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Research Article

Combination of inulin-shellac as a unique coating formulation for design of colonic delivery dosage form of ibuprofen

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ABSTRACT

A combination of inulin and shellac, when applied as a film coat, has a potential value as a colon-specific delivery system for ibuprofen. The inulin-based multi-unit pellet system is prepared by coating inulin and shellac sequentially around drug-loaded cores in a fluid-bed coater. The outer shellac coating protects the system against gastrointestinal environment and dissolves rapidly in small intestine, where a lumen pH around 7 triggers the dissolution of the enteric polymer. The inner inulin coating works as a time-controlled retardant and offers additional protection of the pellets until it is degraded by microbial enzymes at the colon region. *In vitro* data indicates that inulin is a promising coating material to obtain timed and enzyme-triggered ibuprofen release. Pharmacokinetic study in New Zealand rabbit confirmed the *in vitro* data and revealed a delayed absorption of about 3 h but with higher AUC. This result suggests that inulin-shellac system shows potential materials to provide a microbial triggered drug in the colon compartment. The higher AUC of ibuprofen from double coated pellet dosage form may indicate that absorption of ibuprofen in colonic compartment is more extensive than in gastric.

Keywords: ibuprofen; pellet; inulin; shellac; microbe-dependent colonic release; pharmacokinetic profile.

INTRODUCTION

Colon targeted drug delivery has the potential to deliver bioactive agents for the treatment of a variety of colonic diseases. Colon-specific delivery systems should prevent the release of the drug in the upper-part of gastrointestinal tract (GIT) and require a triggering mechanism to affect an abrupt release on reaching the colon. The site specific drug delivery to colon is important for the treatment of diseases associated with the colon, reducing the side effects of the drug and reducing the administered dose (Anekant et al., 2007, Basit 2005, Coveillo et al., 2005).

For a formulation to act as an effective colon specific drug delivery system, the primary condition is that a minimum amount of drug should be released in the environment of the upper gastrointestinal tract, i.e., in stomach and small intestine. The normal transit time in the stomach is 2 h (though this may vary), while in the small intestine it is relatively constant and is around 3 h (Rubinstein 1995). The usual colonic transit time varies from 20 - 30 h (Kinget et al., 1998).This, for a dosage form to be effective as a colon drug delivery system, the drug release is required to be retarded in the upper GIT conditions. Thereafter, the drug release should be complete within the next 20 - 30 h.

Various strategies, currently available to target the release of drugs to colon, include formation of prodrug, coating of pH-sensitive polymers, use of colon-specific biodegradable polymers, timed released systems, osmotic systems, and pressure controlled drug delivery systems. Among the different approaches to achieve targeted drug release to the colon, the use of polymers especially biodegradable by colonic bacteria holds great promise (Anekant et al., 2007).

The aim of this study was to explore the feasibility of the colonic microorganism to develop Colonic drug delivery system (CDDS). Ibuprofen was chosen as an active compound as this NSAID shows severe side effect on gastric when frequently given as a conventional oral preparation. In our previous study, using guar gum and Na alginate-Ca acetate in pellet formulation was not successfully retained the release of ibuprofen at upper part of GIT (data not published). Therefore, applying of polysaccharides for coating the pellet suggested avoiding premature release of the drug in intestinal region. Polymer of monosaccharide retains their integrity because they are resistant to the digestive action of gastrointestinal enzymes. The matrices of polysaccharides are assumed to remain intact in the physiological environment of stomach and small intestine but once they reach in the colon, they are acted upon by the bacterial polysaccharides and results in the degradation of the matrices. A large number of polysaccharides such as amylose, pectin, chitosan, inulin, cyclodextrins, chondroitin sulphate, dextrans, and

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Table 1: The composition of coating solutions

Ingredients	1st film coating	2nd film coating
Inulin (g)	10	-
Shellac (g)	-	10
PEG 6000 (g)	0.4	0.4
Coloring agent (g)	0.5	-
Aquadest (mL)	ad 100	-
Ethanol 95%	-	100

Table 2: The total weight gain developed during subsequent pellet coating

WG	F1	F2	F3
1st coating	5%	10%	15%
2nd coating	20%	15%	10%

dextrin have been investigated for their use in colon targeted drug delivery systems. The most important fact in the development of polysaccharide derivatives for colon targeted drug delivery is the selection of a suitable biodegradable polysaccharide. As these polysaccharides are usually soluble in water, they must be made water insoluble by cross linking or hydrophobic derivatisation. Very important is an optimal proportional of the hydrophobic and hydrophilic parts respectively and the number of free hydroxy groups in the polymeric molecule.

