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ORIGINAL ARTICLE / ÖZGÜN MAKALE

PREPARATION AND *IN-VITRO* CHARACTERIZATION OF FLOATING-PULSATILE HOLLOWBEADS CONTAINING INDOMETHACIN

İNDOMETAZİN İÇEREN YÜZEN-PULSATİL İÇİ BOŞ BONCUKLARIN HAZIRLANMASI VE İN-VİTRO KARAKTERİZASYONU

Emrah ÖZAKAR[1*], Hasan Burak GÜNEŞ[2], Rukiye SEVİNÇ ÖZAKAR[1]

[1]Atatürk University, Faculty of Pharmacy, Department of Pharmaceutical Technology, 25240, Erzurum, Turkey

[2]Pharmacist, 46300, Kahramanmaraş, Turkey

ABSTRACT

Objective: *Objective: Designing matrix structured controlled release systems using polymers or waxy lipids is a popular option today. Hollowbeads are formulations characterized by the formation of an air-filled cavity inside. In our study, indomethacin was chosen as a model drug. Cetyl alcohol was selected to create the hollowbeads structure, and NaCMC was chosen to achieve long-term release. Kollicoat® MAE100P was used to reduce and/or prevent ulcer formation and control release.*

Material and Method: *The formulations were prepared using a new "wax removal" technique. Different concentrations of $ZnCl_2$ and $CaCl_2$ were used as crosslinkers. In the preformulation studies, 24 different formulations were prepared by changing the amount of NaCMC, the amount of crosslinker, and the crosslinking time. The structure, size, encapsulation efficiency, yield, hollow structure, and long-term release capacity were investigated in the formulations. These parameters were statistically evaluated depending on the amount of NaCMC, the type of crosslinker, the amount of crosslinker, and contact times with the crosslinker.*

Result and Discussion: *Hollowbeads were characterized by SEM and FT-IR. In vitro release studies, release kinetics, and release mechanisms were performed in pH 1.2 HCl and pH 6.8 phosphate buffer media. Swelling, and buoyancy studies were performed. The long-term stability, encapsulation efficiencies, drug loading efficiencies, and yields of the formulations were also evaluated. Two promising formulations (F2 and F19) were found to be able to release indomethacin in both the stomach and intestinal media for 24 hours.*

Keywords: *Controlled release, hollowbeads, indomethacin, polymer, wax removal*

* **Corresponding Author / Sorumlu Yazar:** Emrah Özakar
e-mail / e-posta: emrahozakar@atauni.edu.tr, **Phone / Tel.:** +904422315217

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ÖZ

Amaç: *Polimerlerin veya mumsu lipidlerin kullanılması ile matriks yapılı kontrollü salım sağlayan sistemler tasarlamak günümüzde popüler bir seçenektir. İçi boş boncuklar (hollowbeads), içinde hava dolu bir boşluğun oluşması ile karakterize formülasyonlardır. Çalışmamızda, indometazin model ilaç olarak seçilmiştir. Hollowbeads yapısını oluşturmak için setil alkol ve uzun süreli salım elde edebilmek için de NaCMC seçilmiştir. Ülser oluşumunu azaltmak ve/veya önlemek ve salımı kontollü elde etmek için Kollicoat® MAE100P kullanılmıştır.*

Gereç ve Yöntem: *Formülasyonlar, yeni bir teknik olan "wax removol" tekniği kullanılarak hazırlanmıştır. Çapraz bağlayıcı olarak $ZnCl_2$ ve $CaCl_2$'nin farklı konsantrasyonları kullanılmıştır. Önformülasyon çalışmalarında, NaCMC miktarı, çapraz bağlayıcı miktarı ve çapraz bağlanma sürelerini de değiştirerek 24 farklı formülasyon hazırlanmıştır. Formülasyonların yapısı, boyutu, enkapsülasyon etkinliği, verimi, hollow yapısı, uzun süreli salım kapasiteleri incelenmiştir. Bu parametreler NaCMC miktarına, çapraz bağlayıcı tipine, çapraz bağlayıcı miktarına ve çapraz bağlayıcıyla olan temas sürelerine bağlı olarak istatistiksel olarak değerlendirilmiştir.*

Sonuç ve Tartışma: *Hollowbeads'ler, SEM ve FT-IR ile karakterize edilmiştir. pH 1.2 HCl ve pH 6.8 fosfat tamponu ortamlarında in vitro salım, şişme ve yüzme çalışmaları gerçekleştirilmiştir. Salım kinetikleri ve salım mekanizmaları açıklanmıştır. Formülasyonların uzun süreli stabiliteleri, enkapsülasyon etkinlikleri, ilaç yükleme etkinlikleri ve verimleri de değerlendirilmiştir. Umut vadeden iki formülasyonun (F2 ve F19), 24 saat süreyle hem mide hem de bağırsak ortamında indometazin salımı gerçekleştirebildiği tespit edilmiştir.*

Anahtar Kelimeler: *İndometazin, kontrollü salım, mum yerdeğiştirme, oyuk boncuk, polimer*

INTRODUCTION

The oral route is the most common route of drug administration as it is multifunctional and convenient. Different release systems have been developed to increase bioavailability by reducing toxicity and side effects in drugs with the advances in pharmaceutical technology [1]. Modified-release dosage forms have always been a more effective therapeutic alternative to conventional or immediate dosage forms. The application purpose of modified release dosage forms is to regulate drug absorption from the gastrointestinal (GI) tract by controlling the release of the therapeutic agent. Such a dosage form can keep the concentration of the therapeutic agent in plasma stable for a longer period of time, effectively reducing dose-related side effects [2]. Modified release systems provide great benefits for patients with reduced dosing frequency and dose amount. Also, they provide the therapeutic advantages of drugs with less fluctuation in plasma blood levels. It is a popular option today to design matrix structured controlled release systems using polymers or waxy lipids in order to achieve controlled release [3, 4]. It is now possible to change the release rate of drugs by adjusting the amount of polymer [5].

Polymers have become an integral part of drug delivery systems with their improved pharmacokinetic properties. They can be targeted more specifically to tissues. Diffusion-based drug delivery systems are an important area in investigating the use of polymers in the delivery of solvent-activated drugs. In these systems, the drug is dissolved in a fully swollen matrix that does not dissociate. These solvent-activated systems swell when exposed to aqueous media and release the drug. They are also hydrophilic by nature [6-8].

In recent years, polysaccharide-based polymers have been widely used in biomedical and pharmaceutical applications due to their biocompatibility and biodegradability. It is studied in various fields

such as chemical engineering, medicine, pharmacy, food, and agriculture [9]. Sodium carboxy methyl cellulose (NaCMC) is a water-soluble, low-cost polysaccharide derivative that swells on contact with the GI system. Their viscosity and solubility change depending on the molecular weight and the length of the polymer chains [10]. They are used in many studies in formulation development and drug delivery systems [9-11].

