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RESEARCH ARTICLE

EFFICIENT DRUG CARRIER FOR ACETYLSALICYLIC ACID FROM CHITOSAN-BASED COMPOSITES PREPARED WITH MONTMORILLONITE, CELLULOSE AND HYDROXYAPATITE

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Abstract

In this study, chitosan-based hydroxyapatite/chitosan (HAP/CHI), cellulose/chitosan (CEL/CHI) and montmorillonite/chitosan (MMT/CHI) composites were synthesized and characterized by Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and Thermogravimetric Analysis (TGA). Acetylsalicylic acid (ASA) was used as a drug for loading and desorption studies to determine the release behavior of the synthesized composites. The maximum adsorption capacities (qe) were obtained as 251.5 mg/g, 197.7 mg/g and 288.95 mg/g for HAP/CHI, CEL/CHI and MMT/CHI, respectively. In vitro release studies of ASA from the composites HAP/CHI, CEL/CHI, and MMT/CHI were carried out phosphate buffer solution (PBS) and gastric juice (GJ). In the intestinal medium (PBS) controlled drug release continued for 72 hours (4320 minutes), and burst release was observed in the first 5 minutes in all composites. 19.16%, 47.15% and 37.32% of the active ingredient from HAP/CHI, CEL/CHI and MMT/CHI composites, respectively, were released in the first 5 minutes. After 5 minutes, the release slowed down and became more controlled for all three composites. At the end of the release, the highest releasing composite was CEL/CHI, with 95.77% ASA release. A total drug release of 87.48% was achieved with MMT/CHI and 87.37% with HAP/CHI. In the gastric environment (GJ) Controlled drug release continued for 72 hours (4320 minutes), and burst release was observed in the first 5 minutes in all composites. 52.51%, 72.30% and 44.87% of the active ingredient from HAP/CHI, CEL/CHI and MMT/CHI composites, respectively, were released in the first 5 minutes. After 5 minutes, the release slowed down and became more controlled for all three composites. At the end of the release, the highest releasing capacity was found with the CEL/CHI composite, with 96.05% ASA release. A total drug release of 93.26% was achieved with HAP/CHI and 84.89% with MMT/CHI.

Keywords

Chitosan,
Drug delivery,
Composite,
Acetylsalicylic acid,
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1. INTRODUCTION

Recently, biopolymer composite minerals have attracted considerable research interest for biomedical and pharmaceutical applications [1]. Compared to synthetic polymers, natural polymers have benefits like biocompatibility, biodegradability, and biologically identifiable components that promote cellular functions [2]. Chitosan (CHI), one of the biopolymers, is a potential option for creating artificial bone scaffolds because of its superior hemostasis, adsorptive qualities, biocompatibility, and antibacterial activity. These qualities are widely used in biomedical, cosmetic, and water treatment applications [3-5]. Although chitosan has advanced the development of safe and effective drug delivery systems [6], its properties, including adsorption capacity, weak mechanical strength, irregular size, lack of process repeatability and chemical resistance need to be enhanced. Consequently, in order to produce chitosan beads, microspheres, films, or fibers with a stable size and enhanced mechanical qualities, physical and chemical modifications have been suggested [7].

According to recent research, clay minerals such magnesium aluminum silicate, montmorillonite, and laponite enhance chitosan's mechanical and thermal resilience as well as its rate of drug release and encapsulation. This is mostly because of their advantageous characteristics, which include mechanical stability, high specific surface areas, biocompatibility, huge pore volumes, and chemical inertness [1]. The inorganic calcium phosphate substance known as hydroxyapatite (HAP) is a crucial part of human bones and teeth. HAP can be obtained from natural sources or through chemical synthesis and is a biodegradable material with very good bioactive properties. Due to its numerous beneficial properties, including excellent biocompatibility, bioactivity, osteo conductivity, and anti-inflammatory properties, HAP has been extensively studied for biomedical applications [8,9]. Considering these properties, HAP, which can be obtained synthetically using a suitable calcium and phosphate source, is used as a good adsorbent for many substances such as proteins, antitoxins, antibiotics and growth factors.

