Journal of Applied Polymer Science

Ultrasonic atomization and polyelectrolyte complexation to produce gastroresistant shell–core microparticles

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ABSTRACT: In this study ultrasound-assisted atomization technique was combined with two-stages polyelectrolyte complexation to produce enteric shell–core microparticles encapsulating a non-steroidal, anti-inflammatory gastrolesive active ingredient indomethacin. In particular, a solution of the anionic biopolymer alginate, containing indomethacin, was sprayed in fine droplets which were complexed with a cationic (meth)acrylate copolymer, Eudragit® E 100, which, in turn, was complexed by the anionic copolymer Eudragit® L30D-55. The first complexation stage was applied to achieve a high drug encapsulation efficiency; the second one to assure good gastroresistance feature. The novel protocol has been found more effective in terms of loading, encapsulation efficiency, and enteric properties during *in vitro* release tests, than conventional procedures which involved alginate cross-linking by charged ions. Furthermore ultrasonic atomization–polyelectrolytes complexation preparation approach was performed using mild conditions, aqueous solutions, in the absence of organic solvents and chemical cross-linkers. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 42976.

KEYWORDS: biopolymer; cross-linking; drug delivery systems

Received 23 August 2015; accepted 25 September 2015
DOI: 10.1002/app.42976

INTRODUCTION

Many researches in the pharmaceutical field are focused on drug delivery systems to enhance therapeutic objectives and decrease side effects. The pharmacological response to a drug is directly related to its bioavailability at the target site of action. Conventional dosage systems could cause a non-specific distribution which leads to high drug concentration in healthy organs, tissues, and cells, because of toxicity.[1] Well-designed controlled drug delivery systems overcome the drawbacks of the conventional pharmacological therapies.[2] Microparticles are one of the multiparticulates delivery systems usually used to control the drug release in site and in time enhancing the therapeutic effects.[3,4] They can improve bioavailability of drugs and offer, with respect to conventional formulations, greater effectiveness, limiting fluctuation within therapeutic range; lower toxicity, reducing side effects; more lasting stability; a reduced dosing frequency, improving patient compliance.[5] Biocompatible polymers are the most diffuse materials used to produce microparticles, and many techniques selected also on the basis of the properties of the drugs to be administered, are adopted to achieve micro-sized structures.[6]

Indomethacin is a non-steroidal anti-inflammatory drug (NSAID), widely used for the treatment of the inflammation and pain caused by arthritis.[7] It can also have chemoprotective effects against tumors, reducing the risk of colon cancer.[8] It has poor water solubility, a short biological half-life of about 2.6–11.2 hours with an usual oral dosage (the administration route with highest patient compliance) for adults of 25 or 50 mg, for two to three times a day.[9] Indomethacin has physical properties which may cause severe side effects on the gastrointestinal tract, such as stomach irritation, intestinal bleeding or ulcers, and can increase blood pressure and decrease kidney function.[10]

Many studies have reported attempts to encapsulate indomethacin and/or indomethacin derivatives, to protect the gastrointestinal (GI) tract against mucosal damage, in systems composed by biodegradable natural (polysaccharides including chitosan, amylose, pectin, starch, guar gum, egg albumin, dextran, and alginate) and synthetic (Eudragits, ethyl cellulose) polymers or

their combinations. In addition to the classic but less effective method of tablet coating with pH-sensitive polymers, different kinds of carriers were used: for example, lipid nanocapsules,[11] magnetic nanoparticles,[12] nanocolloids,[13] granules,[14] beads,[15] and especially microparticles. About these latter, recent interesting techniques, used to indomethacin delivery purposes, are reported in literature. Electrospraying process was successfully used to prepare indomethacin-loaded microparticles of inuline acetate, a natural polysaccharide.[16] Indomethacin sustained release microcapsules were prepared by solvent evaporation from an oil (O)/water (W) emulsion using ethyl cellulose and hydroxy propyl methyl cellulose phthalate (release retardants).[9] The pH-sensitive Eudragit L-100 microspheres containing indomethacin and encapsulating magnetic nanoparticles were prepared by a solvent evaporation method for biomedical, tissue engineering, and magnetic resonance applications.[17] Furthermore pH-sensitive Eudragits, that is, Eudragit L or Eudragit S, were used[18] to encapsulate indomethacin by applying an emulsion–solvent evaporation method.[18] The need to combine different polymers to encapsulate indomethacin was evident in Marreto *et al.*,[19] that used pectin cross-linked with casein, to reduce its aqueous solubility in order to achieve a better encapsulation efficiency, as polymer in microparticles prepared by coacervation and then dried by spray or spouted bed methods.[19] Furthermore, similar principles were proposed by Luo *et al.*[20] and successfully used in formulation of casein/pectin nanocomplexes as potential oral delivery vehicles. Nandy *et al.*[7] used a combination of the release retardant ethyl cellulose and Eudragit RS 100 mixed with Eudragit S100 to produce microparticles by a quasi-emulsion solvent diffusion technique. Similarly Chandran *et al.*[21] carried out the solvent evaporation from an O/O emulsion to make indomethacin encapsulating microsystems composed of a polymeric system based on a mixture of a pH-sensitive Eudragit (L100 or S100) and the release delaying polymer ethyl cellulose.

The combination between more polymers showed to be useful for encapsulation of NSAIDs in comparison to polymers modification processes (by copolymerization or derivatization), which could be drawback of introducing new chemicals with an unknown toxicological profile.[22] In particular, mixing cationic and anionic polymers in order to form interpolyelectrolyte complexes (IPEC) including those involving countercharged Eudragit types copolymers, were examined in several reviews[23,24] and seems to be an interesting strategy of encapsulation purposes in order to control to control NSAIDs release. The literature was focused essentially on tablet formulations based on IPEC between sodium alginate (anionic) and the cationic Eudragit EPO[22,25,26] or Eudragit RL[27]; and between chitosan (cationic) with different types of anionic Eudragits.[28,29] Production of large sized beads of chitosan/succinic acid/Eudragit RS/RL was also carried out.[30] However, the use of different carriers, such as microparticles, based on IPEC between natural polymers and Eudragits, was not too much investigated. Few articles were devoted to the possibility of involving IPEC carriers of similar structure as a nano- (basic drugs-IPEC Eudragit E100/Eudragit L100)[31] and micro- (naproxen alginate/oligochitosan/Eudragit L 100-55 "sandwich" IPEC)[32] particulate systems for pH-triggered delivery.

