Combination of inulin and time dependent polymethacrylates as a coating system to achieve colonic delivery of indomethacin

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ABSTRACT

Background: In the previous study it was shown that films prepared from inulin (In) in combination with Eudragit RS (ERS) and RL (ERL) were susceptible to inulinase. *Purpose:* The aim of this work was to assess the suitability of these combinations for colonic delivery of indomethacin.

Methods: Indomethacin was loaded onto non-pareil seeds using fluidized bed apparatus to produce pellets with 20% w/w drug load. Drug loaded pellets were coated with In-ERS in the ratios of 20:80 and 30:70, or In-ERL in the ratio of 20:80 to different coating loads. The release of drug was examined in simulated gastric (for 2 hrs) and small intestine and in the presence of inulinase in simulated colonic medium (for 12 or 24 hrs).

Results: The results of this study revealed that incorporation of inulin as a bacterially degradable polysaccharide into ERS or ERL could modulate drug release. Coating level up to 15% significantly affected drug release from In-ERL or In-ERS coated pellets. However further increase in coating load to 20% had no significant effect on drug release from In-ERL coated pellets (f1=9.39). Drug release from In-ERL coated pellets was faster and showed some pH dependency.

Conclusions: Formulation coated with In-ERS (20:80) and coating level of 20% was considered more appropriate for colon delivery of indomethacin, as drug release was pH independent and formulation was resistant to drug release in the upper GI media for up to 7 hrs. This formulation was also susceptible to inulinase and released about 40% of indomethacin in the simulated colonic media.

Keywords: Colonic drug delivery; Indomethacin, Inulin; Eudragit RS; Eudragit RL

INTRODUCTION

Oral colon-specific delivery system is a valuable method for the treatment of colon-related diseases such as ulcerative colitis or colon cancer (1,2)whereby high local concentration can be achieved while minimizing side effects. Different approaches namely pH responsive, time responsive and bacterially responsive delivery systems have been used for colonic delivery of drugs. Eudragit S polymer has been the polymer of choice for design of pH responsive systems for delivery of 5-aminosalicylic acid in some formulations (Asacol[®], Ipacol[®], Claversal[®]). However formulations based on Eudragit S suffer from drawbacks of variability in sites of disintegration or no drug release at all (3). This is due to the need for a threshold pH of about 7 for polymer dissolution and inter and intra subject variability in pH of gastrointestinal tract. Akhgari et al. using factorial design showed that the use of combination of Eudragit S and L in proper

ratio could manipulate drug release within the pH range of 6-7 (4). However the proper performance of these systems could not be assured at different physiological conditions or disease states like ulcerative colitis with lower colonic pH. To overcome this problem, pH responsive system was evaluated in combination with time responsive system in order to alleviate the pH dependency of former systems and ensure drug release under different physiological conditions (5). Improvement in safety and reduction in toxicity when treating local or systemic chronic diseases is another benefit for using combination of time and pH responsive system (6). It has been shown that addition of Eudragit RS as a sustained release polymer to Eudragit L and S in the coating formulation could modify drug release after a suitable lag time in different media (5). These systems although have some benefits over pH responsive ones still may show some limitations when one encounters different gastric emptying and

intestinal or colonic transit times.

The use of polymers which are specifically sensitive to colon environment may provide better selectivity for drug release in the large intestine. Due to high sitespecificity of polysaccharides and their sensitivity to degradation by colonic enzymes, they have been widely taken into consideration in design of colonic drug delivery. To control premature swelling and dissolution of polysaccharides and also to make it possible to use them in coating process, sustained release polymers have been used in combination with the polysaccharides. Combination of amyloseethyl cellulose (7), guar gum-Eudragit L and RL (8), pectin-HPMC (9), pectin-ethyl cellulose (10), and pectin-Eudragit RL and Eudragit NE (11) have been examined in this regard. The results of these studies revealed that polysaccharides incorporated in the polymers are susceptible to bacterial enzymatic attack in simulated colonic conditions.

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 (12). In the previous study on free films it was revealed that films prepared from inulin in combination with time-dependent polymethacrylates were susceptible to enzymes present in the colon and addition of inulin to Eudragit® RS or RL films increased the permeability of indomethacin in simulated colonic medium (13). Moreover, suitability of inulin as a bacterially degradable polysaccharide has been evaluated in free films with Eudragit® RS by other investigations (14, 15). 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 time-dependent polymers as a unique coating formulation for design of colonic delivery dosage form.

