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Research Paper

Development of Mucoadhesive Microspheres of Corticosteroid UsingCamphor as a Penetration Enhancer

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Abstract

Chronic sinusitis, a prolonged inflammation of the sinus cavities, significantly impacts patient quality of life and requires effective, sustained treatment modalities. This study focuses on the development and evaluation of microspheres with mucoadhesion intended for prolonged nasal drug delivery. The microspheres were formulated using a solvent evaporation method, employing biocompatible polymers such as chitosan and alginate to enhance mucoadhesive properties. Details of the microspheres composition involved assessing particle size, surface shape, effectiveness of encapsulation, and in vitro medication release. Additionally, mucoadhesion studies were conducted to evaluate the adherence capability of the microspheres to nasal mucosa. The optimized formulation demonstrated a spherical shape with a mean particle size suitable for superior encapsulation efficiency for nasal delivery, and a controlled release profile over 12 hours. In vitro mucoadhesion tests confirmed strong adhesive properties, suggesting prolonged retention in the nasal cavity. The therapeutic efficacy was assessed using an in vitro chronic sinusitis model, which revealed significant reduction in inflammation and improved mucociliary clearance compared to conventional treatments. These findings indicate that mucoadhesive microspheres are a promising approach for the sustained and targeted delivery of drugs in the treatment of chronic sinusitis, potentially enhancing patient compliance and therapeutic outcomes.

Keywords: Chronic Sinusitis, Mucoadhesion, Microspheres, Orifice-Ionic Gelation, Corticosteroid.

Introduction:

A nasal drug delivery system is a method of administering drugs through the nasal route, utilizing the nasal mucosa as the site for absorption into the bloodstream. There are various types of nasal drug delivery systems, including nasal sprays, nasal drops, nasal powders, and nasal gels. These formulations can be designed to transport medications for regional action in the nasal cavity, such as decongestants or nasal corticosteroids for allergic rhinitis, or for systemic absorption, such as vaccines, hormones, or analgesics.

Microspheres are tiny, spherical particles that usually have a size between 1 and 1000 μm. They can be made of polymers that are synthetic or natural. Because they may target specific areas within the body and manage release rates, microspheres offer a wide range of applications in drug administration. When it comes to administering medication to mucosal surfaces including those of the eye, nose, urinary tract, and gastrointestinal system, they are especially helpful. Drugs can be released from microspheres in a regulated or prolonged way, providing long-lasting therapeutic benefits. Furthermore, microspheres can stick to mucosal surfaces by combining mucoadhesive characteristics, which improves drug absorption and bioavailability. This adherence makes it easier to distribute drugs to the right places in the body.

Mucoadhesive properties refer to the ability of a substance to adhere to mucosal surfaces such as those found in the gastrointestinal tract, nasal cavity, ocular surface, and vaginal tract. This property is particularly advantageous in drug delivery systems as it can enhance the residence time of drugs at the site of action, improve drug absorption, and facilitate targeted delivery to specific tissues or cells. Like Hydrogen Bonding, Electrostatic Interactions, Van der Waals Forces. Entanglement and Intermingling Mucoadhesive materials ^{can} be natural or synthetic polymers or macromolecules that possess suitable adhesive properties. Examples of natural mucoadhesive polymers include chitosan, alginate, hyaluronic acid, and various plant-derived polysaccharides. Synthetic mucoadhesive polymers include poly (acrylic acid), polyethylene glycol (PEG), and various copolymers.

Materials and Methods:

Materials :

The supplier of flunisolide was Central Drug House in New Delhi. The supplier of chitosan was Central Drug House in New Delhi. In Nandesari, Vadodara, Gujarat 391340, Qualikems Lifesciences Private Limited was the seller of HPMC. The supplier of CaCl2 was Qualikems Lifesciences Private Limited, located at Nandesari, Vadodara, Gujarat 391340.The supplier of sodium alginate was Qualikems Lifesciences Private Limited, located at Nandesari, Vadodara, Gujarat 391340.The supplier of camphor was Qualikems Lifesciences Private Limited, located at Nandesari, Vadodara, Gujarat 391340.

Preparation of microspheres by orifice ionic gelation method;

The orifice ionic gelation method is a variation of the ion gelation method that involves the extrusion of a polymer solution through a nozzle or orifice into a cross-linking bath containing ions. This technique is commonly used to produce hydrogel beads or microspheres with controlled size and shape.