Montmorillonite (MMT) supports drug release by strongly adsorbing to the drug molecules and improves the bioavailability and dissolution rate of hydrophobic drugs [10]. Because of their great adsorption capacity, high specific surface area, swelling ability, and ability to create appropriate particle sizes for embolization, MMT particles are perfectly suited for the creation of drug carrier embolization agents [11].

The most prevalent biopolymer in nature is cellulose (CEL), which may be dissolved and transformed into regenerated useful materials in a variety of shapes and sizes, including films, hydrogels, microspheres, and fibers. However, the lack of antimicrobial activity and poor bioactivity of cellulose restricts its use in antibacterial packaging and biomaterials. In order to address this issue and optimize cellulose's use, it is typically combined or altered with other polymers [4].

Aspirin, which was chosen as the model drug in this study, obtained by the acetylation of salicylic acid, has a long history as an antipyretic and analgesic drug. Acetylsalicylic acid (ASA) is a nonsteroidal drug with anti-inflammatory, analgesic, and antipyretic effects. Furthermore, taking a large amount of ASA orally can have unfavorable side effects such as ulcers, bleeding in the stomach, and tinnitus. Consequently, it would be preferable if the medications containing ASA, and its controlled release feature were combined. This would allow the ASA to be released gradually and create the proper drug concentration in plasma. Consequently, a great option for the polymeric drug to generate the controlled release of ASA would be to combine the chitosan drug carrier with the ASA drug [12]. To our knowledge, there are no other studies using chitosan composites for the release of acetylsalicylic acid.

In this study, chitosan-based composites hydroxyapatite/chitosan (HAP/CHI), cellulose/chitosan (CEL/CHI) and montmorillonite/chitosan (MMT/CHI) were synthesized to improve the resistance and drug delivery properties of chitosan for pharmaceutical systems, and the loading capacity and release behavior of acetylsalicylic acid in phosphate buffer solution (PBS) and gastric environment (GJ) medium were investigated.

2. MATERIALS AND METHODS

2.1. Materials

The following materials were used without further purification: Calcium nitrate (Ca $(NO_3)_2$) (98%) from Carlo Erba. Phosphoric acid (H_3PO_4) (85%) from J.T. Baker. Ammonia solution (NH_4OH) (25%), sodium chloride (NaCl) and cellulose (CEL) from Merck. Potassium phosphate monobasic (KH_2PO_4) and sodium phosphate dibasic heptahydrate ($Na_2HPO_4.7H_2O$) from Riedel-de Haën. Acetylsalicylic acid (ASA) (99%) from Alfa Aesar. Cetyltrimethylammonium bromide (CTAB), montmorillonite (MMT) and chitosan (CHI) (high molecular weight) from Sigma-Aldrich.

2.2. Synthesis of Hydroxyapatite (HAP)

The HAP samples were synthesized by the chemical precipitation technique. The surfactants were used at an estimated yield of 1% (w/w) HAP after being dissolved in water. The target Ca/P ratio was 1.67. While the H_3PO_4 solution (0.72 M) was added dropwise, the Ca $(NO_3)_2$ (1.2 M) solution was stirred constantly. A pH of 10 was maintained with NH_4OH (25%) [13]. At this point, the solution became milky and for surface modification 0.1 mM CTAB solution was added, and it was stirred for 30 min before being allowed at room temperature for a day. After filtration, the HAP samples underwent washing with distilled water, followed by drying in oven, and finally calcined at 550 °C for 6 hours, with a heating rate of 2 °C/min.

2.3. Preparation of Composites

All of the composites were synthesized with chitosan solution which is prepared same methodology that given below.

10 mL of 1% (v/v) acetic acid (CH_3COOH) and 100 mg of chitosan were stirred on a magnetic stirrer at 1000 rpm for 30 min until it became transparent, and then treated in an ultrasonic water bath for 5 min.

