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# Simultaneous *in situ* monitoring of acrylic acid polymerization reaction on N-carboxymethyl chitosan using multidetectors: Formation of a new bioadhesive and gastroprotective hybrid particle

Oscar D'Agostini-Junior [a], Carmen Lucia Petkowicz [b], Angelica Garcia Couto [a], Sergio Faloni de Andrade [a], Rilton Alves Freitas [a,*]

[a] Programa de Mestrado em Ciências Farmacêuticas, Núcleo de Investigações Químico-Farmacêuticas (NIQFAR), Universidade do Vale do Itajaí — UNIVALI, Itajaí, CP 360, CEP 88302-202, Itajaí, Santa Catarina, Brazil
[b] Departamento de Bioquímica e Biologia Molecular, UFPR, Curitiba, Paraná, Brazil

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ABSTRACT

Chitosan and polyacrylic acid (PAA) both have weak, short-lasting bioadhesive properties; therefore, a hybrid particle composed of a chitosan derivative and PAA could be used as a new bioadhesive agent. Using simultaneous *in-situ* monitoring with a multidetection system, N-carboxymethyl chitosan was ionically bonded to acrylic acid and then polymerized using potassium persulphate as the initiator (N-$CMC_A$-$D_h$ of 165 nm). The PAA on N-$CMC_A$ was crosslinked using *N,N*-methylene-bisacrylamide (N-$CMC_{AC}$-$D_h$ of 141 nm). During polymerization, the solution developed a milky white appearance, and polymerization kinetics was determined to be $3.2\times10^{-3}\pm4.0\times10^{-6}$ mmol/min. The reaction for PAA alone was 1.7 times faster than that of the hybrid system. The particles showed an increase in thermal stability and reduction of thermal-mass loss compared with the N-CMC alone. The N-$CMC_{AC}$ particles showed the highest bioadhesion onto the stomach. The gastroprotection index of N-$CMCh_{AC}$ particles against ethanol/HCl-induced ulcers in mice was 68.2 ± 6.4%. Similar results were observed for omeprazole (74.2 ± 5.3%). The particles obtained in this work have potential for use in drug delivery to the stomach, perhaps to aid in treating ulceration and inflammation, and can be used as a system for the prevention of ethanol-induced ulcers.



## 1. Introduction

Therapies using polymeric particles could allow the modulation of drug release; with manipulation of the copolymer and/or the molecular weight of polymer used, it is possible to obtain a desirable system for drug delivery [1,2]. Furthermore chemical modifications and/or the formation of hybrid polymers could improve bioadhesion, among other properties [3,4].

The oral route for drug administration using polymeric particles is attractive because it is associated with convenience and low costs for the patient as well as compliance of the patient with the therapy. However, it has been recognized that some drugs are much less effective administered orally rather than parentally [5], the main problems being related to their poor stability in the gastro-intestinal tract (GIT) and shorter residence time due to GIT transit.

The improvement of oral bioavailability of drugs can be achieved using bioadhesive nanoparticles, which increase the retention time of the drug in the gut, thereby improving its absorption. Also, bioadhesive properties could increase the effectiveness of the drug activity on specific sites, such as ulceration and inflammation in the stomach.

Bioadhesion is defined as the ability of certain materials, such as polymers or particles, to adhere to parts of the biological system (the mucous membrane, for example) [6]. It is not a new concept, and it has been described in the literature for at least forty years. Today, however, it is a promising technology for increasing the residence time of drugs [7–10].

Chitosan is a favourable polysaccharide for use in pharmaceuticals such as a bioadhesive due to its biocompatibility and biodegradability [11–16]. Furthermore, chitosan showed adjuvant activities in therapies as an anti-ulcer agent on oral and vaginal mucosa [17]. However, according to Grabovac et al. [8], chitosan has weak, short-lasting mucoadhesive properties and detaches from the mucosa just a few minutes after administration.

