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Indomethacin-containing interpolyelectrolyte complexes based on Eudragit® E PO/S 100 copolymers as a novel drug delivery system

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Abstract

Potential applications of a novel system composed of two oppositely-charged (meth)acrylate copolymers, Eudragit® EPO (EPO) and Eudragit® S100 (S100), loaded with indomethacin (IND) in oral drug delivery were evaluated. The particles based on drug-interpolyelectrolyte complexes (DIPEC), (EPO-IND)/S100, were prepared by mixing aqueous solutions of both copolymers at fixed pH. Particles of drug-polyelectrolyte complex (DPC), (EPO-IND) have a positive zeta potential, pointing to the surface location of free EPO chains and IND bound to EPO sequences. The formation and composition of both DPC and DIPEC were established by gravimetry, UV-spectrophotometry, capillary viscosity and elemental analysis. The structure and solid state properties of the formulated DIPEC were investigated using FTIR/NIR, Raman spectroscopy, XRPD and modulated DSC. DIPEC is a chemically homogenous material, characterized by a single T_g. DIPEC have an IR absorption band at 1560 cm^{-1}, which can be assigned to the stretching vibration of the carboxylate groups (S100, IND) that form ionic bonds with the dimethylamino groups of EPO. XRPD, NIR and Raman-shifts confirm that during the preparation of this formulation, IND is converted into its amorphous form. The release of IND from DPC EPO/IND (3:1) and DIPEC EPO/L100/IND (4.5:1:1) is sustained and is completed within 7 hours under GIT mimicking conditions. However, S100 within DIPEC makes the release process slower making this system suitable for colon-specific delivery. Finally, DPC and DIPEC with indomethacin were used to prepare tablets, which can be potentially used as oral dosage forms for their slower indomethacin release in case of DIPEC which could be suitable for sustained delivery.

Keywords

Drug-interpolyelectrolyte complexes; drug-polyelectrolyte complexes; Eudragit® EPO; Eudragit® S100; Indomethacin; Oral drug delivery.

1. Introduction

The advantages of interpolymer complexes as polymeric carriers in oral controlled drug release have been reported elsewhere (Kemenova et al., 1991; Hartig et al., 2007; Khutoryanskiy, 2007; Lankalapalli and Kolapalli, 2009; Pillay et al., 2013, Bourganis et al., 2017). In the last years, our research group has developed polycomplex matrices based on interpolyelectrolyte complexes (IPECs) using different oppositely-charged Eudragit® copolymer combinations as new oral delivery systems able to deliver the drugs into site-specific gastrointestinal tract (GIT) regions (Mustafin and Kabanova, 2004, 2005; Moustafine et al., 2005, 2006, 2011, 2013; Moustafine and Bobyleva, 2006; Mustafin et al., 2010a, 2010b, 2011). Moreover, the advantages of Eudragit® copolymer combinations for controlled drug delivery purposes have been reported elsewhere (Siepmann et al., 2008; Obeidat et al., 2008; Sauer and McGinity, 2009; Alhnan and Basit, 2011; Bani-Jaber, et al., 2011; Wulff and Leopold, 2014, 2016).

The comprehensive analysis of the effects of intermacromolecular interactions between chemically complementary Eudragits® on the drug release from oral drug delivery systems (DDS) was examined in recently published reviews (Gallardo et al., 2008; Mustafin, 2011, Moustafine, 2014; De Robertis et al., 2015). However, further studies are needed to address more complex systems involving oppositely-charged Eudragits® forming IPECs in the presence of ionic drugs. Only a few papers reported the possibility of using drug-interpolyelectolyte complexes (DIPEC) as three-component systems for development of drug delivery dosage forms (Palena et al., 2012, 2015; Bigucci et al., 2015).

Recently, a novel self-organized nanoparticulate carrier, based on drug – IPEC Eudragit® E100/L100 combination was successfully prepared using a simple aqueous dispersion method (Palena et al., 2012). In this study, the authors have reported that freeze-dried complexes were easily redispersed in water and DIPEC dispersions behaved as zwitterionic macromolecular systems that may change zeta potential values from negative to positive by changing the polymer composition. The authors have used atenolol, propranolol and metoclopramide as model drugs, which could be formulated using these nanoparticulate systems. Recently four additional anti-inflammatory drugs (salicylic acid, benzoic acid, ketoprofen and naproxen) were also studied (Palena et al., 2015). The DIPECs exhibited interesting properties useful for the design of nanoparticulate DDS for oral and topical administration.

Furthermore, a similar principle was successfully used in a chitosan/carboxymethylcellulose polyelectrolyte system via electrostatic interaction between the amino groups of chitosan and chlorhexidine (cationic drug) with the carboxyl groups of sodium carboxymethylcellulose, used for the preparation of vaginal inserts (Bigucci et al., 2015).

The objective of this study was the preparation and physicochemical characterization of drug-interpolyelectrolyte complexes (DIPEC) as micro-sized particles formed between indomethacin and Eudragit® S100 with oppositely-charged Eudragit® EPO. These microparticles were found to be highly promising materials for designing pH-controlled systems for oral delivery to target the colon. Colon-specific drug

delivery systems are of interest for the therapy of different local conditions such as ulcerative colitis, Crohn's disease, irritable bowel syndrome, chronic pancreatitis, and colonic cancer (Basit, 2005; Gazzaniga, 2006; Van den Mooter, 2006, Maroni et al., 2013; Amidon et al., 2015; Hua et al., 2015). Different approaches have been traditionally used in drug delivery for colon targeting, including the use of prodrugs, pH-responsive matrix systems, timed-release formulations, bioadhesive materials, microparticulate vehicles and enteric coatings (Amidon et al., 2015). Our approach involves the use of conventional enteric coating polymer Eudragit ® S100 that already provides gastric resistance properties; additionally, in our work we utilised the ability of this anionic polymer to form interpolyelectrolyte complexes with cationic Eudragit ® EPO. The functionality of both polymers provided an opportunity of forming polycomplex particles with indomethacin and formulate tablets with sustained drug release.

2. Materials and methods

2. 1 Materials

Eudragit® E PO – a terpolymer of *N,N*-dimethylaminoethyl methacrylate (DMAEMA) with methylmethacrylate (MMA) and butylmethacrylate (BuMA), (PDMAEMA-*co*-MMA-*co*-BMA) (mole ratio 2:1:1, MW 150 kDa) was used in this study as a cationic copolymer. Eudragit® S 100 (a copolymer of methacrylic acid (MAA) with methylmethacrylate (MMA), P(MAA-*co*-MMA) (mole ratio 2:1, MW 135 kDa)) was used as a polyanion. Different types of Eudragit® (EPO, S100) were generously donated by Evonik Röhm GmbH (Darmstadt, Germany). The copolymers were used after vacuum drying at 40°C for 2 days. The solutions at different pH values, simulating the gastrointestinal conditions, were prepared for release tests by using hydrochloric acid, sodium phosphate tribasic dodecahydrate, potassium dihydrogen phosphate, and sodium hydroxide (Sigma-Aldrich, Bornem, Belgium). IND was used as a model anionic drug and was purchased from Sigma-Aldrich (Bornem, Belgium).

2.2. Methods

2.2.1 Preparation of solid DPCs and DIPECs with different macromolecular composition

The optimal conditions for the interaction between chemically complementary grades of a polycation (Eudragit® EPO) and a polyanion copolymer (Eudragit® S100) in the presence of ionized IND molecules were studied in aqueous salt media. EPO solutions were prepared by dissolving the copolymer in 1 M CH_3COOH. This solution was diluted with demineralized water to the desired volume and titrated with 1 M NaOH to the required pH 6.5. S100 and IND solutions were prepared by dissolving the copolymer and the drug in 1 M NaOH. This solution was diluted with demineralized water to the desired volume and titrated with 1 M CH_3COOH to the required pH 7.2. The EPO solutions were slowly poured into S100/IND solutions, and the

mixture was stirred at 1000 r.p.m. for 2 days using a magnetic stirrer RET control visc-white (IKA®, Staufen, Germany). The solutions of copolymers and IND were mixed in different molar ratios. The yields of precipitate formed were first determined gravimetrically after centrifugation for 1 h at 5000 rpm at 5 °C in a SL16R laboratory centrifuge (Thermo Scientific, U.S.A.). The specific viscosity of the supernatant solution was determined using an Ubbelohde viscometer (Schott®, Germany) at 25.0±0.1 °C. The quantity of the non-bonded IND present in the supernatant solutions and the encapsulation efficiency (EE) were investigated UV-spectrophotometrically at 266 nm (Evolution 220, Thermo Scientific, U.S.A.). For gravimetric determination, the sediment was dried under vacuum (vacuum oven VD 23, Binder, Germany) for 2 days at 40 °C until constant weight.