Inulin as a polysaccharide has been evaluated in design of colon drug delivery systems. Studies on degradation of cross linked hydrogels prepared by free radical polymerization of methacrylated inulin in the presence of inulinase showed that degradation of inulin increased by increase in enzyme concentration and incubation time (Vervoort et al., 1998). Moreover, suitability of inulin as a bacterially degradable polysaccharide has been evaluated in free films with Eudragit® RS by other investigations (Vervoort et al., 1996, Cavalcanti et al., 2002). The results of these studies indicated that inulin incorporated in Eudragit® RS or RL films can be degraded by colonic bacteria and increasing the amount of inulin renders the film more permeable. However there has been no report on the use of inulin in combination with shellac as a unique coating formulation for design of colonic delivery dosage form. Shellac is a naturally occurring material obtained from lac, a resinous secretion of the insect *Laccifero lacca* Kerr. *(Coccidae)* (Kibbe 2000). It is an inexpensive and abundantly available polymer and is used for enteric coating of tablets and beads.

To demonstrate the potential application of subsequent coating inulin-shellac on colonic delivery of ibuprofen, we performed pharmacokinetic study of this pellet in New Zealand rabbit.

Material and Method

Materials

Ibuprofen (kindly gift by PT Kimia Farma, Bandung-Indonesia), Avicel PH 101 (BratacoChemica, Bandung-Indonesia), Natrium alginate (BratacoChemica, Bandung-Indonesia), Calcium acetate, guargum (BratacoChemica, Bandung-Indonesia), polyvinylpirrolidone (PVP, BratacoChemica, Bandung-Indonesia), inulin (BratacoChemica, Bandung-Indonesia), shellac (PT Kimia Farma, Bandung-Indonesia), polyethileneglycol (PEG 6000, Merck, Darmstadt-Germany), acetonitrile (HPLC grade, J.T. Baker, USA.), glacial acetic acid (Merck, Darmstadt-Germany). Other materials used in this study are either proanalytic grade or pharmacopoeia quality.

Methods

Preparation and evaluation of pellet

Pellets were prepared by the process of extrusion-spheronization. The previous optimized formulation (not published) comprised 33.33% ibuprofen, 43% microcrystalline cellulose (Avicel1 PH101), 17.41% guar gum, 0.97% Na alginate, 0.97% Ca-acetate, and 4% PVP K-30. The pellets were dried in an oven for 90 min at 60^{o}C. The dried pellets were then sieved and those with 0.9 mm were used in further studies. Prior to coating pellet, a series of pellet evaluation were performed such as particle size and distribution, particle morphology, friability, flow rate, and bulk density.

The pellets were coated subsequently with inulin as inner film coating and shellac as outer film coating. In both coating materials, PEG 6000 (0.4%) was added as a plasticizer. Coating was performed using a fluidized bed coater (Jiangsu Jiafa Granulating drying equipment, Changzhou, China). The coating procedure involved maintaining the inlet temperature at 55-60^{o}C, spray rate at 1 mL/min for inulin and 5 mL/min for shellac, and atomizing pressure at 1.5 bars. One hundred grams batches of pellets were coated each time. A series of coated products were produced with different film thicknesses and quantified by the total weight gain (%TWG). The proper coated pellet for colonic release was chosen based on parameters evaluated such as particle morphology, *in vitro* as well as *in vivo* release profiles.