One of the polymers used to prepare floating systems in the stomach is acrylic acid derivative polymers. Among these polymers is Kollicoat® MAE 100P. Drug delivery systems with mucoadhesive properties and pH-dependent release can be designed using this polymer [10]. The release of the drug from the polymeric matrix is controlled by three main mechanisms: diffusion, disintegration, and swelling. Efforts continue to increase the bioavailability of drugs by adhering to the mucosa, swelling, collapsing, expanding, gas-producing or super porous systems to increase the residence time of the dosage form in the stomach [12]. In recent years, scientific and technological advances have been made in the research and development of rate-controlled drug delivery systems by overcoming the disadvantages such as short residence times in the stomach and unpredictable gastric emptying times [13, 14]. Gastroretentive systems are dosage forms that have the feature of self-retention in the stomach to increase the absorption of the drug released from the acidic media in a controlled manner. Four types of gastroretension can be achieved with high-density systems, modified systems, mucoadhesive systems, and floating systems. Floating drug delivery systems have a lower density than gastric fluid. Therefore, they manage to remain floating in the stomach for a long time without being affected by the gastric emptying rate [10, 15].

Hollowbead systems are characterized by the formation of an air-filled space inside the dried beads after preparation [16]. It has been reported that hollowbeads are obtained by combining drug-containing polymeric structures with waxy components and injecting them into the system containing the organic phase [14, 17].

Indomethacin was chosen as the model drug in our study. Indomethacin is a nonsteroidal anti-inflammatory drug (NSAID) with antipyretic, analgesic, and anti-inflammatory activity [18, 19]. However, its serious gastrointestinal side effects limit its use and its poor solubility in biological fluids requires the design of special formulations [20]. It generally has a plasma concentration of 2 to 3 μg/mL and a biological half-life of 5 to 10 hours [21]. Indomethacin has been associated with ulcers it causes in relation to its residence time in the stomach [22]. Indomethacin exerts a higher ulcerogenic effect than other NSAIDs [23]. Our aim is to design and develop indomethacin-containing hollowbeads formulations floating in stomach contents with the “Wax Removal” technique [17]. Thus, both the ulcer-forming potential of indomethacin is reduced and formulations that can provide analgesic and anti-inflammatory effects have been reached for a longer period. In this study, 24 different hollowbeads formulations were studied to determine the effect of crosslinker type, crosslinker amount, mixing times, and polymer ratio.

The hollowbead formulations containing indomethacin were characterized by SEM and FT-IR. In vitro release studies were carried out in release media containing pH 1.2 HCl (USP30-NF25) and pH 6.8 phosphate (USP30-NF25) buffer. Also, swelling and floating experiments were performed containing pH 1.2 HCl buffer media. The release kinetics of each formulation were determined and the release mechanism was explained. Long-term stability, encapsulation efficiencies (EE%), drug loading capacities (LC%), and yields (Y%) of the formulations were also evaluated.

MATERIAL AND METHOD

Materials

Indomethacin was purchased from Alfa Aesar, Germany. Kollicoat® MAE 100P and Ethanol were purchased from Sigma-Aldrich®, USA. NaCMC was purchased from Doğa İlaç, Turkey. Cethyl alcohol and $ZnCl_2$ were purchased from Merck, Germany. $CaCl_2$ was purchased from J.T Baker, Germany. Ultra-pure water with resistivity higher than 18.2 MΩ cm was used in all experiments.

Development and validation of the quantity assay method

The quantity assay method for indomethacin was developed using UV-spectrophotometer (Beckman DU 730, Germany). 100 µg/mL stock solution was prepared of indomethacin in ethanol, and UV spectra were obtained in the wavelength range of 200-400 nm by making various dilutions from this stock solution. The wavelength at maximum absorbance (λmax) was determined. A calibration curve was created, and the assay method was validated for ICH parameters. All experimental studies were conducted in the dark to protect the indomethacin from light.

Experimental design

In our study, 24 different formulations were developed and the relationship between dependent variables and independent variables was statistically examined. The formulation design and data on the variables are given in Table 1. The effects on the independent variables (size, encapsulation efficiency, drug loading efficiency, yield, buoyancy, swelling, and release mechanism) were investigated by changing the dependent variables (the amount of NaCMC, $ZnCl_2$-$CaCl_2$, and mixing times).

Table 1. Formulation design and variables

Dependent Variables			
NaCMC amount (mg)	**$ZnCl_2$ amount (g)**	**$CaCl_2$ amount (g)**	**Mixing time (h)**
50	0.5	0.5	0.5
100	1.0	1.0	1
-	1.5	1.5	-

Preformulation studies

In our study, formulations were developed using the "Wax Removal" technique [17]. For this purpose, 25 mg of indomethacin, 100 mg of Kollicoat® MAE 100P, and 100 mg of cetyl alcohol were dissolved in a flacon with 2.5 mL of ethanol. In a separate vial, it was dissolved in 2.5 mL of distilled water by adding 50-100 mg of NaCMC. The ethanolic mixture was added to the mixture containing NaCMC and vortexed at 2000 rpm for 5 min. Then, 10 mL of solution containing different amounts of $ZnCl_2$ or $CaCl_2$ was dropped dropwise with a 22G injector while mixing at 550 rpm. Stirring was continued for 5 min after dropping. At the end of this period, the hollowbeads were filtered with filter paper and dried in an oven at 50 °C for 4 hours [24]. Obtained hollowbeads in dry form were stored in a moisture-free environment for further experiments. Each formulation was studied in triplicate. The prepared formulations and formulation parameters are given in Table 2. Blank hollowbeads without indomethacin were also prepared as described above without the addition of indomethacin.

Size, EE%, LC% and Y% of hollowbeads formulations containing indomethacin

The sizes of hollowbeads formulations containing indomethacin were measured manually using a caliper. Sizes were calculated as mean ($\bar{X}$) and standard deviation (SD), over at least 50 randomly selected beads from each formulation. The images were taken with a digital camera.

100 mg of dry beads of each formulation were powdered in a mortar and mixed in a vial containing 5 mL of ethanol for 4 hours at 750 rpm. The beads were completely fragmented by passing through a mechanical homogenizer (IKA®, T-18 Digital Ultra-Turrax, Germany) for 5 minutes at 10,000 rpm. Samples filtered through 0.45 μm membrane filters were quantified using a validated assay method. EE%, LC%, and Y% of the formulations were calculated as $\bar{X}$±SD using the formulas below. Each formulation was studied in triplicate [25].