The optimal composition was prepared in a laboratory reactor system LR 1000 control equipped with pH-/temperature controlling units under continuous and simultaneous agitation at 10,000 r.p.m. using T25-digital Ultra-Turrax® homogenizer (IKA®, Staufen, Germany). The feeding rate was approximately 2 mL/min. After isolation of the precipitates of DPC and DIPEC particles from solutions, they were washed with ultrapure water (Smart2Pure UV/UF, Thermo Scientific, U.S.A.), frozen at -18 °C (Labconco® Shell Freezer, MO, U.S.A.) and subsequently freeze-dried for 2 days (Labconco® Freeze Dry System, FreeZone 1 L, MO, U.S.A.). The solid samples were stored in tightly-sealed containers at room temperature.

2.2.2 Elemental analysis

The composition of freeze-dried DPC (EPO/IND) and DIPEC (EPO/L100/IND) samples and physical mixtures were investigated by elemental analysis using a Thermo Flash 2000 CHNS/O elemental analyzer (Thermo Scientific, UK). Physical mixtures were obtained by mixing copolymer powders and IND at EPO:S100:IND molar ratio of 4.5:1:1.

2.2.3 Fourier Transform Infrared Spectroscopy (ATR-FTIR)

ATR-FTIR-spectra were recorded using a Nicolet iS5 FTIR-spectrometer (Thermo Scientific, U.S.A.) equipped with a DTGS detector. The untreated freeze-dried samples of solid DPC (EPO/IND), DIPEC (EPO/S100/IND) and physical mixtures were directly mounted over the iD5 smart single bounce ZnSe ATR crystal. The spectra were analyzed using OMNIC spectra software.

2.2.4 Near-infrared (NIR) spectroscopy

NIR-spectroscopy of freeze-dried samples of solid DPC (EPO/IND), DIPEC (EPO/S100/IND) and physical mixtures was performed using a Nicolet iS10 XT NIR/FTIR-spectrometer (Thermo Scientific, U.S.A.) equipped with Smart DRA diffusion reflection accessory. The spectra were analyzed using OMNIC spectra software.

2.2.5 Particle characterization

Particle sizes and zeta potential (ZP) of DIPEC particles in aqueous dispersion were evaluated using a Zetasizer Nano ZL (Malvern Instruments Ltd., Worcestershire, UK). Solid state particles characterization of freeze-dried

DIPEC (EPO/S100/IND) samples was performed on the Morphologi G3SE-ID automated system (Malvern Instruments Ltd., Worcestershire, UK) equipped with fiber-optics Raman-spectrometry (RamanRxn1™ Analyzer, Kaiser Optical Systems, INC., Germany).

2.2.6 Thermal analysis

Modulated DSC (MDSC) measurements were carried out using a Discovery DSC™ (TA Instruments, New Castle, DE, U.S.A.), equipped with a refrigerated cooling system (RCS90). TRIOS™ software (version 3.1.5.3696) was used to analyze the obtained data (TA Instruments, New Castle, DE, U.S.A.). Tzero aluminum pans (TA Instruments, New Castle, DE, U.S.A.) were used in all calorimetric studies. The empty pan was used as a reference and the mass of the reference pan and of the sample pans were taken into account. Dry nitrogen at a flow rate of 50 mL/min was used as a purge gas through the DSC cell. Indium and *n*-octadecane standards were used to calibrate the DSC temperature scale; enthalpic response was calibrated with indium. The modulation parameters used were: 2 °C/min heating rate, 40 s period and 1 °C amplitude. Calibration of heat capacity was done using sapphire. Samples were analyzed from 0 to 250 °C. Glass transitions were analyzed in the reversing heat flow signals.

Thermogravimetric analysis (TGA) was performed using Discovery TGA™ (TA Instruments, New Castle, DE, U.S.A.). Samples (10-15 mg) were placed on an aluminum pan and heated from 25 to 190 °C at 10 °C/min. Resulting weight-temperature diagrams were analyzed using TRIOS™ software (version 3.1.5.3696) to calculate the weight loss between 25 and 170 °C.

2.2.7 X-ray powder diffraction

X-ray powder diffraction (XRPD) was performed on the freeze-dried samples of solid DIPEC (EPO/S100/IND) and physical mixtures. An automated XPERT-PRO diffractometer system (PANalytical, Almelo, the Netherlands) was used in reflection mode. All samples were measured without crushing or any other sample processing. A copper tube with the generator set at 45 kV and 40 mA was used. Using a transmission spinner, it was possible to improve the counting statistics by spinning the sample using a rotation time of 4.0 s. In the incident beam path, 0.04 rad soller slit and a programmable divergence slit of 10 mm were applied. In the diffracted beam path, 0.04 rad soller slit and programmable anti-scatter slit were installed. The detector used for data collection was an X'Celerator RTMS detector, with an active length of 2.122°. The data were collected in continuous scan mode with a scan range of 4.0040-40.001° and a step size of 0.0167°. The counting time was 499.745 s. X'Pert Data Collector version 2.2a (PANalytical, Almelo, the Netherlands) was used for data collection and X'Pert Data Viewer version 1.2.a (PANalytical, Almelo, the Netherlands) was used for data visualization and treatment.

2.2.8 Release of indomethacin from the particles under GIT mimicking conditions

The release of IND from the DDS was performed under sink conditions at 37.0±0.1 °C using the USP II Apparatus (the off-line dissolution tester DT 828 with an auto sampler ASS-8, a fraction collector FRL 824 and

a peristaltic pump ICP-8 (Erweka, Heusenstamm, Germany)). The paddles rotation speed was 100 rpm. The release was investigated for 7 hours under GIT mimicking conditions, where the pH of the release medium was gradually increased: 1 hour in 0.1 M hydrochloric acid (pH=1.2), 2 hours in phosphate buffer solution (pH=5.8), 2 hours in phosphate buffer solution (pH=6.8), and finally in phosphate buffer solution (pH=7.4) until the end of the experiment (Lorenzo-Lamoza et al., 1998).

A weighted amount of the DDS (50 mg; estimated to contain approx. 10 mg IND) was suspended in 400 mL of 0.1 M hydrochloric acid, then 400 mL of 0.02 M dibasic potassium phosphate trihydrate were added in the release media after 1 hour. Then the pH of the resulting solution was adjusted to the desired pH (5.8, 6.8, and 7.4) with sodium hydroxide. Final volume was kept at 850 mL. pH control was carried out in each vessel with a portable pH meter Orion Star A 325 **(**Thermo Scientific, U.S.A.**)** using the Orion™ ROSS Ultra™ low maintenance pH/ATC Triode™ **(**Thermo Scientific, U.S.A.**)**. At fixed time intervals, 5 mL of the solution was withdrawn, filtered through a syringe filter with a pore diameter of 0.45 microns (Supelco Iso-Disc Filters N-25-4 Nylon 25 mm) and the amount of IND released was analyzed by UV spectrophotometry (Lambda 25, Perkin Elmer, U.S.A.). IND presence in all performed tests was detected by recording the full absorption spectra in the wavelength range from 200 to 400 nm and identifying the peak height closest to 330 nm to avoid incorrect measurements due to the shift in λ_{max}: a spectrum fitting procedure was adopted instead of the simple reading of the absorbance at given wavelength, being much more effective to eliminate any possible interferences due to copolymers (Dalmoro et al., 2016) or DPC and DIPEC formation. An equal volume of the same dissolution medium was replaced to maintain a constant volume. The experiments were performed in triplicate.

2.2.9 DIPEC particles characterization under GIT mimicking conditions

Measurements of the size and zeta potential of the DIPEC particles under conditions, mimicking the release process was also performed using the Zetasizer Nano ZS equipped with multi-purpose titrator MPT-2 and degasser accessories (Malvern Instruments Ltd., Worcestershire, UK). Samples of DIPEC particles were redispersed in 0.1 M hydrochloric acid (pH 1.2). Then 0.1 M sodium hydroxide solution was gradually added to the dispersion of DPC by using an automatic titrator, until a pH of 7.4 was reached. During the titration, the zeta potential and size of the polymer-drug complex were measured between pH 1.2-7.4.

