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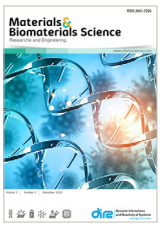

Effect of acidification on biological proprieties of sodium carboxymethylcellulose

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ABSTRACT

Sodium carboxymethylcellulose is frequently used in medicine for its excellent biological properties. In addition, it is known that the polymer chain modification can lead to the change or improvement of its biological properties, for this reason Sodium Carboxymethylcellulose (NaCMC) was subjected to an acidification reaction by hydrochloric acid. Characterization and identification of acidified carboxymethylcellulose (HCMC) was made by Fourier transformed Infrared spectroscopy (FT-IR), Elemental analysis (XRF) and Thermal analysis (TGA - DSC). The opacity measurement shows a marked difference in powders structure. The blood-clotting tests demonstrate that HCMC is more hemostatic than NaCMC. Mucoadhesion exhibits a pronounced increase in adhesion times of HCMC contrary to NaCMC on the gastric and intestinal mucosa. Besides, biodegradability is established.

Introduction

Introduction of synthetic or natural polymers, have seen special attention in recent times and have been heavily used in the biomedical field [1,2]. Sodium Carboxymethylcellulose (NaCMC), the most interesting cellulose derivatives ,is produced by chemical treatment (hydroxyl group carboxymethylation) of natural cellulose, to obtain a biodegradable and biocompatible anionic polymer [3,4]. This water soluble cellulose ether [5] is obtained by reacting alkali cellulose with monochloro-acetic acid or its sodium salt [6]. NaCMC is a widely applied material in industry equipped with excellent green credentials [7] which gives it a wide range of applications in drag reduction, detergents, flocculation, paper, food, drugs, textiles, and oil well drilling operations [8]. On the other hand, it has been discovered that NaCMC has a good biocompatibility, by this fact, it can be considered as the biomaterial of the future in the biomedical field [9]. moreover, natural biopolymers have relatively weak mechanical properties and uncontrollable degradability, which considerably reduces their clinical use. Bioartificial combination and chemical modification processes have been carried out to enhance the properties of natural biopolymers [2].

In this work, we have studied the change in biological properties of carboxymethylcellulose after its acidification with hydrochloric acid. Acidification of NaCMC was confirmed by Fourier transformed infrared (FT-IR) spectroscopy, TGA, DSC analysis and X-ray fluorescence (XRF). Blood clotting, mucoadhesion, and biodegradability were also investigated.

Experimental

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Na CMC (MW= 250 kDa, 8% moisture Sodium content, average viscosity of 400–800 cps), HCl (hydrochloric acid) (37%), $CaCl_2$ (Calcium chloride) and pure ethanol have been bought from Sigma-Aldrich (USA). Human anti-coagulated blood acid citrate dextrose (ACD) was supplied by the blood bank, Setif University Hospital.

Simulated body fluid (SBF) that has ion concentrations almost equal to those of human blood plasma was buffered with, 2.2682 g of $NaHCO_3$, 6.5456 g of NaCl 0.373 g of KCl, , 0.3049 g of $MgCl_2{\times}6H_2O$, 0.1419 g of Na_2HPO_4 0.3675 g of $CaCl_2{\times}2H_2O$, 0.071 g of Na_2SO_4, 6.057 g Tris [$=(CH_2OH)_3CNH_2$] in 1 L of distilled water and HCl (1M) at 37°C and pH 7.40.

Buffered Saline solutions (PBS) at pH 6.8 (0.1M) was prepared by mixing 51 mL of NaH_2PO_4 (0.2 M) and 49 mL of

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Fig. 1. Acidification reaction of NaCMC using ethanol.

Na_2HPO_4 (0.2 M) at T = 27 °C with distilled water to 200 mL. Other reagents were analytical grade and used without further purification. All aqueous solutions were prepared in distilled water.

Acidification of NaCMC

Preparation of acid form of NaCMC (Fig.1) was made according to Roshan et al. [7]. In a beaker of 500 mL, 1 g of NaCMC was dissolved in 100 mL of ethyl alcohol solution (80%) and 10 Ml of HCl. Contents of the beaker was left under gentle stirring at room temperature (27°C) for 30 min. Then, the reaction mixture is filtered and washed with ethanol to neutralize the solution. The precipitate was allowed to air dry at room temperature to obtain the white powder of HCMC.

