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Self-assembly of Zein-based microcarrier system for colon-targeted oral drug delivery

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Abstract

The advances in pharmaceutical technology allow for the development of various region-selective delivery systems for oral administration to optimize local and systemic therapy. In this paper, micronization associated with a polymorph modification approach was proposed for improving the solubility of hydrophobic drugs for developing a Zein-based colon-targeted delivery system. A microcarrier based on self-assembled structures of Zein was fabricated via a built-in ultrasonic dialysis process, which displayed high payload of a model drug, indomethacin (Indo), with its optimal crystal form. The possible self-assembly mechanism of Zein/Indo forming porous structure in the ultrasonic dialysis process was attributed to the results of intra- and/or inter-molecular interactions between Zein and Indo. The designed microspheres, Zein-Indo@PDA, with a surface coating of polydopamine (PDA) not only rendered them enhanced stability and mechanical resistance, but also hindered the premature drug release at undesired sites. This innovative formulation design may offer better chances of colon-targeted release.

Keywords: Zein, self-assembly, colon-targeted, polydopamine, micronization, polymorph modification.

1. Introduction

Colon-specific targeted delivery has attracted wide attention mainly due to local therapy of inflammatory bowel disease (IBD). A major challenge in the therapy of IBD is the prevention and the reduction of drug-related side effects, since short- and long-term adverse drug reactions may deteriorate the health-related quality of life and thus may counteract a successful therapy.^{1,2} Therefore, local delivery of a therapeutic drug to the **inflamed colonic regions** is preferred to minimize systemic adverse effects. In the case of colon-targeted delivery, drug carriers are responsible for preventing the release of the active ingredients during gastric residence as well as throughout small intestinal transit.³ Numerous polymer-based formulations used in the construction of “smart” materials for colon-specific delivery have been reported to achieve controllable drug release using various stimuli including pH-sensitive,^{4, 5} redox-sensitive⁶ or colonic bacterial-driven drug release mechanisms.^{7,8} However, rare formulations can be enrolled in the clinical translational research, which might be related to highly individual variability in vivo conditions as well as undefined safety and cost to effectiveness ratios. Thus, screening more universal carrier systems with achievable safety and cost-effectiveness still remains to be a major issue.

Nowadays, pharmaceutical industry is keen on finding natural materials in high volume at low cost to replace conventional synthetic polymers. Zein, one of the natural and edible biomacromolecules which is readily available from maize, makes it possible to be a sustainable alternative to common synthetic polymer. One interesting feature to highlight is the absence of toxic effects when Zein-based particles are orally administered.⁹ Since 1985, Zein has been considered as a GRAS (generally regarded as safe) excipient for film coating of oral pharmaceuticals by the US FDA.¹⁰ For drug delivery purposes, Zein provides various advantages involving biodegradability and its origin in renewable resources, especially an interesting capability to interact with a wide variety of compounds (e.g. drugs and biomacromolecules) in a relatively nonspecific way.¹¹ Due to the hydrophobic nature of Zein, higher payloads are usually obtained for hydrophobic molecules than for hydrophilic or ionizable ingredients. When orally administered, Zein is relatively resistant to digestive

enzymes, which is associated with the fact that Zein particles display a long residence time within the gastrointestinal (GI) tract before being degraded.¹² Because of these advantages, Zein-based particulate systems have received extensive attention as promising delivery vehicles specifically for hydrophobic active molecules.¹³⁻¹⁶ However, to the best of our knowledge, there has been few reports on the application of Zein-based formulation as a colon-targeted delivery system so far.^{17, 18} The reason could be derived from at least two aspects: (1) the issues of premature drug release or incomplete disintegration of drug carriers are likely to be encountered, and (2) the designed trigger-release mechanism may fail to work due to possible aggregation of Zein colloidal particles. To address these problems, further surface functionalization of zein-based vehicles is necessary to achieve desirable outcomes. There have been attempts to coat the drug carriers using polydopamine (PDA), which can be formed by simply dispersing them in a dopamine solution at pH 8.5.^{19, 20} With its outstanding properties, PDA served as an appealing coating material which has been rapidly incorporated into a range of drug carriers including metal-,²¹ graphene-,²² proteins-²³ and polymer-based systems²⁴ over recent years.

Although diverse functional oral delivery systems could partially hinder premature drug release and facilitate drug delivery to proper sites, the pharmacokinetic and pharmacodynamic issues associated with the drug e.g. poor water solubility and low bioavailability still exist. To overcome the limitations of poor solubility, one of the simplest but most effective methods is to produce micro- or nanoparticles, because the solubility and dissolution rate can be enhanced by the decrease of particle size, i.e. the increase of the specific surface area.^{25, 26} Furthermore, the crystal engineering approach has been considered as another potential method for increasing the apparent solubility.^{27, 28} Generally, the metastable or amorphous form of pharmaceuticals, being thermodynamically unstable, exhibits a higher solubility and dissolution rate compared to its stable form.²⁹ Hence, it is desired to combine multiple strategies to achieve improved bioavailability together with controlled absorption properties, providing an opportunity for this drug carrier to become a promising candidate in clinical application.

In this paper, we report a Zein-based microcarrier system for colon-targeted oral drug delivery by introducing polydopamine onto particle surfaces to achieve delayed drug release. Indomethacin (Indo), a nonsteroidal anti-inflammatory drug, was chosen as a poorly water-soluble model drug, and their solid state form and dissolution behavior after recrystallization by the BUDP were studied. The microstructure and mechanical properties of Indo-loaded particles were investigated by means of scanning electron microscopy (SEM), transmission electron microscopy (TEM) and a micromanipulation technique. Moreover, the self-assembly mechanism of Zein/Indo in the ultrasonic dialysis process was explored by looking into the molecular interactions between Zein and Indo. The drug release behavior of the designed delivery system based on the microparticles in the simulated GI fluids was also evaluated.

2. Materials and Methods

2.1. Materials

Indomethacin (Indo, mass purity > 99%), Zein, Trizma base, sodium bicarbonate and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma-Aldrich (UK). Ethanol (Fisher Scientific, UK) was the analytic reagent. Dopamine hydrochloride (purity > 99%) was purchased from the Aladdin Co. Ltd., China. A dialysis bag with a diameter of 49 mm (MWCO 12000 Da, Sigma-Aldrich, UK) was pre-treated with a large volume of 2% sodium bicarbonate and 1.0 mM EDTA at 80 °C for 30 minutes before usage. The corresponding chemicals were obtained from Sigma-Aldrich (UK) and used directly without further purification. Distilled water produced by a Milli-Q Synthesis System was used in all experiments.

2.2. Preparation of Zein-Indo@PDA particles and α form Indo

Zein-Indo microspheres and α form Indo were prepared by the built-in ultrasonic dialysis process (BUDP) according to our previous work with modifications.³⁰⁻³² A simple schematic diagram for preparation of Zein-Indo is illustrated in Fig. 1. Firstly, 200 mg of Zein and 20 mg of Indo were dissolved into 70% (v/v) ethanol-water mixture of 40 mL, and the solution was poured into a pre-treated dialysis bag and dialyzed against distilled water (800 mL) ultrasonically using a Sonics Vibracell

probe sonicator (Sonics & Materials Inc., Newtown, CT, USA), pulsing 2 s on and 1 s off at an amplitude of 50% with the input power of 125 W for 2 h. Next, the above Zein-Indo suspension (60 mL) was added to 60 mL of freshly prepared dopamine solution with a concentration of 0.50 mg/mL in Tris buffer (10 mM, pH 8.5). Here, the dopamine solution at a low concentration (0.25 mg/mL) was selected in order to minimize the formation of self-polymerized PDA particles. After being stirred at room temperature for 1 h, the particles were collected by centrifugation (4025 \times g for 15 min) and re-dispersed with distilled water twice. For preparation of the α form Indo, 80 mg of purchased Indo were dissolved into 40 mL of 70% (v/v) ethanol-water mixture, and the solution was processed using the same BUDP method aforementioned. Finally, all particles were harvested by lyophilizing (Edwards, EF03, UK) for 24 h.

