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Colonic drug-targeting: in vitro release of ibuprofen from capsules coated with poly(ether-ester) azopolymers

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Abstract

For the purpose of colonic drug-targeting, poly(ether-ester) azopolymers were synthesized and used to coat capsules containing ibuprofen. The release of ibuprofen was studied from coated capsules incubated in the medium prepared by suspending rat caecal contents in phosphate buffer pH 6.8 (RCCRM) and in capsules incubated in plain phosphate buffer (PB). The release of ibuprofen was higher in RCCRM than in PB. This was due to the presence of azoreductase in RCCRM, an enzyme which breaks down azopolymers by reducing azo bonds. Furthermore, the release of ibuprofen was a function of the thickness of the coating, the release being higher the thinner the coating. Addition of polyethylene glycol in the coating solution resulted in capsules with a higher drug release due to enhanced hydrophilicity of the coating. A two-layer coating gave a too early release, while a thick four-layer coating impeded drug release for 24 h. Medium thickness poly(ether-ester) coating containing 15% polyethylene glycol exhibited potential usefulness in colon-targeting of drugs.

Keywords: Colonic-targeting; Biodegradeable polymers; Azopolymers; Ibuprofen; Azoreductase

1. Introduction

Colonic drug-targeting has several therapeutic advantages. Like any other organ specific targeting, only a small dose of the drug is required, which subsequently results in fewer adverse drug reactions. Diseases of the colon such as irritable

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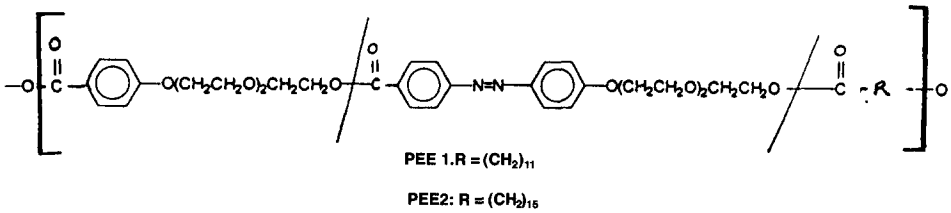

Fig. 1. Structural presentation of the poly(ether-ester) azopolymers.

bowel syndrome, Crohn's disease and ulcerative colitis are effectively treated when the drug is applied directly to the affected area. Likewise, vermicides and colonic diagnostic agents need only be applied in smaller dosages when placed directly in the colon. Recently, colon targeting has attracted much interest due to an unprecedented rapid development of biotechnology and genetic engineering, resulting in availability of peptides and proteins at reasonable costs. But peptide and protein drugs are destroyed by stomach acid and/or enzymes of the pancreas. These drugs are usually administerd by the parenteral route, which is inconvenient. With negligible activity of pancreatic enzymes and much less brushborder membrane peptidase activity, the colon is more suitable for delivery of such drugs (Lee, 1991; Ikesue et al., 1991). Thus colonic delivery of analgesic peptides, contraceptive peptides, oral vaccines, insulin, interferons and interleukins has been attempted (Saffran et al., 1988; Mackay and Thomlinson, 1993).

In addition colonic targeting of drugs would prove useful where intentional delayed drug absorption is required such as nocturnal asthma (Quadros et al., 1995).

The approaches utilized in achieving colonic delivery of drugs include use of prodrugs (Friend, 1992), pH-sensitive polymer coatings (Touitou and Rubinstein, 1986; Peeters, 1990; Peeters and Kinget, 1993), time-dependent formulations (Gazzaniga et al., 1994), bacterial degradable coatings (Saffran et al., 1991; Van den Mooter et al., 1992, 1993; Milojevic et al., 1995) and biodegradable polymeric matrices, hydrogels or bioadhesives (Rubinstein et al., 1993; Brondsted et al., 1995; Kopecek et al., 1992).

This study deals with in vitro release of ibuprofen from capsules coated with a mixture of poly(ether-ester) azopolymer and polymethylmetacrylate. Two polymers having a chemical structure as shown in Fig. 1 were used and are designated as PEE1 and PEE2. The synthesis and in vitro demonstration of biodegradation of the polymers is explained elsewhere (Samyn et al., 1995). The azopolymers had poor film forming properties which neccessitated mixing them with a good film forming pH-insensitive polymer to obtain a better coating. It was not possible to compress the polymers into tablets, because of their poor compression properties.