Morphology of pellets by scanning electron microscopy (SEM)

The surface as well as the inner part morphology of pellets was examined using scanning electron microscope. The samples were fixed on a brass stub using double-sided tape and then gold coated in vacuum by a sputter coater. The pictures were taken at excitation voltage of 10 KV and at 80 and 500X magnification by using JSM-6360LA scanning Microscope, Jeol-Japan.

Determination of ibuprofen content in the pellet

Pellets were weight and powdered. Crushed powder of pellets equivalent to 100 mg ibuprofen was taken and transferred to 100 mL volumetric flasks containing phosphate buffer pH 6.8. The flask was subjected to magnetic stirring with speed of 500 rpm for 1 h. Afterwards, the solution was filtered and diluted 100x using same buffer. Finally absorbance of the resulting solution was measured at the maximum at about 221 nm. All assays were carried out in triplicates. The absence of interference of formulation additives with absorption of ibuprofen at 221 nm was confirmed by taking the UV absorbance of solutions of blank pellet.

Drug Release Study

In Vitro Drug Release Study was carried out in simulated fluids of gastrointestinal tract (GIT). Pellets were evaluated for the *in vitro* drug release subsequently in simulated GI fluids (SGF) and simulated colon fluid (SCF). The drug dissolution test of pellets was performed by USP apparatus 3 (Hanson Research Co, USA). Pellets were dissolved over the surface of 250 mL of dissolution media (SGF and SCF). The content was dipped at 15 dpm at 37°C ± 0.5°C. Perfect sink conditions prevailed during the drug dissolution study period. The simulation of GI transit condition was achieved by altering the pH of dissolution medium at different time intervals. The pH of the dissolution medium was kept 1.2 for 2 h, then 10 mL of sample was withdrawn and replaced with equivalent volume, subsequent dissolution was performed at phosphate buffer pH 6.8 for 3 h and again 10 mL of sample was withdrawn and then replaced to maintain the volume. At the end of time period, dissolution was continued in SCF (1% and 4% w/v rat cecal content) for coated pellet and 0% of rat cecal for uncoated pellet. At different time intervals, the samples were withdrawn and replaced with same volume of fresh medium. The withdrawn samples were pipetted into a series of 10 mL volumetric flasks, and volumes were made up to the mark with PBS and centrifuged. The supernatant was filtered through 0.45 µm membrane filter and the filtrate analyzed for ibuprofen content at 221 nm using UV spectrophotometer method. All the experiments were performed in triplicate.

Preparation of SCF

Rat cecal content was prepared by the method reported by Van den Mooter et al. In brief, the rat cecal content was homogenized and then suspended in PBS (pH 7.4) to give the desired concentration (1% and 4% w/v) of cecal content, which was used as simulated colonic fluid. The suspension was filtered through cotton wool and ultra sonicated for 10 min in an ice bath at 40% voltage frequency using a probe sonicator (QSonica, USA) at 4°C to disrupt the bacterial cells. After sonication, the mixture was centrifuged (Hsiangtai, Taiwan) at 2,000 rpm for 20 minutes.

Pharmacokinetic study

Pharmacokinetics of inulin/shellac double-coated pellets were evaluated and compared with that of suspension preparation. The formula tested for this study was F1 which showed the best performance in *in vitro* release among other 2 formulas. Male New Zealand rabbit was used in the experiments received care in compliance with the ''Principles of Laboratory Animal Care'' and ''Guide for the Care and Use of Laboratory Animals. The pellets were orally administered to rabbits (4-5 months, 2-2.5 Kg) via a polyethylene cannula (diameter: 2mm) with 1mL of water, at a dosage equivalent to 5mg/Kg of ibuprofen. As a control, ibuprofen suspension was used. The pharmacokinetic study was done with two-cross over design with 7 days of washing period between the two periods. Blood samples (0.5 mL) were collected from marginal vein into heparinized tubes at specific time points. The heparinized blood samples were immediately centrifuged at 10,000g for 10 min on a tabletop centrifuge, and the plasma was separated and transferred to microcentrifuge tubes. An aliquot (0.2 mL) of plasma sample was measured into a glass tube with a teflon-lined cap, followed by the addition of 0.6 mL of acetonitrile. The mixture was vortexed for 5 min and centrifuged at 10,000g for 5 min. The supernatants were obtained through this procedure, which was dried under a stream of nitrogen and redissolved in 0.2 mL of mobile phase, vortexed for 1 min and centrifuged at 10,000g for 3 min. Supernatants (0.02 mL) were subjected to HPLC analysis of ibuprofen under the conditions as described below.

