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Synthesis of a Novel Chitosan-Based Polymer and Application as a Matrix for Controlled Drug Delivery

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ABSTRACT

The Ugi reaction was used to prepare hydrophobically modified chitosan through covalent tethering of benzaldehyde, phenyl acetic acid, and cyclohexyl isocyanide. The new polymer was characterized by infrared spectroscopy and differential scanning calorimetry. The generated semi-synthetic polymer was employed to prepare a drug-loaded matrix that was evaluated *in vitro* as potentially orally administered sustained-release delivery system. Caffeine was used as the loaded model drug. The generated matrix proved to be successful in prolonging drug release with near zero order release kinetics.

Keywords: chitosan, Ugi reaction, drug delivery, caffeine, polymer, matrix.

Abbreviation: (CBPI) chitosan benzaldehyde-phenyl acetic acid-cyclohexyl isocyanide

INTRODUCTION

The last two decades have witnessed great interest in the biodegradable, nontoxic, polycationic natural polymer chitosan as one of the most widespread polysaccharides for various pharmaceutical and biological applications.[1,2] Chemically, chitosan is poly-2-amino-2-deoxy-D-glucose units linked through β-(1 → 4) bonds.[3] It is semi-synthetically produced from the natural biopolymer chitin via a partial deacylation reaction. However, the many merits of chitosan over chitin, such as enhanced solubility and biodegradability, have highlighted its superior status in agrochemical, medical, environmental, and food applications.[4]

In the area of drug delivery, chitosan has shown useful properties as a carrier system for different active pharmaceutical ingredients (APIs) prompting its use in a variety of pharmaceutical formulations.[5] For example, the anticancer drugs cisplatin and doxorubicin were loaded in carbon nanotube-chitosan-folate carrier systems to allow sustained anticancer activities.[6,7] Moreover, chitosan was used in the preparation of both micro- and nanoparticles-based drug delivery systems.[8] Various peptides/proteins have been loaded in chitosan based microparticles, e.g., for fast release of insulin.[9] Recently, Saber et al. were able to prepare three safe structurally different chitosans for effective delivery of neomycin to the inner ear.[10]

Drug loaded chitosan hydrogels have been accomplished using diffusion, entrapment and tethering techniques.[11,12] The simplest being the diffusion systems which release their loaded active ingredients through slow diffusion.[13] In this context, Shiraishi et al. prepared chitosan tripolyphosphate–based microspheres and loaded them with indomethacin. The resulting beads exhibited sustained release patterns upon oral administration to Beagle dogs.[14] Larger APIs are usually entrapped within the chitosan matrices via chemical tethering. For example, paclitaxel-albumin complex was tethered to chitosan to allow slow release and to avoid an initial burst release effect.[15] Similarly, chitosan hydrogels with immobilized chondroitin sulfate successfully enhanced cartilage formation in animal models.[16] In another example, bone morphogenetic protein (BMP)-7