2.3. General characterizations

A laser diffraction particle size analyzer (Mastersizer 2000, Malvern, UK) was used to measure the particle size and its distribution. Zeta potential measurements were performed on a Zetersizer Nano ZS particle analyzer (Malvern, UK). Samples were platinum-coated with a SC7640 Sputter Coater (Quorum Technologies Ltd., UK) to improve the electrical conductivity of sample surface before their morphology was observed by scanning electron microscopy (Philips XL30 ESEM-FEG). The elemental components were recorded by using an Oxford Inca 300 EDS. Transmission electron microscopy (TEM, JEM-2100F, JEOL, Japan) analysis was performed at an operating voltage of 80 kV in bright field mode. The concentration of Indo was determined by the ultraviolet absorption at a wavelength of 320 nm with reference to a calibration curve on a UV spectrophotometer (Cecil 1020, Cecil Instruments, UK). The Powder X-ray diffraction (PXRD, D8 ADVANCE, Bruker AXS, Germany) profiles of samples were detected with Cu-ka radiation generated at 40 mA and 40 kV. Mettler Toledo DSC 2 was applied to record the thermograms after temperature and enthalpy calibration using indium. Dried samples (2-4 mg) were crimped in an aluminum pan (40 μ L) and heated at a rate of 10 $^{\circ}$ C min⁻¹ from 40 to 300 $^{\circ}$ C, under a nitrogen gas flow of 100 cm³ min⁻¹. The CD spectra were recorded

from 190 to 260 nm using a Chirascan spectropolarimeter (Applied Photophysics Ltd., UK). The final concentration of samples was 0.2-0.5 mg/mL, and a quartz cell with a 1-cm path length was used. The secondary structure contents of each sample were analyzed by using CDNN software.

2.4. Determination of drug payload and encapsulation efficiency

For an evaluation of the drug payload (DPL) and encapsulation efficiency (EE), a pre-determined amount (5.5-6.0 mg) of Zein-Indo or Zein-Indo@PDA was dissolved in 10 mL 70% (v/v) ethanol-water after accurate weighing. The supernatant was collected by centrifugation at 4025 ×g for 20 min, and the Indo concentration was determined by a UV spectrophotometer at a wavelength of 320 nm. The baseline was established for running the blank sample of 70% (v/v) ethanol-water containing 0.5mg/mL Zein. The DPL of Zein-Indo or Zein-Indo@PDA and their EE of Indo were calculated using Eqs. (1) and (2), respectively.

$$DPL = \frac{M_1}{M_2} * 100\% \quad (1)$$

$$EE = \frac{M_1}{M_0} * 100\% \quad (2)$$

where M_1 is the weight of Indo in Zein-Indo or Zein-Indo@PDA; M_2 is the corresponding weight of Zein-Indo or Zein-Indo@PDA and M_0 is the weight of total Indo. Each experiment was carried out in triplicate.

2.5. In vitro release rate study

The profiles of drug release from carriers to different fluids were investigated using a British Pharmacopoeia (BP) dissolution apparatus (Agilent 708-DS, UK). Briefly, free Indo and Indo-loaded particles with equivalent Indo mass (10 mg) were dispersed into a dialysis bag with 4 mL of simulated gastric fluid respectively. Then an end-sealed dialysis bag was immersed into the 400 mL of a chosen fluid in a vessel at 37 °C, which was agitated with a paddle impeller (Dimensions: 74.5/42.0 × 19.0 × 4.0 mm, top diameter/bottom diameter × height × thickness) at a rotation speed of 100 rpm without baffles. To mimic human GI tract, particles were firstly immersed in the simulated gastric fluid (SGF) for 2 hours, then exposed to simulated small intestinal fluid (SSIF) for another 4 hours. After that, samples were immersed in the simulated

colonic fluid (SCF) for 18 hours. At pre-determined time intervals, 3 mL of release medium was withdrawn and replaced with an equal volume of the corresponding fresh fluid. The Indo concentration of each sample was determined by the UV spectrophotometer at a wavelength of 320 nm.

2.6. Micromanipulation technique

The mechanical properties of single microspheres were performed by a well-established micromanipulation technique (Fig. 2), the principle and details of which are described elsewhere.^{33, 34} Briefly, one drop of suspension with microspheres was placed on a glass slide and left to dry under ambient conditions for 15 min. Single microspheres were observed from a side-view digital camera, and their diameter was measured from their images. A glass probe of 70 μm in diameter connected to a force transducer (Model 403A, maximum scale 5 mN, Aurora Scientific Inc., Canada) was driven down by a stepping motor towards a single microsphere at a speed of 2 $\mu\text{m/s}$. At least 20-30 microspheres from each sample were randomly selected for compression, and the corresponding force versus displacement data and their diameters were obtained.

2.7. Determination of the Young's modulus

Theoretically, the classic Hertz model (Eq.3) is commonly valid to describe the relationship between the imposed force and displacement for small deformations of an elastic object (typically nominal deformation, defined by the ratio of particle displacement to its original diameter < 10%) with frictionless contact, which has been successfully applied to determine the Young's modulus of different particles.³⁵

$$F = \frac{E\sqrt{2R}}{3(1-\nu^2)}\delta^{3/2} \quad (3)$$

where F is the imposed force, E is the Young's modulus of the particles, R is the radius of particles, ν the Poisson's ratio and δ the diametric compressive displacement. From the model, there should be a linear relationship between F and $\delta^{3/2}$, where the value of Young's modulus was determined from the slope of the linear fitting along with a given radius and Poisson ratio (for an incompressible microsphere, ν was assumed to be 0.5).

3. Results and discussion

3.1 Morphology and physical status of Zein-based microspheres

Zein-based microspheres were successfully prepared by the BUDP method, and they were further coated with a shell of PDA by dispersing them in a dopamine solution at pH 8.5 and mildly stirring at room temperature. Their physicochemical properties including the average size, zeta potential, payload and encapsulation efficiency are summarized in Table 1. The average size of Zein particles ($6.4 \pm 0.6 \mu\text{m}$) was much smaller than that of their drug-loaded particles ($9.0 \pm 0.8 \mu\text{m}$). But there was no significant alteration to the particle size after PDA shell formation, which could be mainly attributed to a very thin shell of PDA, probably at the nanometer scale. Indeed, the thickness of the PDA layer on Zein-Indo surfaces ranged from 50 nm to 500 nm as confirmed by TEM images (Fig.3). Zeta potential results (Table 1) manifest that the PDA-coated microspheres exhibited a negative surface charge, which could be ascribed to the deprotonation of catechol -OH groups in the PDA shells.³² Importantly, Zein-Indo and Zein-Indo@PDA displayed high encapsulation efficiency for Indo with a value of $89.1 \pm 1.4\%$ and $83.1 \pm 1.2\%$, which might result from the hydrophobic property of the drug.³⁶ It is believed that molecules with higher hydrophobicity prefer to stay in ethanol phase to aqueous phase, thereby presenting a high loading capacity in the Zein-based microspheres.