The use of ultrasonic atomization to produce fine polymeric droplets, to be used for encapsulating purposes, has been proposed due to the need for reducing energy consumption required in modern manufacturing approaches. This tendency is an emergent issue also in pharmaceutical field where the success of many dosage-system preparations, nowadays, depends both on the ability to introduce innovation in terms of drug functionality, and to perform advancements in manufacturing processes.[33]

Aim of this study was to develop a protocol to produce enteric shell–core micropariclles encapsulating indomethacin, by a solvent-free method based on ultrasonic atomization and two-stages polyelectrolyte complexation. In particular, a water solution of the anionic biopolymer alginate, containing the drug, was sprayed in fine droplets which were complexed with a cationic (meth)acrylate copolymer, Eudragit® E 100, and then with the anionic copolymer Eudragit® L30D-55. The first complexation stage was applied to achieve a high drug encapsulation efficiency, and the second one to assure gastroresistance feature.

EXPERIMENTAL

Materials

Manugel GHB sodium alginate, AL, (medium molecular weight, FMC Bio-polymers, Milan, Italy) was used as encapsulating polymer for both core and shell solutions: it has a high gulur-onic content (63%), giving more rigid gels,[34] moreover it dissolves at pH >7 (thus it can be used as suitable excipient for enteric formulations). Indomethacin, IND, (Sigma Aldrich, Milan, Italy) with a molecular mass of 358 g/mol and a Stokes radius of 0.42 nm, was used as model of poorly water soluble NSAIDs (solubility in water at 25°, 0.002–0.007 mg/mL). Pluronic F127, PF127 (Sigma Aldrich, Milan Italy) was used as alginate mesh size reducer to avoid an easier drug leakage during preparation. Ethanol was selected as solubilizing agent for IND for its low toxic potential (belonging to class 3 of residual solvents in USP). A solution of the cationic Eudragit® E 100, E100 (material kindly donated by Rofarma Italia, Milan, Italy) was used as a new complexing agent for alginate[22,26] and to interact with the anionic drug to raise encapsulation efficiency. A solution of the anionic Eudragit® L30D-55, L30D, copolymer (material kindly donated by Rofarma Italia, Milan, Italy), interacting with the cationic E100 and forming an external gastroresistant layer on the fine droplets, was used.

The other chemicals (Sigma Aldrich srl, Milan, Italy) were: calcium chloride ($CaCl_2$) in water solution at a concentration of 8.9 g/L as cross-linker of alginate (used in conventional procedure) to perform a comparison with the new complexing E100. The solutions at different pH values, simulating the gastrointestinal or physiological conditions, were prepared using hydrochloric acid, sodium phosphate tribasic dodecahydrate, potassium dihydrogen phosphate, and sodium hydroxide.

Methods

Microparticles Production. Shell–core fine droplets were produced by the home-made apparatus as described in a previous work[35] and modified for the purpose of this study (sketched in Figure 1 and briefly described in the follow). The idea was to

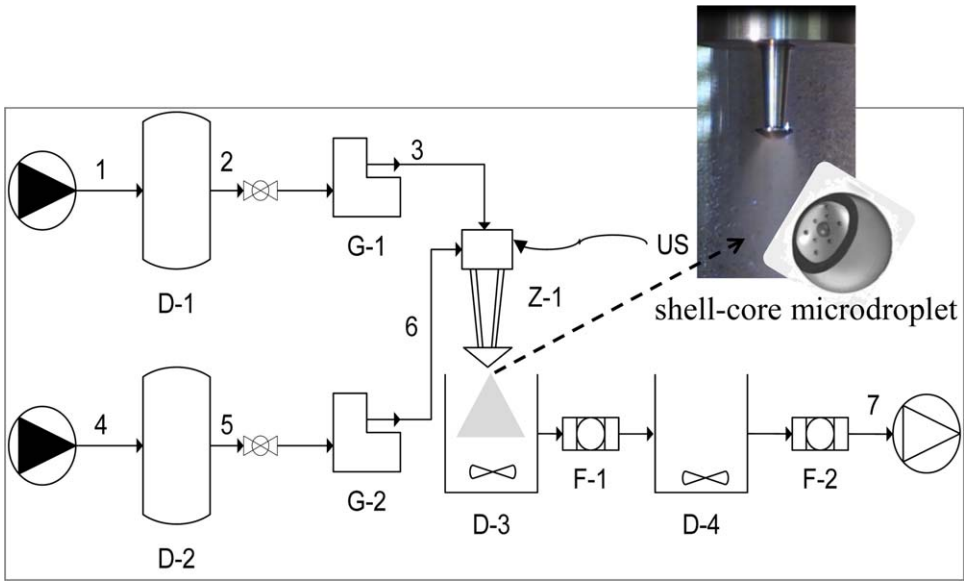

Figure 1. Layout of the experimental set-up: D-1 and D-2 core and shell solutions tanks, respectively; G-1 and G-2 peristaltic pumps; Z-1 double channel atomizer; D-3) cross-linking solution vessel for the first step; D-4) cross-linking solution vessel for the second step; F-1 and F-2 separation by centrifugation; 1–2–3 core feed line; 4–5–6 shell feed line; 7 final product line (product toward freeze drying step). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

load the anionic IND in the anionic natural biopolymer, AL, which, after atomization assisted by ultrasound, must be complexed with the cationic E100, also interacting with the anionic drug, that in turn has to be complexed with the anionic L30D to improve the gastroresistance of the final microparticles (sketched in Figure 2).