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

2.2.10 Tablet preparation and indomethacin release under GIT mimicking conditions

With the aim to study the IND release from tablets as possible oral dosage systems, the produced loaded particles were used to prepare tablets by the following procedures. Tablets with IND loaded particles (DPC and DIPEC) were prepared by compressing about 500 mg of lyophilized particles (estimated to contain approx. 100 mg IND) in a hydraulic press for FTIR (Perkin Elmer, U.S.A.), equipped with flat-faced punches

206 with 13 mm diameter (by a Pike Technologies, U.S.A.) with a compression pressure of 2.45 MPa. The same
207 procedure was applied to 500 mg of physical mixtures and IND powder (the compositions were similar to
208 DPC and DIPEC ratios, respectively). The two kinds of produced tablets were then subjected to in vitro drug
209 release studies applying the method used for IND release from uncompressed particles, previously described.
210 All the experimental determinations were performed in triplicate; the results were expressed as average
211 values ± standard deviation (SD).

212 # 3. Results and discussion

213 ## 3.1 Preparation and characterization of DPC and DIPEC particles

214 EPO is soluble in acidic solutions up to pH 7.0 (Mustafin et al., 2011), due to hydration of protonated
215 dimethylamino groups. On the other hand, S100 is soluble above pH 7.0 due to hydration of ionized carboxyl
216 groups. IND is a non-steroidal anti-inflammatory drug containing an acidic function with a pK_a = 4.5 (Priemel
217 et al., 2013a, 2013b; De Filippis et al., 1991). The possibility of interaction between these two polyelectrolytes
218 and IND was investigated between pH 6.8 and 7.2, where both copolymers and the drug are soluble and
219 partially ionized.
220 EPO-IND polycomplex formation was first investigated using gravimetric analysis of precipitates and UV-
221 spectrophotometry analysis of supernatant solutions, prepared at different molar ratios at pH 6.5. At this pH,
222 the degree of ionization and charge density of EPO is very small. In contrast, the reaction capability of the drug
223 is high. Fig. 1a shows that the maximum of the precipitate yield corresponds to the maximum of bound IND.
224 The maximum of EPO/IND polycomplex yield was found at the unit molar ratio of 3:1. The observed binding
225 molar ratio corresponds to the stoichiometry of the obtained DPC EPO/IND, estimated also by elemental
226 analysis of the dry DPC precipitates.
227 The next step was to determine the optimal composition in DIPEC (EPO/S100/IND) mixtures. Fig. 1b shows
228 the results of precipitate and supernatant analysis, which confirm that the stoichiometric composition of
229 precipitated DIPEC (EPO/S100/IND) corresponds to the molar ratio of 4.5:1:1.

230 ### *3.1.2 Compositional study*

231 Fig. 2 shows the apparent viscosity of the supernatant in EPO/S100/IND mixtures. The decrease in viscosity
232 observed in EPO/S100/IND mixtures showed that the insoluble DIPEC was formed in the investigated medium
233 and was removed by centrifugation (Cilurzo et al., 2000, Moustafine et al., 2005). A minimum in the curve is
234 observed when the mixture of EPO/S100/IND was 4.5:1:1. Thus, the DIPEC is enriched with the less ionized
235 component (charge density on EPO chains > 0). On the other hand, an incorporation of the anionic
236 components (S100 and IND) decreases due to the progressive increase in the fraction of ionized carboxylic
237 acids. This also increases the drug reactivity. In order to confirm the proportion of each component in the

solid DIPEC, elemental analysis of the dry precipitates was performed. The results are summarized in Table 1 and clearly indicate that the molar ratio between EPO, S100 and IND in the triple polycomplex is 4.5:1:1.

3.1.3 Morphological and dimensional analysis

The particle size of freshly prepared DIPEC particles was determined by photon correlation spectroscopy. DIPEC particles showed a mean diameter (MD) of 497±51 nm with a positive value of zeta potential (+17.4 mV), pointing to the surface location of free EPO chains and IND bound to EPO sequences.

Additionally, particle size distribution and morphological analysis of the DIPEC samples was estimated. Three main groups of particle size were observed *(Fig. S1a, Supporting Information)*: small (mean diameter (MD) ≤ 300 nm; 98.06%), medium (300 nm ≥ MD ≤ 10 µm; 1.90%) and large (MD ≥ 10 µm; 0.04%). *Fig. S2b (Supporting Information)* summarized the results of the morphological analysis, in the case of the "large" group, and shows nearly spherical morphology (according to the circularity measurements) of the particles and a low degree of aggregation. Similar morphology was found for the other two groups of particles (data not shown). All of the evaluated particles have circularity values close to 1 indicating nearly perfect spheres. Moreover, identification of the particles included from the "small" group (making up the majority of particles) by Raman-spectrometry showed that these particles consist of DIPEC (94%) and do not contain free IND *(Fig. S2c, Supporting Information)*.

3.1.4 Drug encapsulation

Direct encapsulation of IND was achieved by preparing particles in the presence of EPO and S100 and formation of IPEC between these oppositely-charged copolymers. The residual amount of IND at the end of the particles preparation was evaluated by UV-spectrophotometry. The data showed that encapsulation efficiency (EE) was 75.6% (Table 2). The high EE is most likely the consequence of strong interactions between IND molecules and EPO which is simultaneously bound to the countercharged S100 sequences.

3.2 Evaluation of the DIPEC structure

3.2.1 Mid-infrared spectroscopy

FTIR spectra indicate that IND is present as the γ-form showing absorption peaks at 1714 and 1690 cm^{-1} (Fig. 3a) (Liu et al., 2010, 2012; Chokshi et al., 2005, 2008; Sarode et al., 2013a, 2013b). The FTIR spectra of the physical mixture of IND and copolymers (EPO and S100) in the same as in DIPEC ratio, is virtually a superposition of the spectra of all components (Fig. 3b). However, the DPC and DIPEC show a different absorption band at 1560 cm^{-1}, which is due to the stretching vibration of the carboxylate groups that form the ionic bonds with the protonated dimethylamino groups of EPO (Fig. 3c,d). Although Liu et al. (2010) reported that ionic interactions between ionized carboxylic groups of IND and oppositely charged dimethylamino groups of EPO in IND/EPO solid dispersions result in a broad absorption band at 2479 cm^{-1} which corresponds to ionized amino groups, we did not observe this in spite of similar levels of drug loading. This can be explained since the charge density of the

EPO macromolecules decreases smoothly at the pH of DIPEC preparation. Moreover, in this study, we have a system with a significantly higher complexity since the amino groups of EPO can interact not only with IND but simultaneously with S100. The existence of non-ionized dimethylamino groups (2770 and 2820 cm^{-1}) in DIPEC indicates that in this structure, they are localized mainly in 'defects' together with ionized bound groups of EPO which is largely dependent on the conditions of the DIPEC preparation. The ratio of non-ionized and ionized dimethylamino groups depends on the charge density of EPO macromolecules that is relatively low at pH 6.8–7.2.

The peak of the carbonyl stretching vibration (belonging to the carboxyl group) of IND at 1714 cm^{-1} completely overlapped with a strong band of carbonyl stretching vibration of EPO and S100 at 1730 cm^{-1}. Therefore, we focused on the region of near-infrared spectroscopy in order to evaluate potential IND transformations (from γ-form to α-form or to the amorphous form) (Tanabe et al., 2012; Heinz et al., 2007; Nielsen et al., 2012).

3.2.2 Near-infrared spectroscopy

Due to the complexity of DPC and DIPEC systems, the main differences between the crystalline and amorphous forms were observed from 1650 nm to 1900 nm (Heinz et al., 2007). Indeed, a peak at 1860 nm resulting from the vibrations of the carboxylic group observed in the spectra of γ-form IND was absent both in physical mixtures, DIPEC and DPC (Fig. 4a). Therefore, in the ternary physical mixture and DIPEC, IND could not exist in a γ-form. Moreover, the peak at 1666 nm in IND powder confirms the presence of amorphous form too, which also appeared in DPC and DIPEC, but not in a physical mixture. In case of IND and physical mixture a peak maximum at 1696 nm confirms the existence of γ-form IND, which is absent in DPC and DIPEC. Interestingly, the appearance of a new peak at 1702 nm for DIPEC is also observed in NIR-spectra of the individual copolymers – EPO and S100, but not in their physical mixture (Fig. 4b). NIR-spectroscopy thus confirmed the presence of individual copolymers (EPO and S100) in the structure of DIPEC, due to the appearance of the peaks at 1702 nm, and the amorphous form of IND (the peak at 1666 nm).

