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pH-Sensitive Acrylic Terpolymers for the Coating of Orally Administered Drugs Used for Colonic Release

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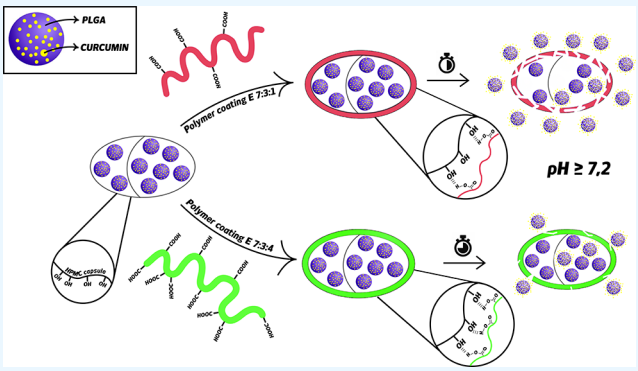
ABSTRACT: Polymeric coatings are a promising option for the development of delivery systems for orally administered drugs. However, the gastrointestinal conditions to which they are subjected, which include low pH and solubility as well as peristaltic movements, can limit their applications. In this work, different formulations of polymeric coatings were produced using pH-sensitive materials consisting of copolymers of methyl acrylate, methyl methacrylate, and methacrylic acid. The polymers were synthesized by the emulsion polymerization technique, obtaining small average particle sizes (56−190 nm), molecular weights between 200,000 and 400,000 g/mol, and a glass transition temperature above 35 °C, which are suitable for film formation at room temperature. Thus, they were assessed as coatings for hydroxypropyl methylcellulose capsules (HPMC) using the immersion method, showing adequate capacity to protect the capsule at gastric pH (pH 1.2) and dissolve at the simulated intestinal pH (pH= 7.2). In particular, the higher the content of the acidic monomer, the higher the release time of the test molecule contained in the acrylic terpolymer-coated HPMC capsules proposed, which was a curcuminoid derivative due to their bright color and potential medical benefits. In addition, a minimum number of immersions was required for coating the HPMC capsules at high acidic concentrations, which further facilitates the delayed release needed for colonic treatment. However, too high proportions of methacrylic acid may result in cytotoxicity issues. Consequently, a biocompatible formulation containing a proportion of methyl acrylate, methyl methacrylate, and methacrylic acid of 7:3:3 is proposed as the most adequate for colonic release. Thus, by chemically modulating the molar percentages of the acrylic monomers, it was possible to obtain tailored acrylic terpolymer coatings with different characteristics and desired properties in order to modulate the release kinetics of an active substance in a colonic environment.



1. INTRODUCTION

Orally administered drugs are an excellent method of incorporating an active ingredient into the body due to their simplicity, convenience, low cost, and safety. However, they are limited by factors in the digestive environment, such as enzymatic degradation, low pH, and solubility, among others.[1,2] In addition, low drug specificity and poor absorption produce the loss of active pharmaceutical substances and the need for high dosages, which can cause adverse effects to patients.[3] To overcome this challenge, drug delivery systems based on nanocarriers, whose function is to contain and only deliver the potent drug at the affected site, have been proposed.[4−7] These systems offer the possibility of broadening the application spectrum of the drugs compared to other conventional treatments by improving their solubility so that they can be stably dispersed in aqueous conditions without aggregation. Additionally, they provide better stability than the free drug and reduced toxicity, exhibiting fewer side or adverse effects during treatment.[8−10] Drug delivery systems have been described for the treatment of colonic diseases such as ulcerative colitis,[11] colorectal polyps,[12,13] and cancer.[14]

Colorectal cancer is the third leading cause of cancer deaths worldwide.[15] Currently, it is mainly treated with chemotherapy, where highly toxic drugs, such as 5-fluorouracil, paclitaxel, and other substances, are administered.[16] Additionally, active drugs with lower toxicity from natural compounds such as resveratrol and curcuminoids have been proposed as interesting options to be explored.[17] In any case, protecting the active ingredients for effective carrier delivery is crucial to

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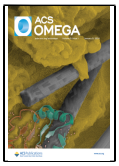

improve drug absorption and bioavailability toward the pharmaceutical target. Delivery carriers can be coated with pH-sensitive polymers to ensure delayed release depending on the chemical functionality, thus potentializing their application in colonic diseases.[18,19]

The design of these polymeric coatings is based on the versatility that can be conferred to specific polymers to modulate their properties, such as molecular weight, hydrophobic and hydrophilic domains, adhesion, and pH-dependent solubility. Thus, the delivery of active pharmaceutical ingredients (APIs) can be modulated in a colonic environment.[20,21]

Polymers of synthetic origin, such as copolymers based on acrylic acids and esters,[22] whose physicochemical properties vary due to the presence of anionic, cationic, or neutral groups, are highly promising, as they allow the API in its solid dosage form to act during the transit through the stomach. This is possible thanks to their high biocompatibility and mucoadhesive properties.[23]

Different formulations that contain polyacrylates have been reported to offer excellent characteristics for drug release control in other pharmaceutical systems.[24] Some polymer families resulted in functional films for sustained release in coated tablets and pellets.[22] Colon-targeted delivery of cyclosporine has been reported by combining dual-functional nanoparticles (NPs) to ameliorate colitis. In this case, copolymers based on pH-sensitive methacrylic acid, methyl methacrylate, and poly(lactic-*co*-glycolic acid) (PLGA) were used to ensure a sustained release of the API that acts as an immunosuppressant, reducing the activity of the immune system as well as the inflammation caused by this condition.[25] In addition, the manufacture of chitosan microspheres coated with copolymers based on methacrylic acids and esters has also been implemented in treating colitis to encapsulate mesalamine, taking advantage of its biodegradability, size, and prolonged residence time.[26]

Other organic compounds have been used in treating conditions such as Crohn's disease with mucosal barrier failure, assisted by copolymers based on the pH-sensitive methacrylic acid, methyl methacrylate, and methacrylate monomers, which prevented the rapid elimination into circulation of drugs using techniques such as spray drying.[27]

In addition, these polyacrylates have been used to study the effect of the coating layer of orally administered drugs and the release medium of capsules coated with the terpolymer Eudragit FS 30 D. The coating was produced by the immersion method to maximize the time needed to erode the thick layers, thus delaying the release of the API.[28]

Finally, studies have been reported on coating hydroxypropyl methylcellulose (HPMC) capsules with different commercial terpolymers such as Eudragit FS 30 D by immersion for enteric use, but the specific composition of the terpolymers was not varied.[29]