“Orifice Ionic Gelation Technique” was chose best fit for this project work. In this methodology, the comparison was done by using 2 different origin polymers. Since this method requires crosslinking process so, Calcium Chloride was used to crosslink the Microspheres. The vehicle used here is distilled water. Below there is procedure mentioned to formulate mucoadhesive

microspheres of flunisolide. The ingredients like, Flunisolide, Chitosan, HPMC, Sodium Alginate, camphor were allowed to pass through sieve of mesh #40 so that we are able to get uniform particle of interacting species. Calcium Chloride was prepared in the concentration of 5% by dissolving sufficient amount of Calcium Chloride in distilled water. Later it was kept aside in a beaker/ flask. As the method selected here is Ionic Gelation Technique, so the ingredients like HPMC, Chitosan and gelling agent i.e. Sodium Alginate were fixed and taken into the proportion mentioned. Our drug being a Corticosteroid its dissolution is the rate limiting step in this process so slowly and carefully the drug Flunisolide was added into the beaker of polymer and gelling agent with adequate amount of homogenization. Homogenization is necessary so that we get a uniform and continuous dispersion. As we have to formulate Microspheres so at this step Calcium Chloride is added to cross link the polymers. Calcium Chloride (5%) is filled in a syringe and a needle of 22# is connected so that the drops of cross linking agent falls through a suitable orifice. This entire assembly is allowed to rotate on a magnetic stirrer at 50 RPM. We can observe that in the calcium chloride solution, spherical beads-like structures, or our microspheres, are forming. In order to allow for full interaction and healing, these microspheres were left in the solution. They were then decanted, set away, and allowed to dry overnight. A naturally occurring chemical substance called camphor is added to the gelatin solution. Camphor functions as a plasticizer, enhancing the gelatin film's pliability and workability. Additionally, it can aid in regulating the rate at which water evaporates, guaranteeing a more consistent film creation.

By reviewing and going through various literature sources available, a formula was obtained by keeping in mind different formulation aspects of designing and developing microspheres. A standardized formula was prepared with the proper amount of drug, polymers, sodium alginate, cross linking agent as $CaCl_2$. The table below represents different interacting molecules along with their ratios.

Table 1- depicting formula of different formulations

Sr. No	**FORMULA CODE**	**SODIUM ALGINATE (mg)**	**CHITOSAN (mg)**	**HPMC (mg)**	**Camphor**	**DISTILLED WATER (ml)**	**$CaCl_2$ (%)**	**DRUG: SA: POLY.**
1	**CM1**	100	100	-	-	25	5	1:1:1
2	**CM2**	100	-	100	25	25	5	1:1:1
3	**CM3**	200	100	-	-	25	5	1:2:1
4	**CM4**	200	-	100	-	25	5	1:2:1

5	**CM5**	200	200	-	25	25	5	1:2:2
6	**CM6**	200	-	200	-	25	5	1:2:2

Figure 1 depicting fabricated mucoadhesive microspheres

Drug-interaction studies

When examining the chemical makeup of materials, Fourier-transform infrared spectroscopy, or FTIR, is a highly effective method. To manufacture the microspheres, the desired formulation would first need to be determined. Based on the particular use and required qualities, these microspheres could be composed of different polymers, such as carbopol, gelatin, or sodium alginate. Following preparation, FTIR analysis would be performed on the excipients and active ingredients. Measurements of wavelength absorption are made by passing infrared light through the sample. Details about the chemical bonds contained in the sample are revealed by the resultant spectrum.

By analyzing the FTIR spectrum, researchers can identify various functional groups present in the microspheres. For example: Peaks in the spectrum corresponding to **O-H** stretching vibrations may indicate the presence of hydroxyl groups, which are common in polymers like chitosan and gelatin. Peaks in the **C=O** stretching region may indicate the presence of carbonyl groups, which are present in many polymers and can provide information about the polymer structure. Peaks in the **C-H** stretching region may indicate the presence of alkyl groups, which are common in many organic compounds.

FTIR spectra of the microspheres can be compared to spectra of pure polymers or other materials used in the formulation. This comparison helps to identify which peaks are associated with the polymer and which may be due to other components, such as drug molecules or excipients.