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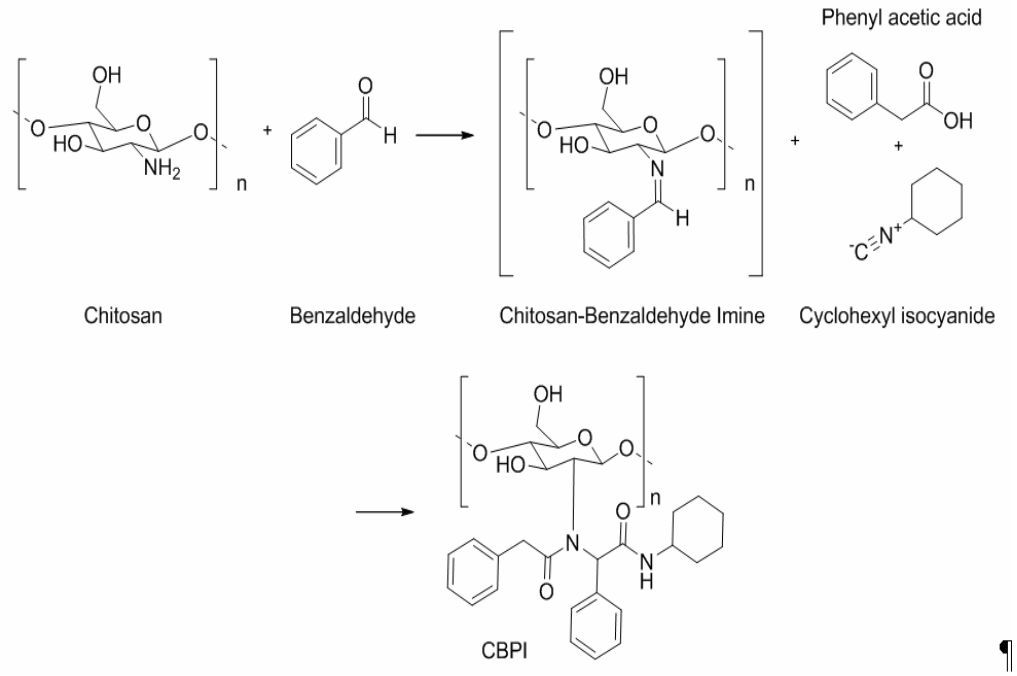
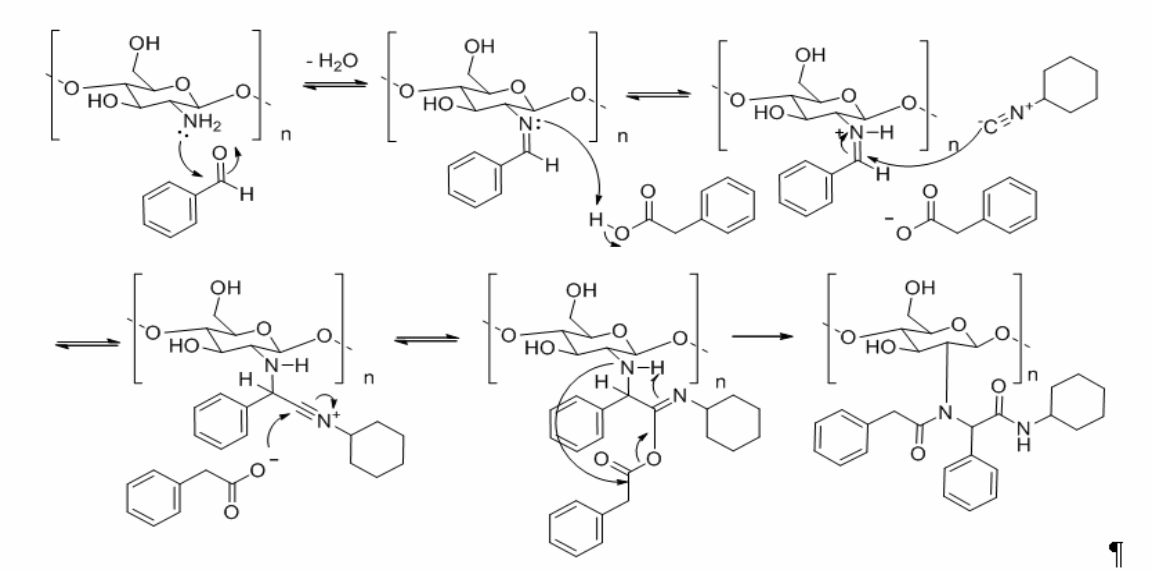
coupled with chitosan hydrogels proved very successful in promoting lesion repair in animal models.[17] Likewise, mixed microsphere systems based on chitosan–albumin adducts have successfully maintained the release of growth hormones over three weeks upon administration as subcutaneous implants in rats. [13,18]