Particles with good physical stability, especially under complex biological conditions, are strongly desired to realize the surface-mediated interaction and controllable drug release behavior. Accordingly, particle size distributions of Zein-Indo and Zein-Indo@PDA were measured in aqueous and GI fluids before and after storage for 48h (Tables S1&S2). The results demonstrate that Zein-Indo microspheres showed weaker stability both in the aqueous and GI fluids than Zein-Indo@PDA, which results in dense aggregates and larger size after the storage (Fig. S1). This was mainly due to the diminished electrostatic repulsion and hydrophobic nature of Zein, especially in the medium at physiological pH with high ionic strength.^{9, 32} On the contrary, Zein-Indo@PDA dispersed well in the aqueous and GI fluids with no significant changes in hydrodynamic size. Optical microscopy

images also illustrate the formation of well dispersed Zein-Indo@PDA without aggregation after 2-days' storage, revealing excellent colloidal stability of the solutions mentioned (Fig. S1). Our observation agrees well with other reports in which the PDA surface modification could serve as an effective strategy to form an ultra-stable nanolayer on solid surfaces via covalent and non-covalent interactions and improve their bio-compatibility and bio-stability.³⁷

In SEM images, pure Zein microspheres showed a spherical and dense structure with a smooth surface (Fig. 4a). It is also interesting to observe the Zein-Indo with porous structure on their surface (Fig. 4b). The alteration of surface structure was supposedly ascribed to the addition of Indo. The presence of Indo might make the molecular interactions quite diverse, which can influence assembling behaviors thereby leading to the changeable surface structure. By comparison, the Zein-Indo@PDA seemed to be relatively rough, and most of the pores were concealed as a result of PDA surface functionalization (Fig. 4c). According to the specific chlorine element present in Indo, it is easy to track the drug distribution on the particle surface by EDX-mapping (Fig. S2). The results show that a noticeable amount of chlorine was detected from Zein-Indo particles, while that was almost unobservable in the case of Zein-Indo@PDA. This is clear evidence that Indo distributed on the outer layer of the Zein matrices has been covered by the PDA shell.

Interestingly, the formation of rod-like Indo at the microscale was observed after recrystallization through the BUDP, while unprocessed Indo, fresh powder form purchased from Sigma, showed irregular polygon-like aggregates with wide size distribution (Fig. 4d & e). The question that arises from these results regarding the varied morphology of Indo is whether it is possible to generate solvent-mediated transformation of solid state during the BUDP. Since polymorphism of pharmaceuticals has significant effects on the physicochemical properties of drug products, e.g. the dissolution rate, solubility, stability and bioavailability, it is necessary to detect the crystal form of Indo in Zein-based microspheres. PXRD was utilized to identify the solid state form of all samples and the results are shown in Fig.5a. The diffraction peaks of Indo obtained by the BUDP method are

distinguishable from that of the starting material. These differences in the PXRD patterns indicate different arrangements of the Indo molecules in the crystal lattice of each form, and solvent-induced crystal structural transformation might occur during the BUDP. The PXRD patterns of Indo were then compared with those of α and γ forms reported by the International Centre for Diffraction Data (ref codes PDF00-058-1165 and PDF00-036-1698), showing the unprocessed Indo was γ form and the Indo prepared via the BUDP was α form. It is also worth noting that there was an obvious reduction in peak intensity when γ form was converted to α form, which might be attributed to the decrease of particle size after recrystallization. The unique diffraction peaks with respect to the Zein/Indo physical mixture (10/1, w/w) exhibited the consistent positions with the γ form, where a decrease in peak intensity was observed when compared to that of the pure γ form. The reason could be that the relative diffraction intensity was strongly related to quantity, thus a small percentage of Indo in the mixture led to the weak peak intensity. Unexpectedly, no unique diffraction peaks of α form were detected from the diffractogram of Zein-Indo microspheres processed via the BUDP. The possible explanation for this observation might be that the payload of Indo in Zein-Indo particles ($11.1 \pm 0.2\%$) is very low, thus leading to the indistinguishable peaks in PXRD patterns. Meanwhile, the weak intensity of α form itself, as mentioned above, might further undermine the recognition of characteristic diffraction peaks. Another reason for the absence of peaks could be that the solid phase of Indo transformed to amorphous form.

In order to further confirm the crystal form of Indo in Zein-Indo microspheres, the thermal behavior of all solid state forms was determined with DSC (Fig. 5b). Thermograms obtained from α and γ samples illustrate that the metastable α form had a melting point onset at 151-152 °C and the highly stable γ -form at 160-161 °C, which are similar to those reported in the previous literatures.^{38, 39} Interestingly, a single mild melting endotherm was also observed at 151.43 °C for Zein-Indo microspheres. This result provided visible evidence of the presence of α form when Indo-loaded Zein microspheres were obtained by the BUDP, although the possibility of amorphous formation could not be excluded. According to an early study⁴⁰, the

plasma level of Indo with α form ($22.6 \pm 1.3 \mu\text{g/mL}$) was significantly higher than that of γ form ($8.4 \pm 0.9 \mu\text{g/mL}$) following the rectal administration, making it possible to have the better bioavailability. Therefore, it is expected to obtain optimal α form-loaded microspheres for achieving potential therapeutic requirements, and the BUDP method offers a facile technique to allow for the modification of crystal form as well as for the purpose of micronization.

3.2 Possible mechanism of Zein-Indo self-assembly

Since we have observed the different surface morphology of microspheres after Indo loaded, it is interesting to explore the possible formation mechanism of porous Zein-Indo structure. Understanding the behavior of Zein-based self-assembly system requires studying the interactions between molecules, involving forces caused by their inherent properties and composition. Zein has an amphiphilic character due to its unusual amino acid sequence. Matsushima and co-workers proposed a structural model of Zein in 70% (v/v) ethanol-water solution, where 9-10 homologous helical segments were aligned in antiparallel, forming a ribbon with the hydrophobic outer surface and hydrophilic edges containing the glutamine-rich bridges.⁴¹ Here, this model was assumed when exploring the possible mechanism of Zein-Indo self-assembly. Self-assembly is considered to be mediated by molecular affinity, for example, van der Waals forces, hydrophobic interactions, hydrogen bonds and electrostatic interactions.⁴² The molecular interactions between a drug and its carrier plays an important role in the development of a drug delivery system since it affects various aspects such as morphology of the carrier, drug payload, release behavior, and distribution. In this case, the possible intra/inter-molecular hydrogen bonds within Zein and Indo molecules are illustrated in Fig. 6a. The benzoyl carbonyl (H-bond acceptor), aromatic protons at the ortho-position of chlorine (H-bond donor) and carboxylic acid (H-bond acceptor and donor) are the three groups capable of participation in the intramolecular H-bonding within the Indo molecules. Zein matrices containing plentiful carbonyl groups (H-bond acceptor) could make contributions to forming the intermolecular H-bonding when coupled with Indo.

It is believed that the interpretation of the C=O peaks in these groups can help to provide insight into the H-bonding arrangements in the binary system, thus further experimental FTIR measurements were carried out (Fig. 6b). Since the alterations of vibrational assignment of C=O peaks within the monomer, dimer or trimer structure are the most visible in the 1500-1800 cm^{-1} region,⁴³ the exact locations of the peaks in these regions were mainly concerned. It can be seen from Fig. 7b that two bands associated with the C=O stretching vibration at 1660 cm^{-1} (H-bonded to carboxylic acid group) and 1751 cm^{-1} (H-bonded to benzoyl C=O group) were detected from α form Indo. Peaks at 1722 cm^{-1} for the α form (with cyclic COOH bonding in the dimer) and 1709 cm^{-1} for the γ form (cyclic COOH bonding) were ascribed to the anti-symmetric acid C=O stretching, which could be the evidence of intramolecular hydrogen bonds.³⁹ It is worthy to note that almost disappearance of the band at 1722 cm^{-1} from the FTIR spectra of Zein-Indo, when compared to that of the α form Indo, suggested absent or weak cyclic COOH bonding within the Indo dimer. This can be explained by that the intramolecular H-bonds within Indo molecules are relatively weak, while the intermolecular H-bonds between Zein and Indo might be more persistent. Thus, H-bonds tended to reconstruct within Zein and Indo molecules rather than within individual Indo molecules. In regard to the Zein/Indo mixture, the presence of peaks at 1632 cm^{-1} and 1711 cm^{-1} , which are typical bands for γ form Indo, revealed that physical mixing of two solid components could not alter the molecular interactions. CD spectra of Zein/Indo samples collected at 0, 30, 60 and 120 min during BUDP were further conducted to observe the conformational transitions of Zein molecules (Fig. 7a), and results showed that there was a sharp decrease in ellipticity at 222 nm, suggesting the conformational transitions of zein molecules. The fractional contents of α -helix, β -sheet and random coil analyzed via the CDNN program (Fig. 7b) illustrated that the α -helix content decreased rapidly from 36.2 to 6.7% during the process, while β -sheet and random coil increased from 15.8 to 34.5% and 30.4 to 42.6%, respectively.