In contrast, we assessed the impact of chemically modulating the molar ratios of the monomers used to produce pH-sensitive terpolymer-based coatings on HPMC capsules. In particular, this study proposes the modulation of the molar ratios of the acrylic monomers of the poly(methyl acrylate-*co*-methyl methacrylate-*co*-methacrylic acid) terpolymer to study their effect on film formation, capsule coating, and the delayed release of a molecular cargo. Thus, the coatings with the synthesized polymers were shown suitable to protect the HPMC capsule at acidic gastric pH (pH = 1.2),[18,28] while dissolving at a simulated intestinal pH (pH = 7.2).[30] In addition, the delivery kinetics of the test molecule used, a curcuminoid, with an intense yellow color and potential medical benefits, could be modulated. In conclusion, optimal layer coating thicknesses of HPMC with adjustable release times of curcumin were achieved by modulating the formulation of the terpolymer.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents. The monomers methyl acrylate (purity: 99%), methyl methacrylate (purity: 99%), methacrylic acid (purity: 99%), the surfactants Tween 80 (purity: 99%), sodium dodecyl sulfate (SDS; purity: 99%), the initiator potassium persulfate (KPS) (purity: 99%), and the plasticizer triethyl citrate (TEC) (purity: 99%) were purchased from Merck (Germany) and used as received. Poly(vinyl alcohol) ($\bar{M}_w$ = 30,000−70,000 Da), poly(lactic-*co*-glycolic acid) (PLGA), commercial Resomer RG 752 S (4000−15,000 Da), HPLC grade acetonitrile, HPLC grade ethanol, and synthesis grade curcumin were also purchased from Merck. Eudragit FS 30 D was supplied by Evonik (Germany). Ultrapure water (type I) was obtained from the Barnstead Smart2pure Thermo Scientific (USA) purification equipment. The vegetarian hydroxypropyl methylcellulose (HPMC) capsules were obtained from XPRS Nutra (USA).

2.2. Synthesis of Poly(methyl acrylate-*co*-methyl methacrylate-*co*-methacrylic acid). The acrylic terpolymer was obtained by an emulsion polymerization technique. First, to synthesize 90 g of the polymer, deionized water was placed in a 100 mL 3-neck reactor with the surfactants Tween 80 (1.2 wt %) and SDS (0.3 wt %) concerning the total mass previously dissolved in the dispersing medium. Next, a 10 wt % seeding of the monomer mixture consisting of methyl acrylate, methyl methacrylate, methacrylic acid, and the initiator KPS (1.0 wt %) concerning the monomers was added. Next, the system was heated to 65 °C with a recirculation equipment (Isotemp, Fisher Scientific; USA) and agitated with an overhead stirrer at 250 rpm for 30 min. After the seeding process, the amount of initiator and the remaining monomer mixture was added with a peristaltic pump (Masterflex, Cole-Parmer Instrument Company; USA) at a speed of 0.1 mL/s for 4 h, and the temperature was increased to 70 °C.

2.3. Characterization of Suspensions of Poly(methyl acrylate-*co*-methyl methacrylate-*co*-methacrylic acid).

2.3.1. Dynamic Light Scattering. The average hydrodynamic diameter (*z*-average) obtained by dynamic light scattering (DLS) was measured on a Zetasizer Pro (Malvern Instruments Ltd., UK) instrument for each NP suspension with two detection angles of 173° and 13°. The suspensions were suitably diluted in deionized water (≈0.1%, m/v). The average of the measurements was obtained by measuring each sample in triplicate.

2.3.2. Zeta-Potential Measurements. ζ-Potential was measured in a Zetasizer Pro (Malvern Instruments Ltd., UK) instrument in a cell Z DTS 1070 at 25 °C. The suspensions were suitably diluted in deionized water (≈0.1%, m/v). All measurements were taken in triplicate.

2.3.3. Brookfield Viscosity. At 25 °C, the aqueous dispersions of the terpolymers were analyzed in a Brookfield LDVE115 Viscometer (Brookfield Engineering Laboratories, USA). The measurements were carried out at 30 rpm by using the S00 spindle.

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2.3.4. Surface Tension Measurements. The measurements were taken in an Interfacial tensiometer KRÜSS K8 (Germany), with a du Noüy Pt−Ir ring. All measurements were run in triplicate.

2.3.5. Scanning Electron Microscopy. Aqueous dispersions of the terpolymers were analyzed by scanning electron microscopy (SEM) using a JEOL JSM-6490 LV microscope (USA), which operates at a voltage acceleration of 15 kV. A drop of the diluted NP suspension was deposited on an aluminum sample holder and kept at room temperature until the water evaporated. Finally, the samples were subjected to a gold bath to visualize the particles. To analyze the coatings on the HPMC capsules, and PLGA NPs with curcumin, a FESEM Thermo Fischer Scientific Apreo 2S LoVac Field Emission Electron Microscope (USA) with a secondary electron detector (ETD) was used to evaluate the surface, morphology, and topography. The approach voltage was 5 kV, and the magnifications were 1000×.

2.3.6. Transmission Electron Microscopy. Transmission electron microscopy (TEM) was performed on a JEOL 1200EX electron microscope (USA) operated at 100 kV. 5.0 μL of the NP suspension with a 1.5 mg/mL concentration in type I ultrapure water was added to a carbon-coated copper grid (Holey Carbon Film, Electron Microscopy Sciences; (USA). The drying process was performed at room temperature in a desiccator. The NPs were then stained with 2.0% uranyl acetate drop-in type I water. Finally, they were dried at room temperature before TEM analysis.

2.4. Characterization of Poly(methyl acrylate-*co*-methyl methacrylate-*co*-methacrylic acid). *2.4.1. Fourier Transform Infrared Spectroscopy.* Fourier transform infrared (FTIR) spectra were obtained on a TENSOR II spectrophotometer with an MCT detector (Bruker, Germany) and the Platinum Attenuated Total Reflectance (ATR) accessory. The spectra were performed with a resolution of 4 cm^{-1} and 64 scans. The associated software for data delivery was OPUS.

2.4.2. Nuclear Magnetic Resonance Spectroscopy. ^1H NMR spectra were obtained on a Bruker AVANCE III HD 600 MHz spectrometer (Germany) with a 5 mm TCI CryoProbe. TMS was used as an internal standard. The signals of the deuterated solvents and the chemical shifts (δ) are displayed in ppm.