HPLC Conditions

The quantitative determination of ibuprofen was performed by HPLC. The HPLC system consisted of a Waters 2487 detector (UV) and an Empower workstation. The separations were performed at 25^{o}C using a LiChrospher®250-4, 10 µm, 4,6 x 150 mm column (RP-18). The mobile phase consisted of a mixture of acetonitrile/acetic acid 0.1% (3:2, v/v). The mobile phase was filtered and pumped at a flow rate of 1.2 mL/min. The column was maintained at a temperature of 25^{o}C. The eluent was detected by UV detector at 220 nm.

RESULT AND DISCUSSION

Characterization of coated pellet

Physical characteristics of core pellet

The physical characteristics of the pellets were presented in table 3. Core pellets met physical requirements for further coating process.

Table 3: Physical characteristics of core pellet

Parameters	value
Flow rate (g/s)	8.64 ± 0.16
Friability (%)	0.96± 0.05
Bulk density (g/mL)	0.65 ± 0.02

Particle size and particle morphology

Pellets with the size of 900-1180 µm were selected for further coating process. This range was selected due to its ability to reduce the electrostatistical force. The higher the surface area because of small particle size, the higher the electrostatics force. Thus, the use of selected range of size is expected to avoid technical problem related to electrostatic force during coating process. The morphology of the pellets was shown in figure 1.

The scanning electron micrograph of pellets coated with inulin-shellac and coating level of 15% and 10% respectively, shows that the coating layer is uniform and formed continuously as depicted in figure 1.

As shown in figure 2, coating process resulted in continuous and tight layer of both coatings which is important characteristic to prevent premature release of the drug before reaching the target due to unexpected leakage of the drug in upper part of GIT.

Ibuprofen content in the pellet

The content of drug in pellets (Table 4) is in the range of 84-90 % of theoretical values. The results presented in this table were used for calculation of the amounts of pellet containing 100 mg ibuprofen for dissolution studies.

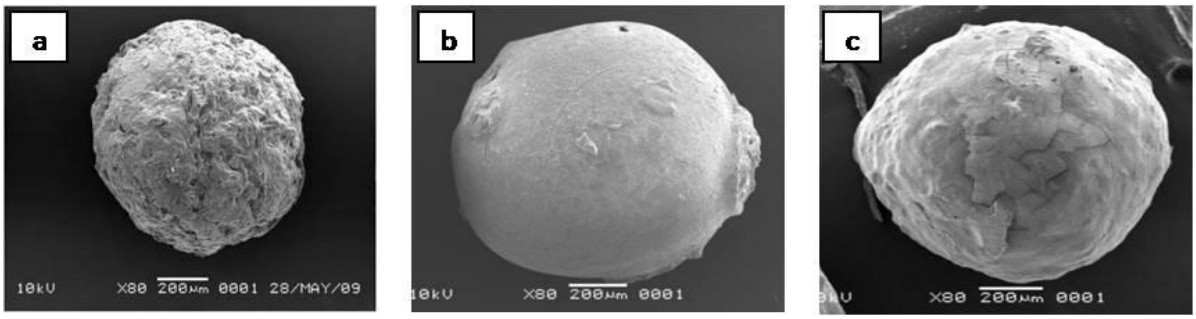

Figure 1: Scanning electron micrographs showing the external morphology of the uncoated (a), single coating (b), and double coating (c) pellets. Magnification 80 x

