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Rheumatoid Arthritis Chronopharmacological Symptom Synchronization with Colon-Targeted Delivery of Drug

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Abstract: The objective of drug delivery research is to create formulations that address specific clinical conditions and fulfil therapeutic requirements. The ongoing investigation focuses on analyzing the influence of several factors, including polymer concentration, emulsifier concentration, stirring speed, and cross-linking agent concentration, to enhance the microspheres for precise and controlled delivery of medication to the colon, specifically for the treatment of rheumatoid arthritis. The current study employed the widely documented techniques for the development of the ultimate formulation. The main focus of the analysis was to determine the legitimacy and compatibility of the drugs and excipients. The impact of these independent variables was noticed on the parameters of particle size, polydispersity index (PDI), and entrapment efficiency. The optimized batch was assessed for several criteria. An assessment of the stability of the final batches was conducted. The findings additionally indicated the existence of a maximum rate at which a specific polymer concentration can be mixed. The needed concentration of pectin is determined accordingly to the polymer concentration. Pectin necessitates a rotational speed of 2000 rpm, xanthan gum and chondroitin sulphate necessitate a rotational speed of 3000 rpm, while chitosan and guar gum require a rotational speed of 4000 rpm. The optimal mixing time for pectin microspheres was found to be 60 min, while chitosan required 2.5 h, and guar gum, chondroitin sulphate, and xanthan gum required 4 h. A emulsifying agent was used to improve the formation of a long-lasting microsphere. The concentration of the emulsifier used determines the average size of the microspheres.

It has been discovered via the ongoing research that the rotational speed also affects the average diameter of microspheres. In order to enhance the particle size of the microspheres, a range of rotational rates, varying from 1000 rpm to 4000 rpm, were investigated. The optimal particle size of the microspheres depends on the rotational velocity.

Keywords: Rheumatoid arthritis, Chronopharmacological, Synchronization, Xanthan gum, Chitosan, Guar gum

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Introduction

The objective of drug delivery research is to create formulations that address specific clinical conditions and fulfil therapeutic requirements. The temporal fluctuation of physiological and pathological activities has led to a novel approach in the design of medication delivery devices. Studies in the field of chronopharmacology have shown the significance of biological rhythms in the administration of drugs (Singh, 2007). If the symptoms of an illness exhibit circadian variation, it is necessary for the release of drugs to similarly change according to the time of day (Kowanko *et al*., 1981). In recent years, there has been a growing use of various technologies in the creation of drug delivery systems that are time controlled, pulsed, triggered, and programmable. Biopharmaceutical and pharmacokinetic studies should be conducted to justify the choice of the optimal administration time for formulations. Furthermore, it should be noted that the circadian variation of physiological function can also have an impact on the time-dependent nature of medication pharmacokinetics. When constructing drug delivery systems for treating diseases, it is important to consider both the fluctuations in the disease itself and the concentration of the drug in the plasma. This ensures that the drug is delivered in the right amount at the right time.

Drug delivery system design has thus far been influenced by the ideas of homeostatic theory. This hypothesis is based on the assumption that biological functions demonstrate temporal consistency. Research in chronobiology has substantiated the presence of circadian rhythms in almost all physiological processes, including heart rate, blood pressure, body temperature, hormone levels, gastric acidity, and kidney function (Kokande *et al*., 2024). The importance of rhythmic processes in the treatment of human diseases is clear, as physiological functions vary across time and disease progression follows circadian rhythms (Tiwari *et al*., 2020). Epidemiological studies indicate a higher likelihood of experiencing signs of sickness during a 24 h period. Human diseases, including asthma, arthritis, angina, myocardial infarction, allergies, inflammation, cancer, and others, have a circadian rhythmic pattern. The exacerbation of such illnesses reaches its peak intensity during specified periods of the day (Khambete *et al*., 2016).

Morning stiffness is regarded as a diagnostic criterion for rheumatoid arthritis. Upon waking up and in the early morning, there is an increase in joint size, stiffness, and soreness, which happens at the same time as the period of lowest grip strength. The diurnal pattern of interleukin-6 levels may synchronize with the pattern of rheumatoid arthritis symptoms (Smolensky and Peppas, 2007).

Chronopharmacotherapy involves the timing of drug delivery in accordance with biological rhythms to get the most therapeutic benefit while minimizing harm to the patient. Optimizing medicine distribution based on circadian patterns of diseases can enhance treatment efficacy and minimise negative effects. If symptoms manifest during the daytime, it is advisable to administer a standard dosage form just before the symptoms intensify (Dyer, 1965).

Materials and Methods

The drug was acquired as a complimentary sample. All excipients utilised in the investigation were of standard grade and were obtained from licensed vendors. All the remaining solvents and ingredients utilized were of analytical grade.

Preformulation studies:

Differential Scanning Calorimetry (*DSC*):

Thermal analysis is a commonly employed method for examining pharmaceuticals and compounds that have pharmacological importance. Differential Scanning Calorimetry (DSC) offers insights into multiple characteristics of pharmaceuticals, such as their melting behaviour, heat of fusion, purity, polymorphism, pseudo-polymorphism, glass transition, compatibility, crystallization, and chemical interactions. Additionally, it aids in evaluating the stability and kinetics of decomposition. This method quantifies the difference in energy inputs between a substance and a reference material based on temperature, while putting the samples to a controlled temperature schedule (Leopold *et al*., 2000).

Fourier transform infrared (*FTIR*):

The structure characterization was performed using Fourier transform infrared (FTIR) analysis. The FTIR spectra of the pure drug, polymers, and their physical mixes were obtained. The samples were collected in a KBr pellet using a Bomen MB series FTIR spectrometer. A total of 5 mg of KBr of high quality for spectroscopic analysis, along with the samples, were subjected to infrared scanning within the range of 500 to 3500 cm^{-1}, with a resolution of 4 cm^{-1} (Thombre *et al*., 2022).

Preparation of microspheres:

Pectin microspheres:

The objective of this study was to develop and evaluate Eudragit-coated pectin microspheres for targeted administration of aceclofenac to the colon. Pectin is mostly constituted of a linear polymer made up principally of α-(1-4)-linked D-galacturonic acid residues, with rare interruptions by L-rhamnose residues linked through a 1, 2 linkage. Because pectin is soluble in water, it cannot effectively safeguard the medication it carries while passing through the stomach and small intestine. Hydrophilic polymer matrix systems are designed to achieve a certain drug release pattern. Pectin is extensively utilized in the pharmaceutical industry because of its biocompatibility, cost-effectiveness, and abundant availability (Shimono *et al*., 2003; Ravi *et al*., 2008).