Indomethacin a sutibale candidate for formulation into colonic delivery system was used as a model drug in this study due to it's effect on inflammatory bowel diseases and decreasing the risk of colorectal cancer (16, 17). In many studies over expression of COX-2 in colon adenomas and colon carcinoma has been reported. The inhibitory effect of NSAIDs on cyclooxygenase, COX-1 and/or COX-2 and therefore decrease in biosynthesis of arachidoinc acid metabolites especially prostaglandins and all prostanoids provides a rational for use of these drugs in those conditions. Several studies have focused on design of either multiparticultae or single unit indomethacin colon delivery system. The use of pH dependent Eudragit S and L (4), combination of pH dependent Eudragir S and L and time dependent

Fable	1.	Coating	parameters	for	indomethacin	layering.	
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Inlet temperature (°C)	60-65
Outlet temperature(°C)	45-50
Nozzle diameter (mm)	1.0
Atomization pressure (bar)	2.0
Spray rate (gmin ⁻¹)	15

Eudragit RS and RL (5), and bacterially degradable chitosan and pectin as a coating onto multiparticulate systems (18) or calcium pectinate as a matrix forming material (19) and pectin, guar gum, Eudragit E as a binder in preparation of indomethacin tablets (20) are some examples of efforts in this regard.

The aim of this work was to assess the suitability of formulations containing inulin and Eudragit RS or Eudragit RL in a unique coating formulation to achieve specific delivery of drug-containing pellets to the colon.

MATERIALS AND METHODS

Materials

Eudragit[®] RS 30D and Eudragit[®] RL 30D (Rohm Pharma, Germany), inulin (Raftiline HP, Orafti, France), inulinase from *aspergillus niger* (Sigma, USA), indomethacin (Darupakhsh, Iran), nonpareils (NP Pharm, France), triethyl citrate (Merck, Germany), polyvinylpyrolidone K30 (Merck, Germany), and talc (Merck, Germany) were used in this investigation.

Methods

Drug layering

Drug containing pellets were produced by layering of indomethacin onto the non-pareils (850-1180 μ m) using fluidized bed coater (Wurster, Werner Glatt, Germany). Suspension containing 30% w/v indomethacin (<90 μ m) was prepared by dispersing drug in 7% (w/v) PVP K30 solution in water. The suspension was passed through a 140 mesh sieve and sprayed onto non-pareils. Coating conditions are listed in Table 1. The drug layering process was carried out to produce pellets with 20% (w/w) drug load. Afterward the pellets were kept in an oven for 2 hrs at 40°C.

Determination of drug content in pellets

The procedure for determination of drug content in pellet was the same as that reported previously (4, 5). Accurately weighed (500 mg) of drug loaded pellets were grounded and transferred to 250 mL volumetric flasks containing phosphate buffer of pH 7.2. The flasks were shaken in a shaking water bath at 25°C for 3 hrs. The indomethacin concentration was determined by spectrophotometry at 318 nm in filtered solutions. All assays were carried out in triplicates. The absence of interference

Ingredients	Inulin-ERS 20:80 Coating formulation (gr/100 mL)	Inulin-ERS 30:70 Coating formulation (gr/100 mL)	Inulin-ERL 20:80 Coating formulation (gr/100 mL)
ERS	12	8.4	-
ERL	-	-	12
Inulin	3	3.6	3
TEC	1.2	0.8	1.2
Talc	1.2	0.8	1.2

Table 2. The composition of coating suspensions.

of formulation additives with absorption of indomethacin at 318 nm was confirmed by taking the UV absorbance of solutions of PVP and non-pareil seeds at the range of 200-400 nm.

Polymer coating

Preparation of coating dispersion

A 5% w/v aqueous solution of inulin was prepared and gently added with stirring to a proper amount of aqueous dispersion of ERS or ERL. Triethyl citrate (TEC) was added to the dispersion as plasticizer (10% w/w related to dry polymer). Talc was passed through a 90 µm sieve and was added to the polymer solution as a glidant (10% w/w related to dry polymer). The resulted suspension was stirred for 15 min. Different coating compositions containing In-ERS in the ratios of 20:80 and 30:70, and In-ERL in the ratio of 20:80 were prepared. Due to poor film forming ability of inulin, formulations containing inulin in levels higher than 30% were not reproducible and therefore the amount of inulin in the coating formulations was limited to 20 or 30%. The formulations which were prepared are shown in Table 2.