Chitosan bioadhesion could be improved by carboxymethylation of the structure or by polymerization using acrylic acid [18–20]. Non-crosslinked polyacrylic acid (PAA) exhibited a stomach retention time of up to 1 h. However, crosslinked PAA diminishes the dissolution rate of the hydrophilic polymer chain in aqueous environments, providing comparatively greater cohesion of polymers, and, consequently, increasing bioadhesion [8].

* Corresponding author.
*E-mail address:* rilton@univali.br (R.A. Freitas).

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The aim of this study is to evaluate, using real-time analysis, the formation of hybrid particles of N-CMCh and PAA obtained by ionic interaction (N-CMCh$_A$) and a more cohesive system obtained after crosslinking these polymers with *N,N*-methylene-bisacrylamide (N-CMCh$_{AC}$). Parameters of *in vivo* bioadhesion and gastric protection were also investigated.

## 2. Materials and methods

### 2.1. N-Carboxymethylation of chitosan (N-CMCh)

Chitosan (Shangyu Biotech Co., Ltd, China, imported by Purifarma®, Brazil) was produced from shrimp shell chitin. The degree of chitosan deacetylation ($87 \pm 1.4\%$) was assayed by Broussignac's method [21,22] The N-carboxymethylation (CM) of chitosan (25%) and the molar mass ($M_w$) $1.05 \times 10^6$ g/mol as well as the FTIR and $^{13}$C NMR spectra, were previously described [22,23].

### 2.2. Polymerization of acrylic acid on N-CMCh

The hybrid bioadhesive particle N-CMCh/polyacrylic acid (PAA) was obtained by polymerization of acrylic acid (AA). In 100-mL polymerization flasks, the N-CMCh was dissolved in purified water (0.5 mg/mL), followed by the addition of AA (molar ratio 1:0.5 of N-CMCh: AA), at pH 4.0. The reaction was purged with nitrogen gas at a flow rate of 20 mL/min for 15 min and the reaction was initiated with 0.1 mM potassium persulphate. A glycerol bath maintained the system at 70 °C.

The hybrid polymers obtained were named N-CMCh$_A$ for the reaction described above, and the crosslinked hybrid (N-CMCh$_{AC}$) was obtained by adding 2.7 mM of *N,N*-methylene-bisacrylamide to the reactor [24].

### 2.3. Real time analysis of polymerization

Before starting the polymerization reaction of AA, purified water was pumped (Shimadzu Pump LC-20AT) at 0.5 mL/min through the reactor system to stabilize the refraction index (RI, Shimadzu), 210 nm ultraviolet (UV, Shimadzu), 90° static light scattering (LS, BI-MwA Brookhaven) and viscometer (VS, home designed). The N-CMCh was then pumped into the mixture with AA (pH 4.0) until the instruments were stable. Subsequently, a 10 mM persulphate solution (100× concentrated) was added to the reactor and monitoring was continued. The same polymerization procedure was performed with AA alone.

Additionally, a Brookhaven BI-90 plus instrument at a 90° detection dynamic light scattering angle was used for the intensity autocorrelation computation and analysis by the standard method of moments, using small samples (3 mL) from the reactor to yield the average $D_z$ and the higher moments. From $D_z$, the z-averaged equivalent sphere hydrodynamic radius $R_h$ was determined by the Stokes–Einstein relationship for spheres [25]. Complementary studies of pH and temperature effects were performed on a NANODLS Brookhaven instrument at 90°.

### 2.4. Thermal analysis

Thermal analysis was performed using a calorimeter Netzsch Proteus Thermal with a heating rate of 10 K/min during the dynamic ramp from 30 to 600 °C, an alumina pan, and an inert atmosphere of $N_2$ (purge 50 mL/min and protection at 20 mL/min).

To evaluate the solid state properties, the samples were spray-dried using a Büchi 290 Mini-Spray dryer with a two-component nozzle and a current flow of 7 mL/min, an aspiration air of 90%, an inlet temperature of 170 °C, a spraying pressure of 5 bar and an air flow height of 40 mm.