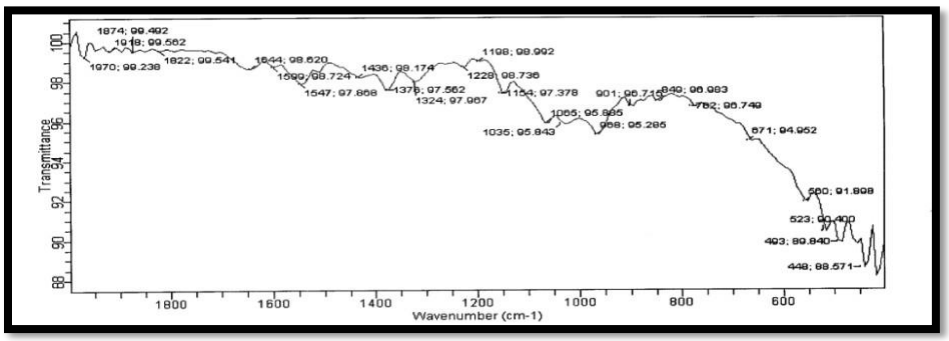

Figure 2 FTIR spectra of HPMC and pure drug

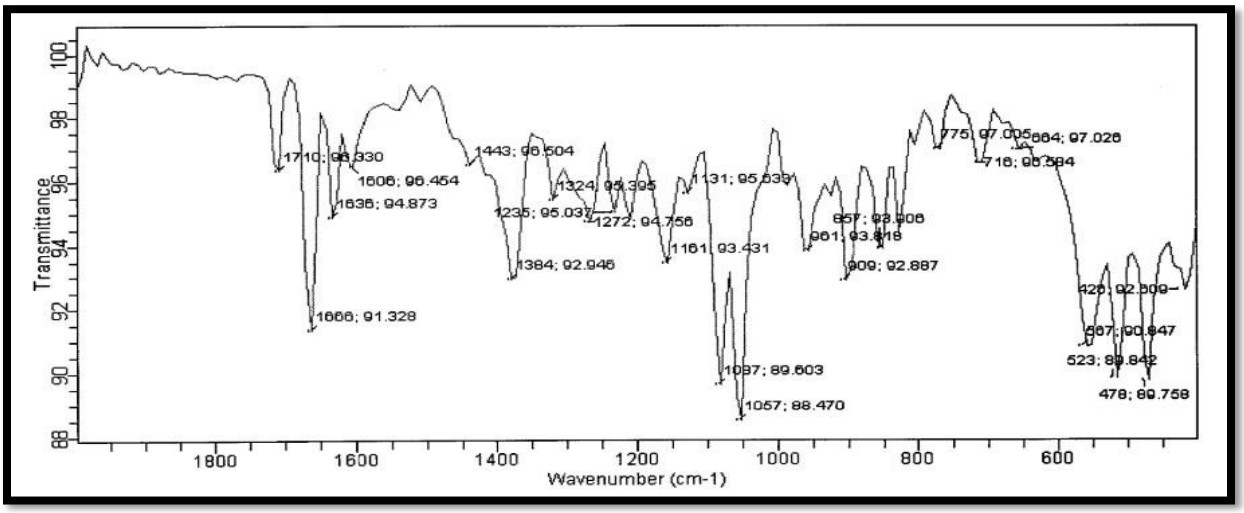

Figure 3 FTIR spectra of Flunisolide

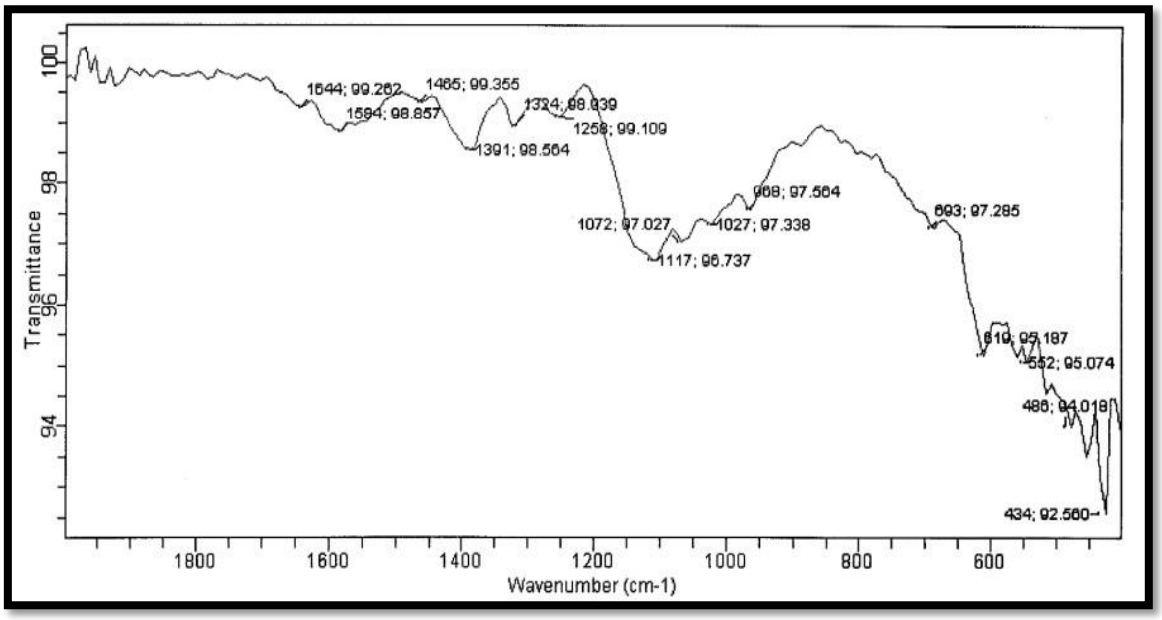

Figure 4 FTIR spectra of pure drug and chitosan

Characterization of microspheres:

Production yield %

Regarding microspheres, the production yield % denotes the effectiveness of the microsphere manufacturing process by showing the portion of the intended product that was achieved relative to the theoretical maximum yield. The formula is used to compute it:

Yield (%) of Production = Actual Yield / Theoretical Yield * 100%

Whereas

Real Yield: The quantity or mass of microspheres that are actually produced during the process.

Theoretical Yield: The greatest quantity or mass of microspheres that could be produced in a perfect world.

A higher production yield percentage denotes a more productive process with little waste, whereas a lower percentage denotes inefficiencies or waste throughout the manufacturing process.

Degree of swelling:

The amount that the microspheres expand in size or volume when exposed to a solvent or other medium is known as the degree of swelling of the microspheres. It is a crucial characteristic for comprehending how microspheres behave and function in a variety of settings, including chromatography and drug delivery systems.

The following formula can be used to determine the degree of swelling (S):

(Ws - Wd)/Wd × 100 = Swelling (%) where Wd is the dry microspheres' weight and Ws is the swollen microspheres' weight.

The degree of swelling is often expressed as a percentage, indicating the increase in weight relative to the initial weight of the dry microspheres. It provides insights into the ability of the microspheres to absorb or retain a solvent, which is crucial for their functionality in various applications.

Morphological studies of microspheres :

Microsphere morphological investigations by electron microscopy entail high-resolution examination of the microspheres' surface morphology and physical structure using transmission electron microscopy (TEM) or scanning electron microscopy (SEM).

Scanning Electron Microscopy (SEM):

SEM provides detailed images of the surface morphology of microspheres. Microspheres are typically prepared by fixing them onto a sample holder, coating them with a thin layer of conductive material (such as gold or platinum) to improve conductivity, and then imaging them under high vacuum. SEM images reveal information about the size, shape, surface texture, and porosity of microspheres. It allows for the visualization of surface irregularities, cracks, pores, and any surface modifications.

In vitro mucoadhesion study:

Conducting an in vitro mucoadhesion study with goat nasal mucosa involves evaluating the adhesive properties of microspheres to the mucosal surface of the goat nasal cavity. Here's a general overview of how such a study might be conducted:

First, the microspheres intended for the mucoadhesion study are prepared using appropriate formulation techniques, such as orifice ionic gelation method. These microspheres may be loaded with a drug or model compound for testing .After that, Fresh goat nasal mucosa is collected from slaughtered goats. The mucosa is carefully excised, cleaned to remove excess blood and debris, and then stored in an appropriate buffer solution to maintain its physiological properties. The goat nasal mucosa is mounted onto a suitable apparatus, such as a Franz diffusion cell or a custom-designed setup that mimics the nasal cavity environment. The mucosal surface should be oriented facing upward to allow for direct contact with the microspheres. The prepared microspheres are suspended in a suitable buffer or simulated nasal fluid and applied onto the mucosal surface of the goat nasal cavity. The concentration and volume of microsphere suspension applied may vary based on the experimental protocol. The microspheres are allowed to incubate with the goat nasal mucosa for a predetermined period, typically ranging from a few minutes to several hours. During this time, the interaction between the microspheres and the mucosal surface is monitored.

Results and Discussions

a. Physical appearance and morphological characteristics:

Table-3 Particle size distribution of microspheres

Sr. No.	FORMULA CODE	AVERAGE PARTICLE SIZE (µm)
1	**CM1**	257
2	**CM2**	233
3	**CM3**	355
4	**CM4**	376
5	**CM5**	481
6	**CM6**	521