Based on the evaporation-induced self-assembling behavior of Zein proposed by Wang and Padua,⁴² we proposed a possible mechanism for Zein/Indo self-assembly

from single molecules to porous microspheres during the BUDP (Fig. 7c). Firstly, stable complexes involving Indo and Zein could be formed due to the abundant presence of hydrogen-bond networks. In a pulse ultrasonic system, physical shear which is caused by acoustic cavitation can improve the mobility of droplets in the solution,⁴⁴ thus resulting in the homogeneous distribution of Zein/Indo units. The BUDP aroused a radial ethanol concentration gradient in the solution as a result of irreversible dialysis, thus leading to solvent-induced α -helix to β -sheet transformation of Zein. Secondly, Zein/Indo complexes in the form of antiparallel β -sheets packed side by side forming a long ribbon or coil. Because of the hydrophobic nature, the orientations of the Indo chains might be close to the main helical segments of the hydrophobic domains from Zein molecules. Then, the ribbon tended to curl into a ring or toroid, continuously growing towards a nanosphere with a pore-scattered structure due to the random arrangement of Indo within Zein lattices. Finally, additional Zein/Indo ribbons were adsorbed onto neighboring nanospheres, resulting in their continuous growth toward porous microspheres.

3.3 Mechanical properties

It is known that the occurrence of fracture/crack at the interface causes a reduction in the overall elastic stiffness (Young's modulus), and architectures of oral formulations therefore tend to be fragile and fail to satisfy the manufacturing requirements in particular for producing a final dosage form of tablet by compaction.⁴² From this point of view, it is necessary to achieve satisfactory mechanical strength and ruggedness for a designed formulation to survive normal processing, handling, packaging, and shipping stresses. The Young's modulus signifies the intrinsic stiffness of an elastic material undergoing recoverable compression.³³ It is intended to use the Hertz model to determine the Young's modulus of Zein-based microspheres at small nominal deformations (up to 10%), hence providing an indication of the elasticity of the particles.

The typical force versus displacement data up to a nominal deformation of 10% obtained from compression of single Zein-based microspheres by micromanipulation

was fitted with the Hertz model (Eq. (3)), and typical examples of such fittings are illustrated in Fig. 8. The mean Young's modulus values of these microspheres are presented in Table S3. The Poisson ratio of Zein was assumed to be 0.5, since Zein was considered an incompressible material. It can be seen that there was no significant alteration in the Young's modulus value after Indo was loaded into Zein microspheres, showing the mean value of 6.1 ± 1.1 GPa for Zein microspheres and 6.7 ± 0.8 GPa for Zein-Indo microspheres. However, a striking increment of the Young's modulus was observed when PDA shell was deposited onto the surface of Zein-Indo particles. The incorporation of the dopamine biomolecules on the surface microspheres might result in good interfacial adhesion between the Indo and Zein matrix.⁴⁵ It is known that interfacial interactions play a key role in mechanical properties of composites.^{46, 47} Thus, the improvement of the stiffness of the Zein-Indo@PDA could be attributed to the better interfacial interaction among the Indo, Zein matrix and the additional surface coating. Besides, the Zein-Indo@PDA microspheres did not show rupture under compression when the maximum nominal stress (ratio of the force to the initial cross-sectional area) of 75.4 ± 3.8 MPa was applied to them (data not shown). It is believed that such mechanical strength is sufficient for the carriers to survive further processing, e.g. tableting, when compared to other oral formulations showing the Young's modulus of a large proportion of particles was inferior to 5 Gpa.⁴⁸

3.4 *In vitro* release behavior

Considering that relevant in-vitro dissolution testing on the formulations is useful for qualitative prediction of their dissolution and availability of orally administered drugs, the release of the model drug from Indo-tethered microspheres or Indo crystals was monitored in a simulated GI environment and data are shown in Fig. 9a. It can be seen clearly that the release of Indo from all samples in the SGF was minimal. This was mainly due to the low solubility of Indo in an acidic environment. In terms of the dissolution behavior of pure Indo crystal, a rapid release (over 50%) of α form was observed after exposing them to the SSIF for 4h, while only approximately 20% of γ

form was released under the same condition. Meanwhile, the cumulative release of α form was much higher than that of γ form after sequential incubation in the simulated media up to 24h. These data demonstrate that α form, which exhibited a higher solubility and associated dissolution rate compared to γ form, can be considered the optimal solid state form in terms of solubility.

Comparison with release behavior of the α form, the percentages of Indo release from Zein-Indo was found to be $28.4 \pm 4.2\%$ within 4 h in a simulated small intestinal environment, which was remarkably lower than that of nude α form. This indicates that the encapsulation of Indo into Zein-based microspheres could facilitate their controlled release as well as the reduction of drug pre-release. The cumulative release of Indo finally reached nearly 80% when exposed to the following release medium for another 18 h. The initial release in SSIF is most likely attributed to some Indo loaded in the outer layer of the Zein matrices where it diffused out easily. Gradually, Indo released from the inner hydrophobic Zein matrices diffused through aqueous channels formed during Zein hydration and swelling,^{49, 50} leading to a retarded release rate of Indo in SSIF. As mentioned above, the elevated ionic strength is a powerful factor interfering with the stability of Zein-Indo in the release medium. Consequently, with larger aggregates of Zein-Indo gradually forming, their release behavior would differ drastically, failing to efficiently release from the aggregated blocks. This may explain that the release almost stopped after 24 h with more than 20% drugs still remaining inside Zein-Indo matrices.

It is worth to note that there is an obvious decline of drug release with respect to Zein-Indo@PDA in SSIF, showing only $16.2 \pm 3.8\%$ of drug released from the Zein-Indo@PDA matrices. This can be considered as a proof that PDA acted as a protective layer which made a contribution to alleviating drug release. Additionally, more than 95% of drug was released from the PDA-coated formulation within 24 h, rather than incomplete disintegration and drug release like Zein-Indo encountered (about 80% of drug release). The fact of this noticeable difference would be related to the good dispersion of Zein-Indo@PDA in simulated GI fluid so that Indo could achieve the sustained release. Furthermore, representative SEM images of

Zein-Indo@PDA after incubation in SGF, SSIF and SCF (Fig 9b-d) provided the evidence that the hydration interaction and swelling of Zein could also directly correlate with the release kinetics. Form the point of morphology, Zein-Indo@PDA particles displayed porous structure at the outer region close to the surface. Rupture or erosion is expected to take place at this boundary, which migrates inwards driven by the hydration process. This could be a consequence of the hydrophilic layer of PDA and associated porosity of the Zein-Indo microspheres. Owing to the low release rate in simulated gastric and small intestinal media, the Zein-Indo@PDA carriers could potentially reduce side effects caused by premature release of Indo during their retention in upper GI tract, and significantly enhance the drug concentration in the diseased colon (Fig. 9e). Moreover, one should be reminded that Zein, as a safe and biodegradable protein, could be susceptible to degradation by the action of colonic microbes. It is believed that the microbial population in the colon is much more abundant than those residing in the small intestine and can catalyze a series of enzymatic reactions, many of which do not take place in the upper intestine.^{51, 52} However, the possible effect of these enzymatic reactions on the release rate of the drug from the Zein@PDA is not clear at this stage, which remains to be future work. By considering all the above results, we can conclude that the designed Zein-Indo@PDA micro-formulation could be served as a promising colon-targeted drug delivery system.