However, the main disadvantage of utilizing unmodified chitosan for peroral controlled drug delivery is its pH-dependent release kinetics. [19] In fact, matrices based on low and medium molecular weight chitosan undergo rapid erosion in the acidic environment of the stomach leading to the rapid release of loaded drug content.[20] This pitfall prompted us to envisage a hydrophobic modification of chitosan via the Ugi reaction, such that a hydrophobic aldehyde (benzaldehyde) and carboxylic acid (phenyl acetic acid) are attached to the amine group of chitosan via an isocyanide (cyclohexyl isocyanide) to yield a chitosan benzaldehyde-phenyl acetic acid-cyclohexyl isocyanide (CBPI) adduct. The introduced attachments should also reduce the ionizability of chitosan under acidic conditions by partially consuming the amino substituents of the polymer by the Ugi reaction, thus rendering chitosan matrices more stable to acidic conditions. To the best of our knowledge, this modification is a completely novel approach to hydrophobically modify chitosan for potential application in drug delivery.

The Ugi reaction is a four-component condensation reaction,[21] in which an aldehyde or a ketone, an amine, an isocyanide, and a carboxylic acid can form a bis-amide. It is considered a prime example of a multi-component reaction (MCRs) which proceeds through the formation of an imine as a result of the condensation of the aldehyde and primary amine. Upon that, the isocyanide and carboxylic acid are added to the imine intermediate which, via an acyl transfer, rearranges to the bis-amide product (Schemes 1 and 2).[22]



Scheme 1. Synthesis of CBPI via the Ugi reaction on chitosan



Scheme 2.The proposed mechanism for the Ugi reaction in the formation of CBPI

RESULTS AND DISCUSSION

Synthesis and Characterization of CBPI

CBPI was prepared using the Ugi reaction. Scheme 1 shows the reaction steps performed on chitosan to yield CBPI. Chitosan was dissolved in an acidic aqueous solution prior to the addition of benzaldehyde. The mixture was stirred at an elevated temperature to allow dehydration to form the expected imine. Protonation of the formed imine from the phenyl acetic acid activates the iminium ion to react with cyclohexyl isocyanide via a nucleophilic addition reaction. This step yielded a nitrilium ion which reacted with phenyl acetate via a second nucleophilic addition followed by a rearrangement to form CBPI. Scheme 2 shows the proposed mechanism of the Ugi reaction towards the formation of CBPI.

Reaction progress was probed using an infrared spectrophotometry and differential scanning calorimetry (DSC). By comparing the IR spectrums of CBPI and chitosan (fig. 1), the C-H stretch in aromatics is observed at 3067 and 3030 cm^{-1}, One can also clearly see that the most distinctive difference is the presence of several C=C aromatic stretching bands at 1565 cm^{-1} indicating more than one aromatic benzene, and an out of plane aromatic C-H bending band at 703 cm^{-1}, both of which correspond to the newly introduced aromatic benzene rings originating from benzaldehyde and phenyl acetic acid starting materials. [23] However, the overwhelming presence of N-H bending vibrations in the region from 1580 to 1650 cm^{-1}, corresponding to the amino substituents of chitosan's poly-glucosamine units, concealed C=O stretching bands of the introduced amidic bonds of CBPI. Nevertheless, the emergence of an extra spike at 1454 cm^{-1} in the FTIR spectrum of CBPI is probably related to the C-N stretching of the newly introduced bis-amidic groups in CBPI.