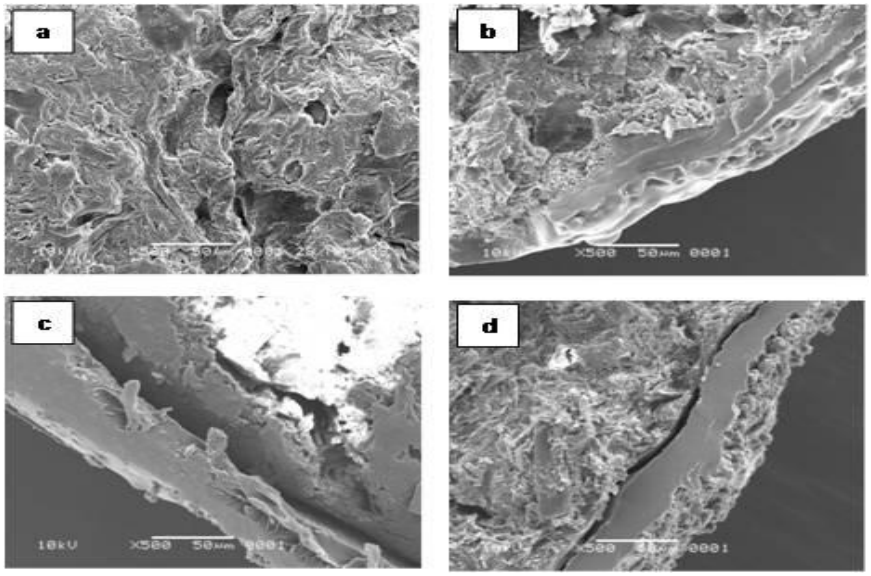

Figure 2: Scanning electron micrographs of the cross-section showing the internal morphology of uncoated (a) and double coated pellet of F1 (b), F2 (c) and F3 (d). Magnification 500x

Table 4: The ratio of experimental to theoretical drug content in coated pellets obtained from the results of determination of drug content test

Samples	Ibuprofen content (%)*
uncoated	85.06±4.42
F1	84.77 ± 0.24
F2	90.59 ± 1.19
F3	90.35 ± 0.13

*Measurement was performed in triplicate

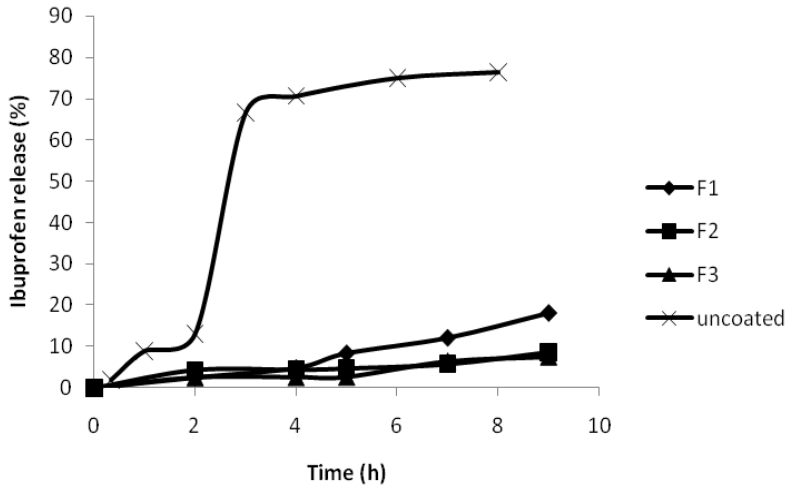

Figure 3: Dissolution profiles for pellets coated with inulin/shellac in the media with pH 6.8 containing 1% of rat cecal