4. Conclusions

A novel formulation design based on the combined strategies of polymorph modification via the BUDP and PDA-related carrier functionalization was developed to fabricate Zein-based microspheres for colon-targeted drug delivery. The α form Indo at the microscale, which featured with higher solubility and dissolution rate, was successfully encapsulated into Zein-based microcarriers with high encapsulation efficiency of $89.1 \pm 1.4\%$ via the BUDP. The results proved that the BUDP technique enabled not only promising micronization, but also solvent-induced crystal form

transformation for the polymorph of the model drug, Indo. The formation of porous Zein-Indo structure in the ultrasonic dialysis process is proposed to be attributed to intereactions between intramolecular and intermolecular hydrogen bond networks within the two-component systems, which can be beneficial to guide the design of artificially engineered Zein-based structures for potential applications.

After surface functionalization by PDA, the resultant particles, Zein-Indo@PDA, presented the increase of the overall physical stability in the GI environment and superior mechanical strength. Less than 20% of loaded Indo was released from Zein-Indo@PDA in the simulated upper gastrointestinal fluid within 6 h, but nearly all of the remaining drug content was released at the colonic condition within the following 18 h. Utilization of a delayed release approach based on Zein-based microcarriers for oral delivery, which rely on GI transit time to realize the colonic delivery, appears to be reliable and reproducible due to the inherent traits of Zein resistant to digestive enzymes and tolerant to changing pH environment. This study provides insight into the construction of a Zein-based colon-targeted delivery system, which is cheap, sustainable and is valuable in the field of pharmaceuticals and biomaterials.

Supporting Information

Materials of the simulated gastrointestinal fluids; Hydrodynamic size of fresh prepared particles dispersed in different media before storage (Table S1); Hydrodynamic size of particles stored in different media for 48h (Table S2); The mean values of Young's modulus for different samples (Table S3); Optical microscope images of zein-Indo and Zein-Indo@PDA in different media (Figure S1); Elemental analysis of Zein, Zein-Indo and Zein-Indo@PDA particles by EDX-mapping (Figure S2).

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Table and Figures

Table 1. Various properties of different prepared particles

	Average size	Zeta potential	Drug payload	Encapsulation
	(μm)	ζ [mV]	(%)	efficiency (%)
Zein	6.4 ± 0.6	12.4 ± 0.5	---	---
Zein-Indo	9.0 ± 0.8	10.7 ± 0.8	11.1 ± 0.2	89.1 ± 1.4
Zein-Indo@PDA	9.5 ± 1.2	-24.9 ± 1.1	10.4 ± 0.1	83.1 ± 1.2

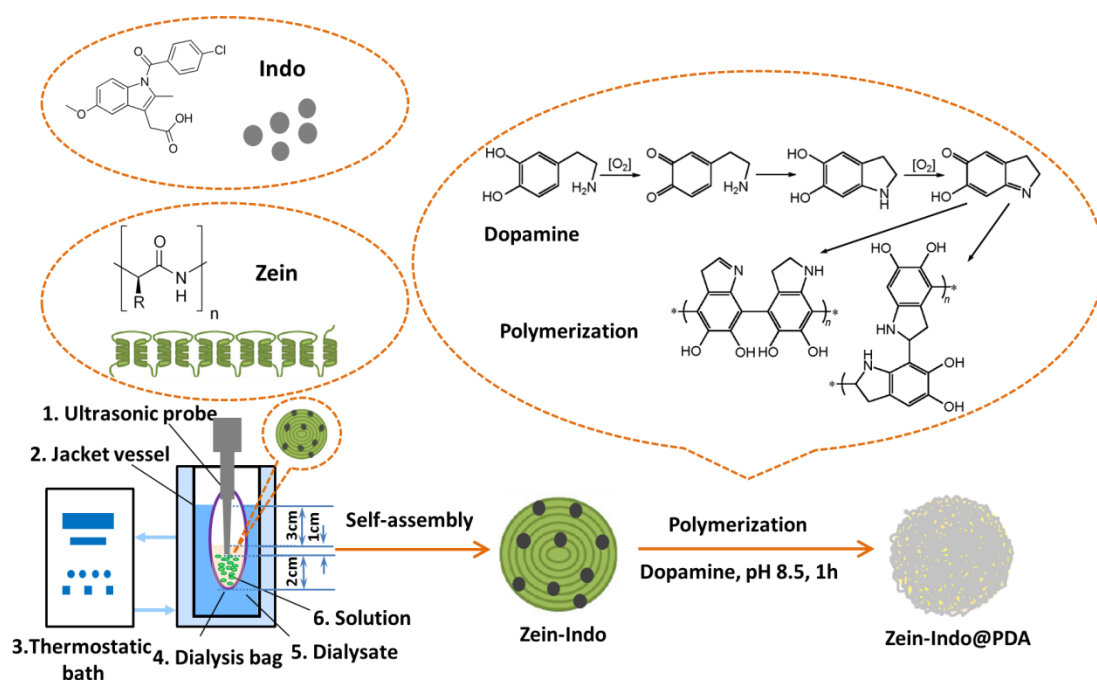


Fig. 1. Schematic diagram for preparation of Zein-Indo@PDA.

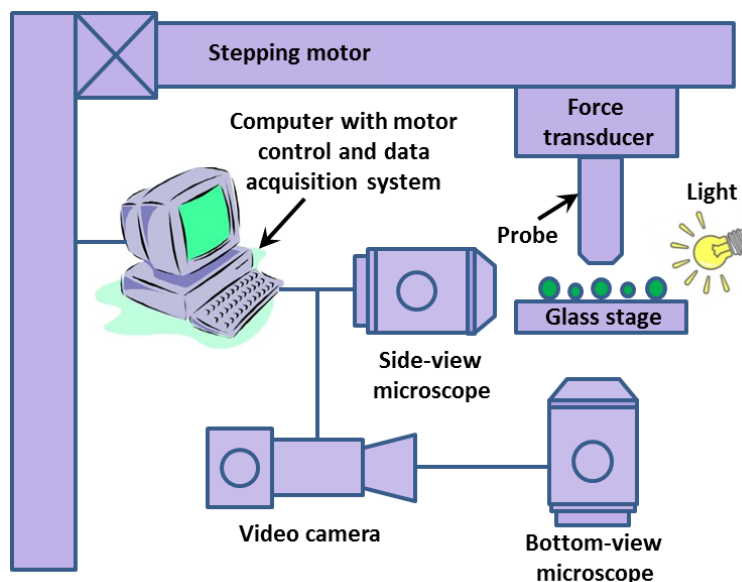


Fig. 2. Schematic diagram of the micromanipulation rig.