2.4.3. Differential Scanning Calorimetry. The DSC Q100 equipment (TA Instruments, USA) was used for glass transition temperature (T_g) measurements. The samples were first heated from room temperature to 300 °C, then cooled to −20 °C maintaining the isotherm for 5 min, and finally warmed again from −20 to 300 °C at a heating rate of 10 °C/min. This last sweep is the one reported here.

2.4.4. Gel Permeation Chromatography. Molecular weights (M_w) were determined by gel permeation chromatography (GPC) on an Ultimate 3000 UHPLC liquid chromatography (USA). Serial coupling was used with a Phenogel 5 μm 10 E3, GPC, 7.8 mm ID × 300 mm column for M_w between 1 and 75 kDa and a Phenogel 10 μm 10 E6, GPC, 7.8 mm ID × 300 mm column, for M_w between 60−10,000 kDa using HPLC grade tetrahydrofuran, at an isocratic flow rate of 1 mL/min, the temperature of 40 °C, and injection volume of 50 μL. The calibration curve was performed with polystyrene standards.

2.4.5. Contact Angle. This measurement was performed after film formation via a Dataphysics Model Dataphysics Oca 14 EC instrument (Germany), with a dosing volume of 5 μL and a dosing rate of 0.5 μL/s. All measurements were performed in triplicate.

2.5. Preparation of Nanocarriers of PLGA with Curcuminoids. Curcumin loaded in PLGA NPs were used as a testing system to evaluate HPMC capsules coated with the synthesized pH-sensitive polymer as a nanocarrier. The technique used to prepare PLGA NPs was simple emulsion-solvent evaporation with ultrasounds.[31] PLGA (100 mg) was dissolved in 5 mL of dichloromethane with curcumin (5 mg) to obtain a translucent and low viscosity solution. This organic mixture was emulsified in 10 mL of a poly(vinyl alcohol) solution (1% w/v) prepared with Milli-Q water. The immiscible mixture formed a pre-emulsion by vortexing for 1 min at 2000 rpm. Then, this pre-emulsion was sonicated using an ultrasound probe for 20 s three times and resting for 10 s between each sonication cycle (3 mm diameter, Digital Sonifier 550, Branson, USA), to prevent temperature rise.[32] The pre-emulsion was kept in an ice bath using a sonication power of 20%.[32] The resulting primary oil in water (O/W) emulsion was added to 30 mL of Milli-Q water. The dichloromethane in the nanoemulsion was evaporated for 24 h from the dispersion with magnetic stirring. The resulting PGLA NP suspensions were washed to remove excess surfactant using a Digicen 21 R centrifuge (Orto-Alresa, Spain) at 1600 rpm with a temperature of 0 °C for 30 min to concentrate the particles. Then, the NPs were resuspended in type I water by vortex mixing. This washing process was performed twice and the NPs were lyophilized in a BIOBASE BK-FD10PT freeze-dryer for 24 h.

2.6. HPMC Capsule Coating by the Simple Immersion Method. The HPMC capsules of size 1, with an approximate weight of 0.08 g per capsule, were coated by the simple immersion method. Only one coating layer was made, which consisted of 15 s immersion for each formulation of synthesized aqueous dispersions (130 μL of triethyl citrate plasticizer was added to 5 mL of each dispersion), and the dispersions were allowed to dry at room temperature for 30 min. Each capsule contained about 15 mg of testing system (PLGA NPs with curcuminoids).

2.7. pH-Dependent Release of Curcumin from Coated Capsules. The coated capsules were soaked in solutions of HCl (0.06 M) with pH 1.2 or phosphate buffer (4.3 g of K_2HPO_4 + 3.4 g of KH_2PO_4) with pH 7.2 with constant stirring. To obtain the release profiles, samples of 500 μL of the medium were extracted every 30 min and diluted by adding 500 μL of ethanol. The quantification of the differentiated curcuminoids was performed by HPLC, using an UltiMate 3000 HPLC system (Thermo Scientific, USA) equipped with a diode array detector and a Pinnacle II C18 5 μm, 250 × 6 mm column. The equipment worked in isocratic mode; the mobile phase consisted of 0.1% v/v H_3PO_4 in type I water:acetonitrile (40:60) with a flow rate of 1.0 mL/min, the diode array detector was used at a wavelength of 426 nm, the oven temperature was 25 °C, and the injection volume was 10 μL.

2.8. Cell Viability. Primary human peripheral blood mononuclear cells (PBMCs) were obtained following an established protocol.[33−35] In brief, PBMCs were isolated using a density gradient centrifugation with Ficoll from buffy coats supplied by "Banc de Sang i Teixits" (Barcelona, Spain) under the approval of the "Ethics Committee on Animal and Human Experimentation" of the Autonomous University of Barcelona (Nr.: 5099).

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Coatings comprising each sample and the plasticizer triethyl citrate at 3% v/v were prepared as films on the bottom of 6-well plates or on top of an HPMC capsule square piece (1 cm^2). The samples were placed under UV light for 30 min for sterilization and were washed three times with phosphate-buffered saline (PBS). Afterward, 3 mL of PBMCs at a concentration of 0.5 × 10^6 cells/mL were incubated with the samples for 3 h at 37 °C and 5% CO_2.

After the incubation of PBMCs with the films or coated capsules, cells were collected, washed with PBS, and stained with a solution of calcein and propidium iodide (PI). Cell viability was then analyzed by flow cytometry with a BD FACSCanto instrument (BD Bioscience, USA). The data was treated using FlowJo software (FlowJo LLC, USA). Calcein +/PI- events were considered alive cells, while calcein-/PI+ events were considered dead cells. Other events were discarded.