Production of guar gum microspheres:

Drugs were encapsulated into guar gum microspheres using the process of emulsification and chemical cross-linking. A 40 g solution of guar gum in water was allowed to undergo expansion for a duration of 2 h. Subsequently, it was combined with 100 g of castor oil, which included 3 g of span 85, using a mechanical stirrer operating at a speed of 4000 rpm. After a comprehensive mixing process, a volume of 0.2 ml of concentrated sulphuric acid and 2% of glutaraldehyde were added to the dispersion. The mixture was then stirred continuously at a fixed rate for a duration of 4 h at a temperature of 50°C. The microspheres were gathered through the process of sedimentation and subsequent decantation of oil. Subsequently, the microspheres were cleansed using multiple portions of isopropyl alcohol (Liu *et al*., 1997; Jin *et al*., 2004).

Preparation of xanthan gum microspheres:

Xanthan gum microspheres loaded with medicines were generated using the emulsification and cross-linking technique. A solution of xanthan gum (40 g) in water (containing 2% w/w of xanthan gum) was mixed with castor oil (100 g) containing 1.5% v/v of span 85. The mixing was done using a mechanical stirrer at 3000 rpm for 2 h, allowing the gum to swell. The concentrated sulphuric acid and 2% glutaraldehyde were completely mixed together in a volume of 0.2 ml. The resulting mixture was swirled constantly at a constant speed for a duration of 4 h at a temperature of

50ºC. The microspheres were acquired using the sedimentation process, followed by the decantation of the oil (Mehregan *et al*., 2005; Emami *et al*., 2007; Musmade, 2007).

Evaluation of Microspheres:

Surface morphology:

The Scanning Electron Microscopy method was employed to analyze the structure and surface characteristics of both unprocessed microspheres and Eudragit S100 coated microcapsules. The particles were subjected to freeze drying, after which they were coated with gold palladium to produce a film of 20 nm in thickness. Later, they underwent microscopic examination. The results section includes scanning electron microscope photographs of both the core and coated microcapsules, as documented by Khatun *et al.* (2004) and Najib *et al.* (2004).

Particle Size:

The particle size was determined using an optical microscope, where the diameter of 100 particles was directly measured using a calibrated eyepiece micrometre. The mean particle size for microcores and the particle size of microcapsules were measured (Tozaki *et al*., 1999; Ahire *et al*., 2020).

Percentage production yield (*PY*):

The formulation was replicated three times and the PY (%) was computed.

$$\% \text{ PY} = \frac{\text{Practical Mass of Microspheres}}{\text{Therorotical Mass}} \text{ X } 100$$

Drug loading/encapsulation efficiency:

The loading capacity refers to the upper limit of drug content that can be contained within the microspheres. The loading capacity was evaluated as the upper limit of aceclofenac content present in 100 mg of microspheres. Encapsulation efficiency refers to the quantity of a medicine that is enclosed within the microspheres during the formulation process.

Assay:

The practical drug content was determined by quantifying a precisely measured amount of microspheres equivalent to 50 mg of aceclofenac. Subsequently, the microspheres were pulverized and immersed in methanol to facilitate the extraction of the medication from the microspheres. Following a 24 h period, the liquid that passed through the filter was analyzed using a spectrophotometer at a wavelength of 275 nm to determine the amount of drug present, with methanol used as a reference. The drug concentrations in the sample were determined by calculating the values from the calibration plot, which was created by doing regression analysis on the triplicate data (Butler *et al*., 1992; Adi *et al*., 2012).

Microsphere equilibrium swelling investigations:

100 mg of microspheres was added to a solution that mimics the conditions of the colon (simulated colonic fluid) with a pH of 7.0. The microspheres were allowed to expand until they reached a consistent weight. The microspheres were removed and desiccated using filter paper, and their weight fluctuations were measured. The magnitude of swelling (α) was later calculated using the following formula (Gowda *et al*., 2006; Van Halm *et al*., 2007).

Mucoadhesion in vitro:

We tested the microspheres' mucoadhesion to sheep nasal mucosa in a controlled laboratory setting. After being bonded to the polyethylene support, the microspheres were dispersed in water and subsequently applied to the nasal mucosa of the sheep. After that, the mucosa was dried for 30 min at room temperature with a relative humidity of more than 80% in desiccators. This was done to facilitate hydration and interaction between the polymer and glycoprotein, as well as to prevent the mucous from drying out. Subsequently, the mucosa was examined using a microscope to determine the quantity of particles adhering to the specific region (Winter *et al*., 1962; Rhim *et al*., 2008).

Dissolution studies:

A 500 ml solution was prepared with 100 mg of aceclofenac, which was measured with precision. The USP rotating paddle dissolving device was used at a speed of 100 rpm and a temperature of 37ºC to study the release of aceclofenac from AGM, ACS, BATCH, AXM, and ACSM microspheres. For two hours, a 0.1N hydrochloric acid solution was used to keep the pH of the dissolving medium (simulated stomach juice) at 1.2. Soluble in a buffer solution with a pH of 7.4, SIF was added to the dissolution medium and left to work for six hours. Rat faeces at a concentration of 4%w/v was added to a pH 7.4 buffer for the experiment. Similar to how much time it takes for food to travel through the colon, the dissolving process lasted between 12 and 24 h. The final volume was always 500 ml. Using a pipette fitted with a micro filter, samples were periodically removed from the dissolving media. The absorbance was then measured at a wavelength of 275 nm. To change the sink condition, we added the same amount of new dissolving media at the same temperature. The drug release percentage was calculated relative to the drug content of the microspheres (Devi *et al*., 2003; Ahirrao *et al*., 2023).

Stability studies:

The chosen formulation of microspheres was stored in amber-colored glass bottles at a temperature of 45ºC and a relative humidity of 75% for a duration of 3 months. During this time, the microspheres were monitored for any alterations in colour, odour, percentage of drug content, and entrapment efficiency. Similarly, the pellets and tablets were evaluated for changes in colour, odour, and percentage of drug content (Jia *et al*., 2003; Khulbe *et al*., 2023).