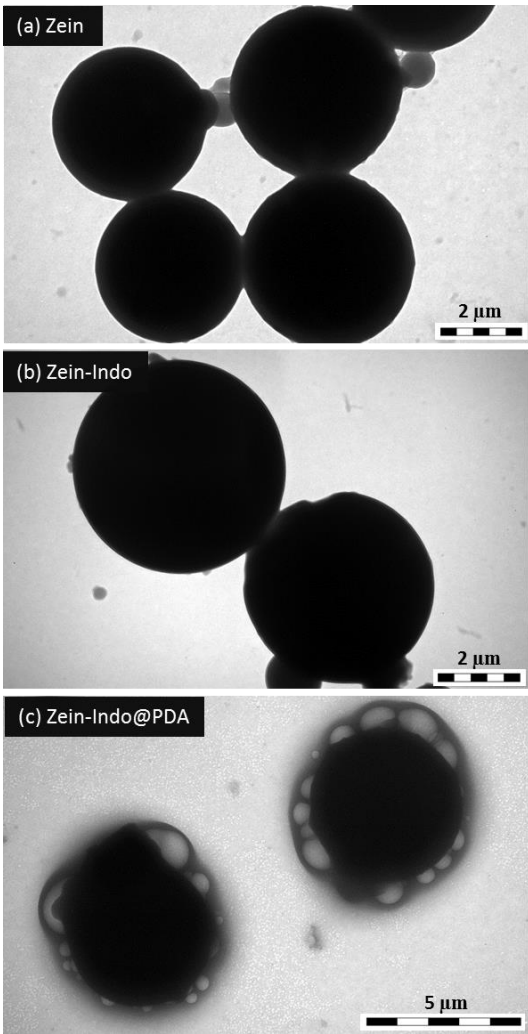


Fig. 3. TEM images of Zein (a), Zein-Indo (b), and Zein-Indo@PDA (c).

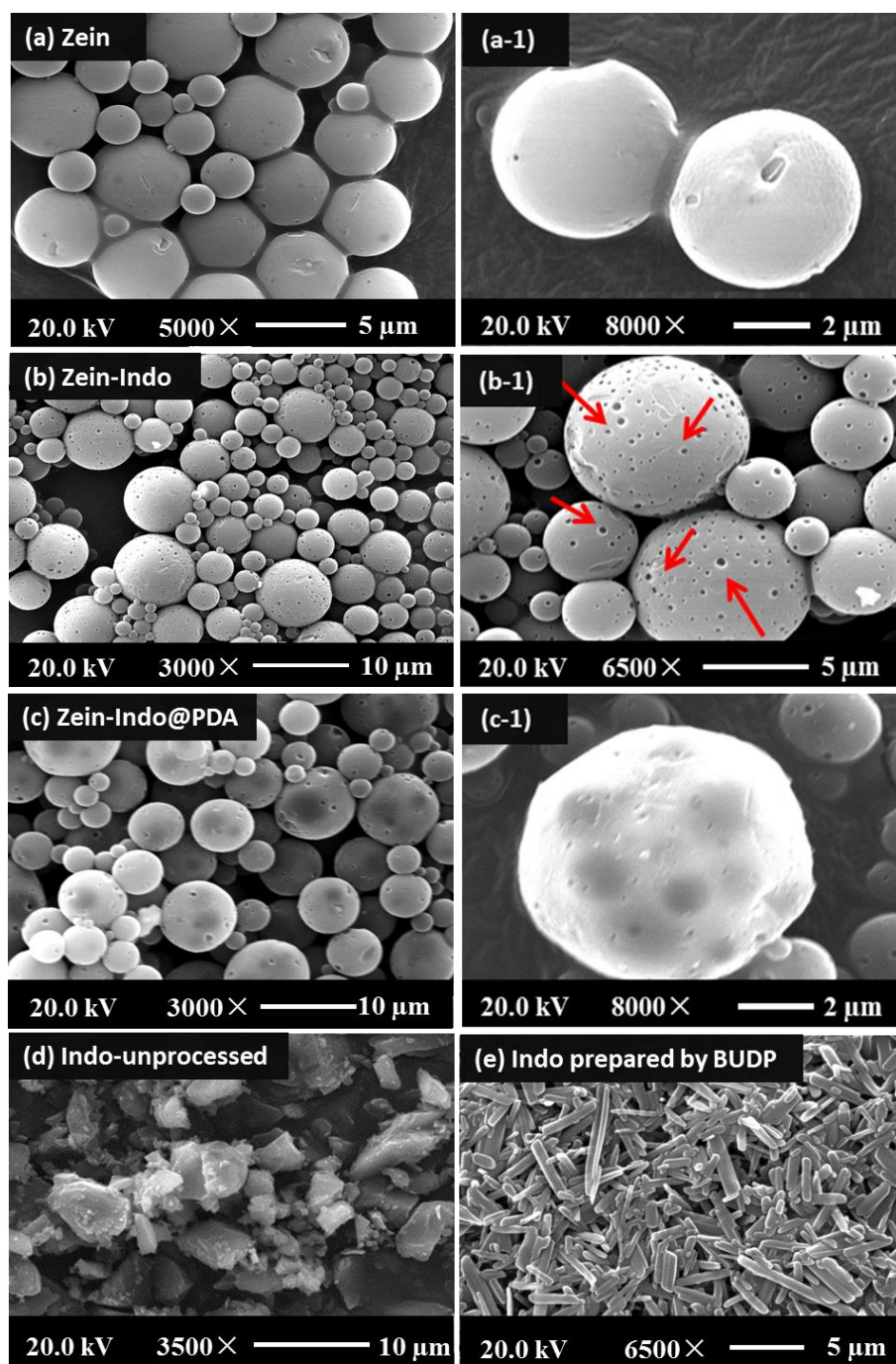


Fig. 4. SEM images of Zein (a), Zein-Indo (b), Zein-Indo@PDA (c), unprocessed Indo (d) and Indo prepared by the BUDP method (e). Images (a-1) to (c-1) represent the higher magnification of (a) to (c), respectively.

