Drug Development and Industrial Pharmacy, 2011; 37(11): 1282–1289

ISSN 0363-9045 print/ISSN 1520-5762 online
DOI: 10.3109/03639045.2011.569934

informa
healthcare

RESEARCH ARTICLE

In vitro evaluation of compression-coated glycyl-L-histidyl-L-lysine–Cu(II) (GHK–Cu^{2+})-loaded microparticles for colonic drug delivery

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Abstract

Glycyl-L-histidyl-L-lysine–Cu(II) (GHK–Cu^{2+})-loaded Zn-pectinate microparticles in the form of hydroxypropyl cellulose (HPC) compression-coated tablets were prepared and their *in vitro* behavior tested. GHK–Cu^{2+} delivery to colon can be useful for the inhibition of matrix metalloproteinase, with the increasing secretion of tissue inhibitors of metalloproteinases (TIMPS),which are the major factors contributing in mucosal ulceration and inflammation in inflammatory bowel disease. The concentration of peptide was determined spectrophotometrically. The results obtained implied that surfactant ratio had a significant effect on percent production yield (1.25 to 1.75 w/w; 72.22% to 80.84%), but cross-linking agent concentration had not. The entrapment efficiency (EE) was found to be in the range of 58.25–78.37%. The drug-loading factor significantly increased the EE; however, enhancement of cross-linking agent concentration decreased it. The release of GHK–Cu^{2+} from Zn-pectinate microparticles (F1–F8) in simulated intestinal fluid was strongly affected by cross-linking agent concentration and drug amount (50 mg for F1–F6; 250 mg for F7–F8), but not particularly affected by surfactant amount. Release profiles represented that the microparticles released 50–80% their drug load within 4 h. Therefore, the optimum microparticle formulation (F8) coated with a relatively hydrophobic polymer HPC to get a suitable colonic delivery system. The optimum colonic delivery tablets prepared with 700 mg HPC-SL provided the expected delayed release with a lag time of 6 h. The effects of polymer viscosity and coat weight on GHK–Cu^{2+} release were found to be crucial for the optimum delay of lag time. The invention was found to be promising for colonic delivery.

Keywords: Microparticles, GHK–Cu2+, compression-coating, pectin, HPC, colonic drug delivery

Introduction

The colonic drug delivery systems would be preferred for the local treatment of the bowel and for the effective protection of peptide drugs that are not stable in the milieu of the stomach and the small intestines[1,2]. The colon itself is susceptible to many disease states including irritable bowel syndrome and more serious disease such as inflammatory bowel disease (e.g. Crohn's disease and ulcerative colitis), carcinomas, and infections[3]. At the present time, these diseases are often poorly and inefficiently managed either by oral drugs that are largely absorbed or decomposed before they reach the colon, or by rectal administration[3,4]. The colon is upside down "u"-shaped organ, which makes the application of drugs so difficult to the ascending and transverse colon via rectal route. For the treatment of a colonic disease, anti-inflammatory agents, chemotherapeutics, or antibiotics need to be present in the colon. Site-specific local delivery may permit their utilization and also permit lower dosing resulting in fewer side effects. Systemic absorption and topical application of many drugs including peptides were achieved in the colon. The way that proteolytic enzymes, which are excreted into the small intestines, are mainly reabsorbed from the distal parts of the ileum makes the application of a peptide possible from colon in comparison with intestinal application[4,5]. Colon as a site offers distinct advantages on account of a neutral pH, a much longer transit time, reduced digestive enzymatic

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(Received 25 October 2010; revised 14 January 2011; accepted 04 March 2011)

activity, much greater response to absorption enhancers, and the presence of large amounts of microbial enzymes[6]. Various systems have been developed for colon-specific drug delivery. These include covalent linkage of a drug with carrier, pressure-controlled delivery systems, coating with pH-sensitive polymers, time-dependent release systems, pH- and time-dependent systems, and enzymatically controlled delivery systems[7–10]. To reach the colon and to be able to specifically deliver a peptide or a protein drug, the dosage form must be formulated taking into account the obstacles of upper gastrointestinal (GI) tract and advantages of colonic environment.