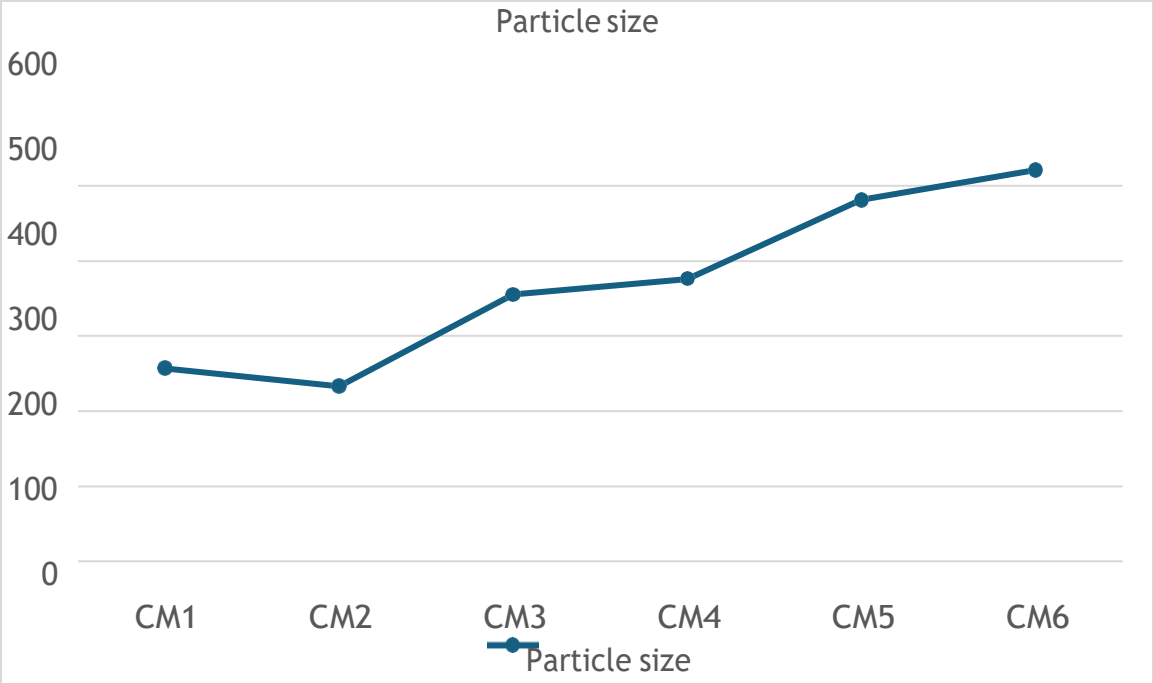

Figure 5 Graphical Representation of Particle size evaluation

b. Drug entrapment efficiency:

Table-4 Percentage drug entrapment efficiency

SR. NO	FORMULA CODE	DRUG ENTRAPMENT EFFICIENCY(%)
1	**CM1**	57.46
2	**CM2**	63.96
3	**CM3**	69.87
4	**CM4**	72.49
5	**CM5**	79.87
6	**CM6**	87.60

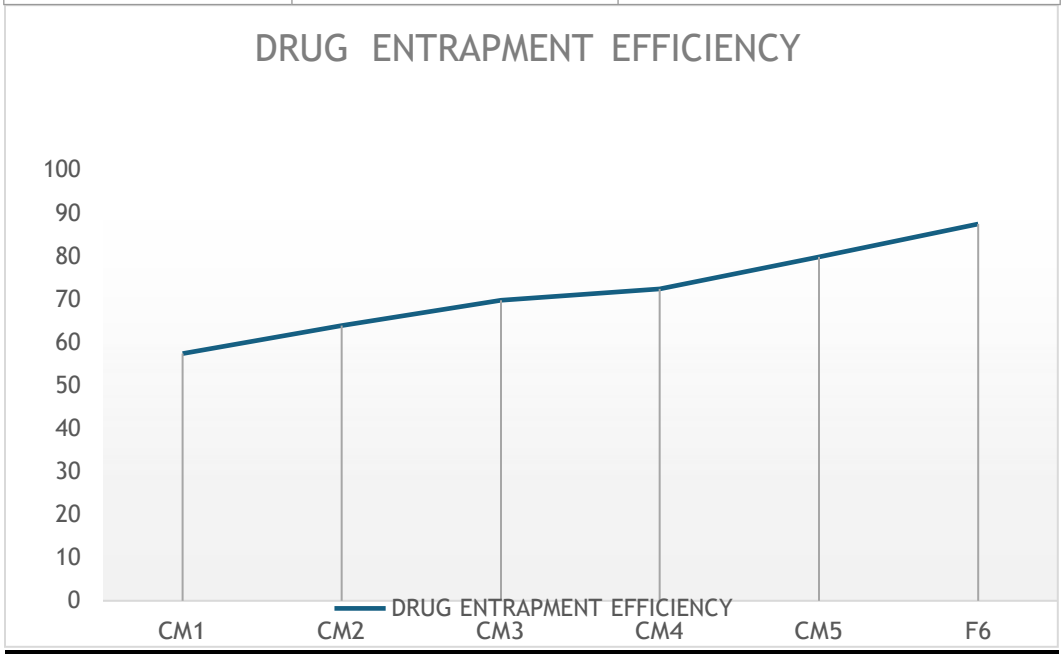

Figure 6 Graphical Representation of Drug entrapment efficiency

Percentage Yield :