EE% = (The calculated drug amount- Experimentally determined drug amount) / (The calculated drug amount) x 100
LC% = (The calculated drug amount- Experimentally determined drug amount) / (The amount of obtained beads) x 100
Y% = (The amount of obtained beads) / (The calcuated total amount of formulation ingredients) x 100

Swelling study of hollowbeads

50 mg of each hollowbead formulations containing indomethacin was weighed and placed in a vial. 50 mL of pH 1.2 HCl (USP30-NF25) buffer was added to them, and swelling was carried out at 100 rpm in a horizontal shaker water bath (Memmert WNB 14, SV-1422, Germany) adjusted to 37 °C for 24 hours. The swelling beads in each formulation were filtered from the medium, and their wet weights were recorded by reading on a precision balance at certain time intervals (0.5, 1, 2, 4, 8, 12, 24 hours). Each sample was run in three repetitions. The results were evaluated statistically. The weights of the beads before the experiment were compared with the weights after 24 hours, and the swelling capacity was determined as percentage [26].

$$\text{Swelling Degree (\%)} = 100 \times (W_2 - W_1) / W_1$$

W_1 = Beads weight before the experiment
W_2 = Beads weight after the experiment

Table 2. Formulations and formulation development parameters

	Formulation Parameters						
	Indomethacin (mg)	**Cethyl alcohol (mg)**	**Kollicoat® MAE 100P (mg)**	**NaCMC (mg)**	**$CaCl_2$ (g)**	**$ZnCl_2$ (g)**	**Mixing time (h)**
F_1	25	100	100	50	-	0.5	0.5
F_2	25	100	100	50	0.5	-	0.5
F_3	25	100	100	50	-	0.5	1
F_4	25	100	100	50	0.5	-	1
F_5	25	100	100	50	-	1	1
F_6	25	100	100	50	1	-	1
F_7	25	100	100	50	-	1.5	1
F_8	25	100	100	50	1.5	-	1
F_9	25	100	100	50	1	-	0.5
F_{10}	25	100	100	50	-	1	0.5
F_{11}	25	100	100	50	1.5	-	0.5
F_{12}	25	100	100	50	-	1.5	0.5
F_{13}	25	100	100	100	0.5	-	0.5
F_{14}	25	100	100	100	-	1	1
F_{15}	25	100	100	100	1	-	1
F_{16}	25	100	100	100	-	1.5	1
F_{17}	25	100	100	100	1.5	-	1
F_{18}	25	100	100	100	-	0.5	0.5
F_{19}	25	100	100	100	1	-	0.5
F_{20}	25	100	100	100	-	1	0.5
F_{21}	25	100	100	100	0.5	-	1
F_{22}	25	100	100	100	-	0.5	1
F_{23}	25	100	100	100	-	1.5	0.5
F_{24}	25	100	100	100	1.5	-	0.5

Buoyancy study of hollowbeads

One hundred beads were taken from each of the hollowbead formulations containing indomethacin and placed in a vial. pH 1.2 HCl (USP30-NF25) buffer was added to them, and floating was carried out at 100 rpm in a horizontal shaking water bath adjusted to 37 °C for 24 hours. The number of floating beads in each formulation was counted and recorded at certain time intervals (0.5, 1, 2, 4, 8, 12, 24 hours). Floated beads were determined at each measurement time as percentage. The time was examined for the beads to come to the surface of the vial and the total time the beads floated. Floating behavior and the number of floating beads were statistically compared with other formulations. This experiment was run in triplicate for each formulation [12, 26].

In vitro release study of hollowbeads

10 mg of dry hollowbeads were weighed and placed in amber-colored vials. 20 mL of pH 1.2 HCl (USP30-NF25) buffer or pH 6.8 phosphate buffer (USP30-NF25) was added to them, and the release study was carried out in a horizontal shaking water bath (37±0.5 °C, 100 rpm). One mL of each release medium was withdrawn and filtered through a 0.45 µm membrane filter at certain time intervals (0.5, 1, 2, 4, 8, 12, 24 hours). The same volume of fresh pH 1.2 HCl buffer or pH 6.8 phosphate buffer was added instead of the amount taken from each formulation to maintain sink conditions. The amount of indomethacin in supernatants was calculated using the validated quantity assay method. % cumulative release results were calculated. Each formulation was studied in triplicate [27, 28].

Release kinetics of hollowbeads

Release kinetics is an essential parameter in understanding the absorption, distribution, metabolism, and excretion of the active substance. Some mathematical processes and equations were used to explain the mechanism of indomethacin release from hollowbeads. R^2 values were taken as a basis in order to determine the appropriate kinetic model. The data for release profile of all the drug lodaded formulations in pH 1.2 HCl buffer (USP30-NF25) and pH 6.8 phosphate buffer (USP30-NF25) was processed by Microsoft Office Excel program in order to determine the best fitted kinetic model (Zero Order, First Order, Korsmeyer-Peppas, and Higuchi models) [12, 29].

FT-IR analysis of hollowbeads

It was carried out in order to determine the interactions between the active substance and excipients and to determine the presence of the active substance in the prepared formulations. For this purpose, infrared spectrums of indomethacin and two formulations (F2 and F19) with the longest floating/releasing time in the stomach, were taken using an FT-IR (Bruker, VERTEX ATR 70v, Germany). FT-IR analysis were carried out in the wavenumber range 4000-400 cm^{-1} for all bead formulations [29].

Examination of morphology of hollowbeads

Shapes and surface properties of the pure indomethacin and prepared hollowbeads examined using a SEM (Zeiss Sigma 300, Germany). The formulations were fixed on metal sheets with two-sided adhesive tape. The hollowbeads were coated with 100 Å thick gold. They were carefully cut in the middle. Their interiors were examined and photographed [30].

Evaluation of the stability of hollowbeads

Stability studies are an integral part of formulation development. It gives an idea to determine the most suitable conditions for the structure of the prepared formulation. The stability study was performed according to ICH guidelines for promising formulations (F2 and F19). The stability test for 12 months

was carried out at 25±2 °C/60 ± 5% RH in a non-hygroscopic package with a certain amount of hollowbeads belonging to F2 and F19 [26]. The stability and effectiveness of the product were evaluated with various parameters at the end of the period [31].

Statistical Analysis

All analysis and experiments were statistically analysed determine whether a significant difference between formulations. Fort his purpose, One-way Analysis of Variance (ANOVA) test was applied. In addition, all experimental data were calculated as arithmetic mean and standard deviation. The confidence limit was accepted as 95%. The $p<0.05$ level was considered statistically significant in the differences between the groups.

RESULT AND DISCUSSION

Development and validation of the quantity assay method

Dilutions were made from the stock solution of indomethacin and the maximum wavelength was found to be 317 nm (Figure 1). The calibration curve was drawn from 7 points in the concentration range of 10-55 µg/mL. Method validation was provided on the parameters of specificity, sensitivity, linearity, accuracy, precision (repeatability, reproducibility) and stability in accordance with ICH guidelines. The method is specific and sensitive for indomethacin. The method was found to be linear within the range of the calibration curve. % Relative Standard Deviation (RSD%) and % Relative Error (RE%) were found below 2%. It was determined that the stock solution remained stable for 3 days at both room temperature and +4 °C. Other parameters of validation are given in Table 3.

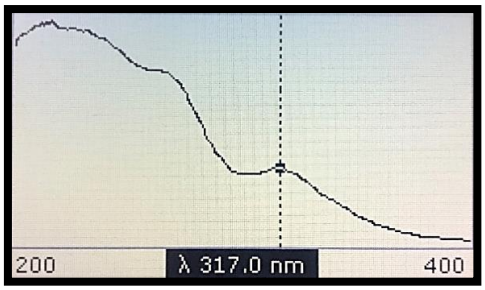

Figure 1. Indomethacin UV spectrum

Table 3. Validation parameters

Equation	y=0.0185x+0.0043
R^2	0.9993
Λ (nm)	317
LOD (µg/mL)	0.857
LOQ (µg/mL)	2.688

* Accuracy and precision calculations were found to be less than ±2%.
** Recovery (%) calculations were not found less than 98%.