The natural tripeptide glycyl-L-histidyl-L-lysine (GHK) is a typical matrikine that modulates new connective tissue formation. It was first isolated from human plasma in 1973[11]. GHK was first described as a liver cell growth factor; later, it was proved to stimulate the growth, differentiation of a number of cell lines, regulation of cell activities, and wound healing activator. It plays an important role in copper ion uptake into cells and it spontaneously forms a high affinity complex with copper (II) ions to form GHK–Cu^{2+} solution[12,13]. In the wound healing process, the copper peptide activity begins immediately following injury during the inflammatory stage. GHK–Cu^{2+} protects tissue by acting as anti-inflammatory agent that limits oxidative damage after tissue injury, and by suppressing local inflammatory signals. Moreover, GHK–Cu^{2+} acts as an activator that signals the removal of damaged tissue and promotes insertion of healthy tissue[14–16]. Recent evidence demonstrated that the increased expression and activity of matrix metalloproteinases (MMPs) may contribute to the intestinal tissue injury and inflammation. Inhibition of MMPs is conducted by a group of endogenous MMP inhibitors, known as tissue inhibitors of metalloproteinases (TIMPS). It is also known that GHK–Cu^{2+} increased the secretion of TIMPS. The MMPs have been implicated as one of the major factors contributing to mucosal ulceration as well as inflammation in inflammatory bowel diseases[17–19].

Based on the information summarized above, MMP inhibition by increasing the secretion of TIPMs by GHK–Cu^{2+} might be another therapeutic approach to control inflammatory response in the colon. In two of our previous studies, we successfully showed the usefulness of compression-coating for colonic drug delivery. The use of compression-coating tablet combined with high-viscosity HPMC would result in a long lag time (6 h) and followed by an immediate release phase achieved with pectin–HPMC mixture[20,21]. In both studies 5-aminosalicylic acid and nisin, a naturally occurring ribosomally synthesized antimicrobial protein, containing core tablets were compression-coated with pectin–HPMC mixture. The main reason for selecting pectin was its biodegradation in the colon by colonic flora. On the other hand, high-molecular-weight HPMC increases the mechanical strength of the tablet wall around a drug core during its transportation in the GI tract. It also provides resistance to GI enzymes. *In vitro* test results suggested that an optimum system for colonic delivery would be obtained using 20% HPMC (100,000 cP) and 80% pectin USP100 mixtures as coating material. A 5% increase of HPMC in the polymer mixture caused 2-h lag time for active moiety. No drug release was observed with such system up to 6 h. Combination of (80:20%) pectin–HPMC had appropriate mechanical strength and also had a desired erosion profile based on a 6-h colon arrival time concept. Hence, in this study it was our hypothesis that with the positive contribution of HPC compression-coating, GHK–Cu^{2+}-loaded Zn-pectinate microparticles would be a good candidate for a new colonic delivery system for the topical treatments of inflammatory bowel disease. We aimed to combine the advantages of enzymatically degradable Zn-pectinate microparticles and time-dependent dissolution of HPC compression coat to design a colonic delivery system that would prevent the drug release before colonic arrival. The limitations of time-dependent release system alone are: it is not able to sense any variation in the upper GI tract transit time, and any variation in gastric emptying time may lead to drug release in small intestine before arrival to colon. On the other hand, the most convenient approach for site-specific drug delivery to colon is enzymatically controlled delivery systems. No drug release can occur unless the system arrives to the colon. However, in our research prepared Zn-pectinate microparticles alone could not keep their drug content up to 6 h was our first reason for combining these two systems together. The other reason for incorporation of GHK–Cu^{2+} into microparticles in the first step was to prevent the tripeptide from the negative effect of compression pressure during the compaction procedure. To our knowledge, it is the first time GHK–Cu^{2+}-loaded pectin microparticles compression-coated with HPC polymer to form colonic drug delivery system. The system was evaluated *in vitro* in order to obtain the basic formulation characteristics.

Materials and methods

Materials

GHK–Cu(II) (GHK–Cu^{2+}) was kindly donated by GL Biochem (Shanghai) Ltd. (China) and Skin Biology (Bellevue, WA). GENU pectin type LM12 CG-Z (DE 30%) was obtained from CPKelco (Skensved, Denmark). Hydroxypropyl cellulose (HPC) (types HPC-SL, HPC-L, and HPC-H: 3.0–5.9 cp, 6.0–10.0 cp, 1000–4000 cp, respectively) was a gift from Nippon Soda Co. Ltd. (Tokyo, Japan). Biscyclohexanon-oxalyldihydrazone (cuprizone) was purchased from Fluka (Buchs, Switzerland). All other chemicals were of analytical grade.

Methods

General procedure for GHK–Cu^{2+} determination

It is possible to detect GHK–Cu^{2+} spectrophotometrically after complexation with cuprizone, which is a

well-known copper chelator and has been widely used for a copper detecting[22]. A buffer solution (pH 10.0) was prepared from ammonium chloride (54 g/L solution; Merck, Darmstadt, Germany) solution by adjusting the pH to 10.0 with ammonia solution (*25*% (w/w) NH_3 *solution;* Merck). A 0.1% cuprizone (Fluka) solution was prepared by dissolving 200 mg of cuprizone in 40 mL of 50% hot ethanol. The obtained solution was diluted with ethanol to 200 mL. GHK–Cu^{2+} was determined spectrophotometrically (UV-mini 1240, Shimadzu, Japan) at 600 nm as reported by Mazurowska and Mojski[22]. In brief, 1 mL of the solution from the stock was transferred into a 10-mL volumetric flask. One milliliters of 0.1% cuprizone solution and 2 mL of buffer solution were added. The mixture was diluted to 10 mL with water in volumetric flask and the absorbance was read at 600 nm[22,23].