100 mg of HAP, CEL, MMT and 100 mL of distilled water were added and then placed in an ultrasonic water bath for 30 min. Composite suspensions were added to the prepared chitosan solution and then stirred on a magnetic stirrer at 1500 rpm for 24 hours. Then 2M NaOH solution was added dropwise with a syringe and stirred on a magnetic stirrer at 500 rpm. After 24 h, the samples were centrifuged at 4000 rpm for 20 min and washed with distilled water until pH neutral. The samples were collected and dried at 60 ˚C for 24 hours. After 24 hours, the samples were completely dried and the composites were collected, stored for further analysis and labeled as HAP/CHI, CEL/CHI and MMT/CHI.

2.4. ASA Loading

To investigate ASA's adsorption capability on the chitosan-based composites, 50 mg of acetylsalicylic acid was dissolved in 100 mL of distilled water, and 300 mg of each composite (HAP/CHI, CEL/CHI, and MMT/CHI) was submerged in a 500 mL ASA solution (0.5 mg/mL) for 24 hours at 25 °C in a shaking water bath. On the chitosan-based composites, the adsorption capability was ascertained. Following the adsorption procedure, the liquid phase was filtered, and a UV spectrophotometer (Shimadzu UV-1800) set to 275 nm was used to measure the concentration of ASA in the solution.

The amount of ASA adsorbed per gram of composite, qe (mg/g), was calculated according to Equation 1.

$$qe = V \times \frac{(C_o - C_t)}{m} \quad (1)$$

Where V is the solution volume (L), C_o is the initial concentration of ASA (mg/L), C_t is the concentration of ASA at a given time, and m is the amount of composite (g).

2.5. In vitro Drug Release Studies

To investigate the ASA release, 10 mL of phosphate-buffered saline (PBS) (pH = 7.4) and GJ (pH = 1.2) were mixed with approximately 20 mg of the samples loaded with ASA HAP/CHI, CEL/CHI, and MMT/CHI. To make the PBS solution, distilled water was mixed with KH_2PO_4, $Na_2HPO_4.7H_2O$, and NaCl. To make GJ solution, 0.01 M HCl solution with pH 1.2 was used. The experiments were conducted in a shaking bath at 37 °C and 50 rpm. A sample was taken from solution, and it was measured with the UV spectrophotometer at a prearranged time interval. The system was then replenished with fresh solution in order to maintain a steady volume. The total amount of ASA released within each time interval was determined based on the absorbance values, allowing for the calculation of the concentration and subsequent mass of ASA released [14,15].

2.6. Characterizations of Composites

For characterization studies of composites, FT-IR, SEM and TGA analyzes were used. Thermoscientific Nicolet IS10 FT-IR, which is outfitted with a universal ATR sample adapter, was utilized for conducting FT-IR analyses. 64 scans of ATR-FT-IR spectra were obtained at a resolution of 4 cm^{-1}, covering the spectral range of 4000-650 cm^{-1}. Using a HITACHI TM 3030 Plus SEM apparatus, pictures were captured to gather data on the materials' surface morphology. The produced samples were subjected to a Thermogravimetric Analysis in nitrogen atmosphere (N_2) using a TGA instrument (TGA 4000, Perkin Elmer) from 40 to 800 °C at a heating rate of 10 °C/min.