3. RESULTS AND DISCUSSION

Before assessing the performance of our coatings based on pH-sensitive acrylic polymers for colonic release, a characterization

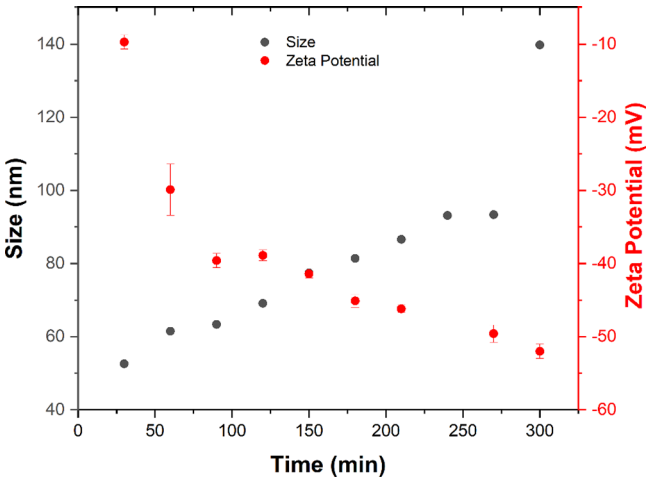

Figure 1. ζ-Potential and particle size of formulation E 7:3:1 during reaction synthesis.

of these materials in suspension is shown given their importance in film formation.[36]

3.1. Characterization of Suspensions of Poly(methyl acrylate-*co*-methyl methacrylate-*co*-methacrylic acid) Terpolymers. The different coating formulations were obtained by emulsion polymerization, with varying molar ratios of the acrylic monomer. In particular, we used the nomenclature E *x*:*y*:*z*, where *x*, *y*, and *z* are the molar ratios of methyl acrylate, methyl methacrylate, and methacrylic acid, respectively. The solids content was around 30 wt %. At the beginning of the reaction, iridescence could be observed during the seeding step (30 min), which evidences the polymerization process. Figure 1 shows the particle size distribution and ζ-potential during the reaction time for system E 7:3:1. The increase of the average particle size with time is due to the monomer delayed addition method; after seeding, very fine particles with an average size of about 53 nm are obtained. With time, the increase in the average particle size is due to the

Table 2. Colloidal Characterization of Formulations

formulation	*Z*-average (nm)	standard deviation (*Z*-average)	polydispersity index (PDI)	ζ-potential (mV)	standard deviation (ζ-potential
E 8:2:1	87.3	0.86	0.071	−57.4	0.49
E 6:4:1	74.2	0.85	0.116	−48.7	0.51
E 5:5:1	77.5	0.38	0.104	−50.9	1.10
E 7:3:4	189.8	0.10	0.070	−57.8	1.0
E 7:3:3	139.3	0.64	0.034	−65.44	0.73
E 7:3:2	116.2	1.09	0.031	−55.34	0.47
E 7:3:1	93.4	0.04	0.056	−50.3	1.18
E 7:3:0.8	81.8	0.66	0.044	−61.7	2.73
E 7:3:0.5	70.68	0.30	0.068	−53.3	0.58
E 7:3:0	55.6	0.27	0.083	−46.3	1.14

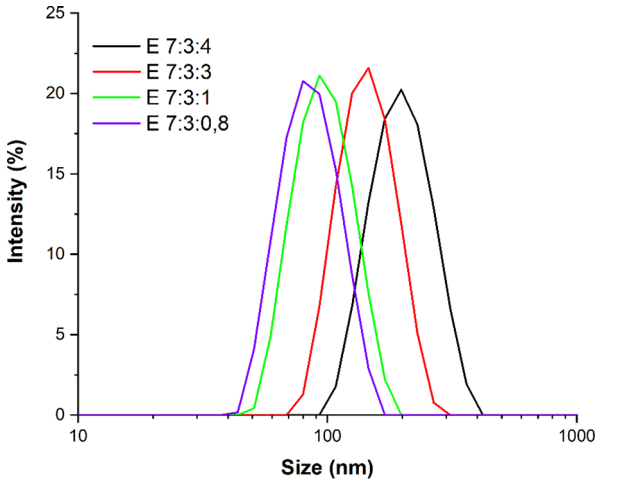

Figure 2. *Z*-average size distribution (nm) of formulations E 7:3:4, E 7:3:3, E 7:3:1, and E 7:3:0.8.

Table 1. Characterization of Poly(methyl acrylate-*co*-methyl methacrylate-*co*-methacrylic acid) Terpolymer Dispersions

formulation	solid content (%)	standard deviation (solid content)	viscosity brookfield (cp)	standard deviation (viscosity brookfield)	surface tension (dyn/cm)	standard deviation (surface tension)
E 8:2:1	29.00	0.07	4.17	0.01	35.6	0.5
E 6:4:1	30.40	0.00	4.07	0.00	37.9	0.1
E 5:5:1	33.62	0.04	4.28	0.01	38.0	1.0
E 7:3:4	38.72	1.30	4.33	0.01	32.3	0.7
E 7:3:3	30.52	0.08	4.14	0.01	35.7	0.3
E 7:3:2	30.81	0.05	4.10	0.00	35.9	0.3
E 7:3:1	32.40	0.20	4.62	0.01	35.0	0.6
E 7:3:0.8	29.05	0.03	3.40	0.02	33.8	0.1
E 7:3:0.5	29.80	0.02	3.23	0.01	36.4	0.1
E 7:3:0	29.90	0.05	3.22	0.01	36.6	0.1

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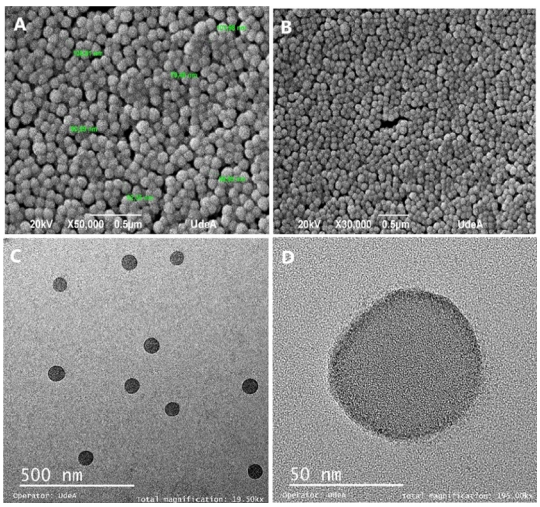

Figure 3. (A, B) SEM and (C, D) TEM micrographs of the formulation E 7:3:1 prepared by the emulsion polymerization technique.

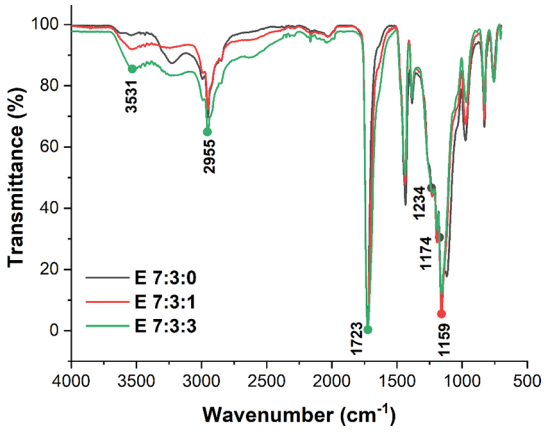

Figure 4. FTIR spectra of formulations E 7:3:0, 7:3:1, and E 7:3:3.

feeding stage, getting a *z*-average of 93 nm after 4.5 h of reaction. The ζ-potential becomes more negative due to the successful incorporation of methacrylic acid. Its carboxylic group partially dissociates, generating carboxylate ions on the NP surface, obtaining highly stable colloidal dispersions with ζ-potential higher than −50 mV.