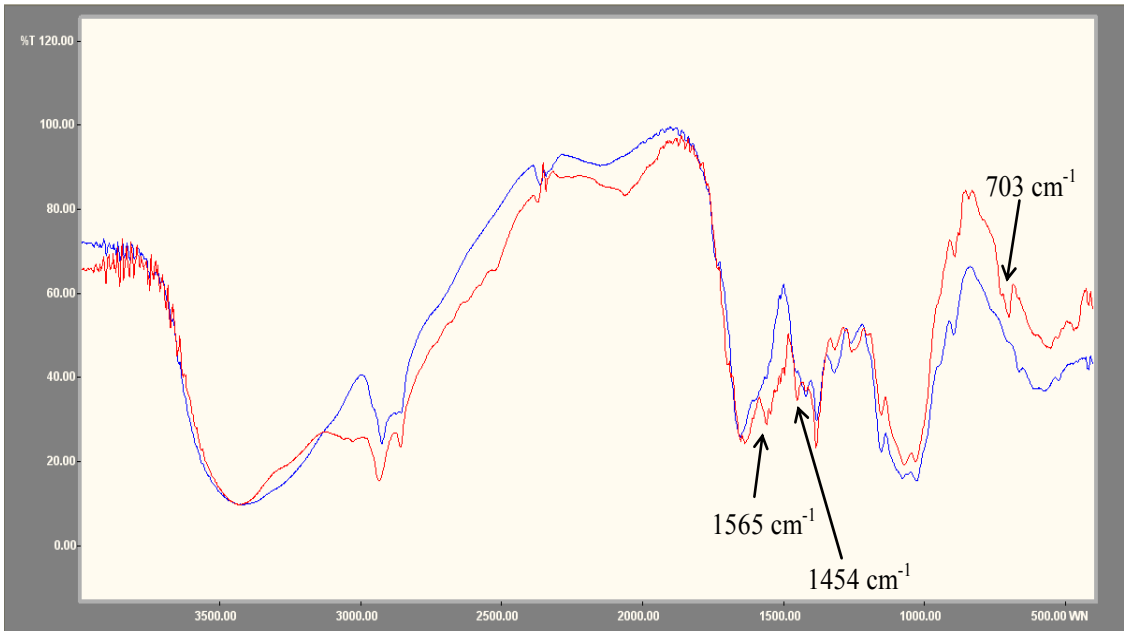


Figure 1. An overlay of the FTIR spectrums of chitosan (blue line) and CBPI (red line)

Figure 2 shows the DSC thermograms of chitosan (base and protonated) and CBPI. Clearly from the figure, protonated chitosan exhibits two significant endothermic peaks, at 85 °C and 203 °C, respectively, while chitosan base showed only a single endothermic band at 85 °C. The first bands probably correspond to the heat of evaporation of the associated water of hydration, while the second band (at 203 °C) represents the heat required to break various intra- and intermolecular hydrogen bonding interactions within the polymeric matrix (i.e., melting). These interactions are particularly enhanced by the ionization of the polymeric amine functions.

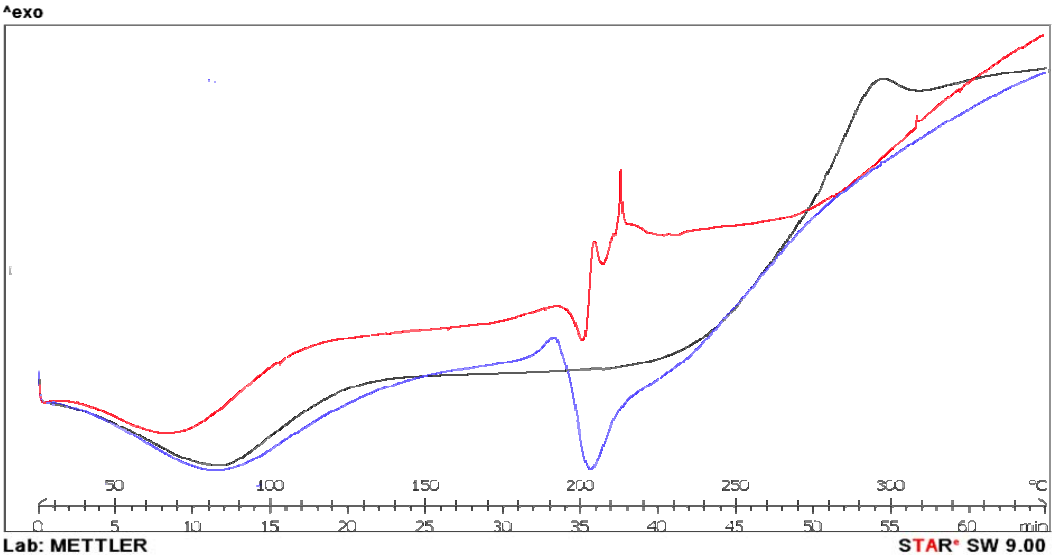


Figure 2. An overlay of the DSC thermograms of chitosan (base "black" and protonated "blue" with 1% HCl) and CBPI "red"