Briefly, both the solutions, core and shell feed, were sent to the coaxial ultrasonic atomizer (20 kHz ultrasonic frequency), where they were nebulized (2 min) and placed in contact with the cross-linking solution (5 min), and mixed in a beaker (at constant magnetic stirring, speed 400 rev/min). AL was chosen as encapsulating polymer for both core and shell solutions in similar concentrations (Table I) because, in addition to its biocompatibility, it does not need solvents different from water for solubilization, giving aqueous solutions with a viscosity able to give fine droplets after atomization. IND was firstly dissolved in ethanol (12.7% v/v) and then emulsified with alginate core solution at a concentration of 0.17% (w/v). PF127 was introduced in the core solution in the same concentration of alginate (1.3% w/v) as both emulsifier and alginate mesh size reducer. The nebulized shell and core solutions, each at a flow rate such that having core wrapped in the shell, were complexed with a solution in 1*M* hydrochloric acid of cationic E100 with a concentration of 0.5% w/v (other concentrations were tested, but this was the best to obtain a good encapsulation efficiency), obtaining microparticles defined as *BATCH B* (main process parameters are summarized in Table I).

Furthermore, *BATCH B* microparticles, having the external shell based on cationic copolymer E100 that dissolving at pH < 5 (so they were not suitable as enteric carriers), after a washing step, were put for 3 min in the anionic L30D copolymer solution (0.1% w/v), allowing a second complexation stage and achieving, thus, an external gastroresistant layer on the fine droplets (copolymer L30D dissolving at pH > 5.5). The Eudragit L30D solution was prepared by first dissolving it in 1*N* sodium hydroxide, then by diluting with water up to a pH of 5. The second complexing step brought to the *BATCH C* (main process parameters in Table I).

To compare the effectiveness of this new approach, the most applied cross-linking procedure, based on traditional cross-linking with a $CaCl_2$ aqueous solution (0.89% w/v) was also performed, giving the microparticles of the *BATCH A* (main process parameters in Table I).

Summarizing, the different batches were obtained in the following ways:

- *BATCH A* by cross-linking AL with $CaCl_2$;
- *BATCH B* by ultrasonic atomization—polyelectrolyte complexation of AL and E100;

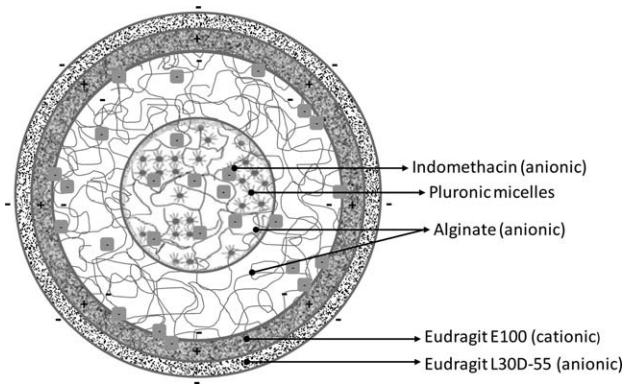

Figure 2. Sketch of the structure of the microparticles produced by ultrasonic-atomization—two stage polyelectrolyte complexation (AL-E100-L30D).

Table I. Feed and Cross-Linking Solutions Compositions and Operative Parameters Selected for the Production of Enteric Shell–Core Microparticles

Parameters	Core feeding	Shell feeding
Alginate, % w/v	1.3	1.5
Indomethacin, % w/v	0.17	-
Pluronic F127, % w/v	1.3	-
Ethanol, % v/v	12.7	-
Flow rate, mL/min	1.1	4.35
Atomization time, min	2	
First cross-linker Eudragit E100, % w/v for ***BATCH B*** (or $CaCl_2$% w/v for ***BATCH A***)	0.5 (0.89)	
Cross-linking time-first stage, min	5	
Second complexing agent Eudragit L30D-55, % w/v (***BATCH C***)	0.1	
Cross-linking time-second stage, min	3	
Magnetic stirring during atomization/complexing steps, rev/min	400	
Separation by centrifugation, min	5	
Drying treatment	Freeze-drying	

- *BATCH C* by ultrasonic atomization—two stages polyelectrolyte complexation of AL, E100, and L30D.

All kinds of produced microparticles at the end were separated from the liquid bulk by centrifugation (R 8C-XS, Bench Top Centrifuge, Remi) and then freeze-dried (LIO 5P 4k).

It is noteworthy that the tested protocols allowed to work at room conditions and pressure with aqueous solutions.

Size and Morphology. Microparticles size analysis was performed by image analysis carried out on the pictures taken by optical microscope (Leica DM LP, equipped by the DFC 280 digital camera) using the public domain software ImageJ 1.40g (Wayne Rasband, National Institutes of Health, the United States). About one hundred of microparticles were used to achieve reliable size measurements.

Fresh microsystems were investigated by the same apparatus and technique.

Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared Spectroscopy (FTIR). DSC (Mettler Toledo DSC-822, Mettler, Switzerland) characterizations were performed on: (1) pure sodium alginate; (2) crushed beads of Eudragit E; (3) a film of Eudragit L 30 D-55 obtained by drying at room conditions a volume of the L30D suspension on a Petri disc; (4) Indomethacin; (5) particles obtained by spraying the core and shell feed in a solution of Eudragit E (*BATCH B*); and (6) particles obtained by first spraying the core and shell feed in a solution of Eudragit E, and then covering them with a solution of Eudragit L 30 D-55 (*BATCH C*). DSC tests were performed, under a constant nitrogen flow rate of 50 mL/min, from 25°C to 330°C at a heating rate of 20°C/min.[32]

FTIR-spectroscopy equipped with iD5 ATR smart accessory (Nicolet iS5 ATR-FTIR-spectrometer, Thermo Scientific, the United States) were performed on: (1) pure sodium alginate; (2) crushed beads of Eudragit E; (3) a film of Eudragit L 30 D-55 obtained by drying at room conditions a volume of the L30D suspension on a Petri disc; (4) Indomethacin; (5) Pluronic F127; (6) particles obtained by first spraying the core and shell feed in a solution of Eudragit E, and then covering them with a solution of Eudragit L 30 D-55 (*BATCH C*).