Preparation of pectin–GHK–Cu^{2+} microparticles

The pectin microparticles were prepared by emulsion–dehydration technique as reported by Esposito et al.[24] Pectin 5% (w/v) and GHK–Cu^{2+} were dissolved in 50 mL of water and stirred overnight to solubilize completely. This drug–polymer solution was dispersed in 100 mL isopropyl isostearate at 50°C containing different amounts of Span 80 to form a w/o emulsion. The cross-linking was accomplished during microsphere production. One minute after the formation of the emulsion, 1 to 5 mL of $Zn(CH_3COO)_2{\cdot}2H_2O$ solution was added to the emulsion under stirring. After 5 min, the solution was rapidly cooled to 15°C and then 100 mL of acetone was added in order to dehydrate the pectin droplets. This system was maintained under mechanical agitation with propeller stirrer at 1000 rpm at room temperature for 60 min to allow the complete solvent evaporation. The pectin particles were isolated from the suspension by filtration. The microparticles were washed with acetone, dried at 30°C, and kept in an airtight container[25,26]. Table 1 shows formulation codes and variables of pectin microparticles.

Particle shape and size determination of microparticles

The shapes of the dried microparticles were determined by optical microscopy (Olympus, SZX7, Japan). Microparticles were sized and the distribution of particle size was determined using laser diffraction method (Malvern Instruments Ltd., Worcestershine, UK).

Determination of percent yield and entrapment efficiency

The yield was determined by dividing the weight of the dried microparticles by the total initial weight of the polymer and GHK–Cu^{2+}. The 25-mg Zn-pectinate microparticles loaded with GHK–Cu^{2+} were dissolved in 10 mL of pH 6.8 phosphate buffer under agitation for 24 h. The solutions were filtered through 0.22 μm Millipore filters, and the amount of GHK–Cu^{2+} was assayed spectrophotometrically at 600 nm. Each determination was performed in triplicate. Percent drug entrapment efficiency (EE) was determined by the following formula:

$$\%\ \text{Drug entrapment} = \frac{\text{Mass of drug present in microparticles}}{\text{Mass of drug used in the formulation}} \times 100$$

GHK–Cu^{2+} release studies from microparticles

Release studies of GHK–Cu^{2+} from Zn-pectinate microparticles were carried out in pH 6.8 phosphate buffer. The 25-mg GHK–Cu^{2+}-loaded microparticles were placed into a container containing 40 mL of pH 6.8 phosphate buffer solution. Containers were shaken at 100 rpm at 37 ± 0.2°C using a water bath (Certomat WR, Braun Biotech International, Germany). At specific time intervals, 1 mL of samples were collected and replaced with 1 mL of fresh buffer. GHK–Cu^{2+} concentrations were determined spectrophotometrically.

Preparation of compression-coated tablets

GHK–Cu^{2+}-loaded Zn-pectinate microspheres were compression-coated with various grades of HPC (HPC-H, HPC-L, and HPC-SL) depending on the design. Before microsphere placement, using a 7-mm flat-faced punch a hole was made in order to make 3-mm coat thickness on the side walls in a 13-mm die to prevent premature release of drug and then microparticles were placed in this cavity. Maximum 75 mg of GHK–Cu^{2+}-loaded microparticles were placed in a 13-mm die cavity of the hydraulic press (ALPA, Turkey) then the second half portion of outer shell was added on top of the microspheres in the die cavity.

Table 1. Compositions of pectin microparticles.

Formulation code	Surfactant concentration (w/w)	GHK–Cu^{2+} amount (mg)	$Zn(CH_3COO)_2$ amount (M)	Percent of microsphere production yield (%)	Entrapment efficiency (%)
F1	1.25	50	1	72.22	72.95 ± 3.46
F2	1.50	50	1	76.38	71.16 ± 4.43
F3	1.75	50	1	80.84	71.88 ± 3.74
F4	1.50	50	1.5	74.85	67.36 ± 4.56
F5	1.50	50	2	73.99	62.96 ± 2.12
F6	1.50	50	2.5	75.04	58.25 ± 2.89
F7	1.50	250	4	71.76	78.37 ± 4.09
F8	1.50	250	5	70.89	73.43 ± 3.84

Experimental parameters were 5% pectin, pectin LM DE (30–35%), a 50-mm diameter vessel, a 35-mm four blade turbine rotor, 1000 rpm constant stirring speed, oil phase isopropyl isostearate.