Interestingly, CBPI's evaporation band has shifted to 67 °C, which can be probably explained by the newly introduced hydrophobic moieties (two benzene aromatic rings and an aliphatic cyclohexyl ring per amine residue). These substitutions should weaken hydrogen bonding interactions connecting water molecules to the polymeric backbone leading to the observed downward shift in the heat of evaporation. Furthermore, the steep band at 203 °C seen for protonated chitosan is much shallower and split in CBPI despite being exposed to the same amounts of HCl (1% v/v HCl, 25 ml). This behavior is probably due to the newly introduced hydrophobic aromatic rings and cyclohexyl groups, which should sterrically separate adjacent polymeric backbones and therefore weaken cross-linking intermolecular hydrogen bonds connecting them and reduce the enthalpic energy required for breaking intermolecular hydrogen bonds within the matrix.

In conclusion, both FTIR and DSC data confirmed that chitosan was chemically modified by the Ugi reaction.

Release of Caffeine from CBPI Matrix Tablets

Figure 3 shows the release profiles of caffeine from chitosan and CBPI tablets. It can be clearly seen from the figure that the CBPI matrix sustained the release of caffeine with ≥ 85 % released after 8 hours. Furthermore, the release profile approached zero order kinetics (after an initial release burst within the first thirty minutes) and was independent of the dissolution medium pH. On the other hand, the chitosan matrix rapidly disintegrated and dissolved under the acidic condition of the simulated gastric fluid releasing all its caffeine contents within thirty minutes.