3.2.3 Raman spectroscopy

Raman-spectra were recorded to further characterize the solid-state of IND in DIPEC, and the possible interactions between sequences of countercharged copolymers (EPO, S100) and anionic drug (IND). For characterization of IND, the 1715–1100 cm^{-1} spectral range was used *(Figure S2a, Supporting Information)*. The vibrational mode occurring at 1699 cm^{-1} confirmed the existence of γ-form of IND (Heinz et al., 2007; Kao et al., 2012; Hedoux et al., 2008), which is also present in a physical mixture. The spectrum of the physical mixture can be regarded as the superposition of the spectra of IND, EPO and S100. However, in the DIPEC particles, a new peak appeared at 1680 cm^{-1}, which corresponds to the amorphous form of IND (Heinz et al., 2007; Kao et al., 2012). Both peaks are assigned to the benzoyl carbonyl stretching vibration (Hedoux et al., 2008). Molecules of γ-form of IND are mostly organized in cyclic dimers linked by hydrogen bonds (Chokshi et al., 2005; Hedoux et al., 2008). The absence of low frequency mode at 200 cm^{-1} *(Fig. S2a, Supporting*

Information) in the Raman spectrum of DIPEC (which is present in IND spectrum) is also a confirmation of the formation of an amorphous phase since this peak corresponds to the phonon of γ-form with long-range crystalline order (Hedoux et al., 2008).

Therefore, both methods (NIR- and Raman- spectroscopy) confirm the transformation of the γ- form of IND into the amorphous form during the preparation of DIPEC particles. However, Raman spectroscopy was not suitable for establishing inter-macromolecular interactions between the copolymers *(Fig. S2b, Supporting Information)*.

3.2.4 Thermal and XRPD analysis

In order to further support the observed appearance of the amorphous IND form established with FTIR-, NIR- and Raman spectroscopy and to bring further evidence that the formation of DIPEC between EPO and IND in the presence of S100 is the result of an electrostatic interaction between these copolymers and the drug, MDSC experiments were performed.

The γ-form of IND shows an endothermic peak at 160.2 °C, corresponding to the melting point (T_m). The glass transition temperature (T_g) of the amorphous form is located at ca. 46.0 °C which is in accordance with the literature (Liu et al., 2010, 2012; Sarode et al., 2013a, 2013b). Eudragit® copolymers are amorphous substances and have a characteristic T_g: EPO (52.1°C) and S100 (160.7 °C).

Physical mixtures made of EPO/S100/IND showed two T_g values, one at 50.8±1.1°C and a second one at 152.5±1.3°C °C related to EPO and S100. Transitions belonging to IND were not observed (data not shown).

Moreover, MDSC was used to confirm the structural differences between DIPEC and physical mixtures identified by FTIR spectroscopy, as well as to evaluate the chemical homogeneity of the copolymer-drug systems by the absence of microdomains of free copolymers and IND. The thermal characteristics of DIPEC vary with their composition and are given in Table 3. The data recorded for DIPEC demonstrates the amorphous nature of this system and copolymer miscibility since a single T_g (70.8 °C) was observed (Sipos et al., 2008). Also, the DPC (IND/EPO) is a miscible amorphous system displaying a single T_g at 43.7 °C.

To ensure that IND did not degrade during the heating, the DIPEC was studied using thermogravimetric analysis. No appreciable weight loss was observed after heating at 170 °C for 10 min in a nitrogen environment (data not shown). Liu et al. also reported that no significant degradation was observed upon heating to prepare solid dispersions of IND and EPO at 170 °C (Liu et al., 2012).

XRPD analysis *(Fig. S3, Supporting Information)* confirmed the MDSC data that IND is present in the amorphous form in PDC and DIPEC.

3.3 Pharmaceutical evaluation of DPC and DIPEC

3.3.1 Indomethacin loaded particles: release tests

In a further set of experiments, we tested the potential of DPC to be used in drug delivery systems to control the release of IND.

In vitro IND release experiments within 7 hours in GIT mimicking conditions for pure IND, DPC and DIPEC showed the potential of DIPEC (EPO/L100/IND 4.5:1:1) to be used as a carrier, suitable for colon-specific drug delivery (Fig. 5).

The results could be understood if we consider the structure of the formed DIPEC in depth. It is well known, that there are two main classes of IPECs: stoichiometric IPECs, which include the polymers in equimolar ratio and non-stoichiometric IPECs that have excessive amount of one of the polyelectrolytes. The last one is also called soluble IPECs because of their solubility in water (Philipp et all., 1989; Tsuchida, 1994; Thünemann et al., 2004; Kabanov, 2005; Pergushov et al., 2012). Moreover, in the structure of IPECs two types of chains can be distinguished: the interacting chains, which belong to both interacting polymers; and the loops, which are also called "defects" of non-interacting chains due to steric hindrances (Kabanov et al., 2005). According to this, the process of DIPEC formation may be divided into three main steps: (1) drug-interpolyelectrolyte complex formation by simultaneous interactions of EPO with oppositely-charged IND and S100; (2) transformation to a thermodynamically stabilized system by migration of ionic bonds; (3) drug-interpolyelectrolyte complex aggregation process and formation of microparticles. The first step is realized through binding via electrostatic attraction forces. The second step involves the formation of new bonds and/or the correction of the distortions of the polymer chains. The third step involves the aggregation of polycomplex particles, possibly through hydrophobic interactions.

The structure "defects" formed during the preparation of DIPEC do not only contain non-ionized dimethylamino groups of EPO and ether groups of both copolymers, as it could be in a stoichiometric IPEC structure, but also ionized dimethylamino groups that interact with carboxylate groups of IND and S100. Moreover, due to the non-stoichiometric structure of DIPEC, containing three-fold excess of EPO, additional sequences of EPO are able to interact with oppositely-charged IND molecules and S100. As a result, the structure of IPEC is changed because the ionic bonds are not fixed and they can migrate from one electrostatic site to another (Kabanov et al., 2005). The only problem is that at a pH between 6.8 and 7.2, the charge density of EPO macromolecules is low. This means that more sequences of EPO are needed to achieve optimal encapsulation efficiency of IND molecules. Moreover, equimolecular amounts of S100 could bind a similar molar amount of EPO during formation of microparticles. Thus, ionized dimethylamino groups are interacting with ionized carboxylic acid groups of IND in the sequences included in the loops and can also form new interpolymer contacts with S100.

The carboxylic groups of S100 that are present in "defects" are ionized at pH 7.0 and consequently increase the degree of ionization, but the dimethylamino groups present in the loops are losing their charge at this pH and lead to an increase in the contribution of the hydrophobic units in the total DIPEC structure. Aggregation of the interacting chains and non-charged fragments in "defects" lead to the formation of hydrophobic entities within the particles. Schematic structures of DPC and DIPEC particles are shown in Fig. 6.

According to the chemical structure of IND we can expect IND-EPO interactions, which will influence the drug release rate (Kindermann et al., 2011, 2012; Quinteros et al., 2011a, 2011b; Gusman et al., 2012).

Based on these results, the explanation of drug release from this system can be understood as follows. In acidic medium (pH 1.2 and 5.8), macromolecules of EPO hydrate and the copolymer partially dissolves. The solubility of the EPO/IND complex is also relatively high, but in the presence of S100 the release of the drug will decrease significantly. The remaining amount of ionized EPO and EPO/IND complex after transfer to a medium with higher pH will continuously lose charges on dimethylamino groups of the polycation chains, leading to the formation of insoluble fibers in the structure of the particles. At pH 6.8, most of the carboxyl groups of IND are deprotonated but sequences of S100 are still insoluble. Therefore, the repulsive forces between the negative charges of IND in DIPEC structure result in the continuous drug release.

The release rate of IND increases when the DPC and DIPEC are transferred into the final medium. According to the above-mentioned explanation, the increase in the release rate in this case at pH 7.4, could be due to the modification of the structure of DIPEC particles during the penetration of dissolution medium into the system. IND molecules, which cannot compete in the interpolyelectrolyte reaction, cannot find free sequences of charged dimethylamino groups in the insoluble fibers of EPO sequences, which will increase drug release. According to FT-IR results observed for polycomplex matrices based on Eudragit® EPO – Eudragit® S100 (Mustafin et al., 2011) we believe that similar processes are possible in the present DIPEC composed of the same copolymers.

In order to prove this, measuring the size and zeta potential of DIPEC particles under conditions, mimicking the release process was performed. During the titration, zeta potential and size of DIPEC clearly changed (Fig. 7). Zeta potential values increased up to pH 3.2 (+27.75 mV) followed by a gradual decrease with increasing pH. On the other hand, the particle size was minimal below pH 4.4 and then it increased up to pH 5.4 and 6.8 and decreased again at pH 7.4. In our opinion, the behavior of DIPEC particles in acidic medium (the largest size, zeta potential value +26.45 mV) corresponds to the dissolved DIPEC with minor release of IND from the system.