Finally, 5 MPa pressure was applied to make the compression-coated tablets (Figure 6). The dwell time was 10 sec.

Physical characteristics of tablets

The prepared tablets were tested for weight variation, thickness, crushing strength, and friability. The weight variation and friability of tablets were determined according to the USP25. The weight variation of tablets was determined using analytical balance (Shimadzu TX-120, Japan). The friability of tablets was determined using a friability tester (Erweka, TA 120/220, Germany). The diametrical tablet crushing strength was evaluated using a tablet hardness tester (Model C50; I Holland, Nottingham, UK). The tablet thickness was measured using micrometer (Bestool-Kanon, Tokyo, Japan). The results were expressed as mean ± SD.

GHK–Cu^{2+} release studies of compression-coated tablets

The release of GHK–Cu^{2+} from compression-coated tablets was investigated using an *in vitro* USP rotating paddle dissolution apparatus (SOTAX A7, Basel, Switzerland). The dissolution studies were performed in enzyme-free simulated gastric and intestinal fluid. First medium was 300 mL of 0.1 N HCl solution. The USP25 dissolution apparatus II was used at 100 rpm at 37 ± 0.2°C. The test was continued for 2 h, at the end of the time period the medium was discarded and refilled with 300 mL of pH 6.8 phosphate buffer solution and the test was continued for 24 h. At various time intervals, a sample of 2 mL was withdrawn and replaced with the equal volume of fresh medium. Withdrawn samples were analyzed spectrophotometrically.

Results and discussion

Evaluation of general procedure for GHK–Cu^{2+} determination

It was clear from Figure 1 that GHK–Cu^{2+} solution in water, in pH 1.2 HCl solution, and in pH 6.8 phosphate buffer solution had no absorption peaks when they were scanned between 200 and 1100 nm without complexation with cuprizone. However, it is possible to detect GHK–Cu^{2+} spectrophotometrically after complexation with cuprizone (*N*,*N*′-di(cyclohexylidene)ethanedihydrazide) in water, in pH 1.2, and in pH 6.8 at 600 nm[23,27,28].

Evaluation of particle shape and size

Figures 2 and 3 show the shape and size distribution of obtained microparticles. The mean diameter of pectin microparticles varied from 35.73 to 45.75 µm for microparticles formulated with increasing GHK–Cu^{2+} amount from 50 mg (F2) to 250 mg (F8), respectively, and they also showed relatively narrow particle size distribution. Particle size distribution decreased with the increasing cross-linking agent concentration and increasing GHK–Cu^{2+} amount. $Zn(CH_3COO)_2$ and GHK–Cu^{2+} amount somehow promoted the formation of narrower particle size distribution (Figure 3). The diameter variation was thought to be because of decrease in polymer/drug ratio. Results were compiled with Sriamornsak and Nunthanid[29]. Choice of oil phase type, pectin type (LM and HM), pectin origin (citrus or apple), surfactant type, polymer/drug ratio were some of the important factors that affected the results of mean size of particle, particle shape, and distribution[30–32].

Evaluation of production yield and EE

Table 1 shows formulation codes, variables, percent product yield, and EE of pectin microparticles. The percent production yield was found between 70.89% and 80.84%. Changing the surfactant ratio had a significant effect on percent production yield. Increasing the surfactant amount from 1.25 to 1.75 increased the yield from 72.22% to 80.84% (F1 to F3). On the other hand, enhancement of the cross-linking agent concentration had no significant

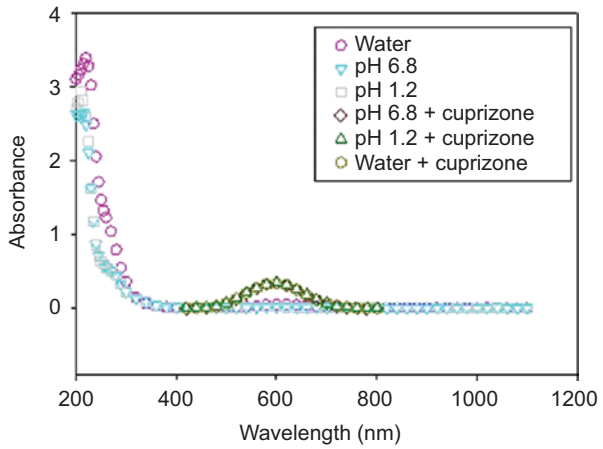

Figure 1. Spectra of GHK-Cu^{2+} with or without 0.1% cuprizone in different milieu.