Formulation development studies

Images of hollowbeads as a result of formulation development studies are given in Figure 2. It has been determined that the hollowbeads in our study were generally spherical and their sizes vary between 1330 μm and 1960 μm. The beads formed when they were dropped into solutions containing Ca^{2+} or Zn^{2+} ions. However, the beads exhibited poor mechanical strength, especially in solution containing Zn^{2+} ions. It was determined that there was no significant change in the dimensions of the hollowbeads with the increasing amount of polymer (NaCMC) ($p>0.05$).

In almost all formulations, it was observed that the use of $CaCl_2$ as a crosslinker decreased the bead size compared to the use of $ZnCl_2$, and this was found to be statistically significant ($p<0.05$). It was also found that as the amount of crosslinker increased (such as 0.5, 1 and 1.5 w/v), the size decreased and this was statistically significant between formulations prepared using the same group of crosslinkers ($p<0.05$). This situation has also been reported in similar studies in the literature. In particular, it has been reported that during the dropping of the polymeric content into the solution containing high amount of crosslinker results in spherical beads due to the excess number of ions surrounding the dripped content [32].

Size, EE%, LC% and Y% of hollowbeads formulations containing indomethacin

Size, EE%, LC% and Y% of hollowbeads formulations containing indomethacin are given in Table 4. It was observed that there was no statistically significant difference among the formulation yields with the change in the amount of crosslinker ($p>0.05$). On the other hand, upon comparison of the cross-linkers, it was determined that the formulations with better yields were those prepared using $CaCl_2$. They showed statistically significant difference ($p<0.05$). In literature, it has been reported that the type and amount of polymer changes the yield in this direction [12]. Another important point is the mixing time. The data indicated that the yield increased as the mixing time was shortened. It was observed that the highest yield values were obtained with 0.5 hour mixing time.

The formulations were evaluated in terms of EE% and LC%, it was observed that LC% and EE% increased statistically with the increase in the amount of crosslinker in the solution (0.5, 1.0, 1.5 g) ($p<0.05$). The $CaCl_2$ and $ZnCl_2$ were compared in terms of LC% and EE%, it was determined that the formulations prepared with $CaCl_2$ had statistically significantly better EE% and LC% ($p<0.05$). In addition, increasing the amount of NaCMC from 50mg to 100mg increased EE% and LC% in all formulations. This situation was found to be statistically significant ($p<0.05$). Similar results have been reported in the literature [12, 33].

Swelling study of hollowbeads

The swelling behavior of the polymer is an important factor in controlling the release of drugs from

the bead systems. The degree of swelling of the formulated beads showed that swelling was associated with different polymer ratios [26]. The swelling study results of our experiments for 24 hours are given in Table 5 as %. The swelling experiments were conducted in the gastric media since the main route of absorption of indomethacin is the stomach and the formulations do not dissolve at gastric pH. Due to the dissolution of Kollicoat® MAE 100P at the intestinal media, no swelling study was performed in the intestinal media.

Figure 2. Images of hollowbeads containing indomethacin

In literature, Giri et al. reported that no formulation swelled above 10% at the end of the first 1.5 hours in the floating study performed on the beads prepared using pectin and xanthan gum [32]. Awasthi and Kulkarni determined that the swelling rate of the beads they prepared was below 3% in the swelling study conducted in a pH 1.2 HCl media for 3 hours [12]. Taranalli et al. designed hollowbeads by experimenting with different polymer combinations. They reported that their formulations floated for 12 hours and 24 hours in a pH 1.2 HCl media, but their swelling rate could not exceed 2% [26]. In our study, it was observed that the formulations swelled in the range of 35-96% even at the end of the first half-hour. The swelling balance was reached in a short time in all formulations. With the doubling of the amount of NaCMC (100 mg), it was determined that the water absorption and swelling rates of the formulations generally showed minor swelling than the formulations prepared using less amount of NaCMC (50 mg) ($p<0.05$). This situation may be since water takes longer to enter the beads due to the increase in polymer amount. It is also thought that the water absorption rate remains low, as the surface in contact with this water erodes and begins to break down over time.

Table 4. Size, EE%, LC% and Y% of hollowbeads formulations containing indomethacin (X̄±SD)

Formulations	Size (mm)	EE%	LC%	Y%
F_1	0.178±0.011	82.15±3.25	3.42±0.41	82.42±3.61
F_2	0.152±0.012	93.68±1.33	5.87±0.78	92.21±1.33
F_3	0.180±0.014	76.73±3.67	2.15±1.11	81.13±3.49
F_4	0.163±0.017	88.19±0.22	4.92±0.78	87.01±1.95
F_5	0.172±0.015	77.68±3.13	2.42±0.55	74.65±3.15
F_6	0.141±0.009	83.41±1.88	3.66±0.71	79.18±1.01
F_7	0.133±0.012	84.56±0.51	3.91±0.58	76.10±0.22
F_8	0.156±0.012	87.75±2.27	4.01±1.03	79.77±0.36
F_9	0.148±0.013	89.13±0.44	4.28±0.54	84.41±.028
F_{10}	0.196±0.013	85.05±1.98	3.88±0.97	81.13±0.75
F_{11}	0.146±0.016	87.42±2.45	4.11±0.48	81.01±1.13
F_{12}	0.206±0.016	82.69±0.79	3.55±2.12	79.15±1.47
F_{13}	0.146±0.017	85.93±2.05	3.75±0.71	88.91±0.18
F_{14}	0.182±0.019	78.17±3.15	2.61±1.09	73.45±2.46
F_{15}	0.144±0.014	91.49±0.85	5.13±0.84	89.81±2.12
F_{16}	0.172±0.014	80.45±2.18	3.88±1.23	76.43±3.08
F_{17}	0.154±0.016	90.81±0.43	4.19±1.65	80.17±0.09
F_{18}	0.176±0.015	78.15±3.59	2.83±0.78	84.12±0.52
F_{19}	0.150±0.014	95.42±2.38	5.94±1.14	93.11±0.43
F_{20}	0.189±0.020	82.42±2.10	3.41±0.22	80.81±0.83
F_{21}	0.154±0.024	89.41±1.33	4.01±2.12	83.15±0.53
F_{22}	0.166±0.015	81.79±0.97	3.01±1.71	81.94±2.54
F_{23}	0.171±0.017	87.43±1.89	3.88±1.47	79.13±0.50
F_{24}	0.188±0.021	90.71±1.41	4.52±1.03	84.15±1.16

In our study, among the groups with the same amount of polymer (F1-F12 and F13-F25), it was determined that the formulations prepared with $CaCl_2$ crosslinker could absorb water significantly less

than the formulations prepared with $ZnCl_2$ (eg, F2 and F19) ($p<0.05$). This may have occurred as a result of the strong interaction of Ca^{+2} ions with the carboxylic end groups on the polymer. This indicates that the type of crosslinker contributes to the hardener strength and toughness on the bead structure. It has also been determined that it allows the water to enter the bead at a later time and to remain in the media for a more extended period of time without disturbing the integrity of the bead. This situation has also been reported in similar studies in the literature [1].