Results and Discussion

Preformulation studies:

DSC studies:

A Differential Scanning Calorimeter (DSC) generates a graph that displays the heat flux (rate) in relation to temperature, using a predetermined temperature rate. Differential scanning calorimetry (DSC) offers insights into the physical characteristics of a sample, such as its crystalline or amorphous nature. Additionally, DSC can reveal potential interactions between drugs and polymers in formulations. The thermogram of the physical mixture of aceclofenac and pectin showed different melting temperatures for various combinations (Figs. 1, 2).

FT-IR:

Fourier transform infrared spectroscopy was used to make sure the medications and polymers did not react chemically. The following outcomes were produced by analyzing the FTIR spectra. Aceclofenac exhibits a pronounced peak at 1771.47 cm^{-1} and a secondary peak at 1716 cm^{-1} in the Fourier Transform Infrared spectrum. Both values are 1589 and 89 cm^{-1}. Functional group bending at 53 cm^{-1} and 1055.9 cm^{-1} is associated with C=O, COOH, NH, and OH. Peaks at 1771.36 cm^{-1}, 1716.93 cm^{-1}, and 1589 cm^{-1} were identified in the FTIR analysis of the physical mixture of aceclofenac and pectin. Two numbers, 89 cm^{-1} and 1055, are given. Along with 33 cm^{-1}, 1771.47 cm^{-1}, 1716.44 cm^{-1}, 1589.12 cm^{-1}, and 1055.49 cm^{-1}, the guar gum and aceclofenac mixture was also monitored at other frequencies. Additional frequencies that were recorded were 1771.69 cm^{-1}, 1716.89 cm^{-1}, and 1589 cm^{-1}. The relative values are 1589.84 cm^{-1} and 1056.24 cm^{-1}. It is clear that the frequencies of the specified functional groups of aceclofenac have not changed much, according to the previous interpretation. This data points to the absence of any chemical interaction between the polymers used in the formulations and aceclofenac. Figures 3–6 show the FTIR charts in that order.

Evaluation of Microspheres:

Eudragit coated pectin microspheres:

Pectin microspheres containing aceclofenac were successfully generated using the emulsion dehydration procedure. The resultant microspheres were nearly spherical, had a uniform surface, and were cross-linked. Microspheres with small size, narrow size distribution, and high drug loading efficiency were

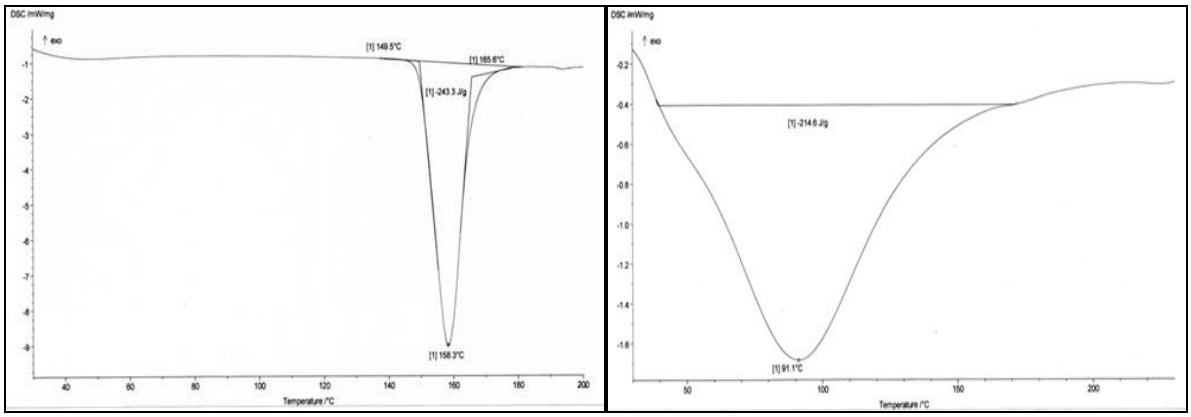


Fig. 1: DSC thermogram of Aceclofenac and Pectin.

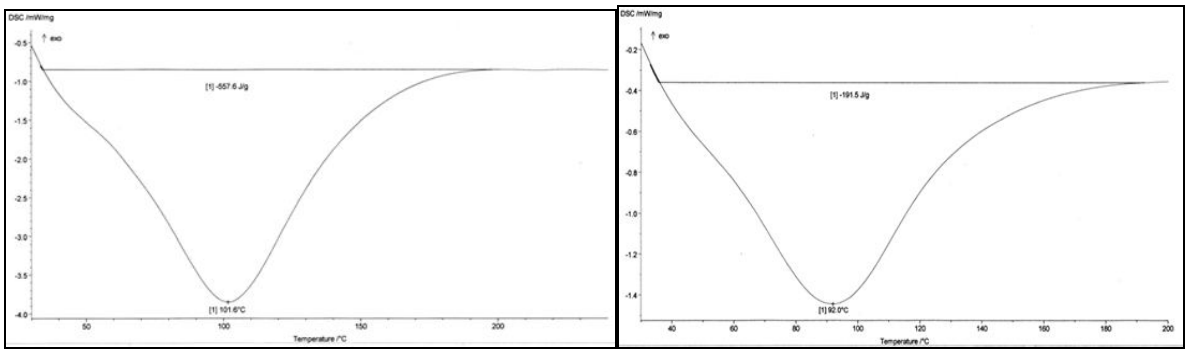


Fig. 2: DSC thermogram of chitosanand Gaur gum.

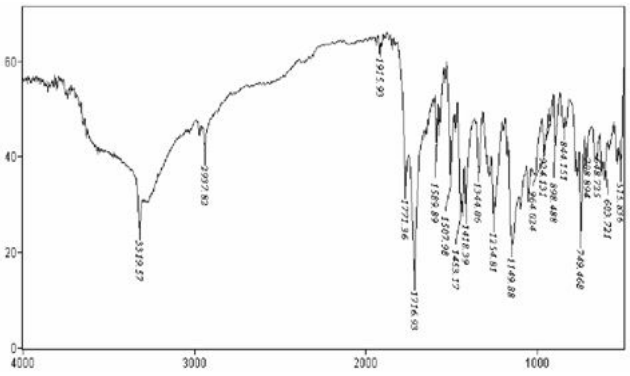


Fig. 3: Aceclofenac and Pectin.