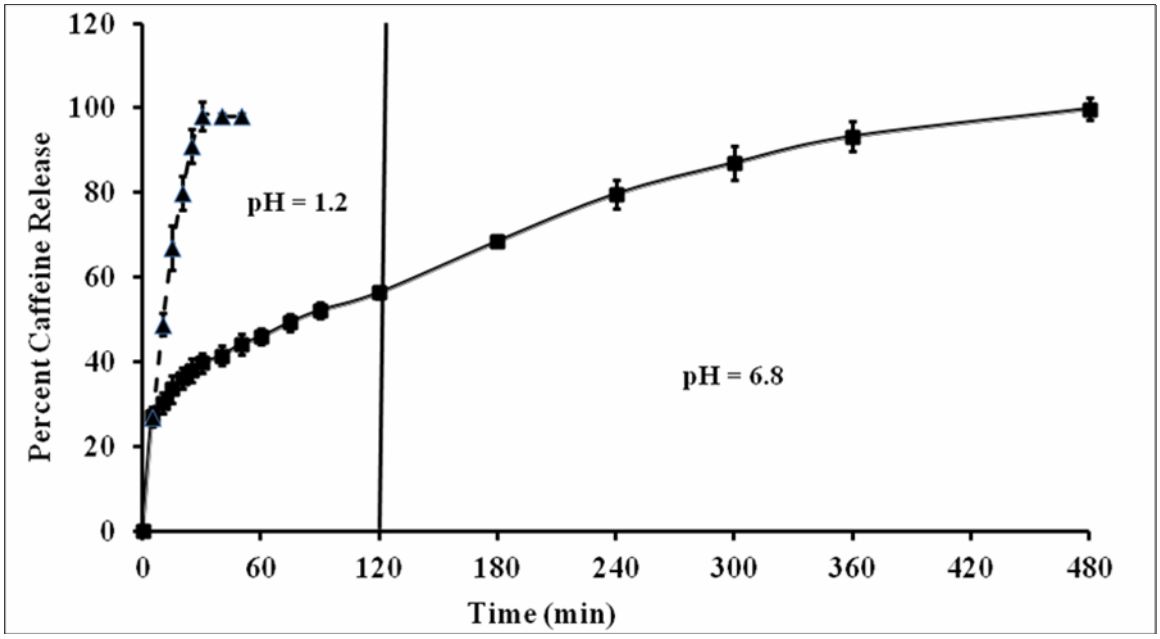


Figure 3. The release profiles of caffeine from chitosan–based matrix (▲) compared to CBPI-based matrix (■). Each profile resembles the release measurements from three separate preparations. The error bars represent the standard deviation of the three measurements.

Apparently, the newly introduced aromatic and cyclohexyl rings in CBPI acted as hydrophobic barriers that hindered water diffusion across the matrix and hence slowed down the release of caffeine molecules during dissolution. Moreover, the newly introduced substituents reduced significantly the number of ionizable amine

moieties rendering the matrix dissolution pH independent. In contrast, the presence of numerous amino substituents in chitosan is responsible for the complete dissolution of chitosan and release of caffeine in the simulated gastric fluid (pH 1.2).

EXPERIMENTAL

Materials

Commercially available analytical grade reagents were used without further purification. Chemicals were purchased from the appropriate commercial sources: benzaldehyde, medium molecular weight chitosan, cyclohexyl isocyanide, phenyl acetic acid, and potassium bromide were purchased from Sigma-Aldrich (USA); caffeine from Fluka (China); acetone and ethyl acetate from TEDIA (USA); absolute ethanol from S&C Chemicals (UK); and methanol from AZ Chemicals (USA).

Preparation of CBPI-Polymer

Benzaldehyde (1.183 g, 11.16 mmol) and methanol (15 ml) were added to a magnetically stirred solution of medium molecular weight chitosan (1.500 g, eq. 9.3 mmol of amine) in a freshly prepared aqueous HCl solution (1%v/v, 37.5 ml). The reaction was stirred at room temperature overnight. Subsequently, the magnetic stirrer was replaced with an overhead mechanical stirrer (Stuart Scientific Stirrer SS3 Soft Start, UK) operating at 500 rpm. Then a methanolic solution (30 ml) of phenyl acetic acid (1.518 g, 11.16 mmol) and cyclohexyl isocyanide (1.217 g, 11.16 mmol) was added drop wise to the reaction mixture over 5 minutes, and the mixture was stirred at 45 °C over 6 hours. Thereafter, distilled water (75 ml) was added to the reaction mixture and stirred at the same temperature for 24 hours. Subsequently, another portion of water (75 ml) was added and the mixture was stirred for a further 24 hour period. Finally, the reaction was cooled down to ambient temperature and acetone (100ml) was added to the gummy mixture. The product was then precipitated using ethyl acetate (100 ml), and the resulting precipitate was pressed against a sieve (mesh number = 325), washed with cold ethanol (100 ml) and acetone (100 ml), filtered and dried overnight at 50 °C to yield CBPI (2.320 g) (Scheme 1) .

FTIR Spectroscopy

The FTIR spectra of chitosan and CBPI were recorded on a Rayleigh WQF-520 FTIR BRAIC spectrophotometer (Beijing, China). The dried polymers were crushed and KBr discs were prepared from the resulting fine powder.

Thermal Analysis

Differential scanning calorimeter thermograms were recorded on a Mettler TA3000 System. The dried matrices (chitosan and CBPI, 10 ± 2 mg) were placed in aluminum pans and heated at a rate of 5 °C/min from ambient temperature to 360 °C.