Drug Entrapment and Process Efficiency, Loading Capacity, and *In Vitro* Release. The process efficiency, that is, how much drug initially introduced in core solution is found after atomization, was firstly calculated only for the novel process based on coupling between ultrasonic atomization and double polyelectrolyte complexation: it is an index of the IND lost due to the atomization process. The process efficiency can be calculated as described in eq. (1):

$$\text{Process efficiency, } \% = \frac{\text{Theoretical IND}}{\text{IND in core solution}} \times 100 \qquad (1)$$

Core and shell solutions were atomized directly in a phosphate buffer solution at pH 7.4 in the same conditions of microparticles production (atomization time: 2 min) in order to evaluate the *theoretical IND* [numerator in eq. (1)] as the amount of drug detected in the buffer solution. The sample withdrawn from the solution was diluted 1:2 with ethanol to extract IND (which is poorly soluble in water) and finally it was subjected to absorbance analysis by UV–vis spectrometer (as described below). The *IND in core solution*, that is, the denominator of eq. (1), was the amount of drug put in the volume of core formulation atomized in 2 min.

The encapsulation efficiency and the loading of IND loaded microparticles was also determined, as in eqs. (2) and (3), respectively:

$$\text{Encapsulation efficiency, } \% = \frac{\text{actual IND}}{\text{theoretical IND}} \times 100 \qquad (2)$$

Table II. Main Properties of the Different Microparticles Produced Batches: Size of Both Fresh and Dried Microparticles (MP), Encapsulation Efficiency (EE), Loading and IND Released at pH 1 Both after 15 min and after 2 h (Gastroresistance Index)

		Size, μm (± SD)				Gastroresistance	
BATCHs	Method	Fresh MP	Dried MP	Encapsulation efficiency (EE), % (±SD)	Loading, %	IND released at 15 min, %	IND released at 2 h, %
BATCH A	Cross-linking AL-$CaCl_2$	72 (±25)	6 (±5)	1.3 (±1)	0.04	-	-
BATCH B	One stage complexation AL-E100	77 (±20)	61 (±13)	75 (±1)	4.00	20	65
BATCH C	Two stages complexation AL-E100-L30D	140 (±19)	140 (±7)	74 (±1)	2.75	10	10

$$\text{Loading, \%} = \frac{\text{actual IND}}{\text{microparticles dry mass}} \times 100 \quad (3)$$

For detecting the *actual IND amount* [numerator in both eqs. (2) and (3)], a fixed amount of dried microparticles [*microparticles dry mass* in eq. (3)] was dissolved in a phosphate buffer solution at pH 7.4; then a sample was withdrawn, diluted 1:2 with ethanol and analyzed by UV–vis spectrometer. The *theoretical IND* is the same of the eq. (1) for the new protocol.

In vitro drug release study was performed as follows: about 200 mg of dried particles were put in 75 mL of 0.1*N* hydrochloric acid (pH 1), that simulates the pH of the stomach, and after 2 h a solution 0.2*M* of tribasic sodium phosphate was added to reach a simulated intestine pH of 6.8, according to United States Pharmacopeia (USP) suggestions. Temperature was kept at 37°C under controlled stirring conditions. About 0.5 mL of dissolution bulk were withdrawn at different time intervals, mixed with 0.5 mL of ethanol (dilution 1:2), as previously described to extract IND, and analyzed by UV–vis spectrometer to obtain the cumulative percent IND release plotted, as function of time under simulated gastrointestinal conditions.

Indomethacin presence in all performed tests was detected by UV–vis spectrometer (Lambda 25 by Perkin-Elmer) recording the full absorption spectra in a wavelength range from 200 to 400 nm and identifying the peak height closest to 330 nm to avoid incorrect measurements due to a shift in λ_{max}: a spectra fitting procedure was adopted instead of the simple reading of the absorbance at a given wavelength, being much more effective to eliminate any possible interferences due to polymers or other substances.[35,36]

All the experimental determinations were performed in triplicate; the results were expressed as average values with standard deviation (SD).

RESULTS AND DISCUSSION

Aim of this work was to produce gastroresistant shell–core microsystems suitable for oral administration of gastrolesive drugs. To this purpose, the indomethacin (selected as model molecule of NSAIDs class) was encapsulated, by assisted ultrasonic atomization, in the anionic natural biopolymer AL, which was reticulated by the cationic E100 complexing agent (also electrically interacting with the anionic drug), that in turn was complexed with the anionic copolymer L30D (*BATCH C* microparticles). To put in evidence the features of obtained microsystems, a comparison among different microparticles BATCHs was done in terms of size, encapsulation efficiency, loading, and gastroresistance, as shown in Table II.

Size and Morphology

Firstly, it is observed that the complexation of alginate with Eudragit E100 (*BATCH B*) did not cause a variation in size of fresh microparticles with respect to the more standard cross-linking with $CaCl_2$ (*BATCH A*): the size was kept at about 70–80 μm. After drying, *BATCH B* microparticles did not change too much their size (medium size of 61 μm) for the presence of the external E100, unlike *BATCH A* microsystems where the shrinkage is too high (final size of about 10 μm). The second complexation, thus the formation of another layer around microparticles, inevitably brought to a larger size (about 140 μm, *BATCH C*) that was kept unchanged after freeze drying.