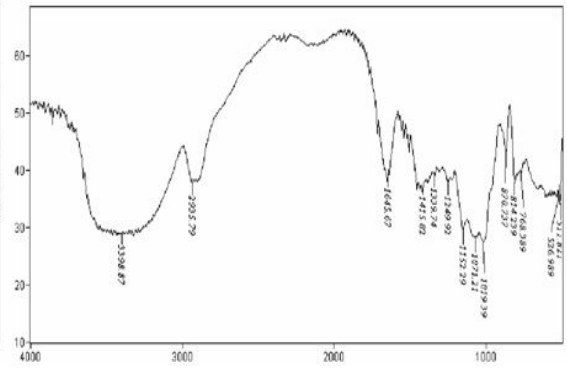


Fig. 4: Aceclofenac and gaur gum.

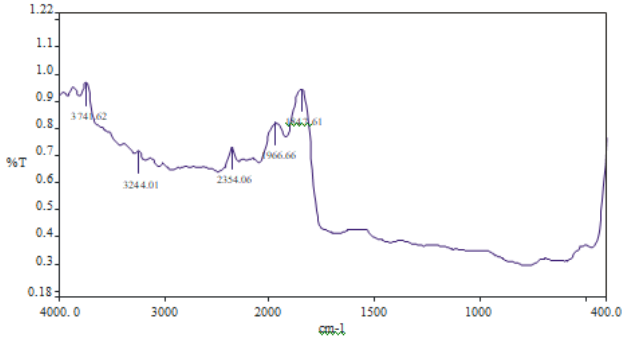


Fig. 5: Aceclofenac and Chitosan.

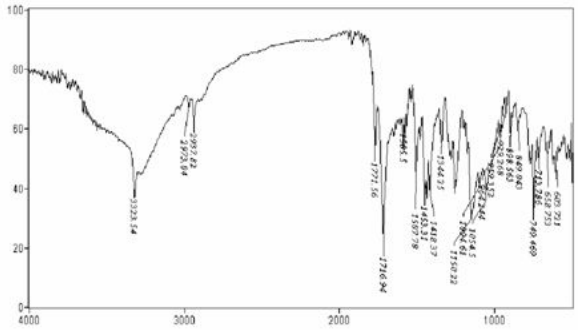


Fig. 6: Aceclofenac and Xanthan gum.

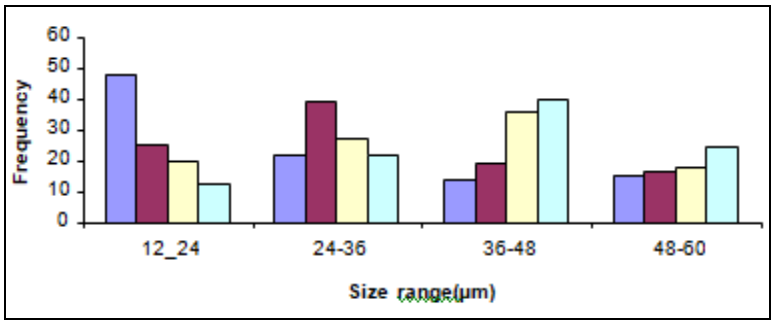


Fig. 7: Particle size distribution.

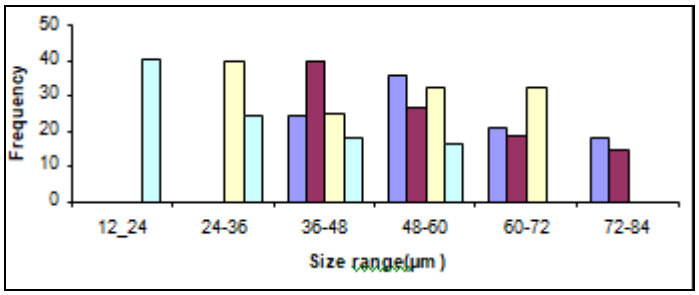


Fig. 8: Effect of emulsifier.

produced by optimizing the process by adjusting the drug to polymer ratio, emulsifier concentration, stirring rate, and stirring time. The objective was to accomplish pH-targeted medication release in the colon (Tiwari *et al*., 2021a).

Particle size:

Polymer concentration effect:

As the drug to polymer ratio grew from 1:3 to 1:6, the average diameter of the pectin microspheres varied between 29.05 ± 1.720 µm and 38.85 ± 0.36 µm, respectively. The production of larger microspheres and droplets was induced by a denser dispersion, which was in turn caused by an increase in polymer concentration (Fig. 7).

Emulsifier concentration and microsphere formation:

As the emulsifier content varied from 0.5 to 2% w/v, the microspheres' average diameters varied from 31.32 ± 0.2 to 57.48 ± 0.35 µm. The results showed that particles with a smaller average diameter were produced when the surfactant concentration was increased. Because the surfactant stops the emulsion droplets from combining, smaller microspheres are formed (Fig. 8).

Particle size and the impact of stirring speed:

This occurred as a result of the elevated shearing force required to divide the oil phase into smaller globules. An optimal speed of 1000 rpm was determined. An elevated stirring speed resulted in the formation of microspheres with an uneven shape, albeit a modest improvement in entrapment efficiency was noted (Fig. 9).

Particle size and the effect of stirring duration:

Moving from 15 to 60 min of stirring speed resulted in a decrease in the average microsphere diameter from 55 ± 0.6 to 31.32 ± 0.20 µm (Fig. 10).

Percentage of production yield:

Batch 9 produced with a stirring speed of 500 rpm achieved the highest production yield of 99.12 ± 1.88%. In contrast, Batch 12, created at 1000 rpm and 30 min, and obtained the lowest yield of 90.1 ± 1.95%. No significant impact of other variables on the percentage output yield was observed.

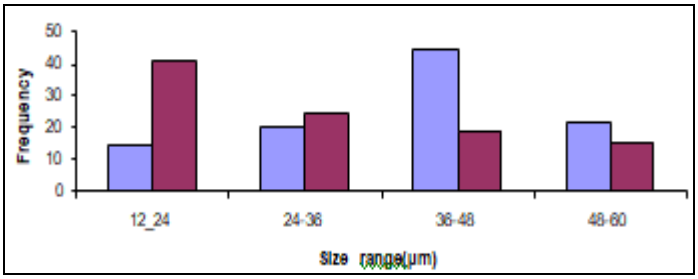


Fig. 9: Effect of stirring speed.