The use of ibuprofen in this study was selected strategically. The drug has a low water solubility. If a highly water-soluble drug was used, diffusion may have started even before the bacterial degradation of the polymer coating started. The drug is also a candidate for an intentional delayed absorption from a dosage form ingested at bed time for the treatment of arthritis, which may have peak symptoms in the early morning.

2. Materials and methods

2.1. Materials

Ibuprofen was Ph. Belg. VI grade (Alpha Pharma, Belgium); naproxen (UCB, Belgium) was used as internal standard.

NADP, glucose-6-phosphate, glucose-6-phosphate dehydrogenase and benzyl viologen (Sigma, Belgium) were used to prepare the rat caecal content release medium (RCCRM).

Eudragit® SR100 (poly(ethylacrylate, methylacrylate, trimethylammonioethylmethylacrylate chloride) 1:2:0.1; Röhm Pharma, Germany) was employed as film forming agent.

All other reagents and solvents were of analytical or HPLC grade. The water used for HPLC was purified with a milli-Q system (Millipore, Belgium).

2.2. Preparation of coated capsules

Gelatin capsules (Nr 4) containing approximately 20 mg of ibuprofen and 125 mg of spray dried lactose were coated by dipping in a solution of the polymer and Eudragit in chloroform. The maximum amounts of PEE1 and PEE2 required to be mixed with Eudragit to form a good film were 40 and 60%, respectively. However, in order to compare the rate of release of the drug between the capsules coated with PEE1 or PEE2, the coating solutions were made using the polymers and Eudragit in the ratio of 4:6. For each polymer, three sets of coated capsules were made with different thickness of the coating. The thickness was varied by dipping twice, three times and four times in the coating solution, respectively. These are hereafter referred to as 'two-layer', 'three-layer' and 'four-layer' capsules. The contributions of the coatings to the total weight of the filled capsule are given in Table 1.

In order to determine the effect of hydrophilicity on the release characteristics of the drug from the coated capsules, polyethylene glycol (PEG 400) was added to the coating solution, and another series of the coated capsules were made using this solution. The amount of PEG 400 added was 5%, 10% or 15% of the weight of Eudragit in the coating solution.

Table 1
Percent of the weight of polymer coating in relation to the total weight of the capsule

Thickness of the coating	Percent capsule weight
Two layers	0.6 (± 0.9%)
Three layers	11.9 (± 1.2%)
Four layers	16.1 (± 1.6%)

2.3. Preparation of the RCCRM

The rat ceacal content release medium (RCCRM) was prepared as follows.

Male wistar rats (300 g) were sacrificed and the caecum was ligated at both ends and removed. Under anaerobic conditions the caecum contents were removed and used to make a 10% w/v suspension in phosphate buffer (0.05 M, pH 6.8). The buffer had been previously bubbled with nitrogen to remove oxygen. The suspension thus made was filtered through glass wool. It was then sonicated for 20 min at 4°C in an ice bath in order to disrupt bacteria cell walls and release the azoreductase. After sonication, the mixture was centrifuged at 20 000 rpm at 4°C for 30 min. To the clear solution the following co-factors were added: benzyl viologen ($1.4 \times 10-10^{-4}$ M); NADP (2.5×10^{-4} M); glucose-6-phosphate dehydrogenase (1 U/ml) and glucose-6-phosphate (8.5×10^{-4} M).

2.4. Analysis of ibuprofen

2.4.1. Instrumentation

Ibuprofen was quantified using isocratic high performance liquid chromatographic method (HPLC) (Geisslinger et al., 1989). The HPLC instrument was composed of a LiChroGraph L-6000 HPLC pump (Merck-Hitachi, Germany); a Rheodyne model 7125 Syringe Loading Sample Injector (Rheodyne, CA, US) equipped with a 20 μl loop; a LiChroGraph L-4000 UV detector (Merck-Hitachi, Germany), set at 220 nm; and a Merck-Hitachi Model D-2500 Chromato-Integrator. The column was a 24.4 × 0.4 cm size, packed with LiChrospher 60 RP-select B (5 μm) (Merck, Germany), and the mobile phase, previously filtered through a nylon membrane filter (0.45 μm) and degassed by ultrasonication, consisted of methanol:water:phosphoric acid (70:30:0.1; v/v/v). The flow rate was 1.0 ml/min.