3. RESULTS AND DISCUSSION

3.1. Characterization of Composites

The synthesized HAP/CHI, CEL/CHI, and MMT/CHI samples were characterized by FT-IR, TGA and SEM analysis. The sample's functional groups were ascertained by FT-IR analysis. All of the synthetic composites' spectra showed characteristic bands at about 3570 cm^{-1}, which corresponded to the distinct stretching vibration modes of the OH- groups. (Figure 1). According to the HAP/CHI spectra, the bands at 3343 and 1654 cm^{-1} belong to adsorbed water, which could be OH- groups present in HAP dust and reacting with atmospheric carbon dioxide [16]. A strong band appeared at 3568 cm^{-1}, which was due to $(PO_4)^{3-}$ ions. The bands at 1028 cm^{-1} were characteristic of phosphate stretch vibrations, and the bands observed at 603 cm^{-1} and 568 cm^{-1} were due to phosphate bending vibrations The peaks around 1400 cm^{-1} in the FTIR spectra indicate the $(CO_3)^{2-}$ groups. The peaks at about 1600 cm^{-1} and 2800 show the spectral features of the -NH groups and -CH group of chitosan [17, 18].

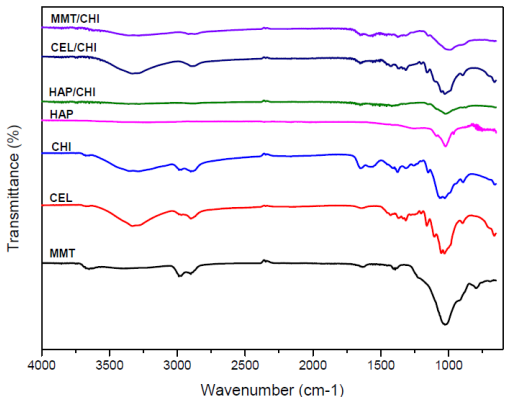

Figure 1. FTIR spectra of HAP, CHI, CEL, MMT, HAP/CHI, CEL/CHI and MMT/CHI

According to the CEL/CHI spectra, the strong vibrational band at 3330 cm^{-1} is considered to be O-H stretching. The band at 2901 cm^{-1} corresponds to the C-H stretching of the aliphatic groups. The vibrational bands at 1656 cm^{-1} and 1457 cm^{-1} in the FTIR spectrum of the cellulose/chitosan composite are expressions of the vibrations associated with the O-H and the N-H group, respectively. Although the molecular structure of chitosan and cellulose is quite similar, the main difference between them can be explained by the spectra of NH stretching at 3330 cm^{-1} and 1457 cm^{-1}, which arise from the NH groups that chitosan possesses [19].

The 3352 cm^{-1} peak in the MMT/CHI composite seen in the FTIR spectrum in Figure 1 is considered to be the vibrational band combined with the N-H stretching vibrational band and the O-H stretching vibrational band. The spectra of the N-H stretching created by the amine groups of chitosan may both account for the peak at 1581 cm^{-1}. While the aliphatic C-H stretching was observed at peaks of 2930 cm^{-1}, the C-H bending was observed at peaks of 1379 cm^{-1} and the C-O stretching at peaks of 1151 cm^{-1}. The peaks for the amide I and amide II bands bending were observed at 1655 cm^{-1} and 1581 cm^{-1}. The peaks at 1013 cm^{-1} which represent the middle OH bending, CO, NH (amine) and NH_2 (amino), respectively, are comparable to those seen in chitosan [12, 20, 21].