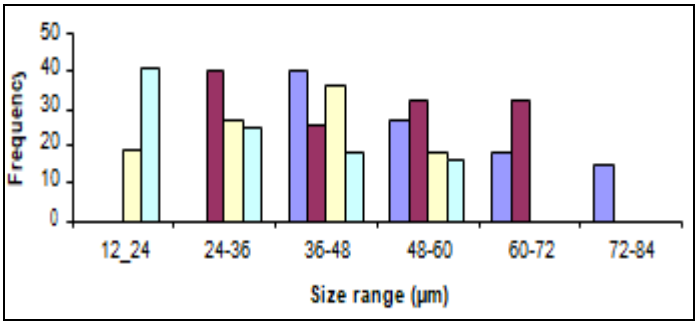


Fig. 10: Effect of stirring time on particle size.

Drug loading:

As the concentration of polymer increased, the drug loading decreased from 21.14 ± 0.26 to 11.3 ± 0.45%. As the emulsifier content was raised from 0.5 to 2.0%, the drug loading increased from 17.67 ± 0.12% to 21.14 ± 1.78%. As the stirring rate was increased from 500 rpm to 2000 rpm, the drug loading percentage shown an increase from 21.75±1.59 to 24.25±2.29%. The percentage loading increased linearly with the stirring period, going from 15 to 60 min. For 15 min, the percentage loading varied between 17.41±0.34% and for 60 min, it ranged from 22.65±1.26% (Table 1).

Entrapment efficiency:

The optimized stirring speed of 1000 rpm resulted in the highest percentage of entrapment efficiency. The entrapment % was lowest when the stirring period was the shortest, specifically 15 min. This indicates that entrapment is achieved more quickly and effectively with a shorter stirring time. The entrapment efficiency reduced from 84.58 ± 1.56 % to 79.1 ± 0.39 %. This is because a higher concentration of polymer results in inadequate mixing of the drug and polymer dispersion into the surrounding phase, leading to a decrease in entrapment efficiency. The emulsifier with the greatest and optimized concentration resulted in the highest percentage of entrapment efficiency.

In vitro drug release studies:

We developed four distinct aceclofenac-containing pectin microsphere formulations, with drug-to-polymer ratios of 1:3, 1:4, 1:5, and 1:6. By studying the microspheres' physiochemical characteristics—including their average size, shape, surface morphology, entrapment efficacy, drug loading percentage, swelling ratio, and mucoadhesive properties—the optimal polymeric concentration was determined. Several simulated gastrointestinal and colonic media were used to conduct the drug release studies *in vitro*, following the typical evaluation technique. The extent of drug release is contingent upon the concentration of the polymer. Higher polymer content results in reduced drug release percentage across all

Table 1: Influence of formulation factors on practical mass and production yield

S. No.	Formulation Batches	Amount of drug (mg)	Polymer (mg)	Theoretic almass (mg)	Production yield %
1	$Batch_1$	400	1200	1000	98.12±1.98
2	$Batch_2$	400	2000	1500	97.32±0.84
3	$Batch_3$	400	1000	2000	96.01±1.47
4	$Batch_4$	400	2000	2500	92.00±0.32
5	$Batch_5$	400	1200	1500	93.02±2.47
6	$Batch_6$	400	1200	1500	96.31±0.87
7	$Batch_7$	400	1200	1500	96.51±0.11
8	$Batch_8$	400	1200	1500	98.44±1.74
9	$Batch_9$	400	1200	1500	98.99±1.78
10	$Batch_{10}$	400	1200	1500	96.56±1.98
11	$Batch_{11}$	400	1200	1500	93.14±1.45
12	$Batch_{12}$	400	1200	1500	91.32±0.66

dissolving media (Tiwari *et al*., 2021b). In the SGF experiment, the batch1 formulation with a drug to polymer ratio of 1:3 had the highest release percentage of 5.84±0.63% after 120 min. In comparison, the batch formed with a drug to polymer ratio of 1:6 had a release percentage of roughly 3.66±0.16% at the conclusion of the 120min period (Fig. 11).

Cumulative % drug release in SGF:

Size, swelling index, and entrapment efficiency are three variables that affect the rate of medication release from microspheres. The CDR is similarly sensitive to the aforementioned values, since the emulsifier concentration impacts all of the aforementioned properties.

Drug release percentage in SIF over time:

The percentage of cell death rate (CDR) in SIF increased from 15.82 ± 0.49% to 26.34 ± 0.12% after 6 h of the investigation. The decrease in particle size seen from batch 5 to batch 8 can be attributed to the rise in emulsifier concentration.

Total SCF drug release %:

The Batch 8, prepared with 2% w/w span 85, achieved a CDR percentage of 100.3 ± 0.92% after 8 h. The greatest percentage of CDR for Batch 5, Batch 6, and Batch 7 was 101.7 ± 0.73%, 101.2 ± 0.93%, and 102.3 ± 2.05%, respectively, at the conclusion of the 24th h of the trial. The Batch 8 was fine-tuned to achieve the most effective emulsifier concentration. The drug to polymer ratio of 1:3 and the concentration of 2.0% span 85 at 1000 rpm were used to optimize the stirring duration. The stirring time varied at intervals of 15, 30, 45, and 60 min. The optimized formulation was selected based on a medication to polymer ratio of 1:3, with an emulsifier concentration of 2% w/w of span 85. The batch was agitated at 1000 rpm for 1 h. Figure 12 depicts the use of this batch for the production of eudragit S 100 coated microspheres.