Table 5. Swelling results of hollowbeads formulations (%)

Formulations	**Swelling rate (%)**						
	0.5 h	**1 h**	**2 h**	**4 h**	**8 h**	**12 h**	**24 h**
F_1	94	98	74	52	47	23	0
F_2	71	81	86	87	84	75	60
F_3	83	99	65	41	19	0	0
F_4	53	62	72	73	72	65	46
F_5	Structure was degraded						
F_6	46	54	48	41	39	34	23
F_7	77	88	62	51	28	20	12
F_8	35	42	51	55	57	42	17
F_9	41	55	60	67	50	28	19
F_{10}	Structure was degraded						
F_{11}	45	26	Structure was degraded				
F_{12}	72	10	Structure was degraded				
F_{13}	63	72	71	62	39	34	34
F_{14}	76	16	Structure was degraded				
F_{15}	52	57	56	44	46	41	41
F_{16}	Structure was degraded						
F_{17}	46	41	41	36	28	22	19
F_{18}	64	27	Structure was degraded				
F_{19}	66	74	68	65	42	43	43
F_{20}	82	17	Structure was degraded				
F_{21}	63	52	49	40	33	32	29
F_{22}	96	41	34	27	18	16	0
F_{23}	Structure was degraded						
F_{24}	42	47	39	32	24	20	18

Buoyancy study of hollowbeads

The results of the buoyancy study of hollowbeads formulations containing indomethacin are given in Table 6, and the sample images of the F2 and F19 formulations at the end of the 24th hour are given in Figure 3.

Table 6. Buoyancy results of hollowbeads formulations (%)

Formulations	Buoyancy rate (%)						
	0.5 h	**1 h**	**2 h**	**4 h**	**8 h**	**12 h**	**24 h**
F_1	100	100	80	50	25	25	25
F_2	100	100	100	100	100	75	75
F_3	100	50	50	25	25	10	10
F_4	100	100	100	100	100	75	75
F_5	100	0	0	0	0	0	0
F_6	100	80	60	40	25	25	25
F_7	100	100	90	50	40	25	10
F_8	100	100	100	75	50	25	10
F_9	100	75	50	50	25	25	25
F_{10}	0	0	0	0	0	0	0
F_{11}	50	25	0	0	0	0	0
F_{12}	50	25	0	0	0	0	0
F_{13}	100	100	100	100	50	40	40
F_{14}	75	25	0	0	0	0	0
F_{15}	100	100	100	50	50	40	40
F_{16}	0	0	0	0	0	0	0
F_{17}	100	75	75	50	25	10	10
F_{18}	50	25	0	0	0	0	0
F_{19}	100	100	100	100	50	50	50
F_{20}	50	25	0	0	0	0	0
F_{21}	100	75	75	50	30	30	25
F_{22}	100	75	50	25	10	10	10
F_{23}	0	0	0	0	0	0	0
F_{24}	100	100	50	25	15	10	10

In the acidic media, the functional carboxyl groups of hollowbeads remain protonated and exert an insignificant electrostatic repulsive force. As a result, the beads swell at a very low rate. However, when higher pH values are reached in the medium, the carboxyl groups of the beads may be ionized and the osmotic pressure inside the beads increases. In this way, swelling and fragmentation of the structure can be observed by absorbing water faster [34]. Further swelling can be observed due to the exchange of positively charged ions (such as Ca^{+2} or Zn^{+2}) in the cross-linked beads with sodium ions in the phosphate buffer in alkaline media. So, it leads to water penetration and swelling [12]. Chemate et al. reported that calcium pectinate beads completed drug release after 10 hours, and the number of floating beads was below 20% at the end of 24 hours [35]. Awasthi et al. designed gastroretentive beads containing sodium alginate, pectin and HPMC. They reported that the floating rate of the formulations was between 60-70% for 12 hours [12]. Somani et al. prepared calcium pectinate hollowbeads and determined that the number of beads floating in the stomach medium was reduced by 50% after 12 hours. At the end of 16 hours, they reported that there was no floating bead [36]. Hsu et al. designed beads in a core-shell structure and reported that they obtained the best floating ratio with 1:4 chitosan:xantham gum. They reported that as the chitosan rate increased, the floating rate decreased [1].

Chauhan et al. also designed floating tablets containing indomethacin and reported that a floating study was performed at 37 °C in pH 1.2 HCl buffer for 12 hours [31].

In our study, it was determined that 13 of the 24 formulations floated even at the end of the 24th hour. It was determined that almost all of the formulations prepared using $CaCl_2$ crosslinker floated after this period. Compared to the formulations prepared using $ZnCl_2$ crosslinker, this difference was significant in terms of floating rate ($p<0.05$). In addition, it was determined that the amount of polymer used (NaCMC) did not make a significant difference ($p>0.05$). It was determined that F2 and F19 formulations floated by 75% and 50%, respectively, even after 24 hours.

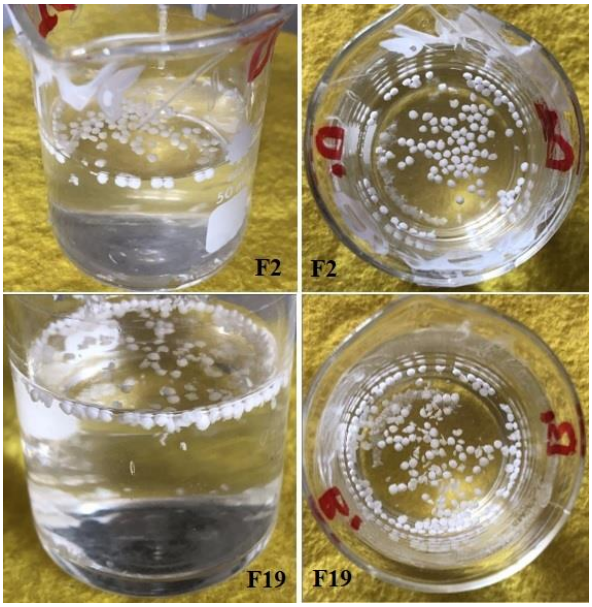

Figure 3. Buoyancy images of formulations after 24 hours (upper: F2; bottom: F19)

Among the obtained hollowbeads, two promising formulations (F2 and F19) were selected as a result of EE%, LC%, Y%, swelling and buoyancy studies. Further studies were carried out on these formulations.

In vitro release study of hollowbeads

The *in vitro* release study results for hollowbeads (F2 and F19) conducted in pH 1.2 HCl buffer and pH 6.8 phosphate buffer media are given graphically in Figure 4 below. While approximately 10% of pure indomethacin was released in pH 1.2 HCl buffer media in 24 hours, this ratio was approximately 3% in our F2 formulation developed using 50 mg NaCMC, and this ratio was approximately 2% in our F19 formulation developed using 100 mg NaCMC. These data indicate that the amount of polymer did not significantly affect the release of indomethacin. Still, both formulations produced significantly less and more extended indomethacin release than pure indomethacin ($p<0.05$).