With increasing pH values the zeta potential begins to decrease, due to gradually decreasing the charge density of the positively charged EPO sequences, but the particles became larger indicating swelling and the start of IND release as a consequence of the dissociation of DIPEC structure. Additionally, drug molecules could simply diffuse through less swollen particles.

3.3.2 Indomethacin loaded tablets: release tests

As described in section 2.2.10, two kinds of tablets were produced: the first by compressing lyophilized DPC or DIPEC particles (encapsulated IND tablet) and the latter by compressing physical mixtures with the similar compositions (dispersed IND tablet).

Both types of dispersed tablets prepared from the physical mixtures disintegrated rapidly after 15 min. The explanation can be found in the fact that the copolymers are acting individually and no inter-polymer and drug-polymer interactions occurs. Indeed, EPO which is used as a gastric soluble film coating material, was already dissolved after 30 min in acidic medium and S100 is not soluble in this medium; tablets prepared

409 from this copolymer almost immediately disintegrated. Therefore, tested physical mixtures (dispersed IND
410 tablets) are clearly not suitable as oral sustained release systems for IND. Our findings are in the line with to
411 those previously reported by our group (Moustafine et al., 2005, 2013).
412 Fig. 8 shows the release profile obtained from DPC and DIPEC tablets with IND (encapsulated IND tablet): in
413 the gastric environment IND was not released at all instead of its release from the particles at about 5%. In
414 case of DPC tablets, after 7 hours, the pH change from pH=1.2 to pH=7.4 caused gradual release of the drug
415 up to its 50% amount due to the dissolution of the particles and further continuous dissociation of the DPC
416 structures (the complete tablet disintegration was observed within the first 2 hours). So, in this case the
417 release profile of IND is the same as we observed with DPC particles due to the fast disintegration of the
418 tablet (very low stability of the matrices) in acidic environment and similar mechanism of the drug release
419 after the dissociation of the DPC starts. The different release profiles in case of DIPEC systems between
420 tableted (Fig. 8) and powdered particles (Fig. 5) with IND, is obviously due to the reduction of surface area
421 exposed to the dissolution medium: particles, having a greater surface area than the tablets, are more
422 exposed to the dissolution medium and then the release of the drug is more rapid compared to tablets with
423 IND, in which, instead, the fluid must first penetrate the interstices between the particles placed in close
424 contact to each other, which is in accordance with the literature (Dalmoro et al., 2017). Moreover, a visible
425 transparent hydrogel layer is formed around the less swollen matrix DIPEC tablets in acidic medium (in the
426 first hour). However, the front of the external layer appeared turbid at pH=5.8 as the pH rises. This is in
427 agreement with our previous findings, concerning oppositely charged systems made of Eudragit EPO/L100
428 matrices during swelling in GIT mimicking conditions (Moustafine et al., 2013). The reason for it is the
429 influence of gastroresistant S100 copolymer, which plays an important role as additional hydrophobic layer
430 forming component. This makes it less penetrable to drug diffusion from the swollen DIPEC matrix, stable
431 until the end of the experiment. Additionally, the rate of the drug dissociation within swollen matrices is also
432 significantly decreased under these conditions.
433 Based on the results generated, we can conclude that unique properties of the EPO-S100 interpolyelectrolyte
434 complexes, which could be easily regulated by changing their composition and charge density, should be
435 applicable for the design of precisely pH-controlled drug-interpolyelectrolyte ternary systems for colon-targeting
436 of the encapsulated drugs.

437 ## 4. Conclusions

438 The results of the present investigation confirm the formation of a novel particulate system composed of
439 interpolyelectrolyte complexes between EPO and S100 in the presence of anionic IND. The formation and
440 chemical composition of ternary systems based on drug-interpolyelectrolyte complex (DIPEC) was established
441 by gravimetry, UV-spectrophotometry, capillary viscosity and elemental analysis and confirms that DIPEC is
442 formed in molar ratio EPO/L100/IND of 4.5:1:1. The particles are spherically shaped with a mean particle size

of 500 nm and with a positive zeta potential. Spectroscopic (FTIR, NIR and Raman) and solid state analytical methods (MDSC, XRPD) confirm that IND, included in DIPEC, was in the amorphous state. These particles are able to strongly protect the drug from the gastric environment and could be suitable for colon-targeting purposes. Finally, particles loaded with indomethacin were used to prepare tablets, with a slower IND release, which can potentially be used as oral pH-controlled drug delivery systems for sustained indomethacin release.

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Notes

The authors declare no competing financial interest.

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Figure S1. Particles characterization of DIPEC systems: dimensions (a), morphology (b) and identity (c), according to Raman spectra.

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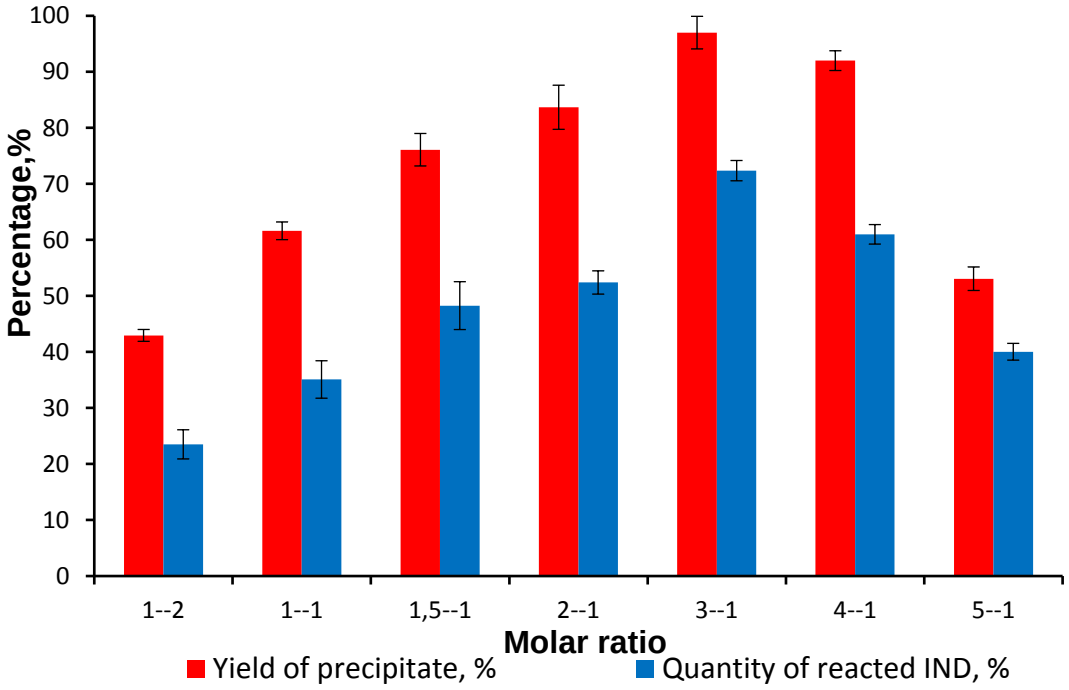
643 # Figures

644 (a)



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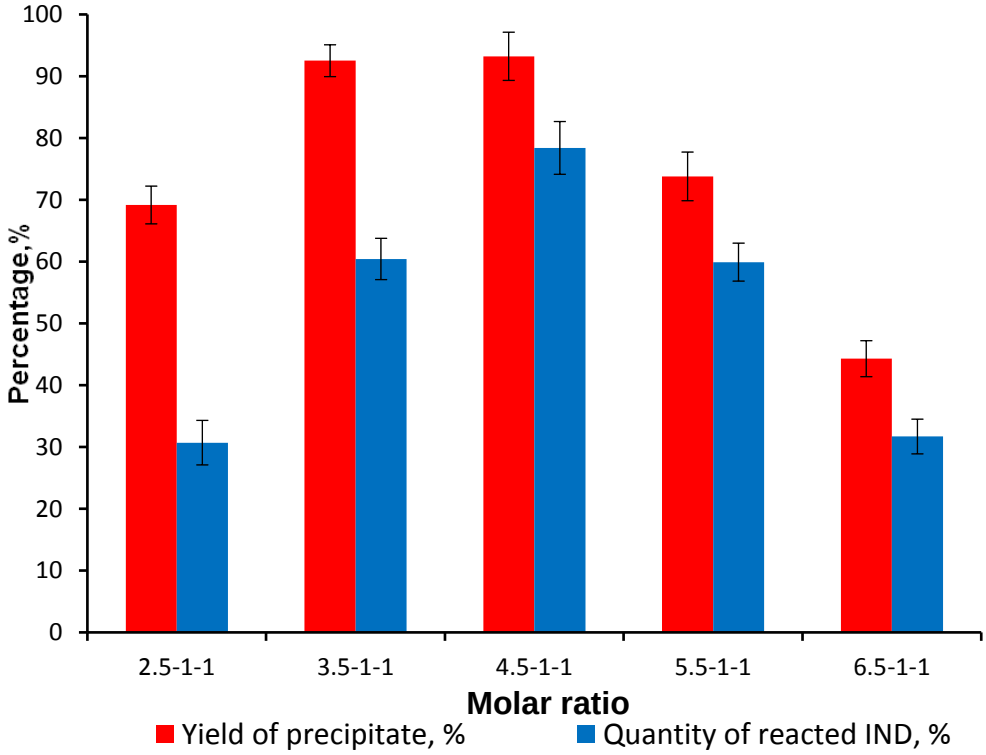
646 (b)