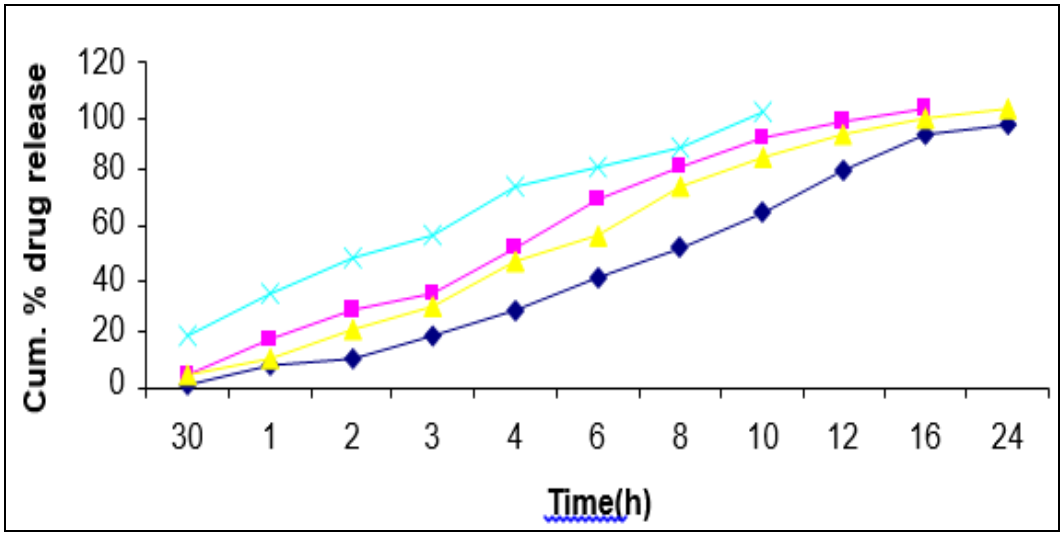


Fig. 11: *In vitro* drug release.

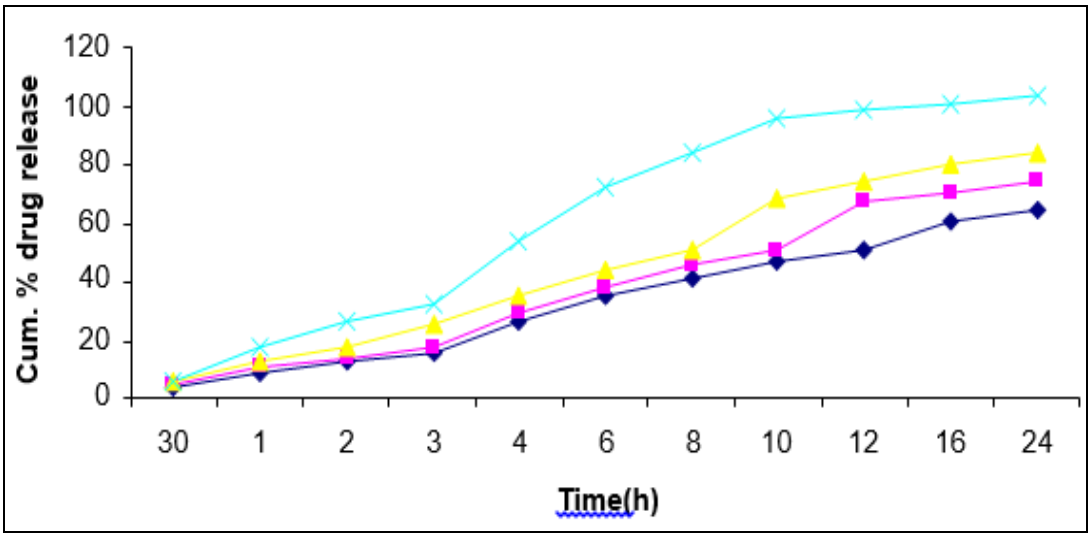


Fig. 12: Cumulative percentage drug release in SCF.

SEM Study:

The SEM of pH-dependent coated microspheres is displayed in Figure 13. The surface exhibited a noticeable lack of smoothness and the core particles were significantly larger in size. They possessed many nuclei.

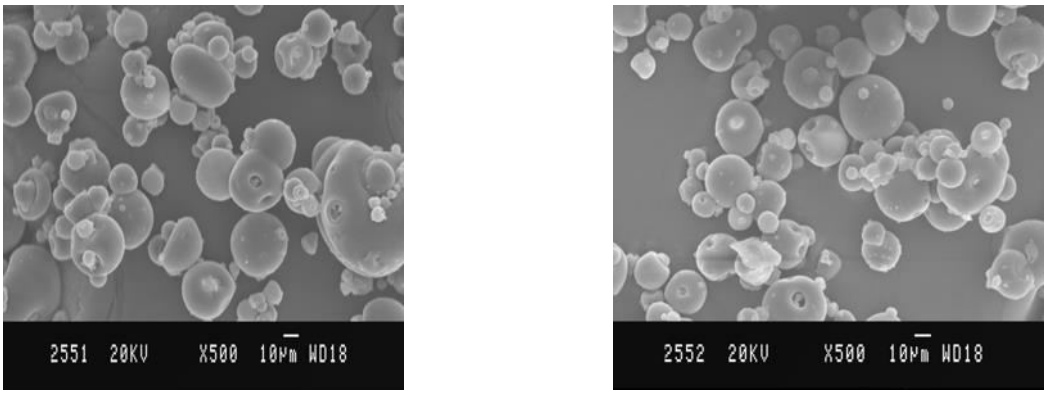
The average particle sizes of the pectin microspheres coated with Eudragit were noticeably greater than those of the original cores, and the production yield was determined to be 82.33%. The particles had a smooth surface and possessed many nuclei.

Stability studies:

Stability experiments indicated that there were no notable alterations in the sensory characteristics and concentration of the medication. Therefore, the formulation remained stable under varying temperature and humidity conditions.

Guar gum microspheres:

The current study aims to utilize the cost-effective and readily accessible guar gum for the targeted delivery of aceclofenac to the colon. Glutaraldehyde was used to cross-link guar gum in order to decrease its swelling qualities, making it



(a) (b)

Fig. 13: (a) Uncoated microspheres, (b) Eudragit coated microspheres.

suitable for use as a vehicle in oral administration formulations. Due to the cross-linking process, guar gum underwent a transformation from being non-ionic to becoming negatively charged. The drug carriers were discovered to be specifically targeted to the colon, taking the form of matrix and compression coated tablets, as well as microspheres. The current study primarily aimed to create and analyze innovative guar gum microspheres for the purpose of delivering aceclofenac specifically to the colon (Tiwari *et al*., 2021c). The process of creating guar gum microspheres included the use of a cross-linking agent. The average diameter of the microspheres grew from 31.05 µm in Batch 10 to 34.5 µm in Batch 11 when the concentration of the agent was raised from 1% to 2%. However, a higher concentration of the cross-linking agent led to a drop in particle size to 32.9 µm. This suggests that there was not a significant influence on particle size and size distribution when the quantity of glutaraldehyde was varied. The particle size distribution data were obtained for formulations with different polymer concentrations, medication concentrations, and cross-linking agent concentrations.