Polymeric coating process and conditions

Two hundred grams of drug loaded pellets were coated in fluidized bed coater (Wurster, Werner Glatt, Germany) by different coating formulations. Coating conditions are listed in Table 3. Samples of coated pellets were removed from the apparatus when the coating loads reached 5, 10, 15 and 20% w/w related to the drug pellets. At each stage the pellets were fluidized for about 5 min and then were transferred into and kept in oven for 2 hrs at 50°C.

Dissolution experiments

Samples of coated pellets containing 80 mg of indomethacin which was determined by the

Table 3. The conditions used for polymer coating.

Inlet temperature (°C)	40-45
Outlet temperature (°C)	30-35
Nozzle diameter (mm)	1.0
Atomization pressure (bar)	2.0
Spray rate (gmin ⁻¹)	10

procedure outlined in determination of drug content was used in dissolution tests. Dissolution studies were carried out in a USP XXIII dissolution apparatus I (Pharmatest, PTWS, Germany) in 900 mL media at 37°C at a rotation speed of 100 rpm. Different dissolution media comprised the media with pH 1.2, 6.4 and 7.2. The drug release studies were also performed at pH 6.4 in the presence of 1, 3 and 6 mL of inulinase enzyme. The times of experiments were 12 or 24 hrs. One of the promising formulation i.e. pellets coated with In-ERS (30:70) with coating level of 15% were also first immersed at pH 6.4 without enzyme for 5 hrs and then were transferred to the media with enzyme. In another dissolution experiment, these pellets were also immersed at pH 6.4 with enzyme for 12 hrs and then were placed in the medium with pH 7.2 for 12 hrs. The percent of drug which was released after 5 hrs (300 minutes) was compared statistically for different formulations using one way analysis of variance. For comparison of drug release profiles a simple model independent approach that uses difference factor was applied. Difference factor (f1) which is a fit factor directly compares the difference between drug release for a pair of dissolution profiles at the same time. Difference factor is defined by

$$f1 = \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum R_t} \times 100$$
 Equation 1

where Rt and Tt are the cumulative percentage dissolved at each of the selected n time points of the reference and test product respectively. Generally fl values up to 15 (0-15) ensures equivalence of two curves (21).

Scanning electron microscopy (SEM)

equation. 1

The morphology of the surface and cross section of pellets was characterized using SEM. The samples were mounted on aluminum stub using double sided adhesive tape, sputter-coated with a thin layer of platinum for 3 min using a sputter coating machine (SC7620 sputter coater, Polaron, England) under argon atmosphere, and then analyzed using SEM (LEO 1450 VP, England). Voltages of 10 and 20 kV

table 4. The fatto of experimental to theoretical drug content in coaled penets obtained from the results of determination of drug content test.					
Coating level	Inulin-ERS 20:80 coated pellets	Inulin-ERS 30:70 coated pellets	Inulin-ERL 20:80 coated pellets		
5%	$94\% \pm 0.02$	$96\% \pm 0.01$	$98\% \pm 0.04$		
10%	$97\% \pm 0.04$	$95\%\pm0.02$	$92\%\pm0.02$		
15%	$95\% \pm 0.02$	$97\%\pm0.04$	$95\% \pm 0.01$		
20%	$93\% \pm 0.01$	$95\% \pm 0.01$	$97\% \pm 0.02$		

were selected for accelerating the electrons from electron gun onto the specimen.

RESULTS AND DISCUSSION

The scanning electron micrograph of pellets coated with inulin-ERL (20:80) and coating level of 20% (Figure 1) shows that the coating layer is uniform and formed continuously. Pellets coated with inulin-ERS also showed the same characteristics.

The content of drug in pellets (Table.4) is in the range of 92-98% of theoretical values. The results presented in this table were used for calculation of the amounts of pellet containing 80 mg indomethacin for dissolution studies.

The effect of coating level on drug release at medium with pH of 6.4 (Figure 2) depicts that increase in coating level of pellets coated with inulin-ERL from 5 to 15% decreased the percent of drug release in each sampling time. The statistical comparison of the percent of drug released after 5 hrs showed significant differences between different coating levels (p < 0.001). Increase in coating level decreased the burst release of drug to high extent. This was due to overlapping of coating layers at higher coating levels and more coverage of pores and increased diffusion path length (22). However further increase in coating level from 15 to 20% did not affect drug release profiles. The f1 value was found to be 9.39 indicating similarity of release profiles for 15 and



Figure 1. SEM of pellets coated with inulin-ERL (20:80) at coating level of 20%.