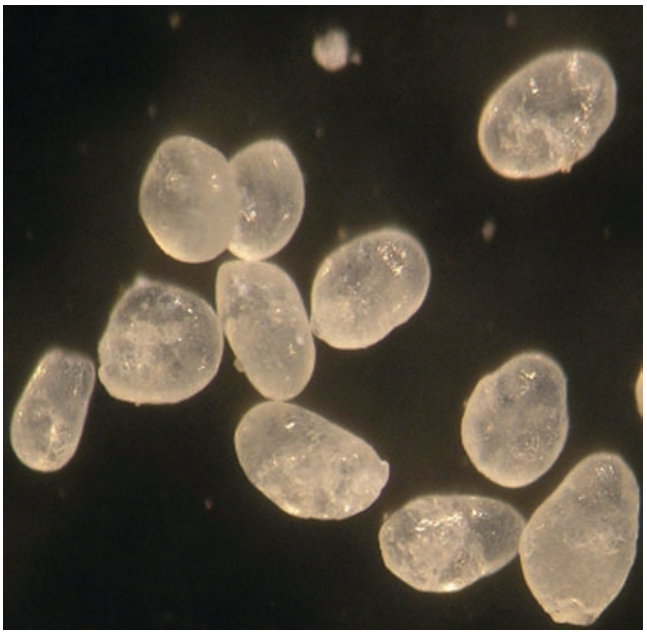

Figure 2. Optical evaluation of Zn-pectinate microparticles using Olympus SZX7 Microscope, Japan.

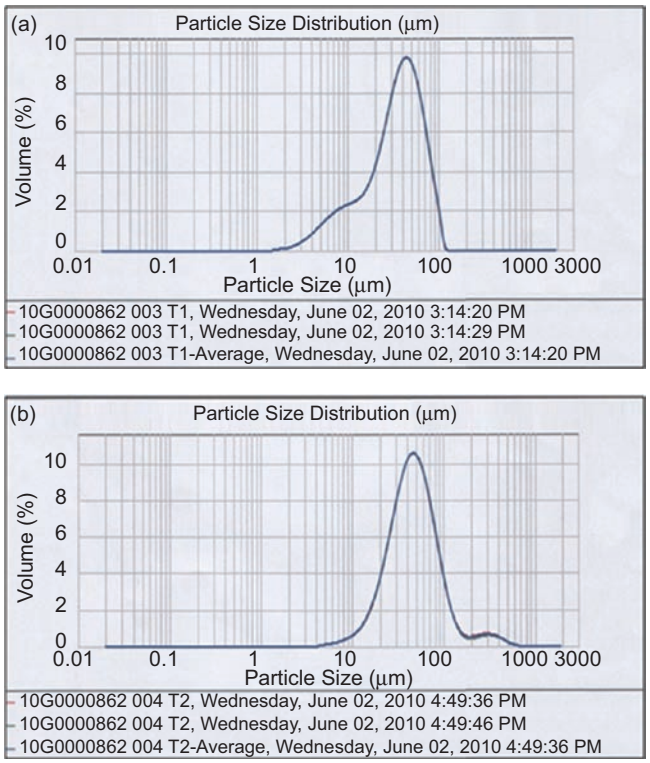

Figure 3. Particle size distribution of microparticles (A) F2 and (B) F8.

effect on yield. Although they had same surfactant ratio with F4–F6, the results obtained from F7 (71.76%) and F8 (70.89%) were lower than that of F4–F6 (74.85–75.04%). It was concluded that this lowering was due to decrease in polymer/drug ratio (Table 1).

With respect to the EE of the microparticles, the crucial role of the drug employed should be considered[30]. In particular, the hydrophilic–hydrophobic balance of drug was found to influence entrapment yield. Indeed, hydrophilic drugs can be more efficiently incorporated into hydrophilic microparticles. In our study, the EE was found to be in the range of 58.25–78.37% as shown in Table 1. It was observed that EE depended upon zinc ion concentration. The EE of microparticles with lower zinc ion concentration were greater than that of microparticles with higher one. Although GHK-Cu^{2+} was a water-soluble molecule, the EE was in the range of 58.25–78.37%. Zinc ions make cross-linking with pectin molecules resulting in relatively hydrophobic molecule when compared with pectin alone, so this situation changes the hydrophilic–hydrophobic balance to hydrophobic side and it prevents proper diffusion of GHK–Cu^{2+} inside the microparticles. When we evaluated EE results of F7 (78.37%) and F8 (73.43%) a little bit closer, it was found that EE was higher than that of F1–F6 (72.95% for F1; 71.16% for F2; 71.88% for F3; 67.36% for F4; 62.96% for F4; 58.25% for F6). The results complied with El-Gibaly[31]. In Table 1, no significant change in the percent EE was occurred with the change of concentration of surfactant. The factors affecting the percent EE were the concentration of zinc and the amount of drug. Obtained results were compiled with Sriamornsak[32,33].