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648

649 **Fig. 1.** Gravimetric analysis of precipitates and UV-spectrophotometry analysis of supernatant solutions prepared at
650 different molar ratios: a) EPO/IND systems, b) EPO/S100/IND systems (n=3; ±SD).
651
652
653
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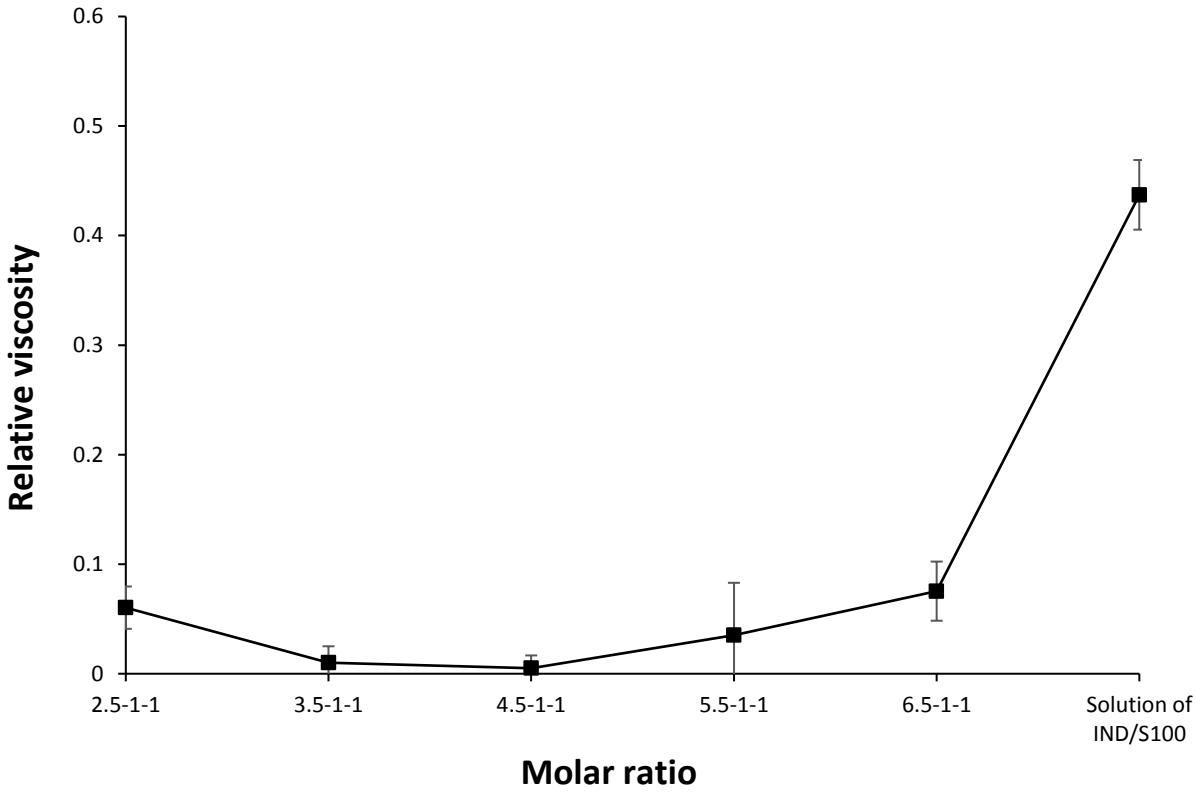
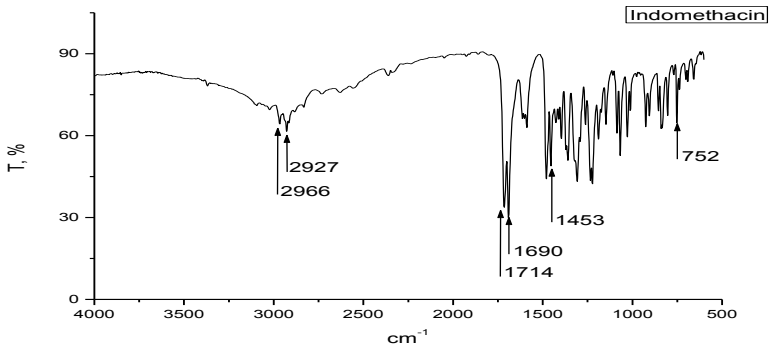
656



657
658

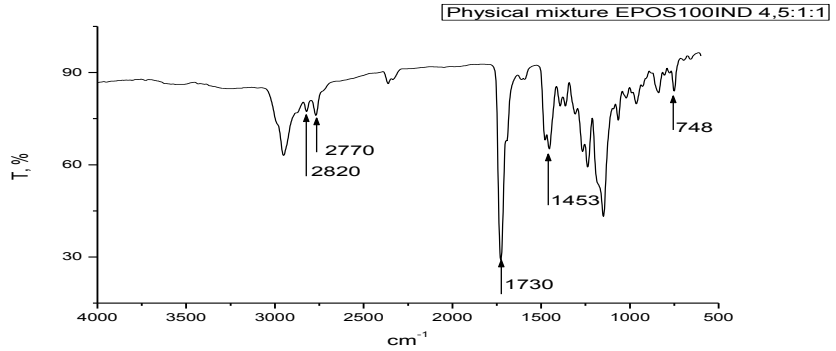
659 **Fig. 2.** Relative viscosity of the supernatant solutions of EPO/S100/IND systems as a function of the molar ratio (n=3;
660 ±SD).
661

662 a)



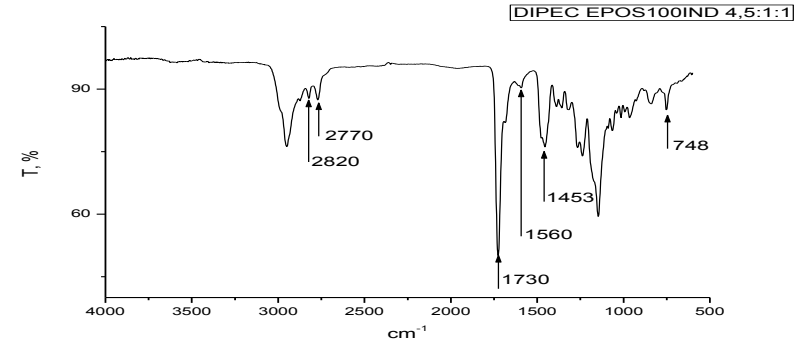
663

664 (b)



665

666 (c)



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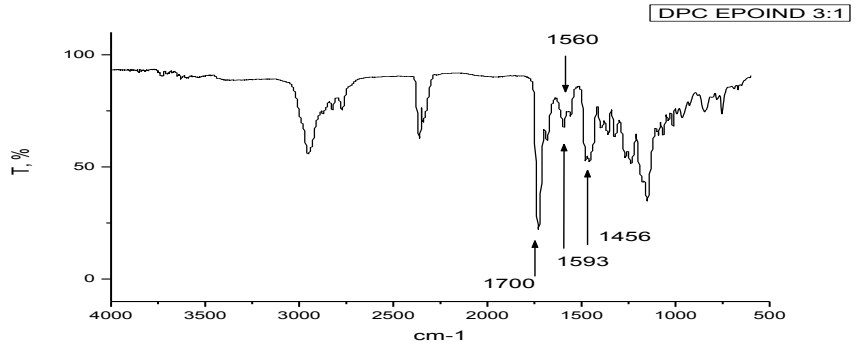
668 (d)