Optical microscopy observations have emphasized that *BATCH A* microparticles shape was as already observed in literature

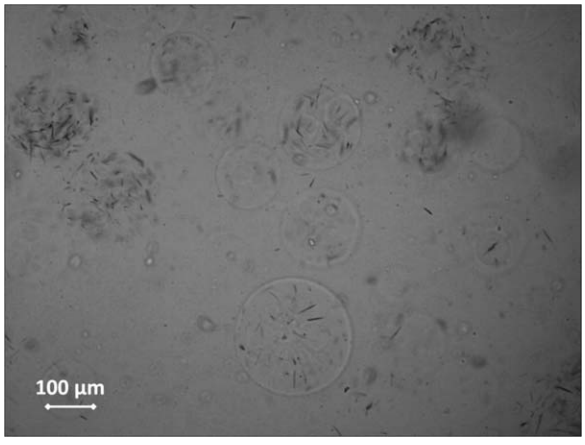

Figure 3. Optical microscope image of microparticles produced by ultrasonic-atomization—two stage polyelectrolyte complexation (AL-E100-L30D).

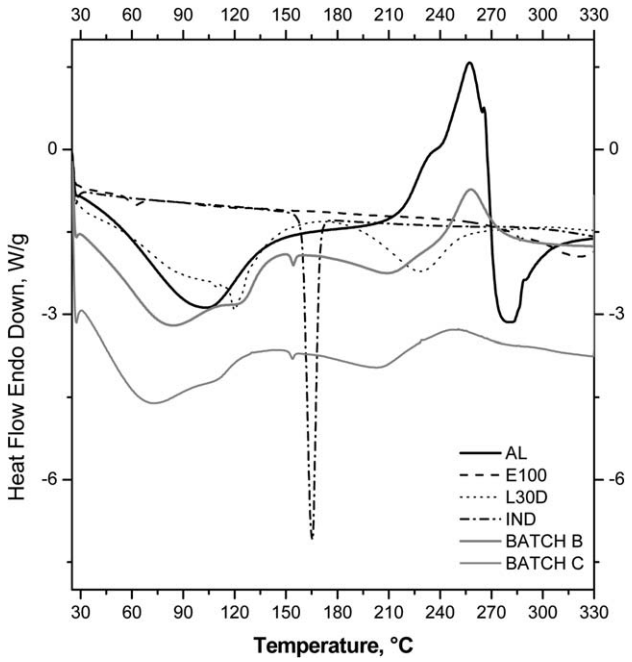

Figure 4. DSC scans of pure sodium alginate (AL), crushed beads of E100, a film of L30D (from dehydration of its suspension), pure indomethacin (IND), microparticles *of BATCH B* and *BATCH C*.

slightly pendent,[36] instead the complexation in both *BATCH B* and *BATCH C* gave a spherical shape to the microparticles (see Figure 3).

DSC, FTIR: Structural Investigations

DSC scans of pure sodium alginate showed first a typical endothermic peak to be attributed to the evaporation of water, then an exothermic behavior starting at around 200°C, with its maximum at about 250°C, highlighting the polymer decomposition.[37] Eudragit E-100 does not present any thermal transition[38] except for glass transition of 45°C. Eudragit L 30 D showed first a typical endothermic peak to be attributed to the evaporation of water; a second endothermic peak according to the literature appeared at 215°C, which was likewise attributable to the anhydride formation.[39] Glass transition at 96°C, in agreement with manufacture's specification, was observed. Crystalline indomethacin has a sharp endothermic melting peak at 165°C in agreement with literature,[40] however it has different polymorphic forms (five crystalline forms) that show melting peak in a different range of temperature. In particular, the α form, that seems to be the most stable, has a lower melting temperature,[40] which can be seen in both DSC scans of particles prepared with and without Eudragit L 30 D-55, data confirmed by optical microscope photos showing needles of indomethacin dispersed in the particles (Figures 3 and 4). Moreover, the melting endotherm preservation with minor changes in terms of sharpening, broadening, or shifting toward a lower temperature may be attributed to mixing process, which lowers the purity of each component in the mixture, thus resulting in slightly lower melting points, but not truly representative of incompatibility.[41]

DSC of both particles with and without Eudragit L 30 D-55 (*BATCH C* and *BATCH B*, respectively) showed, in addition to the typical peak of indomethacin, a behavior similar to alginate, which is the polymer present in larger amount, and, as expected, particles containing L30D (with a low mass percentage of alginate with respect to particles done only with E 100) showed less sharp peaks.

It is important to note that DSC scans performed on microparticles show that ultrasonic atomization applied to achieve the shell–core fine droplets does not affect, by mechanical and thermal stresses, the polymeric structures. This consideration is also sustained by FTIR studies, as described in the following.

According to FTIR spectra the physical state of the used IND, shows absorption peaks at 1714 and 1690 cm^{-1} (Figure 5).[42] Agreeing to the results, in the spectrum of microparticles with Eudragit L 30 D-55 (*BATCH C)* the band at 1692 cm^{-1} still exists that confirm that IND not transformed into other polymorph forms, which is also evident from DSC results. Moreover, Liu *et al.*[42] reported that ionic interactions between ionized carboxylic groups comes from IND and countercharges dimethylamino groups of Eudragit E in IND/Eudragit EPO solid dispersions, could be lead also a broad absorption band at 2479 cm^{-1} corresponds to ionized dimethylamino groups, but in our case we did not observed it, in spite of close drug loadings. This confirms that loaded IND did not interact with Eudragit E-100 in prepared microcapsules. These findings are in agreement with DSC results (the melting peak of the drug has closer to pure IND value). Moreover, in this study, we have more complex system and dimethylamino groups of Eudragit E-100 could has possibilities to interact with both oppositely charged polyanions (sodium alginate, Eudragit L 30 D-55), but according to the results without involving of IND in spite that drug molecules has the same charge.