Table no. 5 depicting percentage yield

SR. NO	FORMULA CODE	PERCENTAGE YIELD
1	**CM1**	58.6
2	**CM2**	65.5
3	**CM3**	77.3
4	**CM4**	72.4
5	**CM5**	83.7
6	**CM6**	87.5

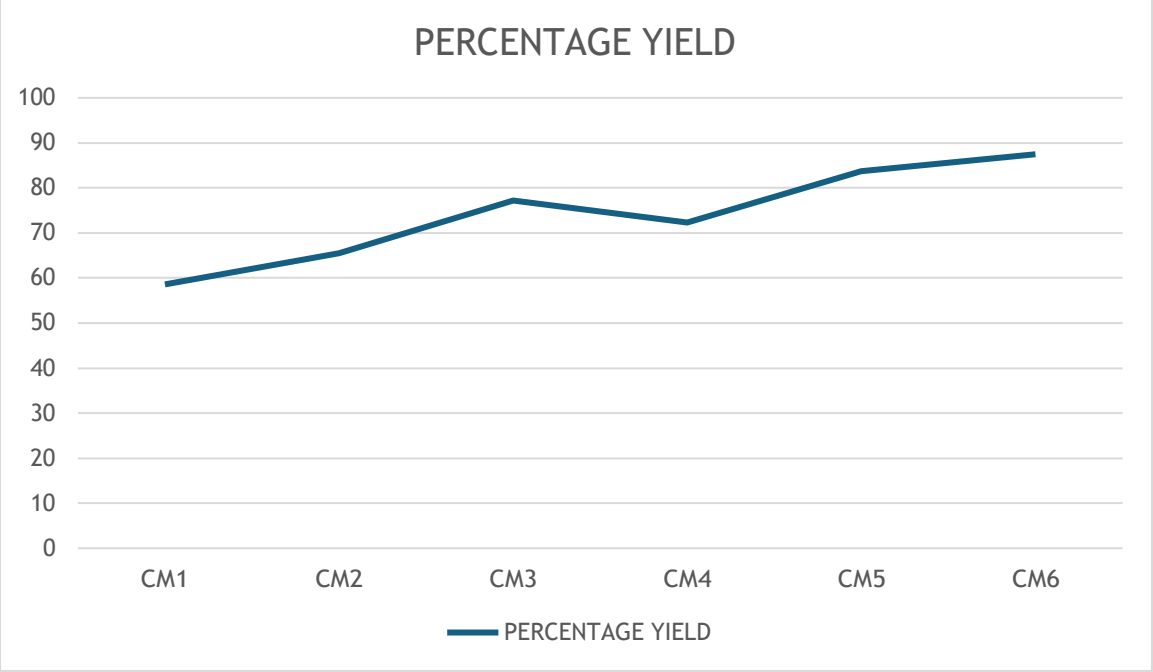

Figure 7 Graphical Representation of Percentage Yield

Swelling Index :

Table-6 Swelling index data

SR. NO	FORMULA CODE	SWELLING INDEX
1	**CM1**	4.25
2	**CM2**	6.57
3	**CM3**	5.03
4	**CM4**	7.57
5	**CM5**	5.67
6	**CM6**	8.99