Evaluation of GHK–Cu^{2+} release studies of microspheres

It was well-stated in literature[20,21] that pectin alone has the disadvantages of swelling and dissolving in aqueous environment, which makes it difficult for the use of colonic drug delivery. To prevent premature release of GHK–Cu^{2+} from pectin microparticles before colonic arrival, ionic cross-linking agent $Zn(CH_3COO)_2$ was added. Release studies were carried out to examine the suitability of Zn-pectinate matrix microparticles for colonic drug delivery. The release pattern was represented by plotting the percent GHK–Cu^{2+} release against time. The surfactant amount, cross-linking agent amount, and the amount of GHK–Cu^{2+} were investigated and their effects on the release of GHK–Cu^{2+} were shown in Figures 4 and 5. It was shown in Figure 4 that the release of GHK–Cu^{2+} from Zn-pectinate microparticles was significantly slower than the dissolution of GHK–Cu^{2+} powder alone. Pure GHK–Cu^{2+} powder dissolved within 30 min. The result was due to the application of a rate-controlling polymer matrix. It could be clearly seen from Figures 4 and 5 that the GHK–Cu^{2+} release from the microparticles were decreased with increasing cross-linking agent concentration. In formulation F1–F3, the concentration of the cross-linking agent was 1 M and microparticles released 80% of their GHK–Cu^{2+} load within 4 h. On the other hand, in F4–F6 the concentration of the cross-linking agent increased gradually with a level of 0.5 M and microparticles released 50–65% of their GHK–Cu^{2+} load within 4 h. The same results can be seen from Figure 5 (F7, F8). It was concluded that the release of GHK–Cu^{2+} from microparticles

was dependent upon zinc ion concentration. It was found that changing the surfactant amount did not significantly affect the release of GHK–Cu^{2+} from microparticles. The release profile presented in Figure 5 shows the effect of GHK–Cu^{2+} amount added in microparticles. F7–F8 contains five times higher GHK–Cu^{2+} amount compared with that of F1–F6. It was concluded that release of GHK–Cu^{2+} from microparticles F7 reached above 90% within 4 h and GHK–Cu^{2+} release from microparticles F8 reached above 60% within 4 h although they had higher amount of $Zn(CH_3COO)_2$ concentration. The fast release profile might be because of higher GHK–Cu^{2+} load or increase in drug/polymer ratio. Therefore, it appeared that the release of GHK–Cu^{2+} from microparticles was also dependent on the amount of GHK–Cu^{2+}. The findings indicated similarity with Sriamornsak and Nunthanid[29] and El-Gibaly[31]. When we considered all dissolution profiles, these formulations could not fulfill the requirements of colonic drug delivery. The suitable colonic drug delivery system should release a maximum 5–10% of its drug load within 6 h. However, our formulations released 50–80% of their GHK–Cu^{2+} load within 4 h. Therefore, we decided to coat the optimum pectin microparticle formulation (F8) with a relatively hydrophobic polymer HPC to get a suitable colonic drug delivery system.

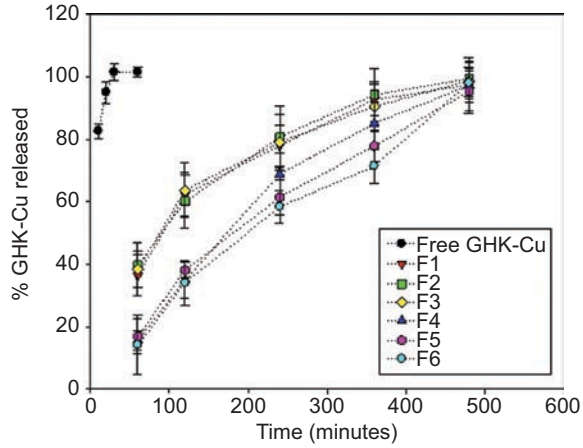

Figure 4. Percent of GHK-Cu^{2+} released from microparticles (F1–F6).

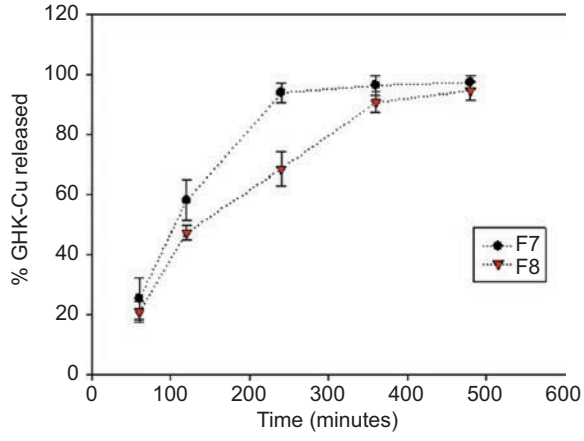

Figure 5. Percent of GHK-Cu^{2+} released from microparticles (F7, F8).