Preparation of NaCMC and HCMC Films

Films were made by solubilizing 1g of obtained powder in 100 mL of distilled water at 80°C under gentle agitation; then, obtained hydrogel is poured into a polystyrene Petri dish (9.5 cm in diameter) and then left in the open air for 3-4 days to dry.

Fourier transformed Infrared spectroscopy (FT-IR)

The infrared spectra were obtained with the Fourier transform SHIMADZU 84005 Spectrophotometer (Japan). The spectra are obtained by solubilizing powders at 3% (w / w) in KBr in a scanning range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1}.

Elemental analysis (XRF)

Powders have been inspected by a RIGAKU ZSX Primus II, wavelength dispersive X-ray fluorescence spectrometer (Japan). The apparatus contains a rhodium X-ray tube, operated at 60 kV and 4 kW at 30 rpm rotation speed with an Al125 X-ray filters. Samples were compressed into pellets of 1 cm diameter and 3 mm thickness. The experienced element was Sodium.

Thermal analysis

Thermal analysis of NaCMC and HCMC was carried out with an Artisan SDT Q 600 thermal analyzer (USA). Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) of samples were carried out from room temperature to 600°C in nitrogen atmosphere at a constant heating rate of 10 °C/min under nitrogen atmosphere.

Opacity test

Opacity was determined using a double-beam UV-Vis spectrophotometer from UNICAN UV 300 (USA). The films of 0.40 mm thickness were divided into rectangular pieces and placed on the sample compartment of the spectrophotometer. An empty compartment was used as a reference [10].

$$Opacity = Abs_{600} / X \quad (1)$$

Where, Abs_{600} is the value of absorbance at 600 nm and X is the film thickness (mm).

Dynamic Blood clotting test

Coagulation times were studied according to Wang et al. .11 The powders of NaCMC or HCMC (50 mg) were placed in beakers thermostatically controlled at 37 ° C for 5 min. After a drop of whole blood ACD (0,25 mL) was deposited on the surface of powders. Afterwards, 0.02 mL of $CaCl_2$ solution (0.2 L mol^{-1}) was added. The blood coagulation test was executed by spectrophotometric measurement using UNICAN UV 300 (USA) at 540 nm after 0, 5, 10, 20, 30 and 50 min. The relative absorbancy of 0.25 mL of ACD whole blood diluted in 50 mL of distilled water was assumed to be 100. The blood clotting index (BCI) of the biomaterial is calculated using the following equation.

$$BCI(\%) = \frac{Absorbancy\ of\ blood\ contaned\ with\ sample}{\text{Absorbancy of solution of distilled water and ACD blood}} \times 100 \quad (2)$$

Mucoadhesion studies

Mucoadhesion gives us information on the residence times of drugs at various sites in the gastrointestinal tract and, therefore, to reduce variability and improve efficiency [12]. The mucoadhesiveness tests were conducted according to the in vitro washing method as detailed by Agarwal et al. [13]. Briefly, fresh parts from a sheep's stomach and colon were supplied by a commercial slaughterhouse and washed with cold water. The tissues (1.5 cm x 1 cm) were immobilized on a glass slide by an adhesive glue while placing the mucous surface upwards. 0.5 g of powders were sprayed onto the mucosa and a load of 5 g was applied to it for 15 min to ensure uniform adhesion of the powder on the mucosa. Afterwards, the powder-laden tissues were immersed in 0.1 N HCl (pH adjusted to 1.2, specific to the stomach) and PBS (pH 6.8, specific to the colon), respectively, in the thickets of the COPLEY tablet disintegrator (United Kingdom). The disintegrator was then turned on to ensure the up and down movement of samples in 1 L of buffer at 37 ° C.

Biodegradation test (in vitro)

Biodegradation of films was estimated by measuring the difference in weights of the dry films before and after their immersion in SBF during specific incubation periods [14]. The samples were immersed in 25mL of SBF for 0 (control), 3, 7 days and 2, 4, 6, 8, 9 weeks, at 37 ° C. at the end of the determined period, the hydrogels were conditioned in an oven for drying for 12 hours after rinsing with distilled water.

$$wt\% = \frac{(Wt-Wo)}{Wo} \times 100 \quad (3)$$

Where,
W_o is the initial dry weight of the sample,
W_t is the dry weight of the sample at time t.
Values are estimated by averaging three tests from the same sample.