During the synthesis process of the different copolymers, a slight increase in viscosity and the generation of milky white polymeric dispersions are evidenced as the concentration of methacrylic acid is increased. As the proportion of acid in the system increases, the cohesive forces increase and, therefore, the viscosity. No significant changes were observed in the surface tension measurements obtained since the proportion of surfactant remained constant in all formulations. These properties are shown in Table 1.

The results of the colloidal characterization are listed in Table 2. A monodisperse distribution is observed in each formulation in Figure 2. The higher the mole fraction of methacrylic acid, the larger is the particle size. The degree of solvation of the polymer is enhanced by the presence of a higher number of carboxylic groups, increasing the hydrodynamic diameter of terpolymer NPs dispersed in water. It also explains the milky color of the suspensions due to the increase in light scattering. These experiments were established based

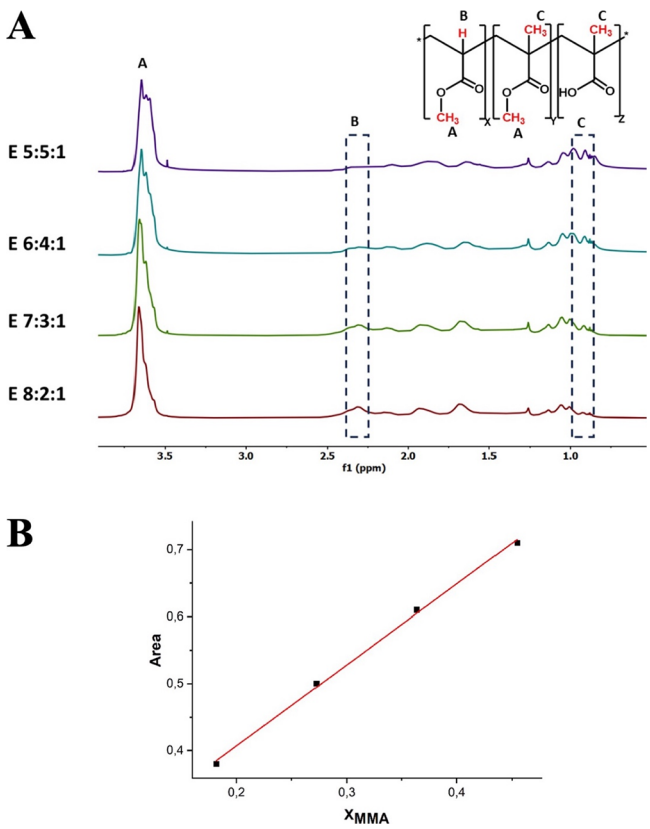

Figure 5. (A) ^{1}H-NMR spectra and (B) area of H (C−CH_3) vs X_{MMA} of the formulations E 8:2:1, E 7:3:1, E 6:4:1, and E 5:5:1.

Table 3. Comparison of the Glass Transition Temperature by the Fox Equation with the Experimental Results and Molecular Weights for Different Formulations

formulation	T_g experimental	T_g Fox	$\bar{M}_n$	$\bar{M}_w$	PDI
E8:2:1	40.0	38.2	195,000	372,000	1.90
E6:4:1	62.1	55.3	292,000	568,000	1.94
E5:5:1	69.5	64.3	304,000	587,000	1.93
E7:3:4	79.2	72.5	294,000	630,000	2.15
E7:3:2	55.9	56.2	384,000	617,000	1.60
E7:3:1	52.7	46.6	238,000	460,000	1.94
E7:3:0.8	65.2	44.6	395,000	532,000	1.35

on the stability of each system since, outside of this design, the systems flocculate and precipitate due to electrostatic interactions.

In the next step, the E 7:3:1 formulation generated by the emulsion polymerization technique was assessed by SEM. As shown in Figure 3A,B, the NPs show a spherical, uniform, and rough morphology, which is consistent with the DLS measurements.

TEM images for the NPs with the same formulation were also in agreement with the DLS and SEM results (Figure 3C, D). In Figure 3D, a rim of uniform thickness surrounding the polymeric core of the particles is observed, which could correspond to the surfactants polysorbate 80 and SDS. This layer could be responsible for the colloidal stability of the NPs.[37]

3.2. Characterization of Poly(methyl acrylate-*co*-methyl methacrylate-*co*-methacrylic acid) Films. Once the terpolymers were studied in aqueous solutions, films with different proportions of the methacrylic acid monomer were

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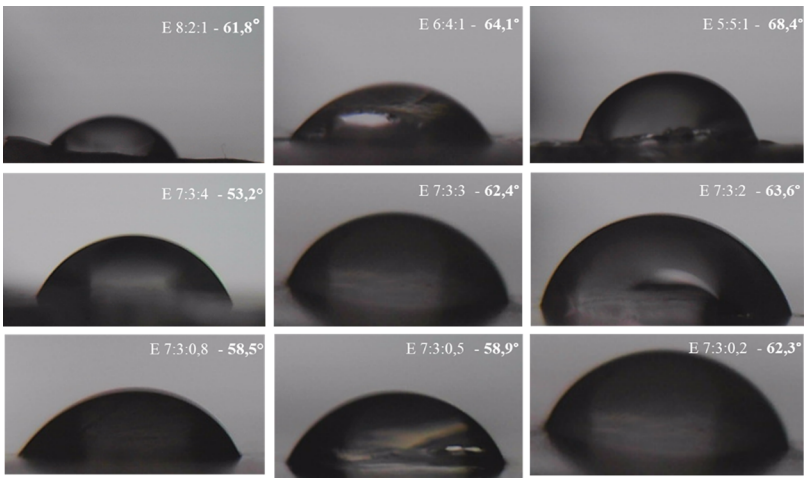

Figure 6. Water contact angles on polymeric films from different formulations.