Evaluation of compression-coated tablet characteristics

GHK–Cu^{2+}-loaded Zn-pectinate microspheres were compression-coated with various grades of HPC (HPC-H, HPC-L, and HPC-SL) depending on the design (Figure 6 and Table 2). The crushing strength of compression-coated tablets was dependent on polymer type and polymer amount seen in Table 3. HPC-H, HPC-L, and HPC-SL have different viscosity. HPC-H has the highest viscosity (1000–4000; HPC-L: 6–10; HPC-SL: 3–6, respectively). The crushing strength increased with the enhancement of polymer viscosity, enhancement of HPC-H ratio in polymer mixture, and finally enhancement of polymer coat weight. HPC provides mechanical strength to the microparticles to be able to form tablets. All tablets complied with the pharmaceutical quality control standards.

Evaluation of release studies from compression-coated tablets

The system was designed based on the GI transit time concept under the assumption of colon arrival time of 6 h. In this study, we used HPC to enforce the tablet during its transit in the GI tract and to partially modify the high solubility of pectin. To mimic GI tract conditions, compression-coated tablets were kept 2 h in 0.1 N HCl solution and further 8–22 h in pH 6.8 phosphate buffer solution USP. Since HPC is an enzyme-resistant polymer, the studies were carried out without colonic enzymes (Figures 7 and 8). Retardation of release from microparticles using matrix type tablets was well-reported in the literature[31]. Although the studied system was matrix type tablet, there was still a release of 7–13% active agent before colonic arrival. In our study, we achieved to retard the release of GHK–Cu^{2+} from microspheres containing compression-coated tablets at least 6 h. When we compared the release of GHK–Cu^{2+} from tablets made with HPC-H, HPC-L, and HPC-SL in Figure 7 (CCT1, CCT2, CCT3, CCT4, and CCT7 has the coat weight of 700 mg HPC), it was found that the tablets coated with HPC-SL released GHK–Cu^{2+} more rapidly than that

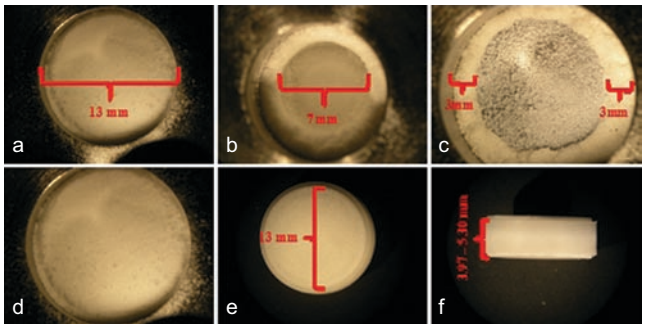

Figure 6. Manufacturing compression-coated tablets of GHK–Cu^{2+} microspheres. (A) First half of outer HPC shell. (B) A cavity made by a 7-mm punch to prevent the spreading around. (C) Placement of microparticles in the cavity. (D) Adding the second half of outer HPC shell. (E) and (F) Compressed tablet.

Table 2. Composition of compression-coated tablets containing GHK–Cu^{2+} microparticles.

Formulation code[a]	Polymers HPC-H	HPC-L	HPC-SL	Polymer ratio (%)	Polymer amount (mg)
CCT1	+			100	700
CCT2	+	+		80:20	700
CCT3	+	+		70:30	700
CCT4		+		100	700
CCT5			+	100	500
CCT6			+	100	600
CCT7			+	100	700

[a]Each compression-coated tablet contains 75 mg GHK–Cu^{2+} microparticle.

Table 3. Physical properties of HPC compression-coated tables.

Formulations[a]	Weight variation (mg)	Thickness (mm)	Hardness (N)	Friability (%)
CCT1	775.92 ± 1.30	5.30 ± 0.01	499.41 ± 8.82	<1%
CCT2	775.45 ± 0.93	5.30 ± 0.02	495.88 ± 9.60	<1%
CCT3	775.70 ± 0.77	5.31 ± 0.02	493.04 ± 9.90	<1%
CCT4	775.50 ± 0.57	5.30 ± 0.02	490.19 ± 6.07	<1%
CCT5	576.18 ± 0.89	3.97 ± 0.01	411.69 ± 4.80	<1%
CCT6	676.05 ± 0.86	4.65 ± 0.01	447.57 ± 7.15	<1%
CCT7	776.36 ± 0.73	5.28 ± 0.01	488.53 ± 4.31	<1%