Efficiency in drug loading and entrapment:

By adjusting various process parameters, we were able to ascertain the optimum entrapment efficiency. With an increase in the drug to polymer ratio from 1:2 to 1:5, the results showed that the entrapment efficiency increased from 61.82 ± 1.11 to 91.38 ± 1.0 %. The 1:4 ratio, on the other hand, was considered ideal because it produced a high yield of 95.64 per cent. The entrapment efficiency, on the other hand, varied between 61.82 ± 1.11% and 91.38 ± 1.0%. This data points to the possibility that the emulsification process released part of the medication into the exterior phase. With an increase in the drug percentage from 25% to 75%, the entrapment efficiency went up from 77.94 ± 1.26% to 79 ± 0.23%. The entrapment efficiency, however, decreased as the drug fraction was increased further. With the addition of a cross-linking agent, the entrapment efficiency reacted in different ways. In particular, as the concentration of glutaraldehyde was raised from 1% to 2%, the entrapment rose from 79.49 ± 2.02% to 81.51 ± 1.14%. Entrapment was reduced to a range of 72.5 ± 1.02% (Batch12) due to the greater content of glutaraldehyde. Results showed that more cohesive spheres were formed with larger concentrations of glutaraldehyde, which prevented further drug entrapment.

% production yield:

The findings demonstrated that the optimal production yield was achieved when the medication to polymer ratio was 1:4. The production yield percentage exhibited an increase from 91.65 ± 1.08 to 94.88 ± 1.29% as the drug concentration was raised from 25 to 75%.

However, a subsequent rise in the drug concentration resulted in a downward trend in the production yield. Furthermore, the rise in glutaraldehyde concentration resulted in a similar variation in production yield as observed in the previous scenario. The percentage climbed from 92.99 ± 2.29 to 93.84 ± 2.24% and thereafter fell to 87.67 ± 1.99% when the glutaraldehyde concentration increased to 3%.

% Drug loading:

By adjusting various process parameters, the optimal drug loading of microspheres was achieved. The findings showed that a drug to polymer ratio of 1:2 resulted in the best drug loading percentage of 22.7 ± 0.4%. Due to an increase in system viscosity, the drug loading decreased from 22.7 ± 0.49 to 16.5 ± 1.29% as the polymer concentration grew. The drug loading went from zero to 42.75 per cent as the drug concentration rose. It can be inferred that when the drug concentration grew, the percentage of drug loading also increased. Drug loading did not correlate consistently with increasing glutaraldehyde levels. At first, when going from 1% to 2% glutaraldehyde concentration, the drug loading went up from 36.63 ± 2.14% to 37.22 ± 1.99%. But when the concentration was raised to 3%, the drug loading dropped to 35.49 ± 1.02%.

In vitro drug release:

Using *in vitro* methods, the dissolving characteristics of aceclofenac microspheres synthesized from guar gum were investigated. In order to study drug release, studies were conducted in various simulated fluids, including SGF, SIF, and SCF, both with and without enzymes. At 120 min, the drug release drops from 5.24 ± 0.32 to 2.22 ± 0.80% when the polymer concentration goes up from 1:2 to 1:5. The drug particles deposited on the surface may have dissolved and diffused out of the system, which could explain this. Because of this, guar gum microspheres can block the stomach's natural release of the medication. As the drug to polymer ratio rose from 1:2 to 1:5, the drug release in the SCF-control group dropped from 23.2 ± 0.12% to 18.04 ± 0.13%. As the polymer content in the colonic fluid increased from 1:2 to 1:5, the release percentage decreased from 85.13 ± 0.13 to 50.99 ± 2.16% during enzyme induction.

Effect of drugs concentration on release %:

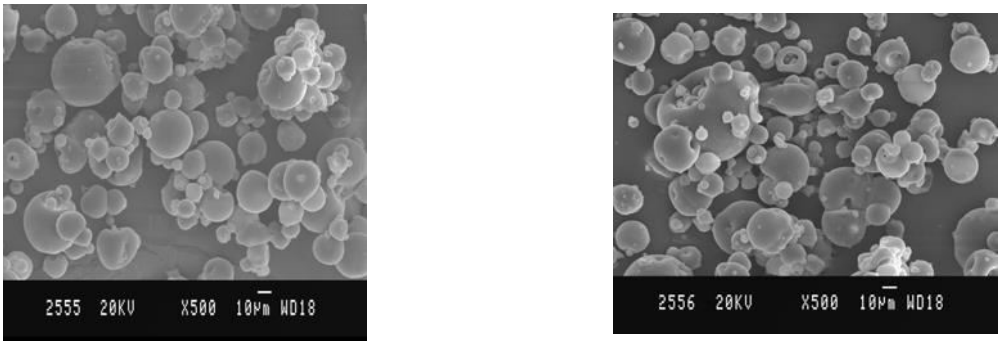
As the drugs concentration increased, the drug release in the simulated gastric fluid (SGF) increased from 3.28 ± 0.20 to 4.82 ± 0.12%. This provides more evidence that the cumulative percentage of drug release is closely related to the drug concentration. A rise from 25% to 100% drug concentration at pH 7.4 at the 6th h resulted in an increase from 7.27 ± 2.13 to 11.98 ± 0.23% cumulative percentage of drug release. When the drug concentration was increased in a pH 7.0 solution without enzyme induction, the total drug release in the situation indicated earlier increased from 15.23 ± 0.26 to 24.2 ± 0.19%. The release percentage varied between 97.57 ± 2.14% and 99.17 ± 1.56% when enzyme induction was carried out with 4% rat caecal material. Batch 8 proved to be the most effective formula for alleviating chonobiological symptoms in arthritis, with material release increasing from 78.15 ± 0.23% at 8 h to 100.88 ± 1.3% at 24 h.

Morphological characters SEM Analysis:

Figure 14 displays the SEM photograph of pH-dependent coated microspheres. The surface exhibited a noticeable lack of smoothness, while the core particles were significantly greater in size. They possessed many nuclei. The average particle size had a significant rise, multiplying by several times, reaching a value of 205 ± 0.26%.