It is interesting to note that the peak normally present at 1635 cm^{-1} in the pure sodium alginate spectrum, attributed to the asymmetric C═O stretching of the carboxylate groups, is shifted to a lower value (1606 cm^{-1}) in the spectrum of microparticles with Eudragit L 30 D-55 (*BATCH C)*, might be assigned to the absorption band of the carboxylate groups of

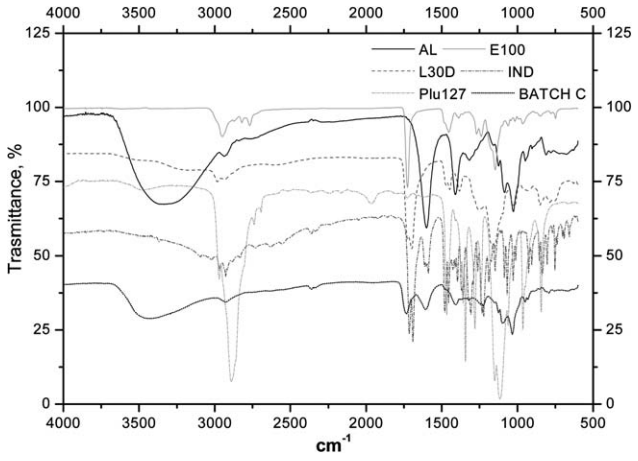

Figure 5. ATR-FTIR spectra of pure sodium alginate (AL), crushed beads of E100 (E100), a film of L30D (from dehydration of its suspension), pure indomethacin (IND) and microparticles *of BATCH C*.

sodium alginate that forms the ionic bonds with protonated dimethylamino groups of Eudragit E-100. It should be noted that according to previously published results[22,25] bounded carboxylate groups of sodium alginate involved in interpolymer ionic bonds appear at 1609 cm^{-1}. Additionally, peak of bounded carboxylate groups of Eudragit L100-55 with the same polycation could characterized by band at 1560–1580 cm^{-1}. Therefore, observed characteristic peak shifting to a lower value (1606 cm^{-1}) in case of evaluated microcapsules possibly could be due to follower mechanism of Eudragit E-100 macromolecules interactions: firstly, with sodium alginate, localized in a core, within inner-layer shell formation and secondly, during covering with Eudragit L 30 D-55 while outer-layer shell preparing, which depends on a sufficiently high charge density of the interacting copolymers.[43]

Moreover, spectrum of microparticles with Eudragit L 30 D-55 (*BATCH C*) show a new wide absorption band at 2720 cm^{-1} which confirms the appearance of bounded ionized dimethylamino groups of Eudragit E-100. These phenomena were in agreement with those observed in previous studies with Eudragit® E/alginate sodium systems[22,25] and with reports from the literature.[26,27,44–46] Furthermore, bands corresponding to the non-ionized dimethylamino groups (2770 and 2820 cm^{-1}) in Eudragit E-100 structure disappeared in evaluated microparticles with Eudragit L 30 D-55 (*BATCH C*). The reason is complete polycation ionization and further ionic interaction with countercharged polyanions (alginate sodium, Eudragit L 30 D-55). Presence of all these bands on the FTIR spectra of the IND loaded microparticles with Eudragit L 30 D-55 (*BATCH C*) confirmed that the drug has been successfully encapsulated in its original, unaltered state, which is in complete agreement with findings of the thermal analysis.

Drug Entrapment and Process Efficiency, Loading Capacity, and *In Vitro* Release

The conventional cross-linking with $CaCl_2$ (*BATCH A microparticles*) was not able to retain IND in the polymeric network (encapsulation efficiency of 1.3%, loading: 0.04%), despite the presence of Pluronic F127 acting as mesh size reducer, maybe for the repulsion between the two anionic species, AL and IND.[47] As reported in previous works[48,49] the encapsulation efficiency was subjected to several factors, mainly drug molecular weight and solubility and polymeric network mesh-size (PNMS), which play a crucial role on drug losses due to diffusive transport phenomena occurring during reticulation stage of microparticles production. Adding a polymeric mesh-size reducer ingredient (such as the Pluronic) has demonstrated that better encapsulation efficiency can be allowed[50] but this strategy, in general, must be evaluated on the bases of drug an polymer features.

The *BATCH B* microparticles showed a good encapsulation efficiency of about 75% (with a loading of about 4%), using the E100 concentration of 0.5% w/v (Table I). Different E100 concentrations were also tested: 0.1% and 0.25% brought to an encapsulation efficiency of 43% and 60%, respectively. The better encapsulation obtained with the chosen E100 concentration (a concentration higher than 0.5% w/v gave a too much viscous, not practical to use, solution) can be explained on the bases of the availability of binding sites: when the amount of macromolecule increases, there is an increase in the charged binding sites accessible to drug, therefore, larger amount of the drug results bound to the macromolecule.[47]

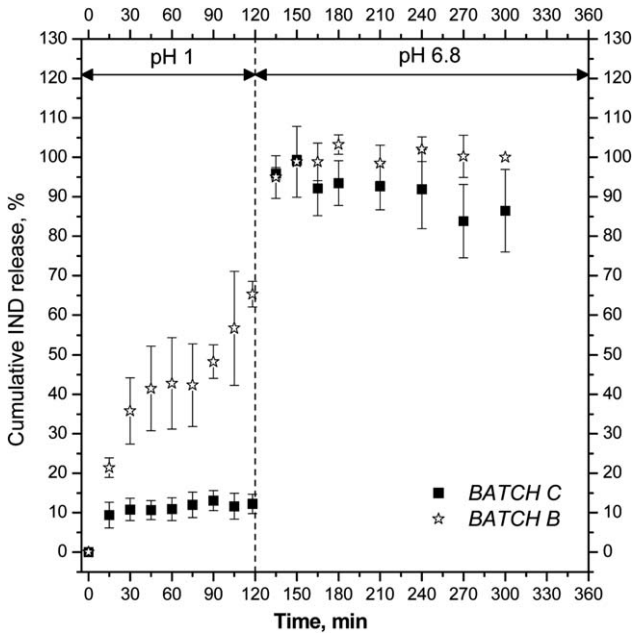

Figure 6. Percentage of indomethacin (IND) released from: shell–core AL-E100 complexed microparticles, *BATCH B* (*stars*); shell–core AL-E100-L30D complexed microparticles, *BATCH C* (*full squares*).