Approximately 100% of pure indomethacin was released in pH 6.8 phosphate buffer in 4 hours. This rate was achieved at approximately 12 hours in our F2 and F19 formulations. It has been determined that our formulations release indomethacin for a long time in pH 1.2 and pH 6.8 buffer media. Especially with the F2 and F19 formulations, which can float in the stomach content even after 24 hours, the release of indomethacin in non-ionized form has been found to be sustainable for 24 hours. This also has been reported by other authors who have designed similar formulations for the gastrointestinal tract. In particular, hollowbeads underwent very little ionization (~2-3%) at pH 1.2 due to the presence of carboxylic acid-containing groups from Kollicoat® MAE 100P. Therefore, the bead structure remained significantly stable at this pH. However, ionization occurred in the pH 6.8 media. The intramolecular and intermolecular electrostatic repulsive forces of the polymer were increased, and the beads were fragmented and released faster. Since pH conditions differ in the stomach and intestinal tract, the use of beads prepared with such pH-sensitive polymers as drug carrier systems provides a significant advantage over other systems [27].

The release of hollowbeads in a pH 6.8 environment was sustained for approximately 12 hours. Although it was observed that the increase in the amount of NaCMC polymer used in F19 compared to F2 prolonged the release somewhat, it did not create a statistically significant difference ($p>0.05$). It was observed that the drug release between the polymeric matrix chains was delayed in both environments with the increase in the amount of polymer. These results have also been reported in similar studies in the literature [32]. It was determined that the F19 formulation produced a longer duration and less indomethacin release compared to the F2 formulation. However, it was determined that both formulations released indomethacin in a longer time compared to pure indomethacin ($p<0.05$). It has also been reported in studies in the literature that the release of the active substance is accelerated when the amount of polymer remains too high or too low [1]. The lower drug loading efficiency in the beads results in larger pore formation resulting in higher swelling and faster drug release. Higher the drug loading efficiency, larger the drug area formed in the beads. This causes the size of the matrix as well as the shrinkage of the pores, resulting in a decrease in drug release. A similar finding has been reported by other authors [32].

Slower drug release from the beads in pH 1.2 HCl buffer media may be due to the limited solubility of the beads in the acidic media as they contain the enteric polymer Kollicoat® MAE 100P, as well as the weak acidic nature of the drug. At higher pH values, Kollicoat® MAE 100P will dissolve and the carboxyl groups of the beads will ionize. As a result, the osmotic pressure inside the beads increases. The result is a higher rate of swelling and faster drug release. In an acidic media, the carboxyl groups cause the electrostatic repulsion to be lost, thereby reducing the swelling of the beads. This slows down the release of the drug. It was also observed that the drug release decreased as the crosslinker ($CaCl_2$) concentration increased. This may have been caused by the reduction of the free volume of the polymer matrix in the presence of higher crosslinkers, thereby inhibiting the movement of solutes through the

polymer matrix [32].

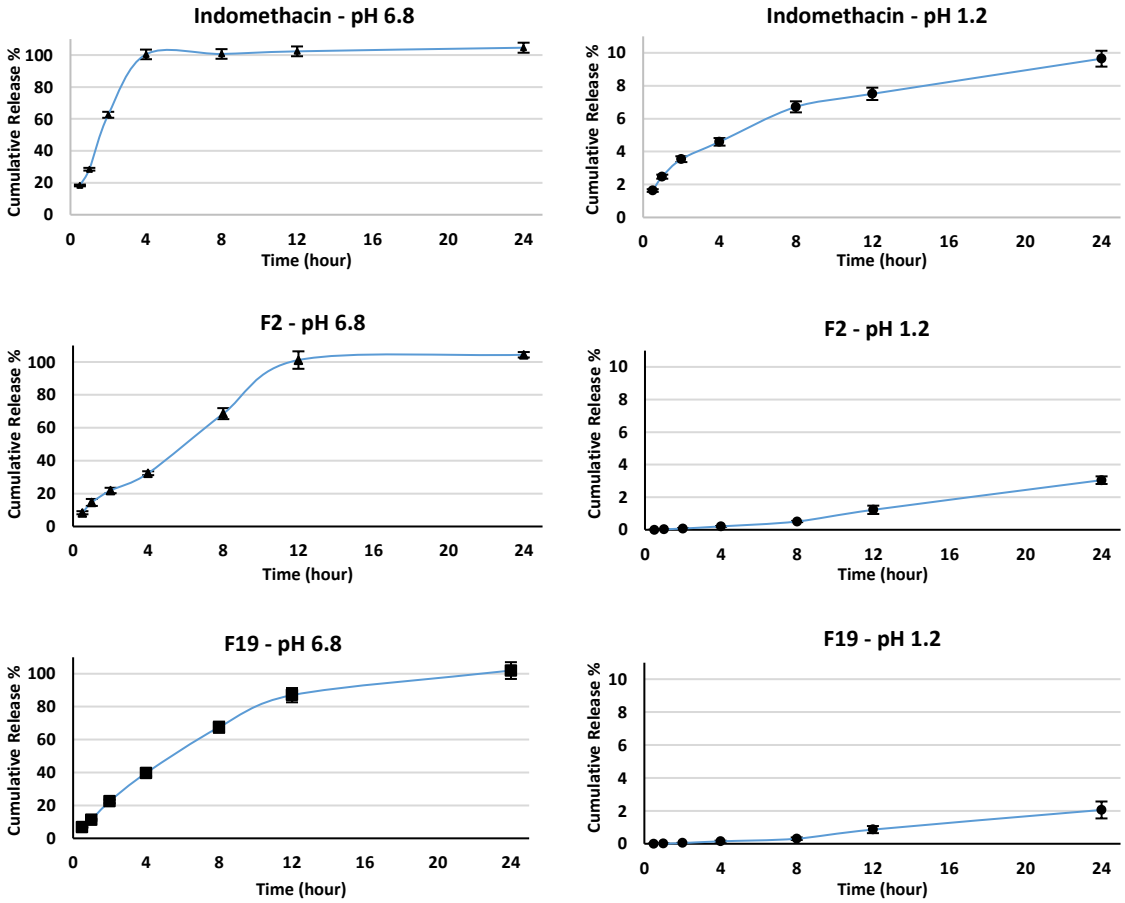

Figure 4. *In vitro* release study of indomethacin, F2 and F19 formulations

Release kinetics of hollowbeads

The release kinetics of F2 and F19 hollowbeads formulations are given in Table 7 and Table 8 respectively, as below. Drug release data were analyzed using Higuchi, Korsmeyer-Peppas, Zero-Order, and First-Order model equations to determine drug release kinetics from hollowbeads.