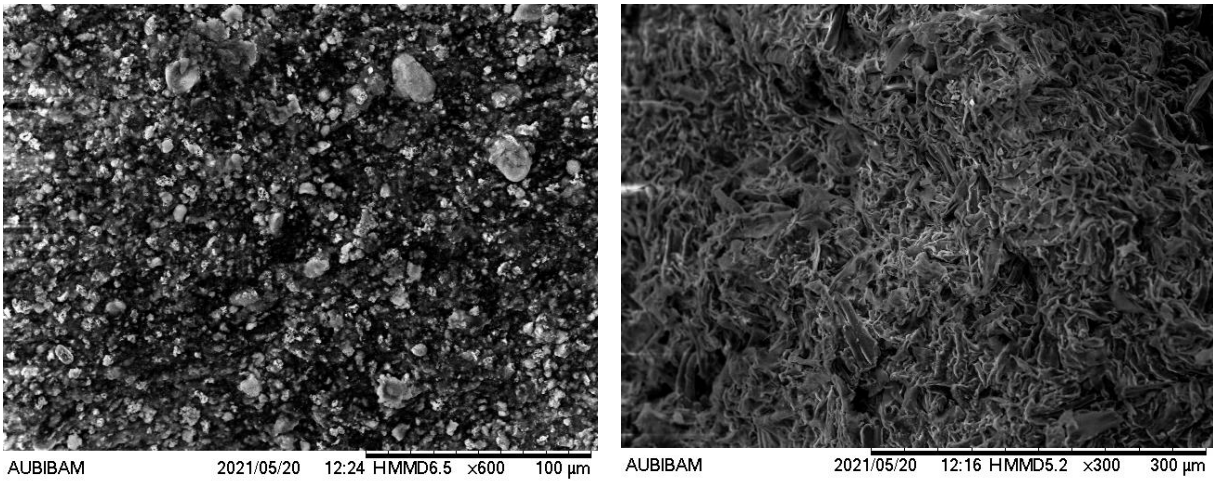

Figure 2. SEM images of HAP/CHI (a) CEL/CHI (b) and MMT/CHI (c)

The SEM images of HAP/CHI, CEL/CHI and MMT/CHI are shown in Figure. 2. As can be seen in the SEM images, HAP/CHI shows a relatively smooth surface with some agglomerates. On the other hand, the surface of MMT/CHI exhibits deep voids and non-uniform pores characterized by long, non-uniform hollow bodies. As we can see MMT/CHI has more pores than the other two composites. It means that using MMT in CHI composites increased the porosity. This increased porosity is the reason why clay adsorbents are preferred for the adsorption process. The CEL/CHI surface shows an irregular and dense morphology with a high pore density and heterogeneity together with reinforced and clearly visible pores.

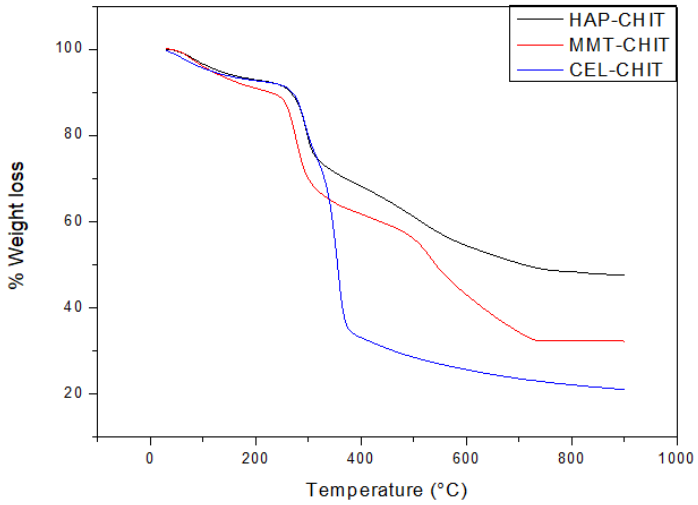

Figure 3. TGA profiles of HAP/CHI, CEL/CHI and MMT/CHI

TGA analyses were performed to observe the thermal behavior of the produced HAP/CHI, CEL/CHI and MMT/CHI materials. As can be seen in Figure 3, the highest mass loss and degradation at high temperatures were observed for CEL/CHI. For the CEL/CHI composite, a rapid mass loss of 60% was observed between 200-400 °C in a single step. From 400 °C to 1000 °C, a mass loss of 10 % was observed. Similarly, for the HAP/CHI composition, a rapid mass loss of 20 % was observed between 200-300 °C in a single step and then a mass loss of 40 % from 300 °C to 1000 °C. For the MMT/CHI composition, the mass loss occurred rapidly in 2 steps. The first step was observed between 200-300 °C and the second step between 450-750 °C. A mass loss of 20 % was observed in the first step and 25 % in the second step. The mass loss of all three composites began to accelerate after 200 °C The first stage (30–200 °C) is associated with the loss of absorbed and bound water and acetic acid residues. The second stage (200–400 °C) is due to the degradation of chitosan [22].