Results and discussions

Fourier transformed Infrared spectroscopy (FT-IR)

Fig. 2(a) illustrates the IR-TF spectrum of NaCMC. It is evident that there is a broad absorption band at 3441 cm^{-1} corresponding to the O-H elongation vibrations, A mean peak at 2928 cm^{-1} due to the asymmetric elongation vibration of aliphatic CH [15], and a strong absorption at 1600 cm^{-1} directly attributed to the asymmetric elongation vibrations of NaCMC carboxylate groups (-COONa) [7]. As for 1423 cm^{-1}, the corresponding peak is assigned to the symmetrical elongation vibration of COO^- while at 1068 cm^{-1}, several authors attribute this absorption to the deformation vibrations of ether glycoside side groups (COC) [3,16].
After acidification of NaCMC (Fig. 2(b)), we observe the presence of a new peak at 1715cm^{-1} which is attributed according to Silverstein et al. [17]. to the functional group C=O of carboxylic acid. Finally, we observe the absence of the peak at 1600 cm^{-1} that confirm the successful acidification.

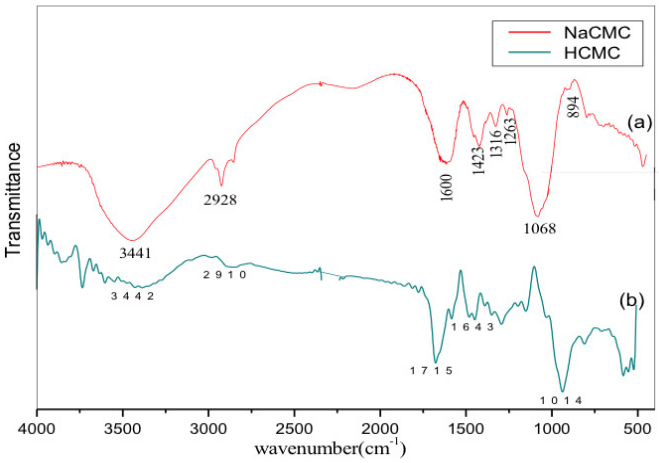

Fig. 2. FTIR spectra of films: NaCMC (a) and HCMC (b).

Elemental analysis (XRF)

Based on Figure 3, we observe that the mass percentage of sodium transported by HCMC decreased up to 3.53%, compared to that of NaCMC which is 7.23%. This implies that the acidification of CMC took place with a percentage of 48.82%.

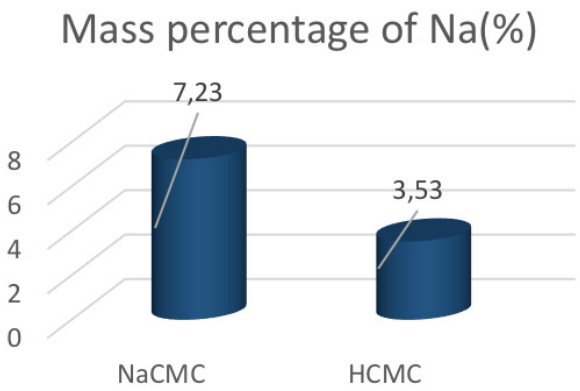

Fig. 3. Mass percentage of Na in NaCMC and HCMC.