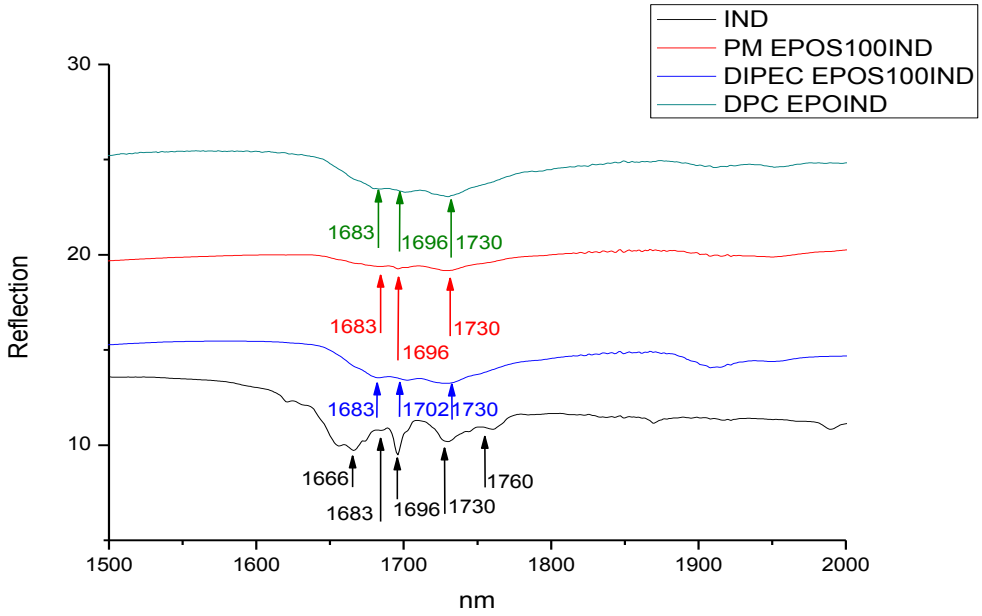


669

670 **Fig. 3.** ATR-FTIR-spectra of Indomethacin (a), physical mixture (b) DIPEC (c) and PDC (d).

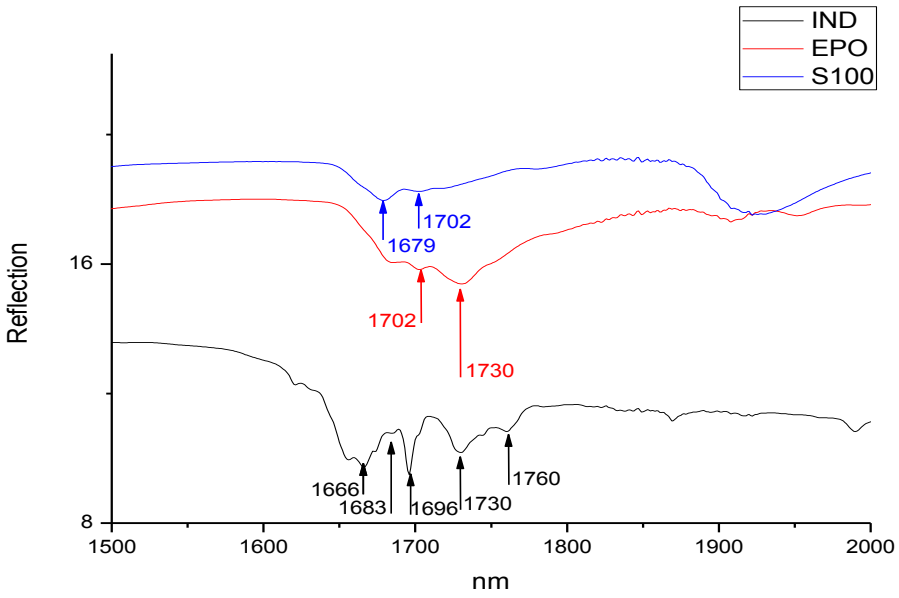
671

672 (a)



673

674 (b)



675

676 **Fig. 4.** NIR-spectra of: IND, physical mixture, DIPEC and DPC (a); IND, EPO and S100 (b).

677

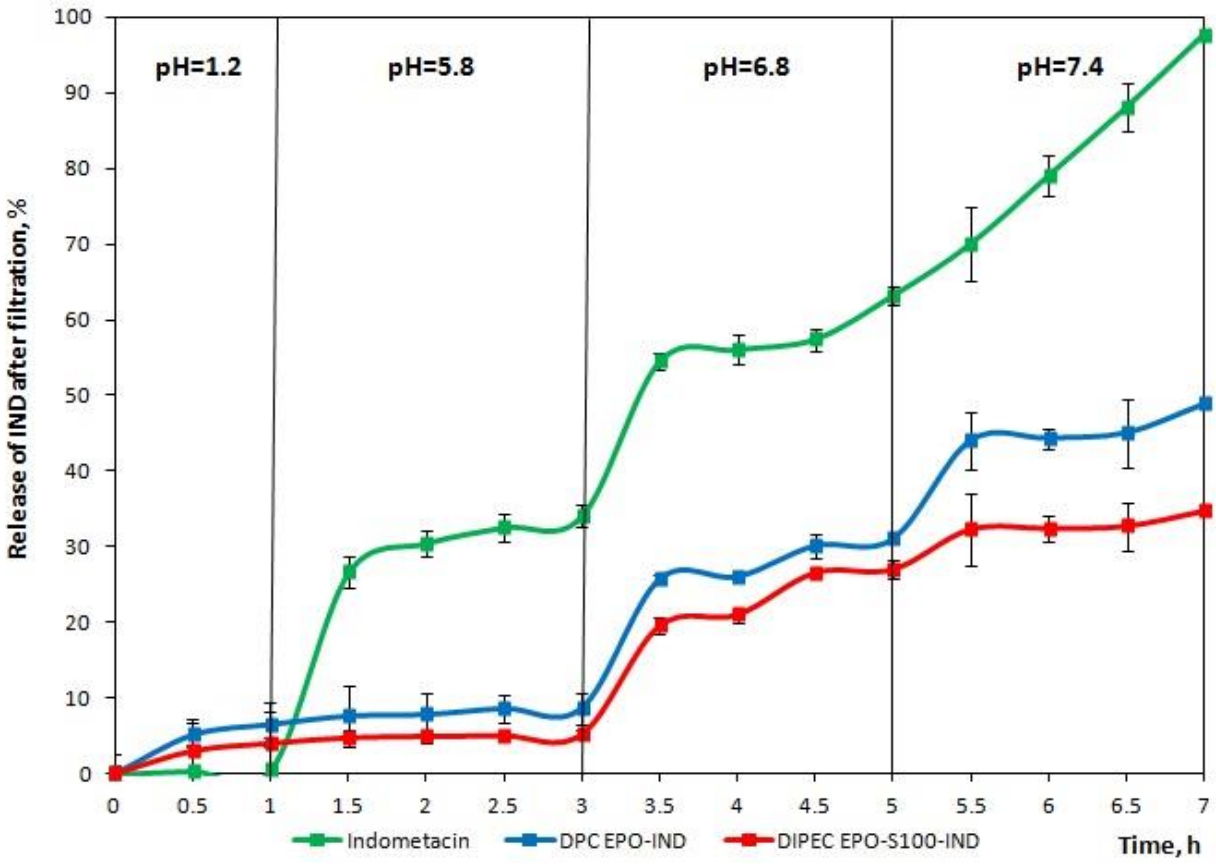


Fig. 5. IND release profiles in GIT mimicking conditions of the pure IND and from particles based on DPC EPO/S100 and DIPEC EPO/S100/IND systems (n=3; ±SD).