3.2. Loading of ASA

The maximum adsorption capacities (qe) were 251.5 mg/g, 197.7 mg/g and 288.95 mg/g for the composites HAP/CHI, CEL/CHI and MMT/CHI, respectively (Figure 4.). The amount of adsorbed ASA increased with time in both composites. The maximum amount of qe was obtained with the composite MMT/CHI. This is related to the functional groups and structural properties of MMT/CHI, which were evaluated in the characterization section.

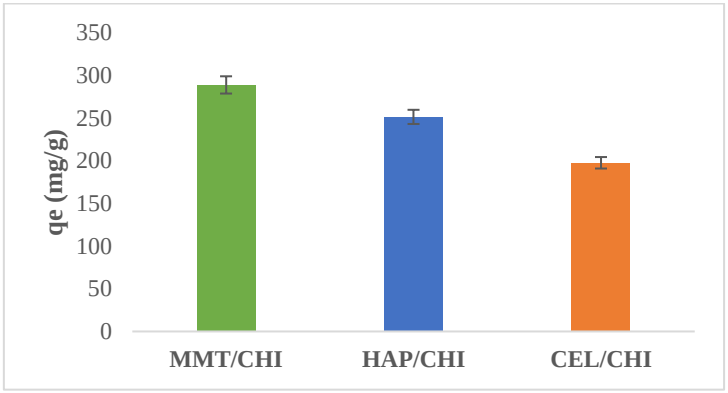

Figure 4. ASA adsorption capacity on the different composites (0.2 mg/mL ASA at 25 °C in 60 min)

3.3. In vitro Release of ASA

3.3.1. In the PBS medium

The release investigations used ASA-loaded HAP/CHI, CEL/CHI and MMT/CHI that were achieved at maximum ASA loading, as mentioned in the section 'Loading with ASA'. Prolonged release in PBS medium was achieved with the composites obtained (Figure 5.). In the study, 1 gram of HAP/CHI, CEL/CHI and MMT/CHI contained 251.5 mg, 197.7 mg and 288.95 mg of the active substance (ASA), respectively. The controlled release of the active substance lasted 72 hours (4320 minutes), with a sudden release observed in the first 5 minutes for all composites. 19.16%, 47.15% and 37.32% of the active substance of HAP/CHI, CEL/CHI and MMT/CHI were released in the first 5 minutes. After 5 minutes, the release slowed down and became more controlled for all three composites. At the end of the release, the composite CEL/CHI was the one with the highest release with 95.77% ASA release. A total release of 87.48% was achieved with MMT/CHI and 87.37% with HAP/CHI. When investigating the release profiles, it was assumed that HAP/CHI is more suitable for controlled release due to its less explosive release and more controlled release compared to the other two systems.

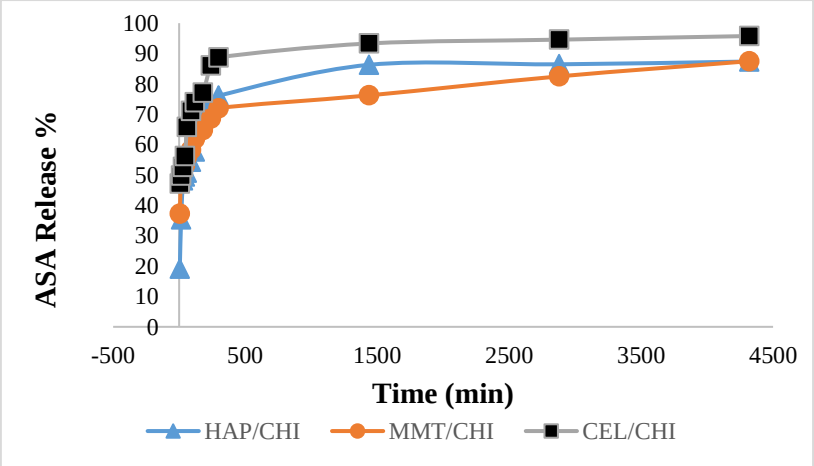

Figure 5. ASA released in the PBS medium with the HAP/CHI, CEL/CHI and MMT/CHI composites.