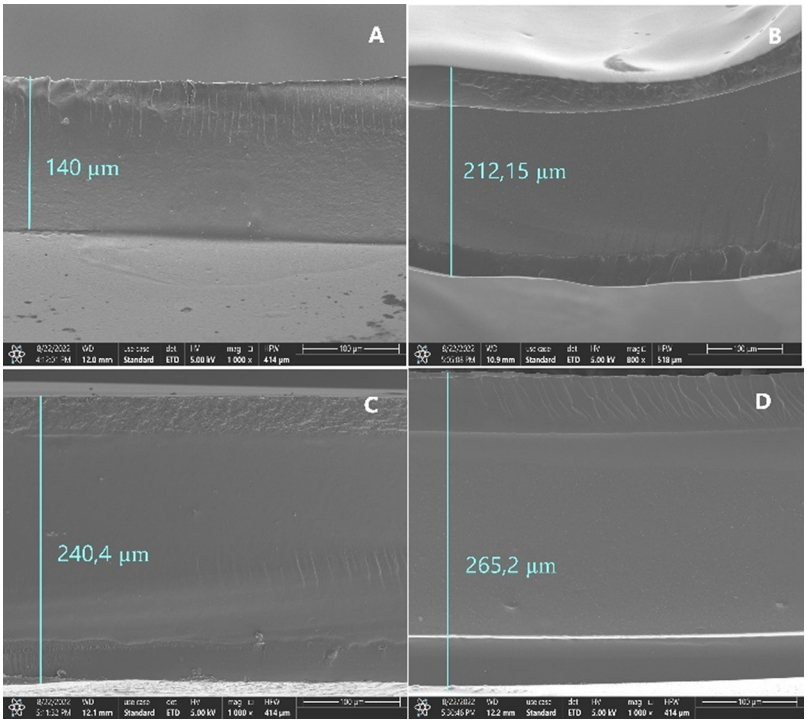

Figure 7. HPMC capsules (A) uncoated, (B) coated with three immersions E 7:3:1, (C) coated with one immersion E 7:3:4, and (D) coated with three immersions E 7:3:4.

synthesized and characterized by IR spectra (Figure 4) as they show sensitivity to pH and, therefore, capacity to be used as coatings for drug release.

The carbonyl characteristic peak of the ester groups can be observed at 1723 cm^{-1} (C=O stretching), whereas at 1234 and 1159 cm^{-1} bands are associated with C−O stretching. Around 3530 cm^{-1} there is the band corresponding to the O−H stretching of the methacrylic acid, while symmetric stretching of CH_2 and CH_3 group vibrations appear at 2900−3000 cm^{-1}. As the content of acid groups increases, the symmetric stretching of the O−H groups becomes slightly more intense, while the signal of the carbonyl becomes a little wider due to the increase in hydrogen bonding with the acid content in the polymer.

In the next step, we performed ^1H NMR spectra of synthesized terpolymers (Figure 5), with a constant mole fraction of methacrylic acid to ensure proper polymerization and facilitate monomer identification (Figure 5A). These spectra show a signal at 3.6 ppm, which is the characteristic chemical shift of the protons of the methoxy group[38] (label A) and as expected, it remains constant in all formulations. This can be explained as the mole fractions of methyl acrylate and methyl methacrylate remain constant at 10 throughout all formulations. On the other hand, the signal corresponding to the C−H at the backbone chain of methyl acrylate appears at 2.3 ppm (label B) and becomes more intense as the ratio of methyl acrylate increases with respect to methyl methacrylate, while maintaining the methacrylic acid constant. Finally, the CH_3 β signal from the carbonyl group at 1.1 ppm (label C) becomes more intense as the mole fraction of methyl methacrylate increases. In fact, varying the mole fraction of methyl methacrylate (MMA)/methyl acrylate while keeping

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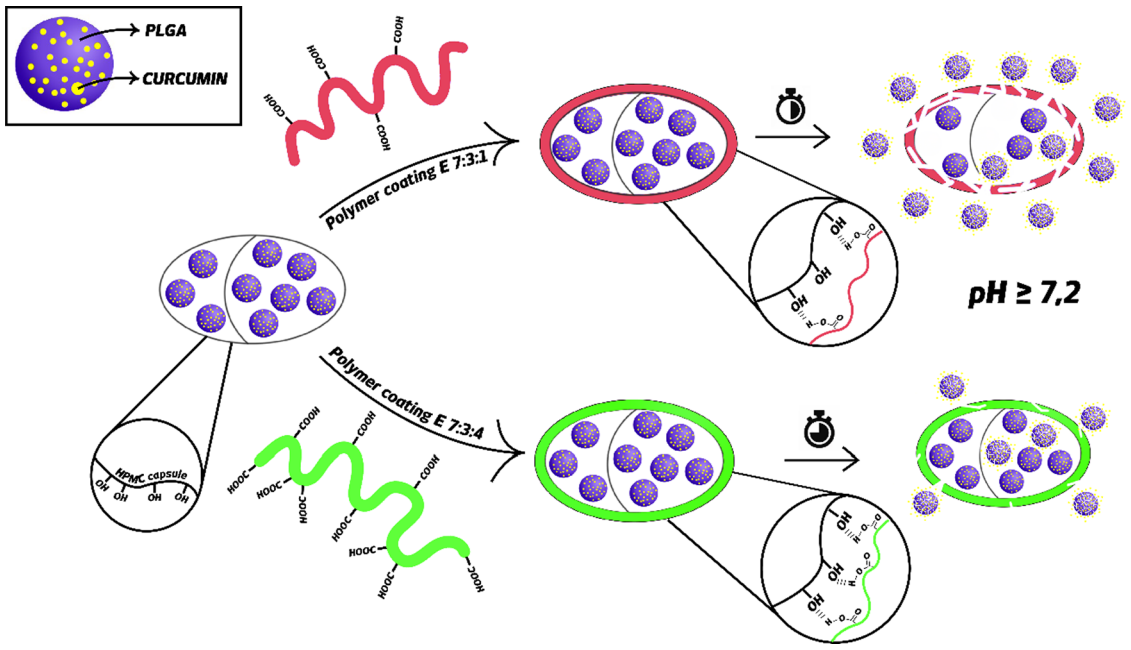

Figure 8. Schematic representation of the release of test molecules in HPMC capsules coated with the terpolymers with different fractions of acid groups at pH ≥ 7.2.