[a]Each compression-coated tablet contains 75 mg GHK–Cu^{2+} microparticle; diameter of punch used in compression-coated tablets was 13 mm.

of tablets coated with HPC-L; HPC-H:L polymer mixture, and HPC-H. This was because of lower molecular weight of HPC-SL compared with others. Release rate of drug increased as viscosity of HPC decreased. HPC-SL dissolved more quickly than other polymers. Drug release from HPC polymer such as those used in the current study will be controlled by the rate of hydration of polymer and the swelling properties of the gel formed on hydration, which influence the drug diffusion and gel dissolution. Rapid hydration is required to establish the gel layer and prevent the release of an initial burst of drug. On the other hand, polymer amount was also an effect on the release of (Figure 8) GHK–Cu^{2+} from compression-coated tablets (500 mg for CCT5; 600 mg for CCT6; and 700 mg for CCT7). The thicker the gel layer, the longer is the diffusional path and the stronger the gel formed around the microparticles. The lag time of drug release from HPC coating layer was delayed because of the coat that interacted with water and formed a hydrogel. Based on the obtained results, (Figure 8) a 100 mg increase of HPC-SL amount in the coat caused a 2-h lag time for GHK–Cu^{2+} release. The 100% HPC-SL and at least 700 mg coat amount was necessary for an optimum colonic drug delivery system. On the other hand, 100% HPC-H, HPC-H:L (80:20), and HPC-H:L (70:30) compression-coated tablets did not release GHK–Cu^{2+} during predetermined time of the dissolution study (Figure 7).

Conclusion

GHK–Cu^{2+}-containing microparticles were compression-coated with HPC polymer. *In vitro* test results suggested

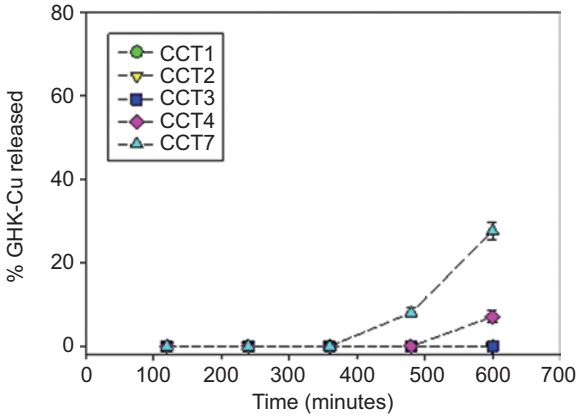

Figure 7. Percent of GHK–Cu^{2+} released from tabletted microparticles that were compression-coated with various HPC polymers.

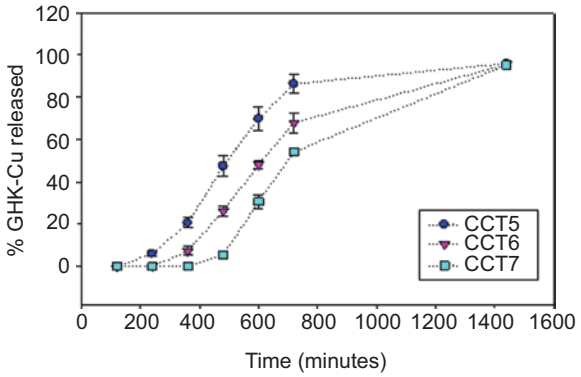

Figure 8. Percent of GHK–Cu^{2+} released from tabletted microparticles that were compression-coated with different coat weight of HPC-SL polymers.

that an optimum system for colonic delivery would be obtained using 700 mg of HPC-SL as coating material and had a 3-mm coat thickness on the side walls in a 13-mm die. No drug release would be expected from such a system up to 6 h. Under the influence of GI tract milieu, the coat erosion controlled the release of GHK–Cu^{2+}. Finally, pectin microparticles alone were not effective or sufficient for the colonic drug delivery. Using compression-coating, manipulation of dissolution was relatively easy. Changing both the molecular weight of the coating polymer and the amount of coat, one can modify the dissolution rate from compression-coated tablets. GHK–Cu^{2+}-loaded Zn-pectinate microparticles compression-coated with HPC would be a good candidate for designing a colonic delivery system for the topical treatments of inflammatory bowel diseases.

Acknowledgements

The authors would like to thank to GL Biochem (Shangai) Ltd., China for kind donations of GHK–Cu^{2+} and acknowledge the generous donations of CPKelco, Denmark and Nippon Soda Co. Ltd., Japan, respectively.

Declaration of interest

This study was supported by the Scientific Research Project Unit of Marmara University (Project Number: SAG-A-080410-0066). The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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