3.3.2. In the GJ medium

Since the presence of an acidic solution and pepsin in the simulated gastric juice allows the acetylsalicylic acid released from the drug-loaded particles to break down into salicylic acid, both acetylsalicylic acid and salicylic acid were found at pH 1.0, and only acetylsalicylic acid at pH 6.8. As a result of our studies, prolonged release was achieved with the obtained composites in both GJ and PBS medium (Figure 5 and Figure 6). In the study, 1 gram of the composites HAP/CHI, CEL/CHI and MMT/CHI contained 251.5 mg, 197.7 mg and 288.95 mg of active substance (ASA), respectively. The controlled release of the active substance lasted 72 hours (4320 minutes), whereby a sudden release was observed for all composites in the first 5 minutes. 52.51%, 72.30% and 44.87% of the active substance of HAP/CHI, CEL/CHI and MMT/CHI were released in the first 5 minutes. After 5 minutes, the release slowed down and became more controlled for all three composites. At the end of the release, the composite CEL/CHI was the one with the highest release with 96.05% ASA release. A total release of 93.26% was achieved with HAP/CHI and 84.89% with MMT/CHI. Examination of the release profiles revealed that MMT/CHI was more suitable for controlled release due to its less explosive release and more controlled release compared to the other two systems. The reason for the higher explosive release and more uncontrolled release compared to the PBS environment is that chitosan dissolves at low pH and does not tend to dissolve at high pH. Due to this property, the chitosan will dissolve quickly in the GJ environment and the active component in the composite will be released faster and uncontrolled into the environment.

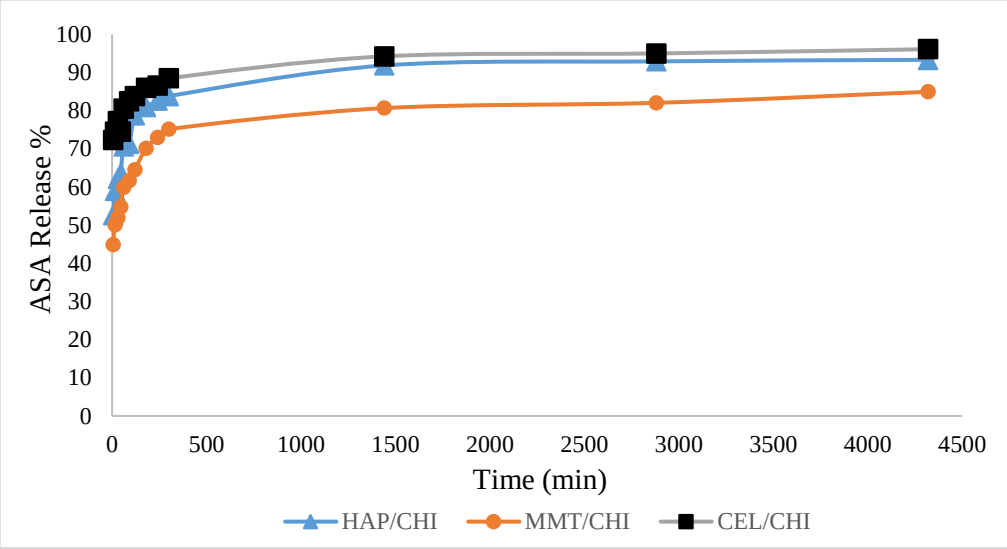

Figure 6. ASA released in the GJ medium with the HAP/CHI, CEL/CHI and MMT/CHI composites.