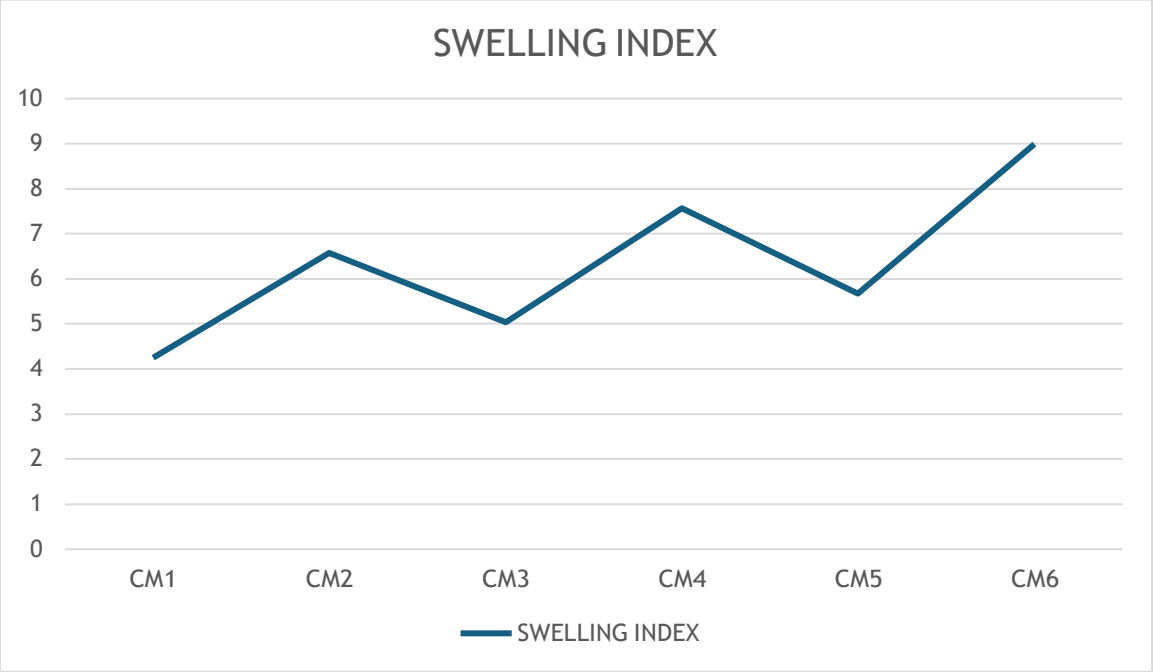

Figure 8 Graphical Representation of Swelling Index

***In Vitro* studies:**

Table no. 7 depicting *In Vitro* studies data

TIME (HRS)	CM1 %	CM2 %	CM3 %	CM4 %	CM5 %	CM6 %
0.5	4.58	5.70	4.29	6.25	6.69	7.62
1	10.59	11.85	8.57	12.59	11.18	13.57
2	21.56	22.65	23.63	24.10	25.32	26.21
4	30.53	31.25	32.20	33.90	35.28	37.49
5	52.98	53.52	58.15	60.85	61.82	64.58
6	66.58	69.21	70.21	71.82	75.96	81.02

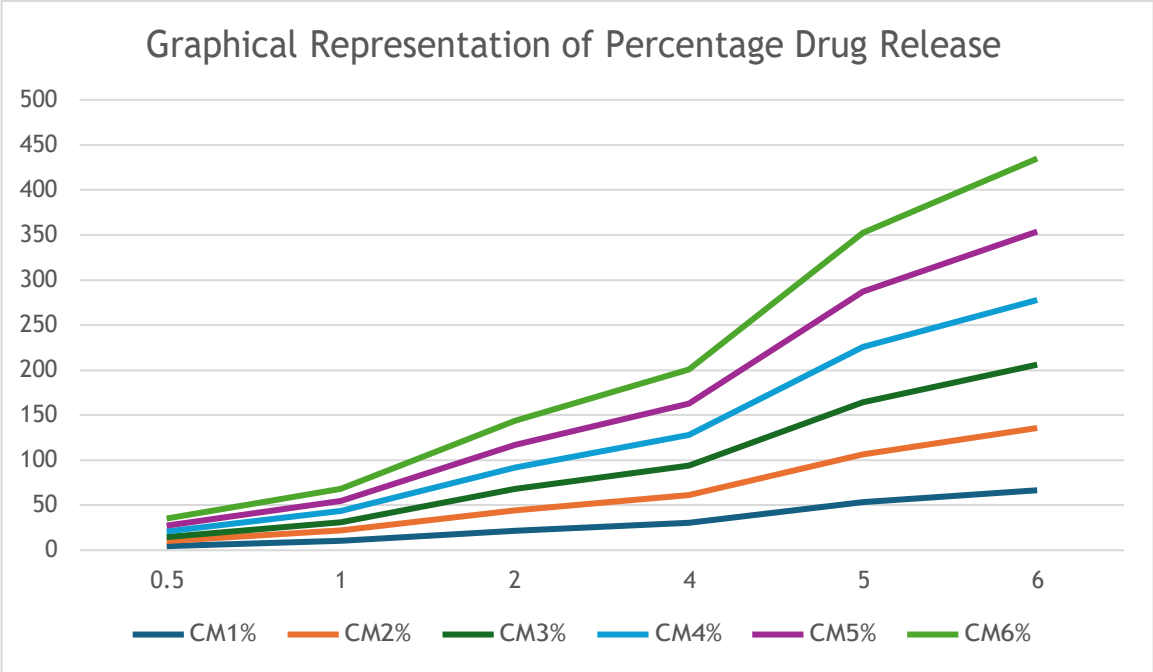

Figure 8 Graphical Representation of Percentage Drug Release

In vitro mucoadhesive test :