The encapsulation efficiency kept unchanged after the two stages complexation in *BATCH C* microparticles (EE about 74%, loading: 2.75%) reasonably because IND was already "blocked" in the polymeric network during the first complexation. It is worth to note that the efficiency of the novel process, as defined in eq. (1) in section "Methods," was about 100%: all the theoretical IND was detected (by spectrometry) in the particles.

The gastroresistance of the prepared microparticles was tested by performing *in vitro* release tests, results of which are shown in Figure 6. *BATCH B* microparticles cannot be defined as enteric systems because the external E100, complexed with AL, does not prevent the indomethacin release in gastric simulated environment (E100 has a dissolution pH < 5). In particular, an IND release percentage of about 65% was observed after 2 hours at simulated stomach pH.

BATCH C shell–core microparticles were characterized by only 10% of drug released during the acidic step, and a complete release at pH 6.8 (after a couple of samplings at pH 6.8, where about 100% of IND is assayed, a decreasing in IND is detected probably due to an incomplete indomethacin extraction by ethanol caused by the presence of too much polymer in the dissolution bulk). The features of these obtained microparticles suggest an interesting use in the production of smart enteric tablets based on shell–core microparticles tableting. By their use a possible surface crack or exposure to unexpected high values

of pH in the stomach[51] will damage only the microparticles located on the tablet's surface, keeping intact the internal ones, at the contrary of common tablets, where a surface damage can cause the leak of the drug in a site different from the target.[52]

BATCH A microparticles were not tested due to the very low, thus not useful, indomethacin load achieved.

CONCLUSIONS

A new method to produce enteric shell–core microparticles encapsulating gastrolesive drug, such as indomethacin used as model molecule in this study, was successfully developed. In particular, the main feature of this work was the development of an operative protocol, based on the coupling between the ultrasonic atomization and complexation of polyelectrolytes, that allowed to obtain enteric microparticles at mild process conditions (room temperature and pressure), without solvents or chemical cross-linkers. This procedure can address toward the production of smart enteric tablets based on microparticles tableting. The produced microparticles are in fact able to strongly protect the gastric environment in case of sudden damage of tablet's surface. The protection was due to the active ingredient preservation inside the microsystems composing the tablet and it is much more effective than the conventional coated tablets.

ACKNOWLEDGMENTS

This work is developed in the frame of the activities the Cooperation Agreement stipulated between the Kazan State Medical University and the University of Salerno, 2014-2019. It is, in part, financially supported by the Russian Science Foundation via grant 14-15-01059 (to A.Y.S. and R.I.M.) and by the Ministero dell'Istruzione dell'Università e della Ricerca (PRIN 2010/2011, contract grant number 20109PLMH2).