20% of coating levels. The percent of drug released after 5 hrs was not also significantly different between these two coating levels (p > 0.05).

Nearly the same results were observed for formulations coated with inulin-ERS. However in



Figure 2. The effect of coating level on drug release from pellets coated with (a) inulin-ERL (20:80), (b) inulin-ERS (30:70) and (c) inulin-ERS (20:80) in the medium with pH 6.4. (Error bars indicate SD; n=6).



Figure 3. Dissolution profiles for pellets coated by different formulations with coating level of 20% at media with (a) pH 6.4 and (b) pH 7.2 (error bars indicate SD; n=6).



Figure 4. Dissolution profiles for pellets coated with inulin-ERL (20:80) and coating level of 20% in the media with pH 6.4 without or with different amounts of enzyme (error bars indicate SD; n=6).

this case the effect of coating level was even more apparent. Indeed, burst release of drug at lower coating levels (5 and 10%) was not observed for formulations with coating levels of 15 and 20% and increase in coating level decreased drug release rate to high extent.

Drug release from all formulations at pH 1.2 was extremely low (data are not shown). This was due to low solubility of indomethacin in acidic medium. Indomethacin with pKa of 4.5 is more soluble at higher pH compared to lower pH values (23). Drug release profiles for pellets coated with combination of inulin and either ERS or RL with coating level of 20% at pH 6.4 and 7.2 are shown in Figure 3 as an example. The percent of drug release was significantly higher in the case of pellets coated with ERL (p < 0.001) compared to ERS. A burst release of drug could also be observed for pellets coated with formulations containing ERL with coating level of 20%. These results could be attributed to higher permeability of ERL compared to ERS (24). Increase in pH of dissolution media from 6.4 to



Figure 5. Dissolution profiles for pellets coated with (a) inulin-ERS (20:80) and (b) inulin-ERS (30:70) with coating level of 20% in the media with pH 6.4 in the presence of absence of 6 mL inulinase (error bars indicate SD; n=6).



Figure 6. Dissolution profiles for pellets coated with inulin-ERS (30:70) with the coating level of 15% at pH 6.4 in different conditions (24 h in the presence of enzyme, or 5 h pre-exposure in the media without enzyme and following 19 hrs with inulinase) (Error bars indicate SD; n=6).

7.2 increased the percent of drug release at each sampling time for pellets coated with formulation containing ERL. Percent of drug release after 5 hrs was significantly lower at pH 6.4 (p < 0.05). The major difference in drug release was observed at early stage of dissolution test. This phenomenon was not observed for pellets coated with ERS. The effect of pH on formulations coated with the other

coating levels was the same, and change of pH was only effective on pellets coated with ERL and inulin. The pH-dependent drug release from beads coated with ERL and ERS has been previously explained with an anion exchange process (25). The higher cationic ammonium groups present in ERL, may explain the more pH-dependency of drug release for this polymer.



Figure 7. SEM of pellets coated with inulin-ERS (30:70) and coating level of 20% in the medium containing inulinase with pH 6.4 (a) after 4 hrs and (b) after 7 hrs.

Figure 4 shows dissolution profiles for pellets coated with inulin-ERL (20:80) at coating level of 20% in the media with pH 6.4 and different levels of inulinase. Drug release was faster in the media with inulinase, especially at the amounts of 6 (f1= 69.3) and 9 mL (f1= 87.1) of enzyme. The percent of drug released after 5 hrs was significantly higher in the presence of 6 or 9 mL enzyme compared with those without enzyme (p < 0.001). This result can be explained by the enzymatic degradation of inulin in the presence of inulinase (26, 27) and formation of aqueous filled pores in the coating layer. The effect of different amounts of enzyme on drug release profiles was almost the same for the other coating levels (data are not shown). The early stage of drug release in the media in the presence and absence of enzyme was similar (Figure 4), however after about 200-300 min differences in the profile of release could be observed. This might be due to the time that is required for inulinase to diffuse into the coating thickness and affect the inulin enzymatically. The other probable reason could be slow leaching of degraded inulin which provides pores and channels for drug release. Similarly the retardation in drug release in the

media with enzyme for pectin/ethyl cellulose film coated tablets was attributed to time-dependent dissolution of pectin (10).