2.4.2. Extraction procedure

The quantity of ibuprofen in the RCCRM was determined using a method devised by Geisslinger et al. (1989). Samples of 500 μl were acidified by adding 100 μl of 2-*N*-hydrochloric acid. Then 100

μl of naproxen solution (40 μg/ml in hexane-diethylether 80:20; v/v), the internal standard, was added, followed by 5 ml of hexane:diethylether 80:20 v/v. The mixture was vortexed and centrifuged for 5 min at 4000 rpm. From the organic layer, 3 ml was removed and evaporated under a stream of air at room temperature. The residue was dissolved in 1 ml of the mobile phase and analysed using HPLC.

2.4.3. Ibuprofen release experiments

2.4.3.1. Recovery of ibuprofen from rat caecal content release medium. The release study was performed in the Compact Anaerobic Workstation (DW Scientific, U.K.) at 37°C. Each azo-coated capsule containing ibuprofen was introduced in 200 ml of either freshly prepared cell-free extract medium (RCCRM) or phosphate buffer (PB) (pH 6.8, 0.05 M). The aim was to compare the release characteristics of ibuprofen from capsules coated with azopolymers in the medium containing azoreductase to that from the same medium without azoreductase. The experiment for each capsule lasted 24 h and samples were taken 2, 4, 6, 8, 12, 18, and 24 h after introducing the capsule into the medium. At the end of each experiment, the capsule was mechanically crushed in order to get a 100% release.

For statistical purposes, each set of experiments was done six times, the average of which was plotted in the 'percent release versus time' graphs. A two-tailed unpaired t-test was carried out at each time point and a difference was considered statistically significant when $p < 0.05$.

3. Results and discussion

3.1. Release of ibuprofen from the azopolymer coated capsules

3.1.1. Release from capsules coated with azopolymer/eudragit solution without PEG 400

The thickness and hydrophilicity of the coating on the capsules appeared to influence the release characteristics of ibuprofen from the capsules.

The two-layer capsules (without PEG 400) released the drug within 6–8 h in the PB and within 4–6 h in the RCCRM (Fig. 2A and B). The release was complete within 10 h. Apart from a higher initial drug release in the RCCRM, the rate and extent of release of the drug was the same both in the RCCRM and PB. This means that the release of the drug was only affected through simple diffusion, without the influence of azoreductase. Visual observation revealed that the coating was weak and non uniform. Small flakes of the coating were noticed about 6 h from the beginning of the release experiment. The large standard deviations are probably due to the instability and non-uniformity of the thickness of the coat.

Minimal release of ibuprofen was apparent in the three-layer capsules. Within 24 h, the capsules coated with PEE1 (without PEG 400) released only 12.5% $\pm$ 1.8 of the drug when placed in the RCCRM, and 5.7% $\pm$ 1.6 when placed in PB (Fig. 2C). The rate of release increased slightly in the capsules coated with PEE2. After 24 h, 14.2% $\pm$ 1.1 of the drug was released from the capsules in the RCCRM, and 7.5% $\pm$ 1.4 was released from the capsules in the PB (Fig. 2D). In both cases it was observed that the release of the drug was higher in the capsules placed in RCCRM as compared to those in PB, and the difference was statistically significant.

The four-layer capsules did not release any drug after 24 h. This was the case even when 15% PEG 400 was added to the coating solution.

3.1.2. Release from capsules coated with azopolymer/eudragit solution with PEG 400

The addition of PEG 400 in the coating solution had a significant effect on the release characteristics of ibuprofen from the coated capsules. PEG 400 was added as a plasticizer and to promote hydrophilicity on the coat (Röhm Pharma Monographs; Marshal and Rudnic, 1990). The integrity of the coating was a function of the amount of PEG 400 added.