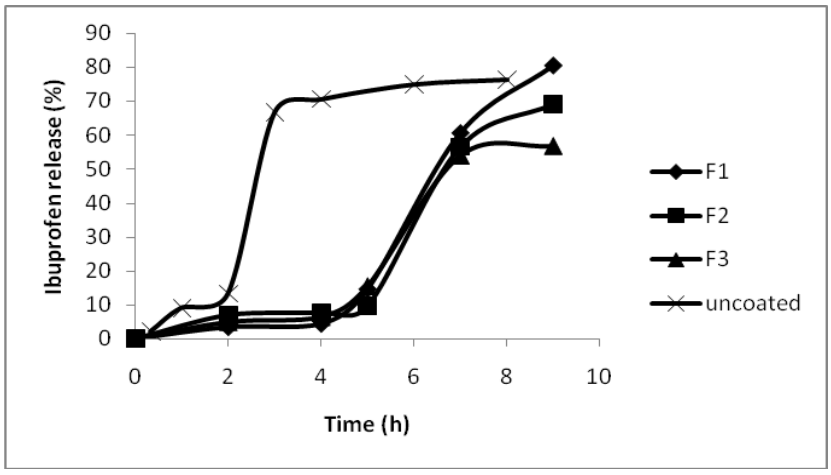

Figure 4: Dissolution profiles for pellets coated with combination of inulin/shellac in the media with pH 6.8 containing 4% of rat cecal

Effect of coating variable on ibuprofen release

In order to achieve tighter protection of the drug-loaded cores, several coating variables of the inulin/shellac layers (table 2) were investigated. *In vitro*-release profile of pellets coated with inulin/shellac of various weight gain are shown in Figure 3 and 4.

As shown in figure 3, uncoated pellet tested in dissolution medium without rat cecal revealed low release in the gastric simulation fluid (GSF) followed by remarkable release after contact with either intestinal simulation fluid (ISF) or colonic simulation fluid (CSF) which is indicating an unexpected premature release of the drug before reaching the colon. In contrast, double coated pellets in ISF showed retarded drug release as compared to uncoated pellet suggesting that inulin/shellac coating prevented the ibuprofen release. The use of shellac as an outer coating was able to protect the ibuprofen release from the pellet in the intestinal medium. The ability of shellac to resist drug release can be explained by the fact that after introduction into a medium of pH 6.8, due to slow solubilization of shellac, the formation of channels took some time which in this case was 3–4 h. The coating level of shellac seems not affecting the ibuprofen release, especially at 2-5 h. However, the release of ibuprofen from double coated pellet in CSF was affected by the level of inulin coating as well as the content of rat cecal. Coating using low level of inulin coating level (5%) resulted in more rapid release of the drug. This was due to that at higher coating level the pores are more covered and the diffusion path length increased. The percent of drug released after 5 h was significantly higher in the presence of rat cecal (4%) compared with those with less content (1%). This result can be explained by the enzymatic degradation of inulin in the presence of inulinase produced by the microbes of rat cecal and formation of aqueous filled pores in the coating layer. This finding was in agreement with other results of studies performed on free films containing inulin-ERS (Li et al., 1997, Wong et al., 1997). They described that the addition of inulinase to the dissolution media increased drug release in colon compartment. The results confirm that inulin is a potential polysaccharide for delivering drugs to be released in the colon region. Inulin belongs to the glucofructans and consists of a mixture of oligomers and polymers containing 2 to 60 (or more)*β*2-1 linked D-fructose molecules. It is not hydro-

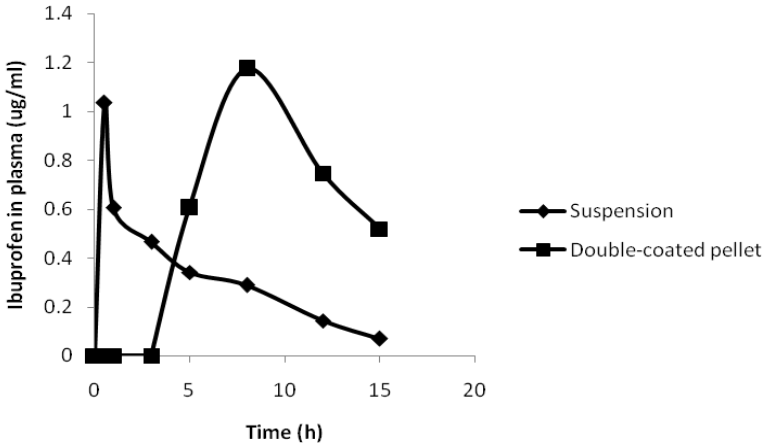

Figure 5: Mean plasma concentration of ibuprofen in rabbits following oral administration of ibuprofen suspension and selected double coated pellets. Each point represents the mean±S.D. (n=5)

lyzed by the endogenous secretions of the human digestive tract (Dysseler et al., 1995). However, bacteria harboring in the colon and more specifically *Bifidobacteria* are able to ferment inulin (McKeller et al., 1989, Wang and Gibson 1993, Gibson and Roberfroid 1995).It resisted degradation in the upper GIT but digested in human fecal medium by the action of *Bifidobacteria* and *Bacteroids* (Vervoort and Kinget 1996).