Thermal analysis

The thermal stability of polymers and the study of their decomposition pattern is ensured by thermogravimetric analysis.1 From Fig. 4(a), the first mass loss point of NaCMC can be determined at around 120°C with an 11% mass loss, which is due to the evaporation of water according to literature. Since the sample was preconditioned at 110°C during 1 h, the remaining moisture removed6 from NaCMC was below 3%. The degradation of NaCMC begins between 180°C and 220°C and its weight decreases up to 60% towards 350°C with a DTG minimum at 286 °C (degradation temperature, Td), indicating the disappearance of the polysaccharide structure [18].
For HCMC (Fig. 4(b)), the water evaporation loss around 120°C is 7%, this decrease in weight loss compared to NaCMC is due to the hydrophobic character acquired during the acidification of NaCMC [19]. The remaining moisture removed from HCMC was below 2%. HCMC degradation appears between 218°C and 455°C with a weight loss of 68% and Td was around 310°C.
However, DSC was conducted to investigate the thermal transitions that occur throughout the exposure of the sample to heating under an inert atmosphere. Fig. 4(c) and 4(d) illustrate the differential scanning calorimetry curves of NaCMC and HCMC respectively. NaCMC present a relatively broad endotherm glass transition at 76°C. Depolymerisation and cleavage of glycoside bonds leads to an exothermic peak at about 290°C with enthalpy, Symbol ΔH, of 78.7 J/g, which corresponds to the crystalline transition point,20 peaks at 375°C and 408°C are resulting from combustion of degraded products [8].8
Details observed in HCMC differential scanning calorimetry curve reveal a decrease in the enthalpy value (ΔH=16.44 J/g) of crystalline transition peak at 300°C. The combustion of degraded products appears at 380°C and 480°C by exothermic peaks with enthalpies, Symbol ΔH, of 4.26 and 5.62 J/g.

Opacity test

From Fig. 5, we notice that NaCMC previously transparent with low opacity value (~0.2%) became after its acidification white and opaque. The opacity value increase up to 0.87%.

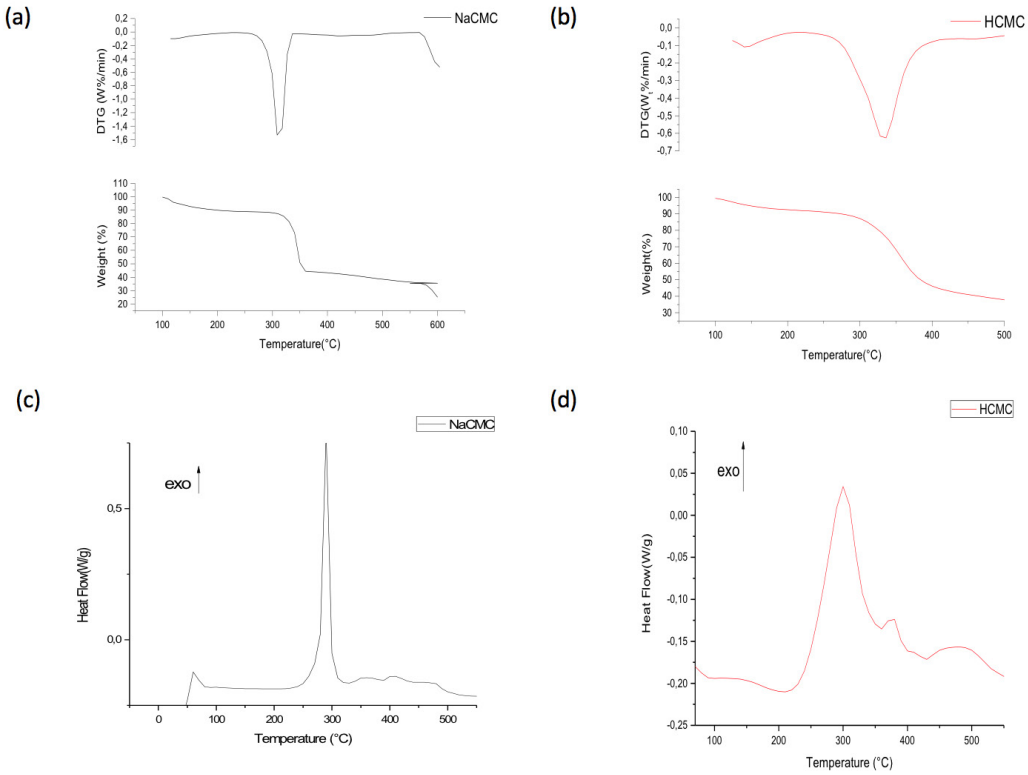

Fig. 4. Thermal curves, (a): TGA/DTG of NaCMC, (b): TGA/DTG of HCMC, (c): DSC of NaCMC and (d): DSC of HCMC.

