside the acid sensitive polymer, which prevented the release of the drug in the media. Still, some of the drug was released in the first two hours, this happened because some of the drug was present on the surface of the microspheresand it wasreleased when the particles came in contact with the gastric fluid. As the polymer to drug ratio was increased, the extent of drug release decreased. A significant decrease in the rate and extent of drug release is attributed to the increase in the density of the polymer matrix, which resulted in increased diffusion path length that the drug molecules had to traverse. The uncrosslinked microspheres (F1, F2 and F3) showed complete release by the end of 8h. Crosslinked microspheres with 0.5% STPP (F4, F5 and F6) showed % cumulative drug release (CDR) between 87 to 90 at the end of 12h. Crosslinked microspheres containing 1% STPP (F7 to F9) showed incomplete release at the end of 12h. The release pattern for each formulation may be due to some possible reasons, as follows.The uncrosslinked microspheres would swell rapidly allowing faster dissolution with complete release, whereas the gel rigidity was enhanced in the presence of crosslinking agent, which did not allow rapid entry of dissolution media, showing slow swelling with a sustained release pattern. As to formulations F7, F8 and F9, the crosslinking was too high, which accounted for the very slow and incomplete release profile.[20] The above release patterns are in agreement with the swelling studies. On the basis of the swelling,

encapsulation efficiency and *in vitro* drug release studies, F7 was chosen as the optimimum formulation. The *in vitro* drug release of various formulations is shown in Graph 6.



Graph 5: Swelling studies of pH sensitive microparticles



Graph 6: *In vitro* drug release of pH sensitive microparticles

CONCLUSION

pH Sensitive microspheres were prepared by the spray drying technique,using different ratios of STPP and N-succinyl chitosan. From the data obtained, it can be concluded that the prepared pH sensitive microspheres can be used effectivelyin the treatment of diverticulitis.

ACKNOWLEDGEMENT: The authors sincerely thank Dr. H. G. Shivakumar, Principal, JSS College of Pharmacy, Mysore, for his support and encouragement. Our gratitude goes to JSS University, Mysore, for providing all the necessary facilities.

REFERENCES

[1] A. Lamprecht and Y. Kawashima, *J. Drug Deliv. Sci. Technol.*, **16**, 26(2006).
[2] H. Takeuchi, T. Handa and Y. Kawashima, *Drug Dev. Ind. Pharm.*, **15**, 1999(1989).
[3] P. Tayade and R. T. D. Kale, *AAPS Pharm. Sci.*, **6**, 12(2004).
[4] K. Kavita and V. A. Raje, *Int. J. Pharm. Sci. Rev. Res.*, **5**, 12(2010).
[5] K. A. I. Tadwee, S. M. Shahi and S. Ankit, *Int. J. Pharm. Res. All. Sci.*, **1**, 24(2012).
[6] L. R. Alysson and P. O. Wanderley, *Powder Tech.*, **171**, 7(2007).
[7] A. F. Peery, P. R. Barrett,D. Park, A. J. Rogers, J. A. Galanko *et al.*, *Gastroenterology*, **142**, 266(2012).
[8] L. Weisberger and B. Jamieson, *J. Fam. Pract.*, **58**, 381(2009).
[9] A. Rubinstein, D. Nakar and A. Sintov, *Int. J. Pharm.*, **84**, 141(1992).
[10] M. Ashford, J. Fell, D. Attwood, H. Sharma and P. Woodhead, *J. Control. Release*, **30**, 225(1994).
[11] V. R. Sinha and R. Kumria, *Int. J. Pharm.*, **224**, 19(2001).
[12] S. Hirano, T. Moriyasu, *Carbohyd. Res.*, **92**, 323(1981).
[13] R. Yamaguchi, Y. Araj, T. Itoh and S. Hirano, *Carbohyd. Res.*, **88**, 172(1981).
[14] M. R. Rekha, C. P. Sharma, *Trends Biomater. Artif. Organs*, **21**, 107(2008).
[15] D. Perumal, C. M. Dangor, A. R. S. Hurbons and K. R. Moopanar, *J. Microencapsul.*, **16**, 475(1996).
[16] A. A. Kahtani, B. S. Scherigara, *J.Mater. Sci.Mater. Med.*, **20**, 1437(2009).
[17] J. Du, J. Dai, J. L. Liu, T. Dankovich, *Funct. Polym.*, **66**, 1055(2006).
[18] N. A. Peppas, *Pharm. Acta Helv.*, **60**, 110(1985).
[19] H. Takeuchi, T. Handa and Y. Kawashima, *Drug Dev. Ind. Pharm.*, **15**, 1999(1989).