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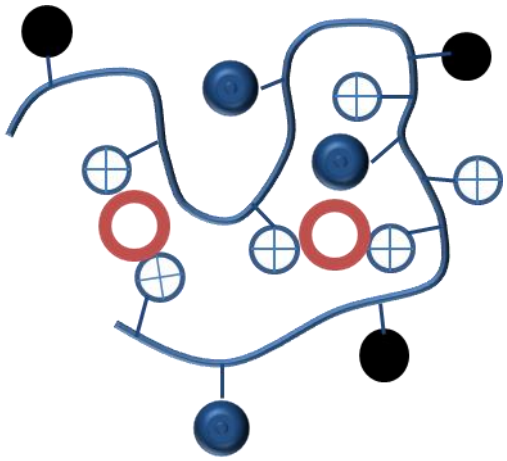
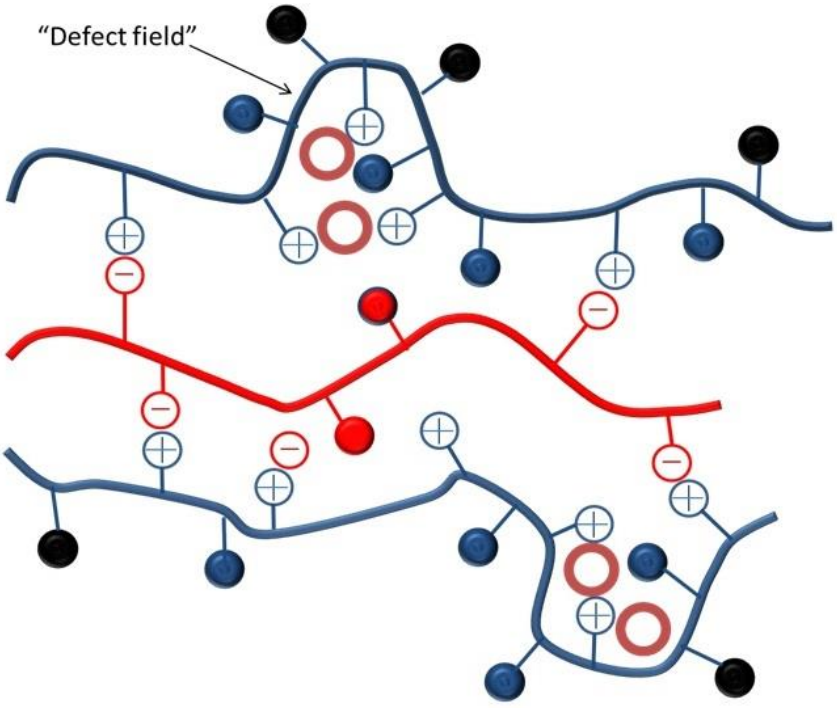
699

700 (a)



701

702 (b)



⊕	$-^{+}NH(CH_3)_2$		⊖	$-COO^{-}$
●	$-CH_3$; $-C_4H_9$	○	●	$-C_2H_5$
●	$-N(CH_3)_2$		●	-COOH
EPO		IND	S100	

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705 **Fig. 6.** Schematic representation of DPC (a) and DIPEC (b) structures.

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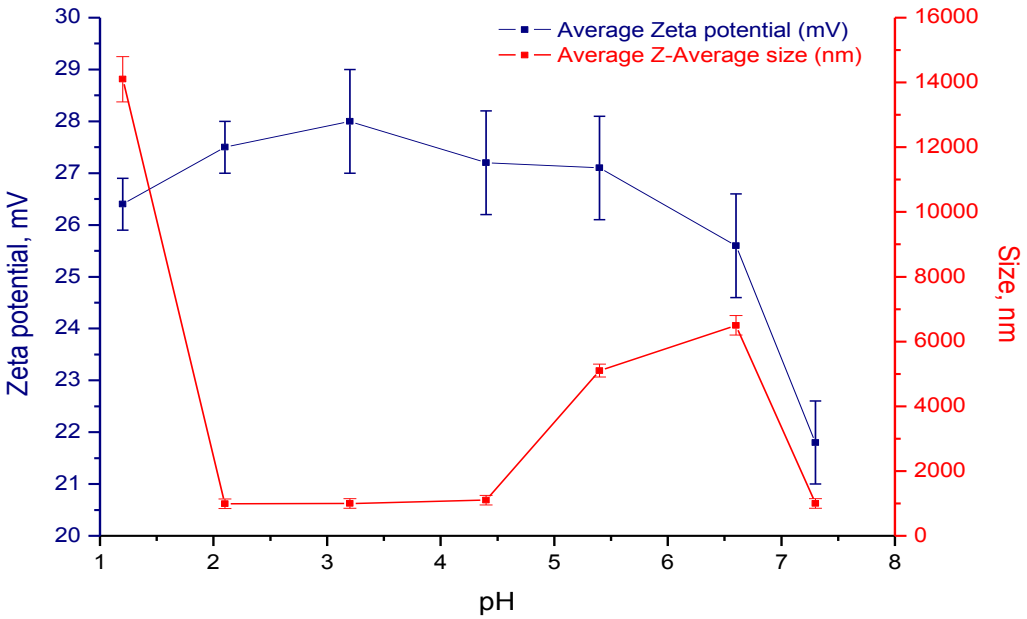

Fig. 7. Zeta potential (blue line) and particle size (red line) of DIPEC dispersions as a function of the pH values during automatic titration technique in GIT mimicking conditions (n=3; ±SD).

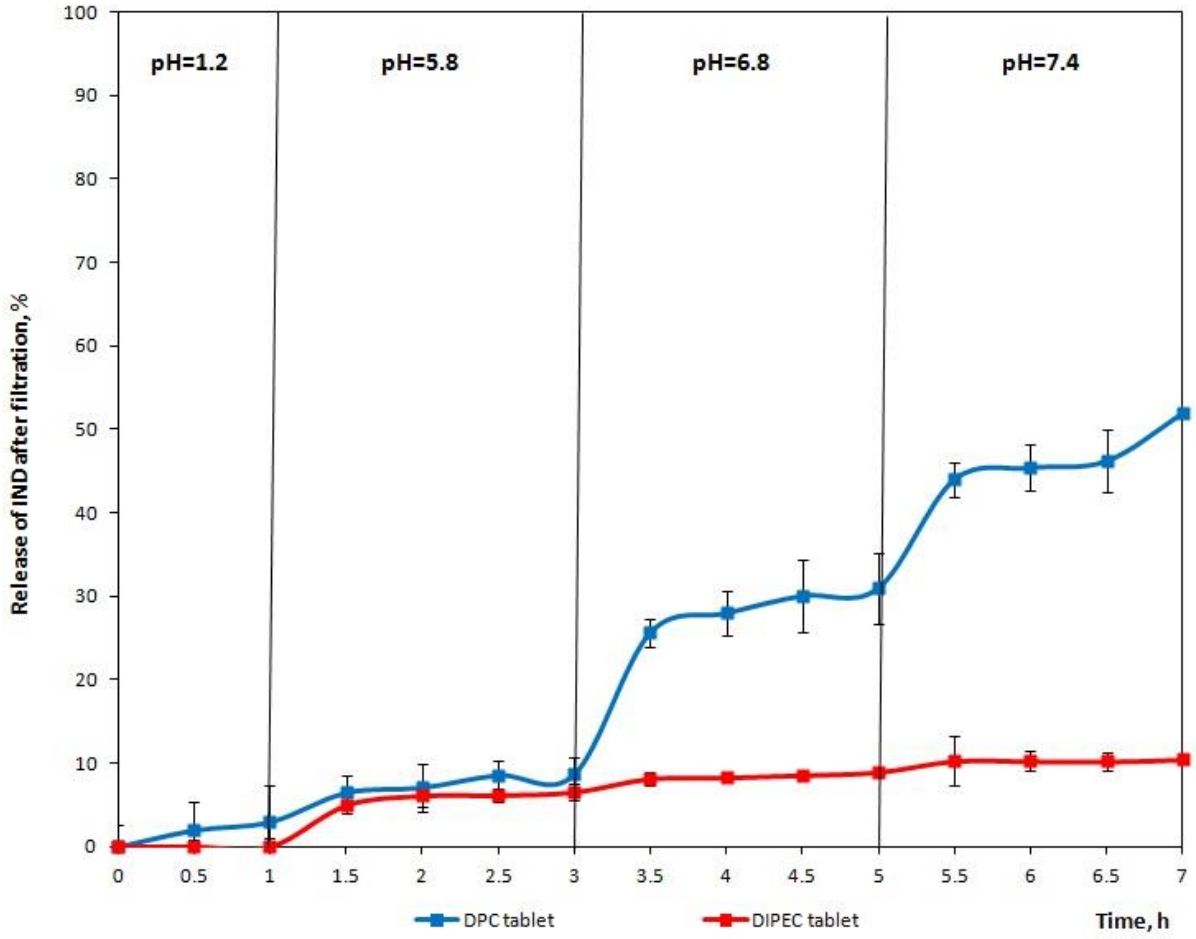

Fig. 8. IND release profiles in GIT mimicking conditions from tablets based on DPC EPO/IND and DIPEC EPO/S100/IND systems (n=3; ±SD).

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