Dynamic Blood clotting test

This essay claims the natural coagulation process and estimates the strength of the clot against hemolysis in the presence of water [21]. An excellent hemostatic effect of the biomaterial translates into a faster blood coagulation time which is manifested by a rapid decrease in absorbance [22]. Generally, the coagulation time is defined by The time at which the absorbance is equal to 0.01; the clotting time is longer when the BCI value is slow over time [11]. Fig. 6 demonstrate the blood clotting ability of NaCMC and HCMC. The coagulation time of HCMC is 5 minutes, and that of NaCMC is 20 minutes, which indicates that the blood coagulation capacity of the two samples shows good hemostatic performance. The rapidity of the hemostatic capacity may be due to the negative charge density conferred by the carboxymethyl group. It is known that the negatively charged surface activates factor XII and platelet factor 3, triggering a series of proteolytic reactions which lead to the inherent clotting of the blood [21]. On the other hand, the gel with its good hydrophilicity can absorb a large amount of blood from the wound. So, it adheres to the surface of latter and consequently blocks the rupture of blood vessels leading to platelet aggregation, fibrinogen deposition and thrombus formation [23].

Mucoadhesion studies

Cellulose ethers are characterized for deionized derivatives by good mucoadhesive properties that is especially due to the interpenetration of polymer chains and mucin molecules, and on the formation of hydrogen bonds [25].
Fig. 7 shows a significant increase in the adhesion times of HCMC (~ 2 hours) on the intestinal mucosa compared to NaCMC that resides only 45 min. The same observation was noted in the gastric environment with an adhesion time of about 3.5 hours for HCMC compared to a total solubilization for NaCMC at the first minutes.

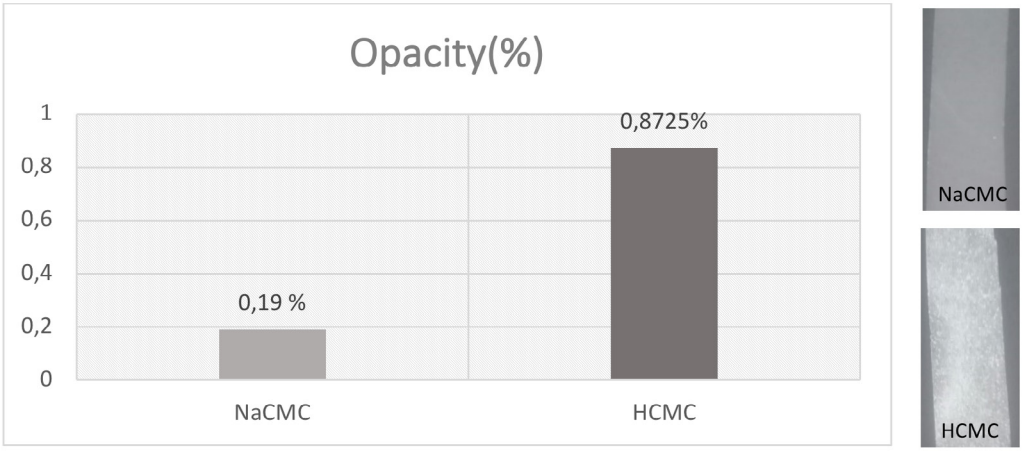

Fig. 5. Opacity values of NaCMC and HCMC films.

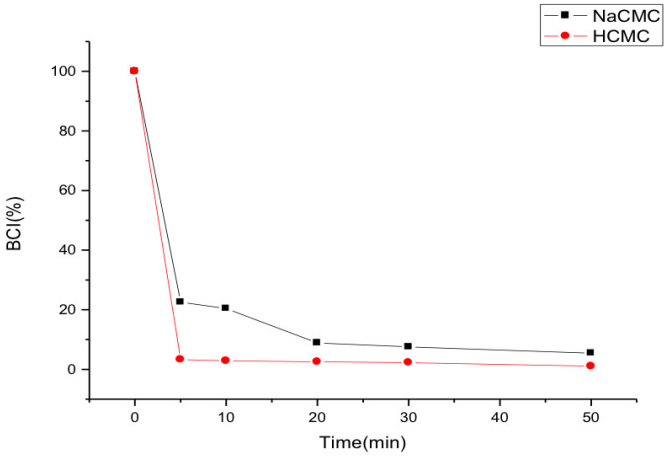

Fig. 6. Opacity values of NaCMC and HCMC films.