Compared to similar studies in the literature, the MMT/CHI composite was found to have a more controlled and prolonged release and a higher loading efficiency than many other studies (Table 1). In the release studies, it is evident that most of the studies in the literature were conducted with PBS medium. Although a cumulative release of 100% was achieved in PBS in the study by Gou and Jiaou [27], the duration of this release was considered insufficient for the drug to exert its therapeutic effect. A longer duration of controlled release is important to reduce side effects and improve bioavailability. Therefore, the total release achieved with MMT/CHI at 87.48% and CEL/CHI at 95.77% in PBS medium at the end of 72 hours exceeds that in similar studies in the literature. In GJ medium, a release of 84.89% was achieved with MMT/CHI, which is significantly more successful than in the studies in the literature. In addition, controlled and long-term release was also achieved in studies conducted in gastric medium. Compared to other studies, our study achieved the most suitable release system for ASA in both gastric and intestinal medium.

Table1. Selection of results from studies on the use of ASA-loaded systems

Material	Drug loading capacity	Drug release medium	Release time	Total drug release (%)	References
MMT/CHI	288.95 mg/g	PBS	72 hours	87.48	Our study
		GJ		84.89	
HAP/Cetyltrimethylammonium Bromide	280.27 mg/g	PBS	50 hours	73.91	[13]
Sporopollenin microcapsules	53.4% (270 mg/g)	PBS	10 hours	81.1	[23]
		0.1 M NaOH		66.9	
		GJ		51.7	
CHI/sodiumtripolyphosphate	90.4%	PBS	24 hours	88.3	[24]
MCM-41	350 mg/g	Degassed water	~16 hours	100	[25]
Dextran-layered double hydroxide	28.2%	PBS	1 hour	100	[26]

4. CONCLUSION

In this study new drug delivery systems based on chitosan were developed that can achieve long-term controlled release in both the stomach and the intestine. In these systems, particles modified with chitosan, cellulose, montmorillonite and hydroxyapatite were effectively synthesized and validated using advanced analytical techniques. In this study, the synthesized composites were investigated for the adsorption and controlled release of acetylsalicylic acid. The adsorption and release profiles of ASA-loaded HAP/CHI, CEL/CHI and MMT/CHI samples were compared. In addition, studies were conducted using SEM, FTIR and TGA analysis methods to better investigate the particle structures and chemical interactions.

The results have shown that the best drug carrier system in terms of adsorption or, in other words, drug loading capacity, is the MMT/CHI particles with 288.95 mg/g. The drug release studies showed that all three drug carriers had a more controlled release profile in the intestinal environment (PBS). On the other hand, a prolonged release of up to 2 days was achieved in both release environments, and at the end of release, the CEL/CHI composite was observed to have a higher drug release in the intestine and stomach than the other composites. However, release spurts were observed for all composites in both release environments. The SEM images showed the irregular morphologies of the beads, which could be effective for the adsorption of metal ions. It can be said that HAP/CHI has a relatively smooth surface except for some clusters, while the surface of MMT/CHI is characterized by long, irregular, hollow structures with deep voids and uneven pores. The increased porosity observed here emphasizes the preference for clay adsorbents in adsorption processes. On the other hand, the surface morphology of CEL/CHI shows an irregular and dense structure characterized by clearly visible pores, together with high pore density and heterogeneity. FTIR revealed the main functional groups of pure chitosan, pure cellulose and chitosan–cellulose beads.

The results of these studies indicate that these chitosan-based composites could be a good candidate for advanced drug delivery systems due to their excellent adsorption capacity and release behavior.

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CONFLICT OF INTEREST

The author(s) stated that there are no conflicts of interest regarding the publication of this article.

CRediT AUTHOR STATEMENT

Dilay Sezer: Investigation, **Zeynep Aktaş:** Investigation, Writing – Original Draft, **Seda Hoşgün:** Writing – Original Draft, Data Curation **Emir Zafer Hoşgün:** Writing – Review & Editing, Supervision, Funding acquisition **Berrin Bozan:** Writing – Review & Editing, Supervision.

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