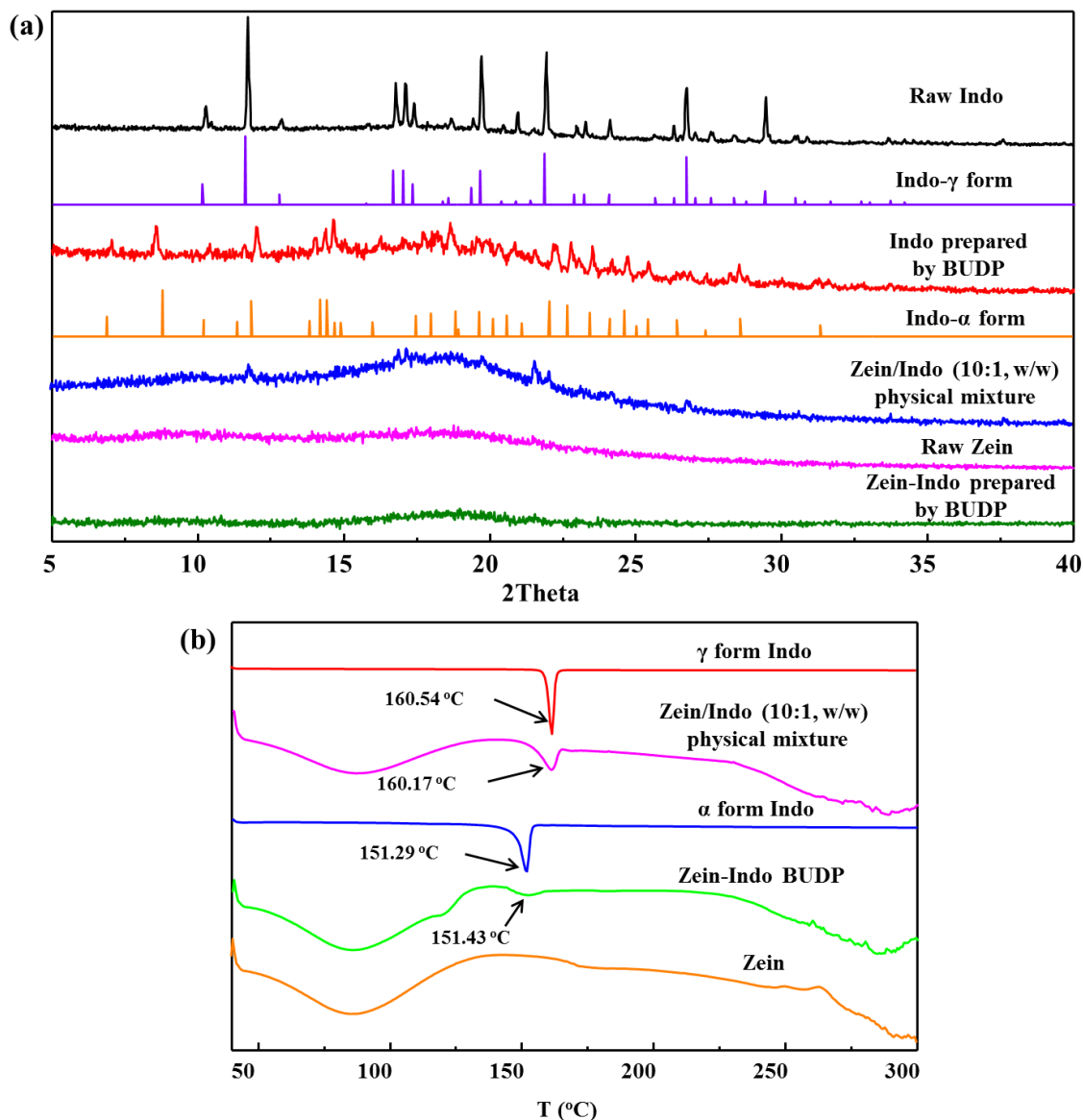


Fig. 5. Powder X-ray diffraction (a) and thermal properties (b) of different solid state forms of particles.

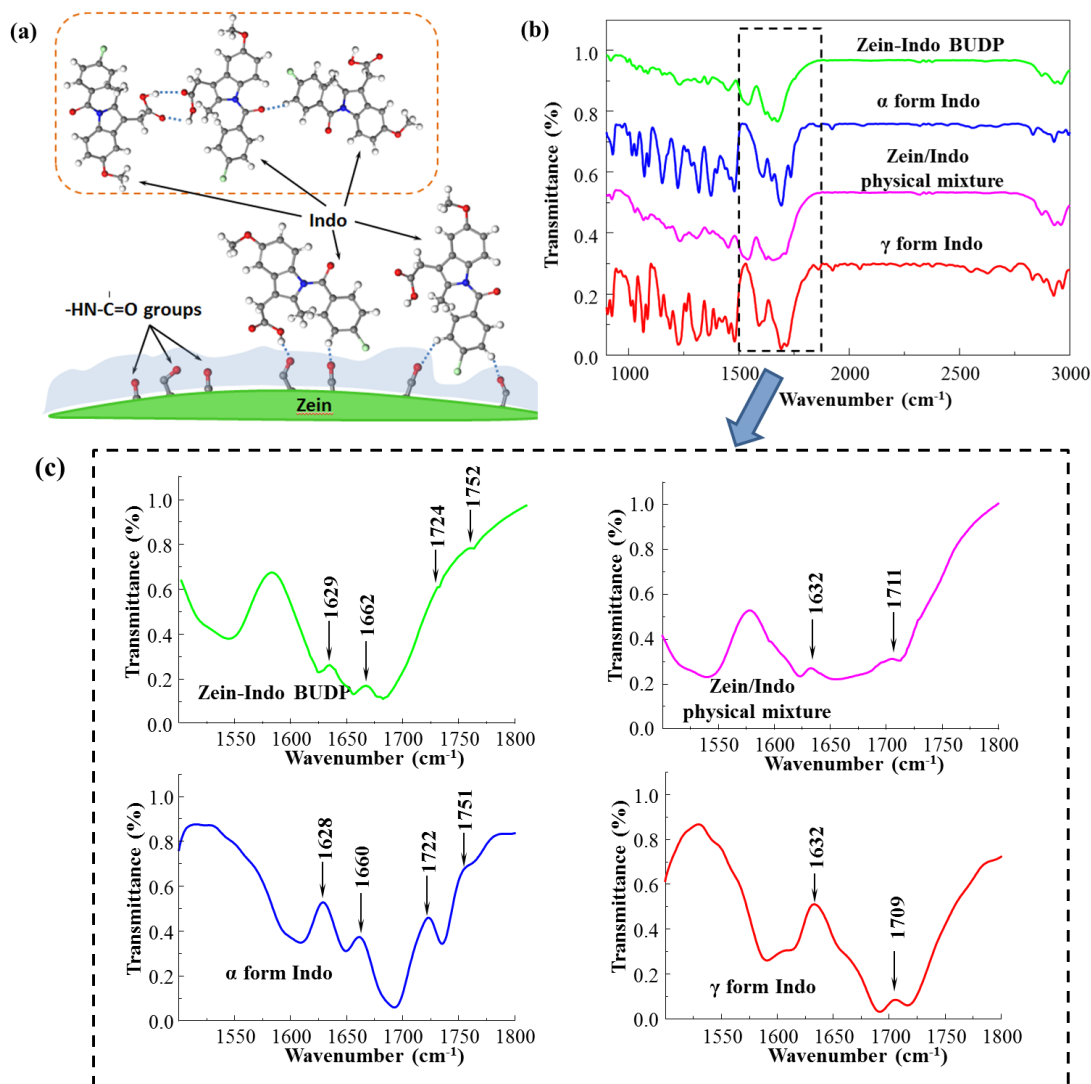


Fig. 6. (a) The possible intramolecular and intermolecular hydrogen bonds between Zein and Indo. (b, c) FTIR spectra of Zein-Indo particles prepared by BUDP and Zein/Indo physical mixture (10:1, weight ratio) as well as α and γ form Indo in the 800-3000 cm^{-1} (b), and 1500-1800 cm^{-1} regions (c).

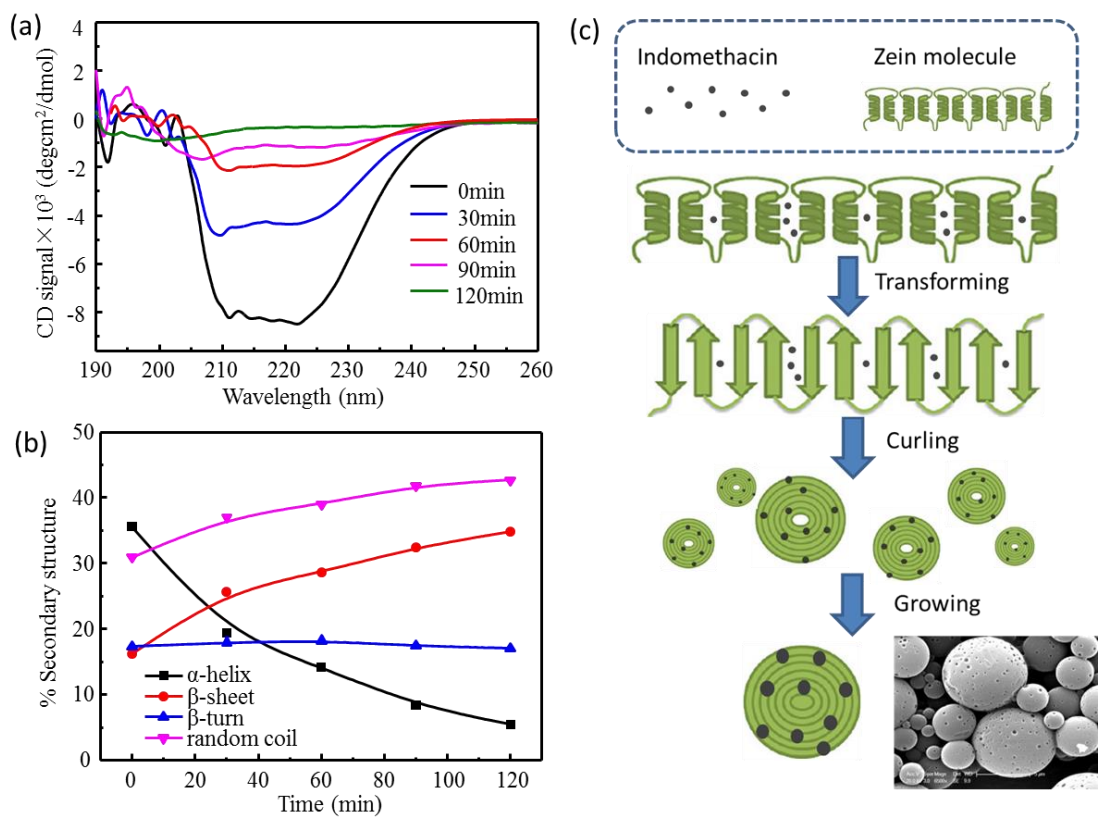


Fig. 7. (a) CD spectra of Zein/Indo samples and (b) their contents of secondary structures at different time intervals during BUDP. (c) Illustration of the self-assembly process of Zein-Indo from single molecules to microspheres.