The two-layer capsules with PEG 400 quickly released the drug in both RCCRM and in the PB. The sample taken 6 h from the beginning of the experiment showed that 42% of the drug from

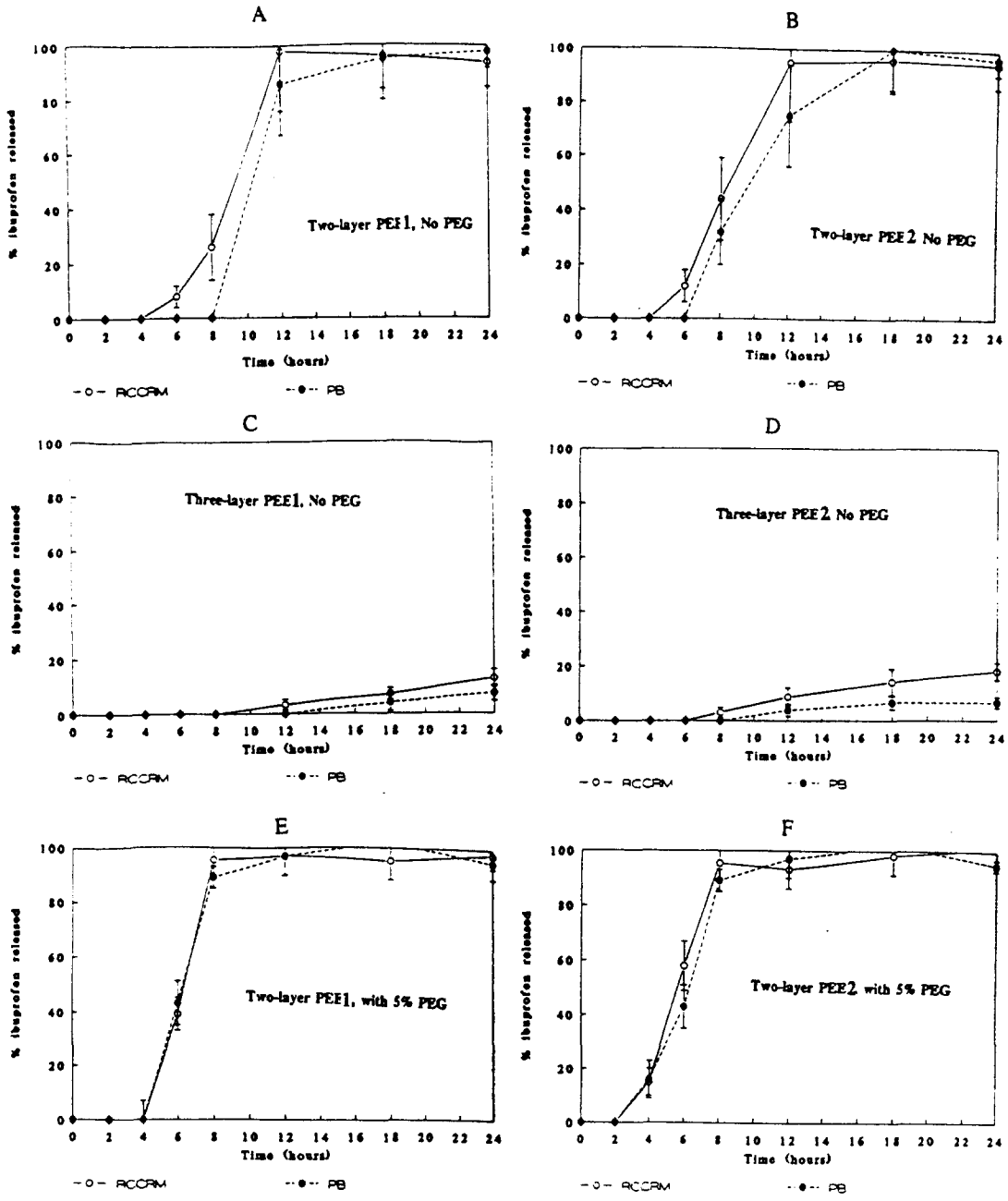

Fig. 2. Release of ibuprofen from capsules coated with poly(ether-ester) azopolymers in RCCRM and PB. Error bars indicate standard deviations.