This noticeable improvement in the mucoadhesive nature of NaCMC either in the gastric or intestinal environment after its acidification is provoked by the high number of COOH groups inducing afterwards an attachment to biological surfaces by hydrogen bonding formation. Additionally, as it has been reported in the literature [25], the presence of unionized groups favors mucoadhesion which explains the higher residence time of HCMC in both mediums.

On the other hand, in agreement with the work of Madsen et al. [26], who assumed that at a lower pH, due to the internal hydrogen bond, NaCMC was found to be in a coiled conformation, limiting the formation of expanded polymer networks. which explains the inability of NaCMC to adhere to the gastric mucosa.

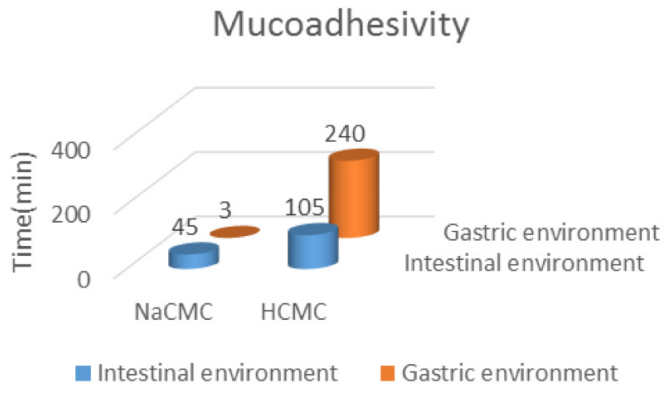

Fig. 7. Mucoadhesivity of NaCMC and HCMC powders.

Biodegradation test (in vitro)

Biomaterial molecular decomposition can be caused by microorganisms or by existing chemical elements in biological media (Fig .8). This phenomenon is called biodegradation. The test carried out in our study is to follow the loss of mass over time in the SBF. Collected results shows that NaCMC and HCMC are easily alterable in SBF and ends with a rate of 100% at 3 weeks for NaCMC Conversely to 2 weeks for HCMC.

These results prove that acidified carboxymethylcellulose can be eliminated from human fluids over a shorter period than NaCMC.

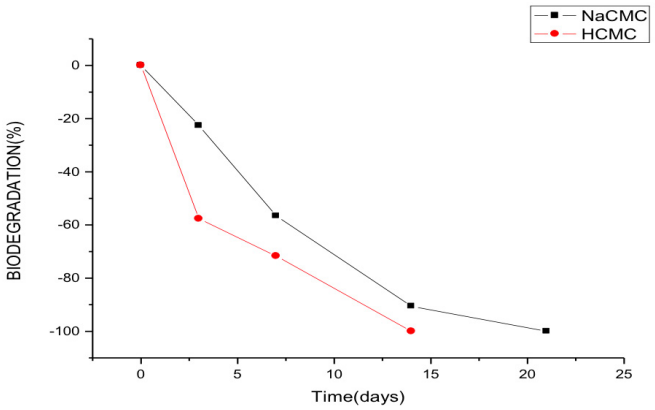

Fig. 8. Biodegradation of NaCMC and HCMC in SBF over time.

Conclusion

This study examines the changes in biological effect of Sodium carboxymethylcellulose (NaCMC) after its acidification by hydrochloric acid.

Firstly, the characterization by infrared spectroscopy reveals that NaCMC has been well acidified. These results are confirmed by the decrease in sodium mass percentage stated by elemental analysis.

Thermal analysis indicates the difference in molecular structure between NaCMC and acidified carboxymethylcellulose (HCMC). Moreover, Opacity test shows a significant increase of the light transmittance after NaCMC acidification.

Dynamic Blood clotting test reveals that HCMC has a highest hemostatic ability contrary to NaCMC, making it a good material for use as a wound dressing.

Mucoadhesion test demonstrates a significant increase in adhesion times of HCMC on the gastric and intestinal mucosa compared to NaCMC, which is completely soluble in gastric environment. This gives to HCMC an opportunity to be used as an excellent excipient in the field of medication formulations of prolonged drug release in both of gastric and intestinal environment. Finally, biodegradation test evokes a better biodegradation capacity of HCMC powders compared to NaCMC that can be removed from physiological fluids for a relatively short time.

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Conflicts of interest

Authors declare no conflict of interests.

Notes

The authors declare no competing financial interest.

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