Preparation of CBPI-Caffeine Loaded Tablets

CBPI (1.900 g) was dissolved in an acetonic aqueous solution (50% v/v, 200 ml) by magnetic stirring at room temperature overnight. Subsequently, a solution of caffeine (0.100 g) in methanol (60 ml) was added to the resulting polymeric solution and left to stir for 24 hours. The mixture was then slowly evaporated and dried in an oven until a constant weight was achieved. The formed solid was then ground using pestle and mortar and passed through a mesh no. 40 sieve. Weighed samples of the resulting powder (0.280 g) were then compressed in a 13-mm diameter round flat-faced punch and die set using a manually-operated hydraulic press (Riken Seiki, Japan) at a high compression force (57 kN) for 10 seconds to ensure minimum attainable porosity. The resulting tablets were used to carry out the dissolution study.

For comparison purposes, tablets were prepared from the solid dispersion of chitosan and caffeine by a similar procedure, albeit using 0.1 N HCl instead of acetonic aqueous solution to dissolve the chitosan.

In Vitro Release Experiments

Caffeine release experiments were performed in a USP Apparatus 1 (basket) dissolution system (Pharma Test PTW 2, Germany) at 50 rpm using 900 ml of HCl (0.1 N) for 2 hours followed by a phosphate buffer (pH 6.8) for 6 hours at 37 ^{o}C (± 0.5). Samples (5 ml) were withdrawn at predetermined time intervals (5, 10, 15, 20, 30, 60, 75, 90, 120, 180, 240, 300, 360, and 480 min), and then filtered. The concentration of dissolved caffeine was determined by UV spectroscopy (Milton Roy

Spectronic 601, USA) at λ_{max} 272 nm.

CONCLUSION

In conclusion, CBPI was successfully prepared. Furthermore, caffeine-loaded CBPI matrix was produced and was shown to retard drug release under physiologically simulated pH conditions. The results suggest the potential usefulness of CBPI as an orally administered and sustained release dosage form. The release profile approximates zero-order release kinetics which further underlines the significance of the new matrix.

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REFERENCES

(1) Mansouri S., Lavigne P., Corsi K., Benderdour M., Beaumont E. and Fernandes J.C. Chitosan-DNA nanoparticles as non-viral vectors in gene therapy: strategies to improve transfection afficacy. Eur. J. Pharm. Biopharm. 2004; 57: (1), 1–8.

(2) Peniche C., Arguelles-Monal W. and Goycoolea F.M. Chitin and chitosan: major sources, properties and applications Monomers, Polymers and Composites from Renewable Resources. Belgacem MN, Gandini A, Elsevier (Editors). Amsterdam, 2008; Chap 25.

(3) Roberts G.A. Chitin chemistry. The Macmillan press, Basingstoke, Great Britain. 1992.

(4) Dasha M., Chiellini F., Ottenbrite R.M. and Chiellini E. Chitosan-A versatile semi-synthetic polymer in biomedical applications. Progress in Polymer Science. 2011; 36: 981–1014.

(5) Park J.H., Saravanakumar G., Kim K. and Kwon I.C. Targeted delivery of low molecular drugs using chitosan and its derivatives. Adv. Drug. Deliv. Rev. 2010; 62: 28–41.

(6) Akbuga J. and Bergisadi N. Effect of formulation variables on cisplatin loaded chitosan microsphere properties. J. Microencapsul. 1999; 16: 697–703.

(7) Huang H., Yuan Q., Shah J.S. and Misra R.D.K. A new family of folate-decorated and carbon nanotube-mediated drug delivery system: Synthesis and drug delivery response. Advanced Drug Delivery Reviews. 2011; 63: 1332–1339.

(8) Agnihotri S.A., Mallikarjuna N.N. and Aminabhavi T.M. Recent advances on chitosan-based and micro-nanoparticles in drug delivery. J. Control Release. 2004; 100: 5–28.

(9) Grenha A., Seijo B. and Remunan-Lopez C. Microencapsulated chitosan nanoparticles for lung protein delivery Eur. J. Pharm. Sci. 2005; 25: 427–37.

(10) Saber A., Strand S.P. and Ulfendahl M. Use of the biodegradable polymer chitosan as a vehicle for applying drugs to the inner ear. Eur. J. Pharm. Sc. 2010; 39: 110–5.