REFERENCES

1. Bernardi, A.; Zilberstein, A.; Jäger, E.; Campos, M.; Morrone, F.; Calixto, J.; Pohlmann, A.; Guterres, S.; Battastini, A. *Br. J. Pharmacol.* **2009**, *158*, 1104.
2. Kirsebom, H.; Galaev, I. Y.; Mattiasson, B. *J. Polym. Sci. Part B: Polym. Phys.* **2011**, *49*, 173.
3. Cohen, S.; Yoshioka, T.; Lucarelli, M.; Hwang, L. H.; Langer, R. *Pharm. Res.* **1991**, *8*, 713.
4. Chen, H.; Langer, R. *Adv. Drug Deliv. Rev.* **1998**, *34*, 339.
5. Dalmoro, A.; Lamberti, G.; Titomanlio, G.; Barba, A.; d'Amore, M. *AAPS PharmSciTech* **2010**, *11*, 1500.
6. Ulery, B. D.; Nair, L. S.; Laurencin, C. T. *J. Polym. Sci. Part B: Polym. Phys.* **2011**, *49*, 832.
7. Chandra Nandy, B.; Mazumder, B. *Curr. Drug Deliv.* **2014**, *11*, 72.
8. Dupeyrón, D.; Kawakami, M.; Ferreira, A. M.; Cáceres-Vélez, P. R.; Rieumont, J.; Azevedo, R. B.; Carvalho, J. C. T. *Int. J. Nanomed.* **2013**, *8*, 3467.
9. Kamal, M. A. H. M.; Ahmed, M.; Wahed, M. I. I.; Amran, M. S.; Shaheen, S. M.; Rashid, M.; Anwar-Ul-Islam, M. *Dhaka Univ. J. Pharm. Sci.* **2008**, *7*, 83.
10. Chinna, G.; Shyam, S.; Varma, V.; Sleeva, R.; Sai, K. *Int. J. Drug Discov.* **2010**, *2*, 8.
11. Bernardi, A.; Frozza, R. L.; Meneghetti, A.; Hoppe, J. B.; Battastini, A. M. O.; Pohlmann, A. R.; Guterres, S. S.; Salbego, C. G. *Int. J. Nanomed.* **2012**, *7*, 4927.
12. Anirudhan, T.; Dilu, D.; Sandeep, S. *J. Magn. Magn. Mater.* **2013**, *343*, 149.
13. Milkova, V.; Kamburova, K.; Radeva, T. *Colloids Surf. B: Biointerfaces* **2013**, *108*, 279.
14. Nagane, K.; Kimura, S.; Ukai, K.; Ogawa, N.; Yamamoto, H. *Int. J. Pharm.* **2014**, *475*, 364.
15. Jung, J.; Arnold, R. D.; Wicker, L. *Colloids Surf. B: Biointerfaces* **2013**, *104*, 116.
16. Jain, A. K.; Sood, V.; Bora, M.; Vasita, R.; Katti, D. S. *Carbohydr. Polym.* **2014**, *112*, 225.
17. Varma, M.; Amareshwar, P.; Devara, R. K. *Int. J. Drug Deliv.* **2011**, *3*, 101.
18. Alhnan, M. A.; Basit, A. W. *J. Pharm. Sci.* **2011**, *100*, 3284.
19. Marreto, R. N.; Ramos, M. F.; Silva, E. J.; de Freitas, O.; de Freitas, L. A. *AAPS PharmSciTech* **2013**, *14*, 1227.
20. Luo, Y.; Pan, K.; Zhong, Q. *Int. J. Pharm.* **2015**, *486*, 59.
21. Chandran, S.; Sanjay, K. S.; Ali Asghar, L. F. *J. Microencapsul.* **2009**, *26*, 420.
22. Moustafine, R. I.; Salachova, A. R.; Frolova, E. S.; Kemenova, V. A.; Van den Mooter, G. *Drug Dev. Ind. Pharm.* **2009**, *35*, 1439.
23. Moustafine, R. *Russ. J. Gen. Chem.* **2014**, *84*, 364.
24. De Robertis, S.; Bonferoni, M. C.; Elviri, L.; Sandri, G.; Caramella, C.; Bettini, R. *Exp. Opin. Drug Deliv.* **2015**, *12*, 441.
25. Moustafine, R.; Kemenova, V.; Van den Mooter, G. *Int. J. Pharm.* **2005**, *294*, 113.
26. Sonavane, G. S.; Devarajan, P. V. *J. Biomed. Nanotechnol.* **2007**, *3*, 160.
27. Khuathan, N.; Pongjanyakul, T. *Int. J. Pharm.* **2014**, *460*, 63.
28. Ofokansi, K. C.; Kenechukwu, F. C. ISRN pharmaceutics **2013**.
29. Moustafine, R. I.; Margulis, E. B.; Sibgatullina, L. F.; Kemenova, V. A.; Van den Mooter, G. *Eur. J. Pharm. Biopharm.* **2008**, *70*, 215.
30. Kaur, K.; Kim, K. *Int. J. Pharm.* **2009**, *366*, 140.
31. Palena, M.; Manzo, R.; Jimenez-Kairuz, A. *J. Nanopart. Res.* **2012**, *14*, 1.
32. Čalija, B.; Cekić, N.; Savić, S.; Daniels, R.; Marković, B.; Milić, J. *Colloids Surf. B: Biointerfaces* **2013**, *110*, 395.
33. Dalmoro, A.; Barba, A. A.; Lamberti, G.; d'Amore, M. *Eur. J. Pharm. Biopharm.* **2012**, *80*, 471.
34. Barba, A. A.; Lamberti, G.; Rabbia, L.; Grassi, M.; Larobina, D.; Grassi, G. *Mater. Sci. Eng.: C* **2014**, *37*, 327.
35. Dalmoro, A.; Barba, A. A.; d'Amore, M.; Lamberti, G. *Ind. Eng. Chem. Res.* **2014**, *53*, 2771.

36. Barba, A. A.; d'Amore, M.; Cascone, S.; Lamberti, G.; Titomanlio, G. *Chem. Eng. Process.* **2009**, *48*, 1475.
37. Simonoska Crcarevska, M.; Glavas Dodov, M.; Goracinova, K. *Eur. J. Pharm. Biopharm.* **2008**, *68*, 565.
38. Malik, K.; Arora, G.; Singh, I. *Sci. Pharm.* **2011**, *79*, 653.
39. Lin, S. Y.; Yu, H. L. *J. Polym. Sci. Part a: Polym. Chem.* **1999**, *37*, 2061.
40. Surwase, S. A.; Boetker, J. P.; Saville, D.; Boyd, B. J.; Gordon, K. C.; Peltonen, L.; Strachan, C. J. *Mol. Pharm.* **2013**, *10*, 4472.
41. Chopra, D.; Sinha, V. R.; Singh, M. *J. Chem.* **2008**, *5*, 316.
42. Liu, H.; Zhang, X.; Suwardie, H.; Wang, P.; Gogos, C. G. *J. Pharm. Sci.* **2012**, *101*, 2204.
43. Mustafin, R. *Pharm. Chem. J.* **2011**, *45*, 285.
44. Jelvehgari, M.; Zakeri-Milani, P.; Siahi-Shadbad, M. R.; Loveymi, B. D.; Nokhodchi, A.; Azari, Z.; Valizadeh, H. *AAPS PharmSciTech* **2010**, *11*, 1237.
45. Laurienzo, P.; Malinconico, M.; Mattia, G.; Russo, R.; La Rotonda, M.; Quaglia, F.; Capitani, D.; Mannina, L. *J. Biomed. Mater. Res. Part A* **2006**, *78*, 523.
46. Gao, C.; Liu, M.; Chen, S.; Jin, S.; Chen, J. *Int. J. Pharm.* **2009**, *371*, 16.
47. Khan, I. A.; Anjum, K.; Ali, M. S.; Din, K. *Colloids Surf. B: Biointerfaces* **2011**, *88*, 72.
48. Barba, A.; Dalmoro, A.; d'Amore, M.; Lamberti, G. *Chem. Biochem. Eng. Q.* **2012**, *26*, 345.
49. Cascone, S.; Lamberti, G.; Titomanlio, G.; Barba, A. A.; d'Amore, M. *Drug Dev. Ind. Pharm.* **2012**, *38*, 1486.
50. Barba, A. A.; Dalmoro, A.; d'Amore, M.; Lamberti, G. *LWT - Food Sci. Technol.* **2015**, *64*, 149.
51. Cascone, S.; De Santis, F.; Lamberti, G.; Titomanlio, G. *Eur. J. Pharm. Biopharm.* **2011**, *79*, 382.
52. Barba, A.; Dalmoro, A.; d'Amore, M.; Vascello, C.; Lamberti, G. *J. Mater. Sci.* **2014**, *49*, 5160.