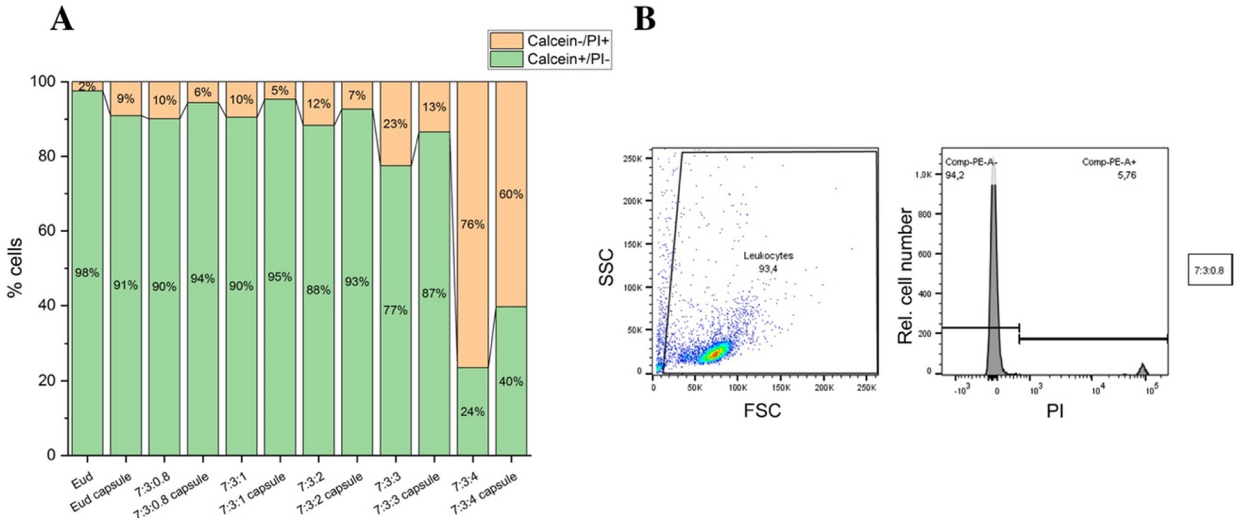

Figure 9. Cell viability test of the various formulations (E 7:3:4, E 7:3:3, E 7:3:2, E 7:3:1, Eudragit FS 30 D, E 7:3:0.8) mixed with the plasticizer processed as films or coated on an HPMC capsule (N_{donors} = 2). Cells were incubated with the different samples for 3 h and then stained with calcein and PI for flow cytometry analysis. (A) Percentage of calcein+/PI− (green bars; alive cells) and calcein−/PI+ (orange bars; dead cells) found in each condition. (B) Representative cytometry graph for the 7:3:0.8 formulation shows the cell population and PI histogram.

the mole fraction of methacrylic acid constant results in a linear increase in the area assigned to the protons of the β CH_3 groups from the carbonyl groups as the concentration of methyl methacrylate increases (Figure 5B). This corroborates the incorporation of MMA into the copolymer structure.

In Table 3, the molecular weights are shown between 200,000 and 445,000 g/mol and the glass transition temperature of some terpolymers. Additionally, the T_g is calculated through the Fox model by using the T_g values of each of the homopolymers and weight fractions in the copolymers:

$$1/T_g = {}^{w_1}/_{T_{g_1}} + {}^{w_2}/_{T_{g_2}} + {}^{w_3}/_{T_{g_3}}$$

It can be seen that the experimental data fit well with the Fox model as the data are within an acceptable margin of error. According to these T_g results, a favorable film formation process from the terpolymers at room temperature is expected. Thus, we could discard the formation of sticky (terpolymers with T_g below the capsule coating temperature) and brittle films (terpolymers with high T_g) that could delaminate from HPMC capsules in all samples, with the exception of E 7:3:0.8.

The water contact angle measurements on polymeric films are listed in Figure 6. As the proportion of methacrylic acid monomer increases, the surface films become more hydrophilic, increasing their wettability. On the contrary, as methyl methacrylate increases, the system behaves more hydrophobic, decreasing its wettability.

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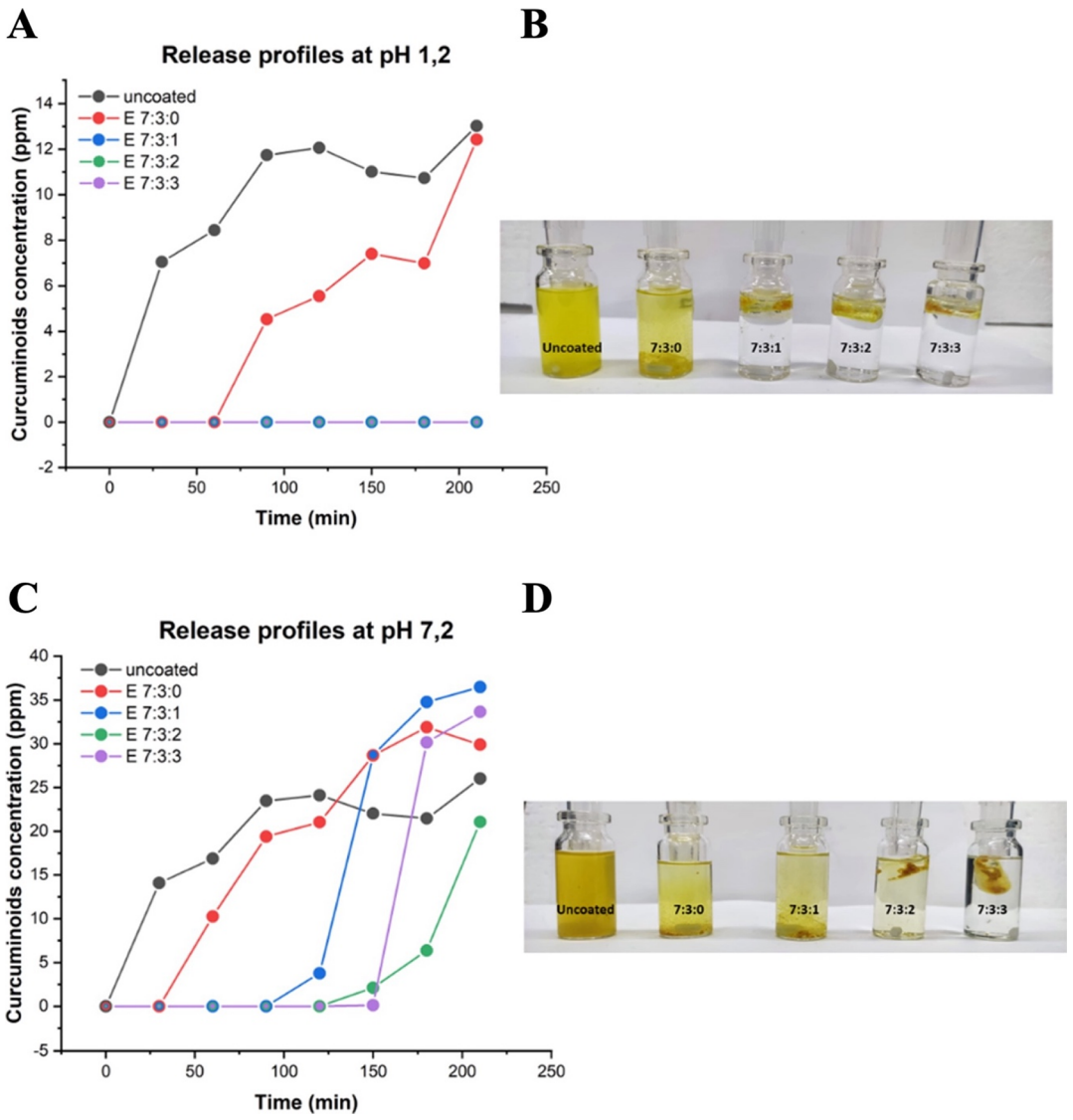