Cumulative % drug release:

The cumulative percentage drug release was measured in simulated gastric fluid (SGF), simulated intestinal fluid (SIF), simulated colonic fluid (SCF) without enzyme induction, and simulated colonic fluid (SCF) with enzyme induction. The cumulative percentage of medication release in simulated intestinal fluid (SIF) and simulated colonic fluid (SCF) for Batch 11. No drug release was seen in the simulated



(a) (b)

Fig. 14: (a) Uncoated microspheres, (b) Eudragit coated microspheres.

gastric fluid (SGF), suggesting the gastric fluid's protective action of eudragit S 100. The drug release in simulated intestinal fluid (SIF) was determined to be 8.78 ± 1.09% after 6 h. In contrast, the drug release in colonic fluid was higher, with a release of 10.96 ± 1.2% after 8 h in the absence of enzyme induction in the simulated colonic fluid (SCF) medium. The drug release in the SCF medium with enzyme present was 50.14 ± 0.74% for the specified duration. However, by the conclusion of the 24th h, the drug release increased to 92.96 ± 0.92%. This indicates that enzyme induction is necessary, as without it, the drug release remains lower.

Stability studies:

Stability experiments indicated that there were no notable alterations in the sensory characteristics and concentration of the medication. Consequently, the formulations exhibited stability under varying temperature and humidity conditions.

Chitosan microspheres:

This entails the formulation of chitosan microspheres that are encapsulated within an enteric polymer. The preparation of aceclofenac chitosan microspheres was achieved using a chemical cross-linking process with glutaraldehyde as the cross-linking agent. The first part of the work involved optimizing formulation factors, such as the drug to polymer ratio, the concentration of the emulsifier, and the concentration of the cross-linking agent, as well as the process variable of rpm. The values relating to the manufacturing yield, percentage of drug loading, particle size, entrapment encapsulation efficiency, and swelling index (Tiwari *et al*., 2020, 2021b).

Optimization of method:

To make the acetate salt, which is soluble in water, the insoluble chitosan can be dissolved in diluted acetic acid. At 30ºC, the viscosity of a 4% weight/volume solution in 5% acetic acid was found to be 850 centipoises. The optimal viscosity for distributing the fluid into droplets containing a high concentration of medicine was determined to be this. Particle size, encapsulation efficiency, swelling index, and the polymer-to-drug ratio were some of the related metrics evaluated. An analysis of the data showed that a medication-to-polymer ratio of 1:2 produced the best core microspheres. The dispersibility in liquid paraffin gets more challenging as the concentration of chitosan/polysaccharide increases because the viscosity rises.

Evaluation:

% production yield:

The batch with the best percentage production yield was the one that was formulated with an optimum medication to polymer ratio of 1:2. Batch 9, on the other hand, had the lowest percentage production yield due to its low polymer content,

emulsifier concentration, and rotating speed. The percentage production yield increased from 90.6 ± 1.17 to 94.72 ± 0.75 as the polymer concentration was increased. A 1:1 and 1:2 drug-to-polymer ratio was used to achieve these yields. On the other hand, the percentage of production yield decreases as the polymer concentration increases. This is due to the fact that the polymeric suspension droplets are unable to penetrate the external phase due to the drug and polymer suspension's enhanced viscosity.

% drug loading:

Experimenting with different drug-to-chitosan ratios, stirring speeds, and emulsifier concentrations allowed us to find the optimal drug loading of microspheres. A drug-to-chitosan ratio of 1:1 produced the best drug loading percentage. At a concentration of 1% w/w emulsifier, the drug loading percentage reached its peak among the various batches, measuring 24.08 ± 0.34%. The drug loading percentage was 24.54 ± 0.66% in Batches 9 to 12, which were stirred at a speed of 4000 rpm. Finally, among the relevant batches, a drug loading percentage of 24.09 ± 0.70% was achieved by utilizing a cross-linking agent.

Encapsulation efficiency:

We calculated the encapsulation efficiency by dividing the total weight of aceclofenac in the microspheres by the total amount of aceclofenac put into the procedure. From 62.02 ± 1.56% to 75.43 ± 1.15%, the percentage of encapsulated medicine rose as the drug to polymer ratio went from 1:1 to 1:3, respectively. Unfortunately, the optimal drug to polymer ratio was found to be 1:2, leading to a manufacturing yield of 94.72 ± 0.75% and an encapsulation efficiency of 71.4 ± 1.16%. The encapsulation efficiency increases from 65.46 ± 1.2% to 72.43 ± 0.86 % when the emulsifier concentration rises. The efficacy of encapsulation decreases as the concentration of the emulsifier rises.

Furthermore, it exhibits an increase when the rotational speed escalates from 1000 rpm to 4000 rpm. The results indicate that a batch produced at 1000 rpm achieved an encapsulation efficiency of 55.5 ± 1.08%, whereas a batch prepared at 4000 rpm achieved an encapsulating efficiency of 73.72 ± 1.35%. The concentration of the cross linker impacts the efficiency of encapsulation. Four different concentrations of glutaraldehyde were utilized in the process of optimising the formulas. The findings demonstrated that an elevation in the concentration of the cross-linking agent resulted in a drop in the entrapment efficiency, reducing it from 72.36 ± 2.10% to 51.56 ± 1.0% when mixed with 15 ml of glutaraldehyde. The prevention of drug particle penetration into the spheres is attributed to the development of complete spheres.

Conclusion

For the purpose of optimizing microspheres for controlled medicine administration in the colon for rheumatoid arthritis, the concentrations of polymer, emulsifier, stirring speed, and cross-linking agent were determined. Rotational speed also affects microsphere mean diameter, according to this study. The optimal particle size of microspheres depends on rotating speed, which was varied from 1000 to 4000 rpm. The results also showed a polymer concentration-dependent mixing rate limit. Pectin needed polymer concentration. Pectin needs 2000 rpm, xanthan gum and chondroitin sulphate 3000, and chitosan and guar gum 4000. It was also shown that stirring duration at a given rotational speed affected microsphere shape and size distribution, probably due to particulate system shear force. Pectin microspheres mix best in 60 min, chitosan in 2.5 h, and guar gum, chondroitin sulphate, and xanthan gum in 4 h. Emulsifiers help produce stable microspheres. The mean microsphere size depends on the emulsifier content. Changing glutaraldehyde concentration did not affect particle size or size distribution, but it did affect surface smoothness, swell ability, and dissolving.

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