Table 7. Release kinetics of hollowbeads in pH 1.2 buffer media

	Zero-Order	First-Order	Higuchi	Korsmeyer-Peppas		Release Mechanism
Hollowbeads	**R^2**	**R^2**	**R^2**	**R^2**	**n**	
F2	0.987	0.770	0.867	0.666	0.940	Super Case-II Transport
F19	0.981	0.600	0.866	0.519	1.026	Super Case-II Transport

Table 8. Release kinetics of hollowbeads in pH 6.8 buffer media

	Zero-Order	First-Order	Higuchi	Korsmeyer-Peppas		Release Mechanism
Hollowbeads	**R^2**	**R^2**	**R^2**	**R^2**	**n**	
F2	0.854	0.645	0.920	0.935	0.799	Non-Fickian diffusion
F19	0.788	0.574	0.885	0.925	0.866	Non-Fickian diffusion

In vitro dissolution data of all series were calculated according to the Peppas equation versus time to find the drug release mechanism and confirm whether the diffusion mechanism is Fickian or non-Fickian. If the "n" value resulting from the Peppas equation is less than or equal to 0.5, it indicates that the drug release mechanism is by diffusion without swelling. However, if the "n" value is greater than 0.5 and less than 1, it is understood that the release is by diffusion based on swelling. If this "n" value is above 1, it indicates a (non-Fickian) release where the release mechanism occurs by abnormal diffusion and Fick's laws cannot be applied [12, 29, 37]. It was clear from the kinetic data that the drug release kinetics followed Peppas' kinetics for F2 and F19 in a pH 6.8 release medium, and the release was via swelling controlled diffusion. The calculated slope values of the Peppas equations gave a value close to but less than 1. This confirmed that the mechanism for the release of indomethacin from hollowbeads was Fickian diffusion with swelling in the intestinal medium. From the kinetic data, it was seen that the drug release kinetics followed the zero-order kinetics for F2 and F19 in the pH 1.2 release medium, and the release mechanism of indomethacin from hollowbeads was confirmed to be Super Case-II Transport in the gastric media. This indicates that indomethacin release from our formulations occurs at a constant rate in the pH 1.2 release medium. The value of "n" greater than 0.85 indicates "Super Case-II Transport" related to polymer relaxation during swelling [19, 32].

FT-IR analysis of hollowbeads

IR spectra were taken from powder samples of selected F2 and F19 hollowbeads formulations and pure indomethacin. FT-IR spectra of indomethacin and formulations are shown in Figure 5. It is seen that the characteristic peaks of indomethacin did not change and were clearly observed in the FT-IR spectra of the formulations. This showed that there was no interaction between indomethacin and excipients [38].

The characteristic peaks of pure indomethacin were compared with the peaks obtained from the formulations. C=O stretching, one of the characteristic peaks of indomethacin, was observed at the frequency of 1711.44 cm^{-1} with the same or slight differences. In the spectra were also seen characteristic peaks at 1689.05 cm^{-1} (C=O stretching vibrations), 1221.34 cm^{-1} (asymmetric aromatic O-C stretching), and 1066.31 cm^{-1} (symmetric aromatic O–H stretching) in the formulations [19, 31].

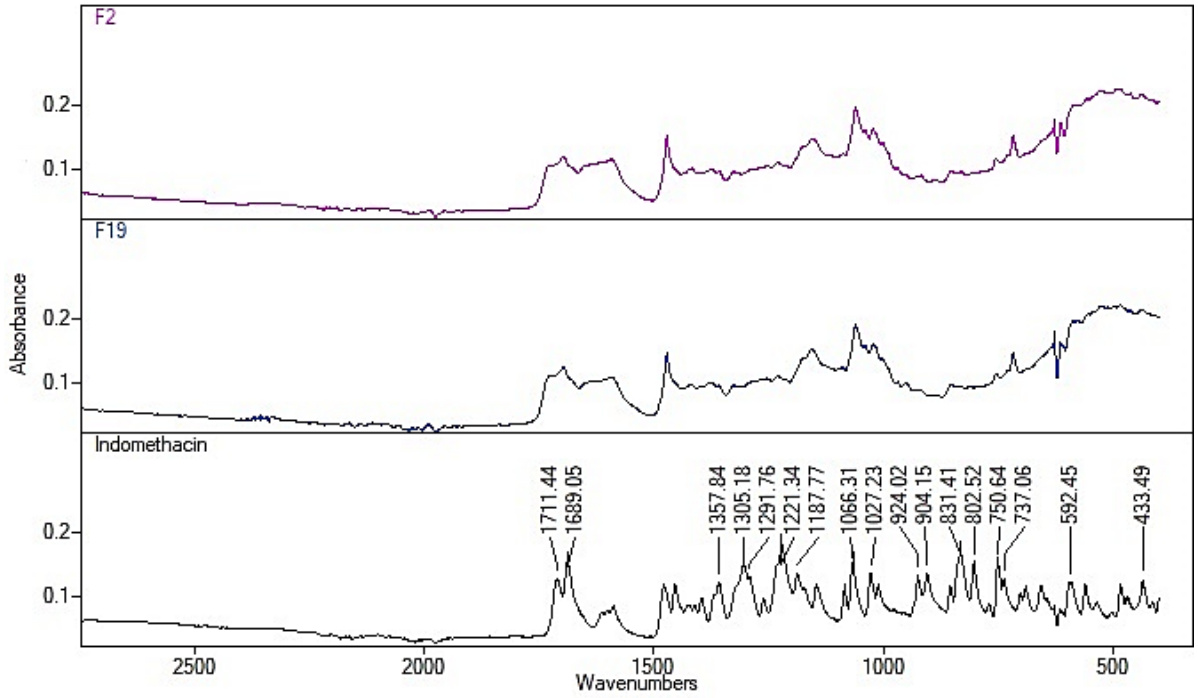

Figure 5. IR spectra of F2 (upper), F19 (middle) and indomethacin (bottom)

Examination of morphology of hollowbeads

SEM images of pure indomethacin, blank, F2, and F19 hollowbeads formulations are given in Figure 6. In the image of blank hollowbeads (upper right), it is seen a very smooth and spherical structure. In the images of F2 (bottom left) and F19 (bottom right), beads were cut in the middle. The cross-section of beads from F2 and F19 showed a hollow core and multiple small hollow pockets in the matrix. The layered structure within the bead wall is also an indication of a long-term release during *in vitro* release studies. The outer surfaces of the beads were very dense, and the inner layers were very porous and pocketed. The fact that the core was completely empty may have allowed the formulations to float for a long time. A similar situation has been reported in studies of hollowbeads and porous beads in the literature [1, 24, 27].

Evaluation of the stability of hollowbeads

Stability studies were carried out on the two promising formulations (F2 and F19) within the scope of formulation development studies. EE%, LC%, buoyancy, swelling, and release mechanisms data were evaluated and comparisons with freshly prepared hollowbeads study results were made at the end of 12 months. The results obtained are given in Table 9 below.

As a result of the stability study performed for 12 months according to the ICH guidelines on formulations that could be potential drugs, it has been shown that no significant changes were observed in the appearance of the formulations, encapsulation efficiencies, drug loading capacity, buoyancy,

swelling and *in vitro* release studies. F2 and F19 formulations were confirmed to be stable after 12 months at 25 ± 2°C/60 ± 5% RH. The obtained results were evaluated and compared to the freshly prepared formulation data. The obtained data at the end of 12 months for all the analyzes mentioned did not show a statistically significant change ($p>0.05$).