According to figure 4 the release profiles were similar in the media with 6 and 9 mL of enzyme (f1= 9.54). This was also confirmed by analysis of variance of percent of drug released after 5 hrs which showed no significant differences (p > 0.05). It seems that 6 mL of enzyme was the maximum amount of inulinase which could be effective on inulin degradation and subsequent drug release in vitro. Therefore, only this amount of inulinase was used for dissolution studies of pellets coated with inulin-ERS.

Figure 5 depicts dissolution profiles for pellets coated with inulin-ERS in different ratios of inulin and coating level of 20% at pH 6.4 with or without inulinase. Addition of inulinase to the dissolution media increased drug release in both formulations (p < 0.001). This was in agreement with the results of studies performed on free films containing inulin-ERS (13, 14). Meanwhile, increasing the percentage of inulin in the coating composition to 30% did not have any profound effect on the drug release (f1= 14.3). The percent of drug released after 5 hour showed no significant differences (p > 0.05). These results were in agreement with studies related to permeability of indomethacin performed on free films (13).

The early stage of indomethacin release for pellets coated with inulin-ERS or inulin-ERL in the presence or absence of and enzyme was similar. The effect of enzyme was observed after about 200 min. In order to investigate whether the swelling of polymethacrylates films could affect the enzyme activity, pellets coated with inulin-ERS (30:70) with coating level of 15% were first immersed for 5 hrs at pH 6.4 without enzyme and then transferred to the media with enzyme. Figure 6 compares the release of drug in two media i.e. 24 hrs in the media with enzyme, and 5 hrs in the media without enzyme following 19 hrs in the presence of inulinase. Preexposure of pellets in the media without enzyme had minor effects on release of indomethacin and therefore penetration of inulinase into the film was not dependent on the swelling of polymethacrylate. This result demonstrated that the slow leaching of degraded inulin could be a key parameter for lag time for drug release. To confirm the time-dependent degradation and leaching of inulin, SEM of pellets coated with inulin-ERS (30:70) and coating level of 15% at different periods during dissolution test were compared in Figure 7. The number of pores in the coating layer increased with increase in dissolution time.

The release of indomethacin from all formulations was not complete at the end of dissolution test. Two probable mechanisms could be responsible for this observation. First, lower solubility of indomethacin at lower pH could slow down drug diffusion. To



Figure 8. Dissolution profiles for pellets coated with inulin-ERS of (30:70) and coating level of 15% in different conditions (\diamond 24 hrs at pH 6.4 with enzyme, \Box 12 hrs at pH 6.4 with enzyme and following 12 hrs at pH 7.2 without enzyme) (Error bars indicate SD; n=6).

investigate this probability, pellets of inulin-ERS (30:70) were placed in the media with pH of 7.2 after exposure to the enzyme at pH 6.4 for 12 hrs and their release profiles were compared with pellets at pH 6.4 for 24 hrs (Figure 8). Minor increase in drug release was observed at pH 7.2 which could be attributed to higher solubility of drug at this pH. The other probable reason for incomplete drug release was a possible ionic interaction between indomethacin and Eudragit polymers which has been demonstrated by the other investigations (28). This interaction which resembles ion-exchange resin reaction could slow down the diffusion of dug and therefore leads to incomplete drug release. Interaction of anionic drugs with Eudragits has been reported in other studies (29-31).

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-ERS (20:80) and coating level of 20% was considered more appropriate, as this formulation showed resistance to drug release in the simulated upper GI media for up to about 7 hrs and the release of indomethacin was not significant at this period. However drug release increased in the presence of enzyme which confirms the sensitivity of this formulation only to colonic medium. Comparing this system with the optimum formulation based on pH-dependent polymethacrylates (4) demonstrated that the system containing inulin and ERS could be more specific for colon delivery of indomethacin. Also, considering the in vivo conditions with perfect sink condition and high amount of enzyme produced by microbial flora of the large intestine the release of drug could be more enhanced and therefore these systems could be more beneficial for colonic delivery compared to system based on combination of timeand pH-dependent polymers reported previously (5).

CONCLUSIONS

The results of this study revealed that incorporation of inulin as a bacterially degradable polysaccharide into ERS or ERL could modulate drug release. It was shown that inulin incorporated in polymethacrylates would be still susceptible to degradation by inulinase enzyme. Drug release from pellets was pH-independent for pellets containing ERS, but coating level had significant effect on the release of indomethacin. With regard to hindering drug release in the upper GI media and susceptibility to the colonic medium, dosage forms coated with inulin-ERS (20:80) and coating level of 20% would be more promising for colon targeted delivery of indomethacin.

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