### 2.5. Bioadhesion studies

The bioadhesion analysis was carried out by labelling the N-CMCh with fluorescein, according to Zhang et al. [26], using fluorescein isothiocyanate (FITC). The unreacted FITC was removed by dialysis for 24 h. The amount of the FITC label was determined by a standard curve using FITC (1 to 100 mM), an excitation of 480 nm, and an emission of 540 nm using a Genius Pro Microplate reader.

Male Wistar rats (220–250 g) were housed under normal conditions with free access to food and water. The animals were kept overnight without feed but were allowed free access to water. The animal protocol was approved by the UNIVALI Ethics Committee (492/08) and is in agreement with EC Directive 86/609/EEC for animal experiments.

The animals were dosed orally by gavage with N-CMCh$_A$ and N-CMCh$_{AC}$ particles (10 mg/mL, dose of 40 mg/kg). The rats were then sacrificed at different time points (1 h, 5.5 h and 8 h) and the entire gastrointestinal tract of each rat was removed and divided into 5 segments: the stomach and four portions of the small intestine. These segments were rinsed with 0.9% saline. The rinsed mucosa segments were cut into portions 2 cm in length and treated with 1 mL of 3 M NaOH for 24 h. The FITC was extracted with 2 mL of methanol, vortexed for 1 min and centrifuged at 4000 rpm for 10 min. Aliquots of 1 mL were diluted with water (3 mL) and quantified at an excitation of 480 nm and an emission of 540 nm using a Genius Pro microplate reader. The standard curve was prepared daily. The kinetic parameters determined for the adhered fraction were the maximal amount of nanoparticles adhered to the gut surface ($Q_{max}$), and the area under the bioadhesion curve ($AUC_{adh}$), evaluated by the trapezoidal rule up to $t_z$, which denoted the last sampling point. The terminal elimination rate of the adhered fraction ($k_{adh}$) and the mean residence time of the adhered fraction of nanoparticles in the mucosa ($MRT_{adh}$) were also determined [27–29].

The statistical analysis was carried out using the Mann–Whitney U-test on the bioadhesion parameters of N-CMCh$_A$ and N-CMCh$_{AC}$ to determine the statistical significance of results. $p<0.05$ was considered to be significant.

### 2.6. Evaluation of the gastroprotection

The gastroprotector activity of the nanoparticle complex was evaluated by the HCl/ethanol-induced model, according to the methodology described by Schmeda-Hirschmann et al. [30]. The mice were divided into groups of 6 animals, which were submitted to fasting 12 h prior to receiving 0.2 mL of the vehicle (distilled water), 30 mg/kg of omeprazole (standard drug) or 30 mg/kg of the nanoparticle complex. All treatments were administered orally by gavage. The dose used was equivalent to the standard dose for purposes of comparison. After 1 h, all groups were orally treated with 0.2 mL of a 0.3 M HCl/60% ethanol solution (HCl/ethanol) to induce gastric ulcers. One hour later, the animals were sacrificed by cervical dislocation and the stomachs removed and opened along the greater curvature. The stomachs were gently rinsed with water to remove the gastric contents and blood clots for subsequent scanning. The images obtained were analyzed using specific software named EARP to measure each lesion point. The ulcers were classified as follows: level I, ulcer area $<1$ mm$^2$; level II, ulcer area 1–3 mm$^2$; and level III, ulcer area $>3$ mm$^2$. The following parameters were determined: i) Ulcerative Lesion Index (ULI) as: 1x (number of ulcers level I) + 2x (number of ulcers level II) + 3x (number of ulcers level III); ii) gastroprotection index (%), which was determined as: $100 - (ULI_{treated} \times 100 / ULI_{control})$; iii) total area of lesion; and iv) percentage of the lesion area in relation to the total stomach area [31].