As depicted in figure 4, the release of ibuprofen from all formulations was not complete at the end of dissolution test (15 h). The probable mechanism could be responsible for this observation is the lower solubility of ibuprofen at lower pH which slowed down the drug diffusion.

Based on this finding, the use of polysaccharides seems to be superiority for delivering the drug specifically to the colon which was also confirmed by other groups (Anekant et al., 2007, Caviello et al., 2005). Polysaccharides retain their integrity and prevent the release of drug during its passage through the GIT. But when it comes in contact with colonic fluid it is confronted by the action of microorganisms and consequently entrapped drug is liberated.

Taking into account that a colonic drug delivery system must remain intact in the upper GI tract and to be able to release majority of its drug content in the colonic medium, formulation coated with inulin-shellac with coating level of 5%-20% was considered more appropriate, as this formulation showed resistance to drug release in the simulated upper GI media for up to about 7 h. Drug release increased in the presence of enzyme which confirms the sensitivity of this formulation only to colonic medium.

Pharmacokinetic study

The mean plasma ibuprofen concentration was plotted as a function of time and is shown in Figure 5.In line with *in vitro* data, the lag time was also observed in *in vivo* release although more rapid. After oral administration of suspension, ibuprofen was rapidly absorbed with a peak time and peak concentration of 0.5 h and 1.036 µg/mL, respectively. Ibuprofen then subsequently was eliminated quickly from plasma and cannot be detected after 15 h. whereas, for double coated pellet, an obvious lag time of about 3 h was observed, and the peak time and concentration were 8 h and 1.173 µ/mL, respectively. At the end of time sampling i.e. 15 h, the presence of ibuprofen in plasma was still highly detected.

The delayed absorption of ibuprofen from the pellet may be ascribed to the shellac coating that can prevent ibuprofen from releasing until to the site where a lumen pH of over 6.8 triggered dissolving of the coat. It seemed that shellac coating may have released the drug ibuprofen early in jejunum, which complies with the discussion of lack of colon-specificity for pH-sensitive CSDDSs (Ashford et al., 1993a and 1993b). Compared to suspension preparation, double coating seemed to achieve tighter controlling on drug release and absorption with an absorption lag time occurred when the pellet was travelling from gastric to intestinal lumen. According to the colon-specific delivery mechanisms proposed in this study, the inulin coat did function as a further protection after dissolving of the outer shellac coat. However, it seemed that the inulin coating provides longer lag time *in vitro* than that *in vivo* of about 5h.Moreover, the fact that ibuprofen could be detected only at limited time intervals showed that the system released the drug quickly and resulted in fast absorption. It is assumed that inulin has been broken down quickly *in vivo* because release in 4% cecal content has already shown obvious triggering effect, not to mention in colonic environment where full cecal content is expected to degrade the outer shellac coating rapidly.

CONCLUSION

Pellet coated with double layer of inulin/shellac was successfully developed to obtain colonic release of ibuprofen. The resultant coat provided a time delay of about 5h *in vitro*. In the presence of rat cecal content, release of ibuprofen was accelerated. In release media at consecutive gradient pH (pH1.2, 6.0, 6.8, and 7.25

for 2, 2, 1, and 4 h, respectively), both pH- and enzyme-triggered release was observed. Pharmacokinetic study in New Zealand rabbit showed that inulin and shellac double-coated CSDDS showed increased absorption of ibuprofen with delayed absorption time as compared to suspension preparation, suggesting that the absorption of ibuprofen even better in colonic compartment than in the gastric. It is concluded that inulin has the potential to be used as a film coating material for colon-specific drug delivery.

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