Table 8 representing data of in vitro studies

TIME (HRS)	CM1 %	CM2 %	CM3 %	CM4 %	CM5 %	CM6 %
0	100	100	100	100	100	100
1	92	88	92	88	88	92
2	80	80	80	84	80	88
4	60	64	72	76	68	80
6	40	56	64	68	52	72
7	32	40	56	60	48	68

8	28	32	40	52	40	60

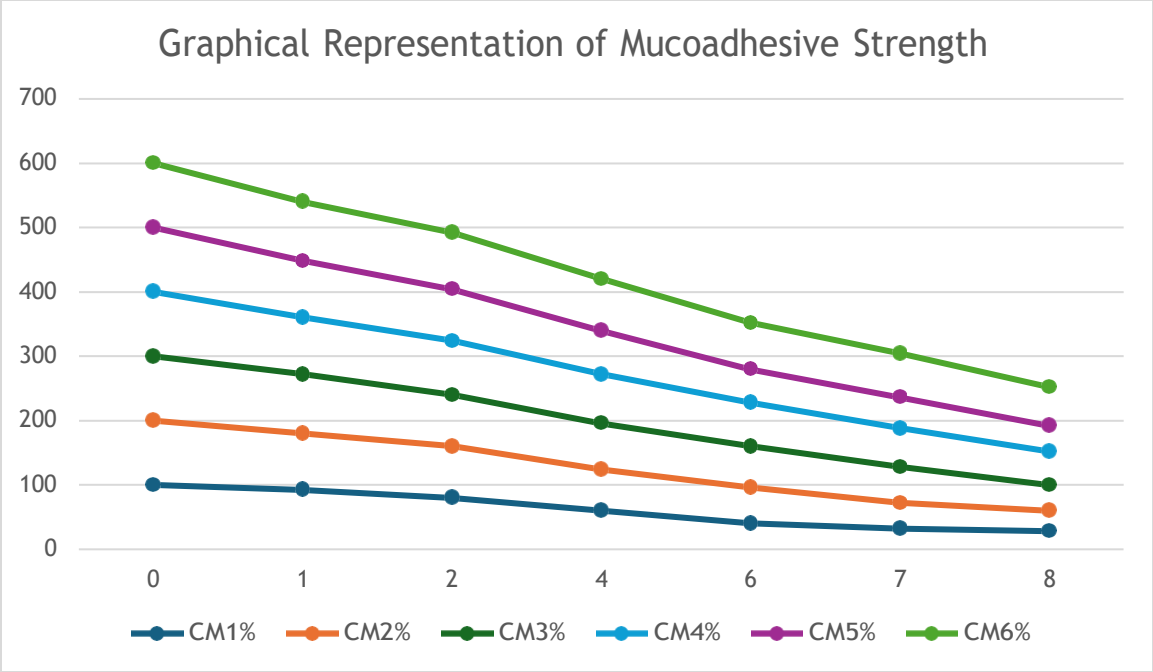

Figure 9 Graphical Representation of Mucoadhesive Strength

Stability studies testing (According to ICH guidelines)

Accelerated stability tests were crucial to the optimum formulation and were carried out in compliance with the International Council for Harmonization (ICH) Q1A requirements. For periods of 30, 60, and 90 days, stability data were gathered. Temperature, relative humidity, light, and moisture content were among the important characteristics measured in these investigations. The assessment parameters were reevaluated to guarantee the formulation's compatibility, stability, and long-term preservation of shelf life after maintaining the improved formulation under the designated conditions for these durations. Post stability tests CM6 emerged as the most stable among all.

Discussion:

Mucoadhesive microspheres significantly enhance drug retention time in the nasal cavity compared to conventional formulations, leading to prolonged therapeutic effects. Studies have shown that localized delivery of antibiotics and anti-inflammatory drugs via mucoadhesive microspheres is more effective in reducing inflammation and bacterial load in chronic sinusitis than systemic administration. By localizing drug delivery, systemic absorption is minimized, reducing the risk of side effects commonly associated with oral or injectable medications. The convenience of reduced dosing frequency and targeted delivery enhances patient adherence to the treatment regimen.

Conclusion:

Chronic Sinusitis, as the name implies is a prolong term disorder, that has to be managed and treated with patience and perfection. As our focus was inclined towards NDDS, we came across Microspheres. And they proved to be very effective in producing targeted and controlled release therapy. Comparing chitosan and HPMC, it was seen that along with sodium Alginate, HPMC showed better and vital results Formula CM6 came out as an emerging formulations among all other 6 formulations, giving us a viable and satisfactory readings. Moreover, most importantly, lab scale preparation was feasible owing to the characteristics of Orifice-ionic Gelation method.

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Conflict of interest: none

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