Figure 10. Release profiles of the test molecule from HPMC capsules coated with E 7:3:0, E 7:3:1, E 7:3:2, and E 7:3:3 and uncoated at (A, B) pH acid and (C, D) pH basic during 210 min.

3.3. Performance Analysis of the Poly(methyl acrylate-*co*-methyl methacrylate-*co*-methacrylic acid) Films. The HPMC capsules were coated with two formulations, E 7:3:1 and E 7:3:4, establishing a comparison of the gain in thickness as a function of the number of immersions. In particular, capsules coated with one, two, and three immersions were compared by using the SEM technique. The uncoated capsule has a thickness of approximately 140 μm. An increase in film thickness of about 52% is obtained in the first immersion for formulation E 7:3:1, which increases to about 70% for the E 7:3:4 formulation. In the successive immersions (two and three), an increase of 20% of each capsule was observed. Consequently, the formulation with a higher amount of acid groups (E 7:3:4) requires fewer immersions to provide the capsule with a thicker layer and thus ensure a more delayed release (Figure 7).

This higher coverage of the HPMC capsules with a lower amount of immersion indicates a higher affinity of the E 7:3:4 formulation on the capsule surface compared to that of the E 7:3:1 system. The HPMC capsules have free hydroxyl groups on the polysaccharide chains that interact favorably with the acid moieties present in the thermoplastic coating through hydrogen bonding (Figure 8), resulting in improved coating wetting. It is also important to mention that the solid content of the final E 7:3:4 dispersion is slightly higher (38.7%) compared to the E 7:3:1 system (32.4%), which should further favor a delayed release when immersing the HPMC capsules.

Before the performance of the delivery capacity of the coated capsules was assessed, the cytotoxicity of the various formulations was determined through a cell viability test using primary human PBMCs to mimic in vivo conditions (Figure 9).

Suspension cells were chosen over adherent cells to avoid requiring an extra surface coating that could interfere with the studied formulations. Moreover, their human and primary origins are appropriate characteristics to resemble the real environment. Additionally, the experiment was kept in incubation for 3 h as a reasonable time frame for a capsule to transit through the digestive system until reaching the intestines. Each film was evaluated alone and also compared with HPMC-coated capsules in triplicate.

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After incubation, cells were stained with calcein/PI, which stained alive and dead cells, respectively. As shown in the graph, good cell viability was observed for all the formulations tested, except for the E 7:3:4. There seems to be a trend of lower cell viability when the methacrylic acid ratio is increased in the formulations. This phenomenon is related to the fact that the formulations containing a higher amount of the acidic compound tend to be more soluble in cell media, thus, resulting in some degree of media acidification. However, this solubility challenge might not be relevant in the context of the intestines due to their large volume and consequent dilution of the acid resulting in nonappreciable pH change.

Finally, performance tests on coated and uncoated HPMC capsules were carried out by immersing the capsules in pH 1.2 and 7.2 solutions for ≥210 min adding hydrochloric acid and phosphate buffer, respectively, to simulate the gastric and intestinal environments (Figures 10 and S1).

The formulations used for the release profiles were E 7:3:0/E 7:3:1/E 7:3:2/E 7:3:3 since the modulation of the molar ratio of the acidic monomer plays a fundamental role in the protection of the capsules when they are subjected to gastric pH.[18] The release of curcumin, the test molecule in acidic pH (Figures 10A, B and S1A, B), occurred only in the formulation that does not contain any acid group, E 7:3:0. This can be explained by the presence of dispersion interactions between the ester groups of the polymer and the OH groups of the capsule, which are weaker than the hydrogen bonding present in the formulations with acidic groups, resulting in improved adherence of the polymer to the capsule. In the uncoated and E 7:3:0 formulations, the amount of test molecule doubles for basic pH compared to acidic pH, which supports the protection nature of the system in acidic pH.

Indeed, Figures 10C, D and S1C, D show how the uncoated capsule disintegrated rapidly in the simulated intestine, whereas the other formulations remained intact after 20 min. As the acid molar ratio of the formulation increases, the adhesion of the polymer to the HPMC capsules improves due to electrostatic interactions and/or hydrogen bonds. The biocompatible formulation with the highest ratio of acid (E 7:3:3) took longer to release the test molecules at pH 7.2. The differences between the released amounts of the test molecules may be attributed to the formation of possible NP aggregates within the capsules. However, the trend of the release profile with the pH is maintained.

4. CONCLUSIONS

This work demonstrates that coatings synthesized from acrylic monomers through the emulsion polymerization technique are promising for the development of drug delivery systems with potential applications in treating colonic diseases. The modulation of the molar ratios of the monomers enabled the fabrication of tailor-made coatings with different characteristics and desired properties, such as the release of an API at the expected time. In particular, we showed that these formulations can withstand gastric pH and release a cargo at intestinal pH and that the release time of it increased with the amount of acid groups. Given the cytotoxicity assays performed, we propose the E 7:3:3 formulation as the most efficient one for colonic delivery due to its retarded liberation and high biocompatibility.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c03437.

Additional release profiles of the test molecule from HPMC capsules coated with E 7:3:0, E 7:3:1, E 7:3:2, and E 7:3:3 (PDF)

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Notes

The authors declare no competing financial interest.

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