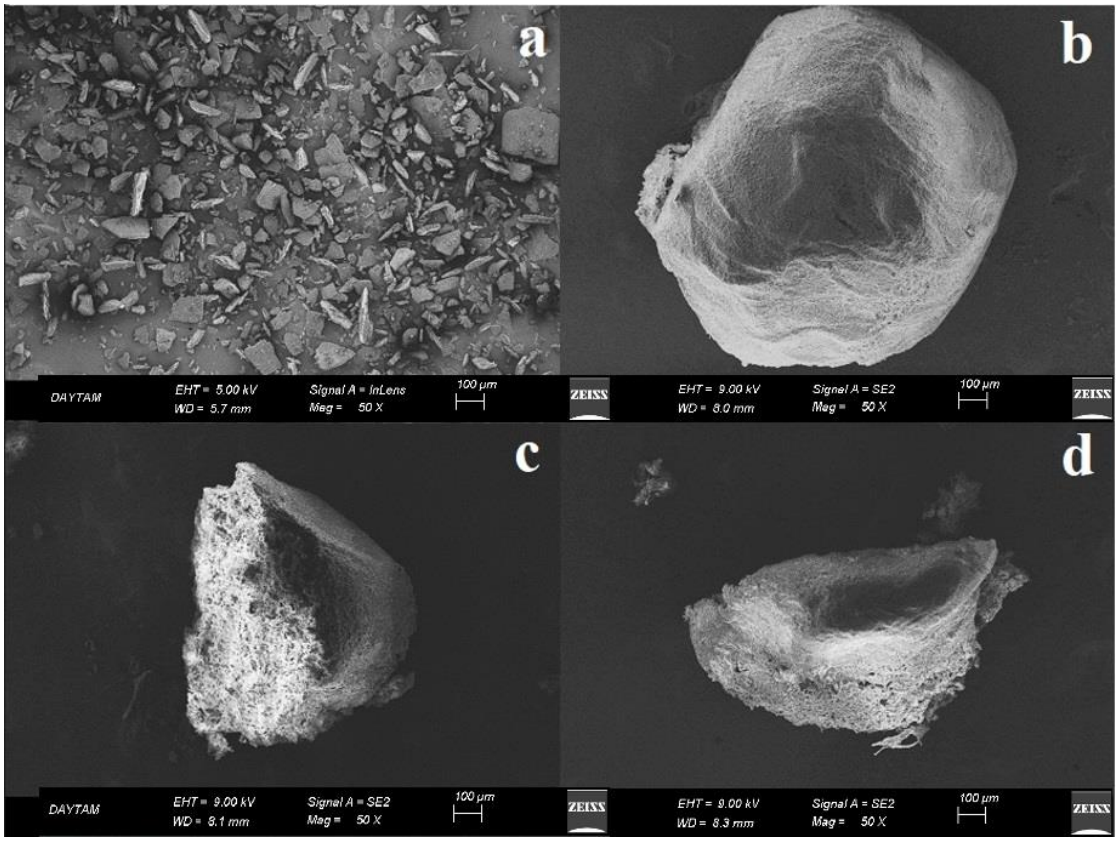

Figure 6. SEM images of hollowbeads and pure indomethacin (**a**:indomethacin, **b**:blank, **c**:F2, **d**:F19)

Table 9. Evaluation of the stability of hollowbeads for 12 months ($\bar{X}$±SD)

		EE%	**LC%**	**Buoyancy rate (%) (24 h)**	**Swelling rate (%) (24 h)**	**Release Mechanism pH 1.2**	**Release Mechanism pH 6.8**
Freshly prepared	F2	93.68±1.33	5.87±0.78	75	60	Super Case-II Transport	Non-Fickian diffusion
	F19	95.42±2.38	5.94±1.14	50	43	Super Case-II Transport	Non-Fickian diffusion
12 months later	F2	92.77±2.49	5.19±0.91	70	61.5	Super Case-II Transport	Non-Fickian diffusion
	F19	93.41±3.18	5.63±0.42	50	46	Super Case-II Transport	Non-Fickian diffusion

Modified release studies of indomethacin were examined in the literature. Similarities or differences of these studies with our study are mentioned below. Ekhodairy et al. designed colon-targeted tablets containing indomethacin and reported that the pectin-based formulation can be released for 16 hours, which is insoluble in the gastric media [19]. In another study, Abbas et al designed nanofibers containing indomethacin targeted to the colon. They were able to increase the release time up to 10 hours with formulations after passing into the intestine [37]. Damiati prepared microparticles indomethacin-loaded and conducted a release study at pH 7.4. He reported that 36% in the first 6 hours and 80% in total indomethacin release at the end of 9 days [33]. Sravani et al. developed pulsatile tablet formulations containing indomethacin. They conducted a release study of the formulations they obtained at pH 7.2 and reported that they achieved nearly 100% indomethacin release at the end of 4 hours [39]. Chauhan et al. reported that nearly 100% release occurred after 12 hours at pH 1.2 in indomethacin floating tablet formulations. The tablets were assumed to float for 12 hours and the study was terminated here [31]. In our study, we were observed that two most effective formulations floated at the rate of 75% and 50% (F2 and F19) even after 24 hours, thus maintaining the release of indomethacin for a longer period of time.

Among the anti-inflammatory drugs, indomethacin is one of the NSAIDs that exhibits the most effective inflammatory response (20 times more than acetylsalicylic acid). It exhibits side effects due to its non-ionization in the stomach medium. In particular, the potential to cause stomach ulcers, the most common side effect, limits the use of this effective NSAID. However, it is very effective in treating chronic inflammatory diseases such as ankylosing spondylitis, osteoarthritis, rheumatoid arthritis, and gout. Against the most critical side effect of indomethacin, our primary goal was to reduce the number of daily doses by prolonging the stay in the stomach (daily dose at least 2x1). Thus, we aimed to develop a formulation in which indomethacin can be used more safely and effectively with a single dose formulation that floats in gastric fluid for a long time and releases nearly 100% indomethacin in the intestinal medium for ~12 hours.

It has been determined that hollowbeads' spherical nature, size, encapsulation efficiency, efficiency, hollowness, and long-term release differ depending on the amount of NaCMC, the type of crosslinker ($ZnCl_2$, $CaCl_2$), the crosslinker concentration and the contact time with the crosslinker. Indomethacin can be released in both the stomach and intestines for at least 24 hours from our indomethacin-containing hollowbeads formulations. These formulations can stay for a long time in stomach and prevent the formation of stomach ulcers. Considering the oral dosage forms of indomethacin in the current drug market, we think that this formulation, which we have developed as an alternative, will be advantageous both in terms of reducing the dose frequency and eliminating the restriction of its use due to its side effects. It could be used safely in the future, when supported by preclinical and clinical studies, for those patients with anti-inflammatory diseases at risk of developing ulcer.

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AUTHOR CONTRIBUTIONS

Concept: *E.Ö., R.S.Ö.*; Design: *E.Ö.*; Control: *E.Ö., R.S.Ö.*; Sources: *E.Ö, H.B.G.*; Materials: *E.Ö, H.B.G.*; Data Collection and/or processing: *E.Ö., H.B.G.*; Analysis and/or interpretation: *E.Ö., R.S.Ö., H.B.G.*; Literature review: *E.Ö.*; Manuscript writing: *E.Ö., H.B.G.*; Critical review: *E.Ö., R.S.Ö., H.B.G.*; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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