(11) Holland T.A., Tessmar J.K., Tabata Y. and Mikos A.G. Transforming growth factor-β 1 release from oligo (poly(ethylene glycol) fumarate) hydrogels in conditions that model the cartilage wound healing environment. J. Control Release. 2004; 94: 101–14.

(12) Lin C.C. and Anseth K.S. PEG hydrogels for the controlled release of biomolecules in regenerative medicine. Pharm Res. 2009; 26: 631–43.

(13) Bhattarai N., Gunn J. and Zhang M. Chitosan-based hydrogels for controlled, localized drug delivery. Adv. Drug Deliv. Rev. 2009; 62: 83–99.

(14) Shiraishi S., Imai T. and Otagiri M. Controlled release of indomethacin by chitosan-polyelectrolyte complex: optimization and *in vivo/in vitro* evaluation. J. Control Release. 1993; 25: 217–25.

(15) Jauhari S. and Dash A.K. A mucoadhesive *in situ* gel delivery system for paclitaxel. AAPS PharmSciTech. 2006; 7: E53/1–E53/6.

(16) Suh J.K. and Matthew H.W. Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: a review. Biomaterials. 2000; 21: 2589–98.

(17) Mattioli-Belmonte M., Gigante A., Muzzarelli, R.A., Politano R., De Benedittis A., Specchia N., Buffa A., Biagini G. and Greco F. N,N-dicarboxymethyl chitosan as delivery agent for bone morphogenetic protein in the repair of articular cartilage. Med. Biol. Eng.Comput. 1999; 37: 130–4.

(18) Elcin Y.M., Dixit V. and Gitnick G. Controlled release of endothelial cell growth factor from chitosan-albumin microspheres for localized angiogenesis: *in vitro* and *in vivo* studies. Artif Cells Blood Substit. Immobil. Biotechnol. 1996; 24: 257–71.

(19) Thomas C. and Chandra P.S. Chitosan matrix for oral sustained delivery of ampicillin. Biomaterials. 1993; 14: (12), 939–44.

(20) Prabaharan M. Chitosan Derivatives as Promising Materials for Controlled Drug Delivery. J. Biomater. Appl. 2008; 23: 5-36.

(21) Ugi I. The α-addition of immonium ions and anions to isonitriles accompanied by secondary reactions. Angewandte Chemie International Edition. 1962; 1: (1), 8–21.

(22) Domling A., Wang W. and Wang K. Chemistry and Biology Of Multicomponent Reactions. Chemical Reviews. 2012; 112: (6) 3083-3135.

(23) Silverstein R.M., Webster F.X. and Kiemle D.J. Spectrometric Identification of Organic Compounds. Infrared Spectroscopy.7th ed., John Wiley & Sons, 2005; p. 80-106.

تركيب لكيتوسان باستخدام تفاعل الأوغي وتطبيقه كمصفوفة دوائية لنظام إيصال الدواء

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ملخـص

تم تعديل الكيتوسان ليصبح كارهاً للماء باستخدام تفاعل الأوغي، وذلك بربط كل من مركبات البنزيلديهايد والفينيل حمض الخليك و حلقة الهكسل ايزوسيانيد بالمبلمر الطبيعي. استخدمت تقنيتي التحليل الطيفي بالأشعة تحت الحمراء والمسح الحراري التباييني لتشخيص خصائص المبلمر الجديد. فعالية هذا المبلمر المعدل كيميائياً كنظام إيصال دواء ممتد الإطلاق عن طريق الفم وقد تم تقييمها مخبرياً، وذلك بإعداد مصفوفة دوائية من المبلمر محملة بالكافيين كنموذج مادة دوائية. المصفوفة الدوائية المعدة نجحت في إطالة إطلاق الكافين بحركية إطلاق تقارب النظام الصفري لحركيات الإطلاق الدوائية.

الكلمـات الدالـة: الكيتوسان، تفاعل الأوغي، المصفوفة الدوائية، نظام إيصال الدواء.

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