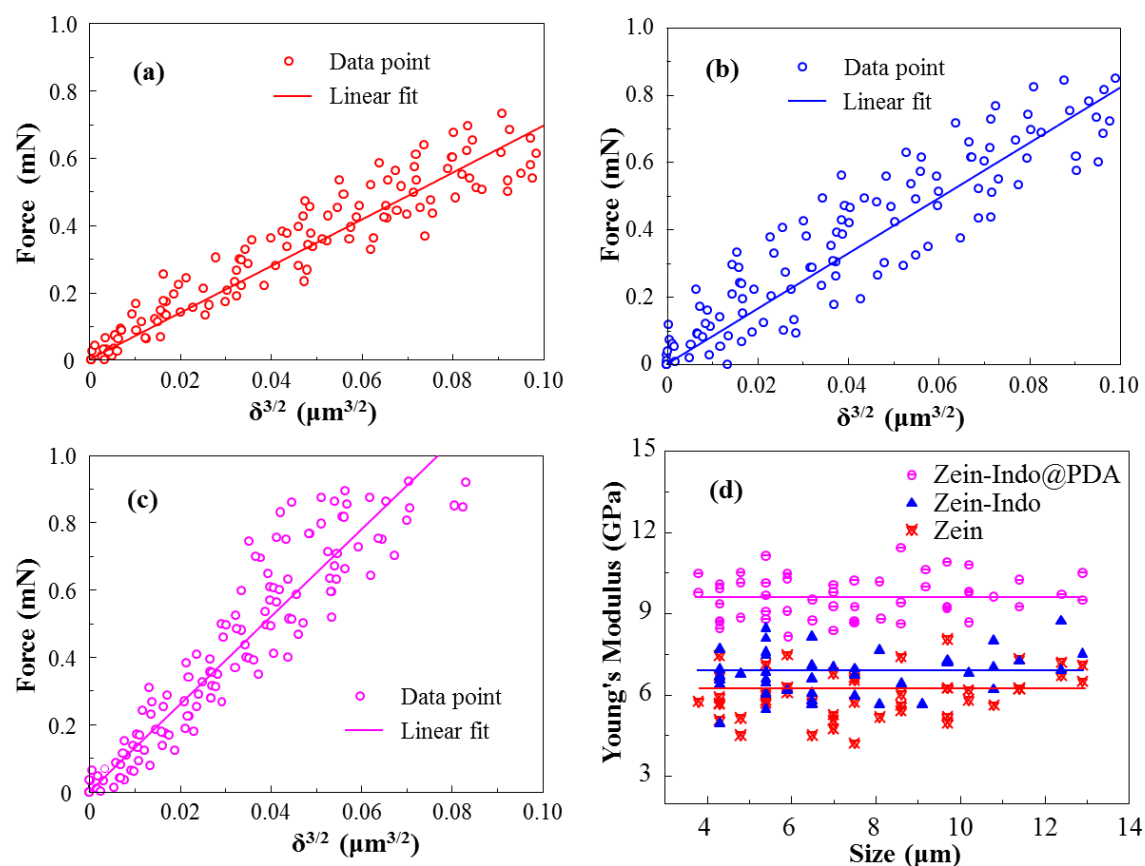


Fig. 8. Force-displacement data corresponding to small deformations fitted with the Hertz model (straight line): (a) Zein; (b) Zein-Indo; (c) Zein-Indo@PDA; (d) Young's modulus value versus particle size. 20 single particles were randomly selected from each sample.

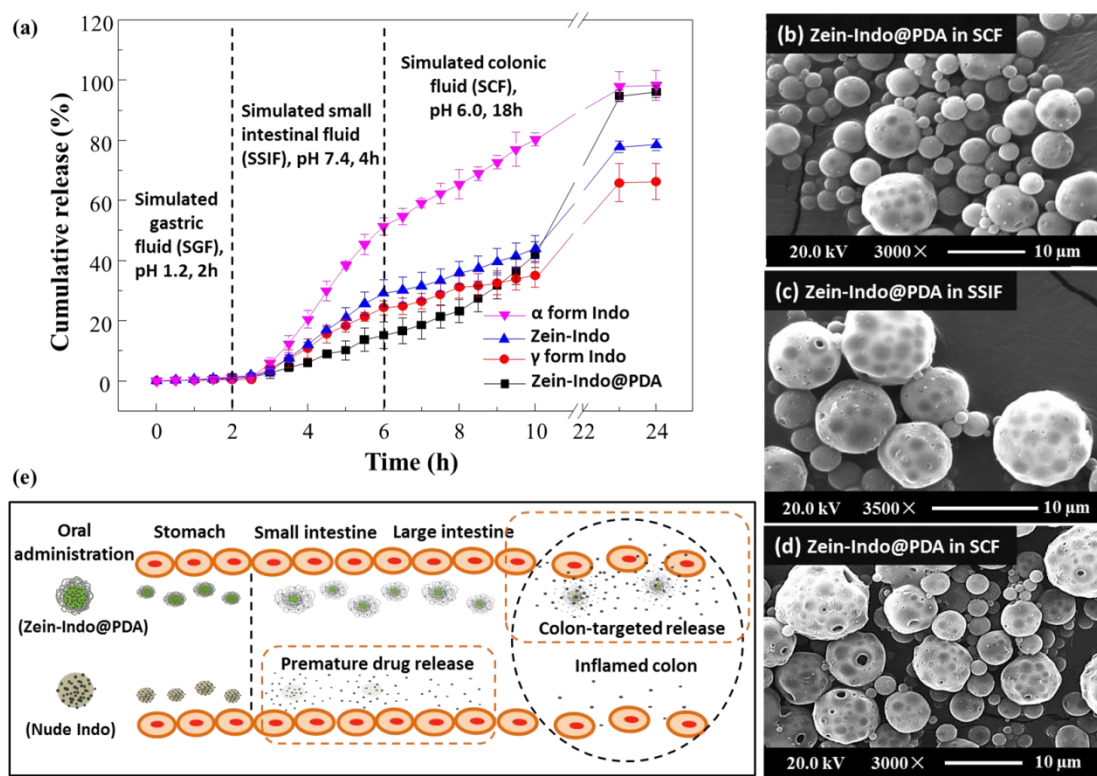


Fig. 9. (a) In vitro release of Indo from Indo-tethered microspheres or indo crystals in simulated gastrointestinal environment at 37 °C, namely simulated gastric fluid (SGF, pH 1.2) for 2 h, simulated small intestinal fluid (SSIF, pH 7.4) for 4 h and simulated colonic fluid (SCF, pH 6.0) for 18 h. (b-d) Representative SEM images of Zein-Indo@PDA after incubation in SGF for 2 h (b), SSIF for another 4 h (c) and SCF for further 18 h (d). (e) Proposed mechanism of drug release from the coated and uncoated Zein@PDA matrices after oral administration, highlighting the importance of Zein@PDA carrier, which prevents premature drug release and achieves colon-targeted drug delivery.

TOC Graphic