PEE1-coated capsules had been released (Fig. 2E). The capsule had completely disintegrated after 8 h. The capsules coated with PEE2 released the drug even faster. Within 4 h of the experiment, about 18% of the drug had been released (Fig. 2F). The difference in the drug release between the capsules in the RCCRM and PB was not statistically significant both in PEE1 and PEE2

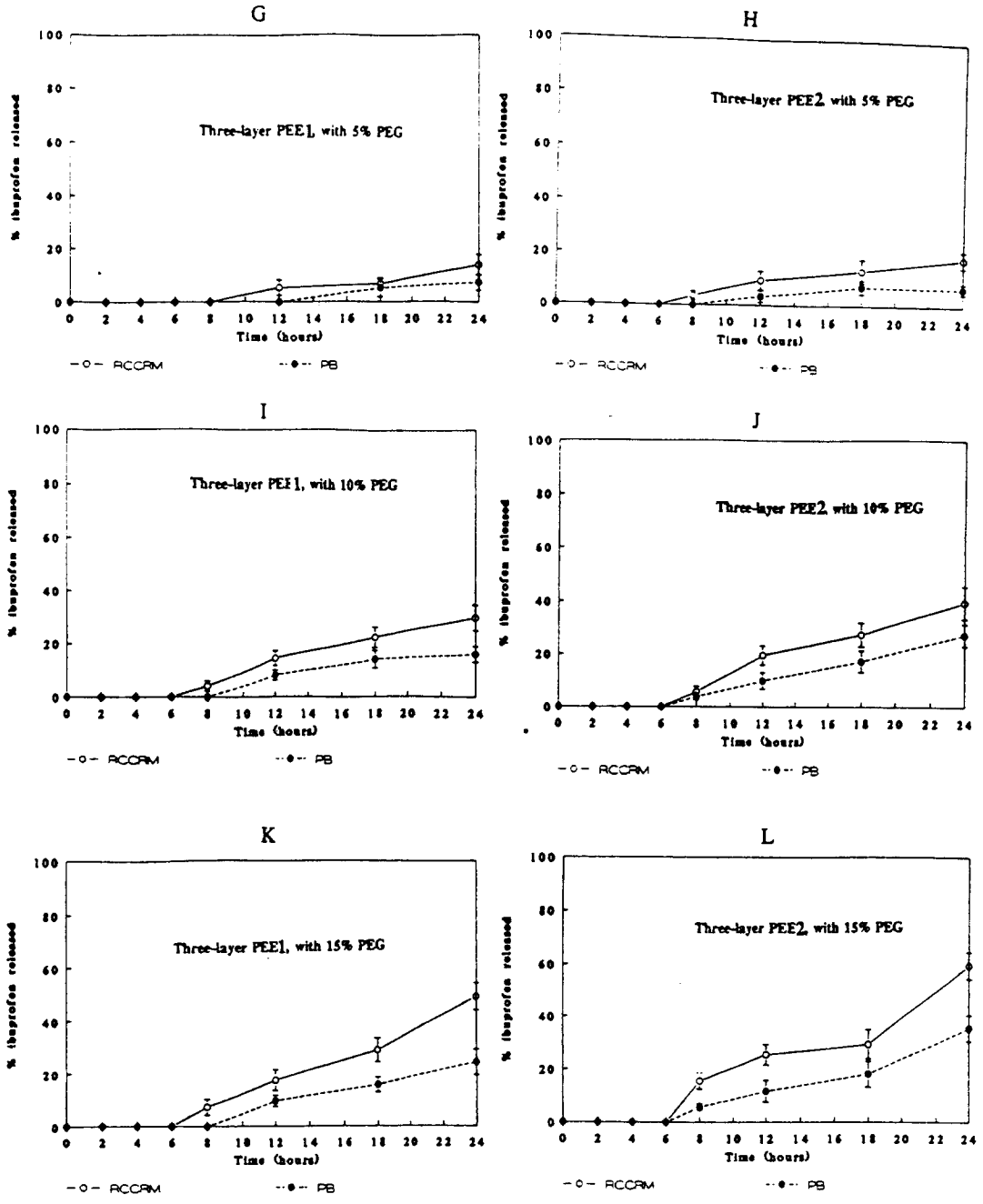

Fig. 2. (G–L).

capsules. This means that azoreductase did not play a significant part in the release of the drug for the two-layer capsules.

The release of ibuprofen from the three-layer capsules with 5% PEG 400 was statistically the same as that of the three-layer without PEG 400,

both for PEE1 and PEE2 (Fig. 2G and 2H). However, when 10% PEG 400 was added to the coating solution, a difference was noticeable. In 8 h, the capsules coated with PEE1 released about 8% of the drug in the RCCRM. The release in PB was apparent between 8 and 12 h (Fig. 2I). After 24 h, about 34 and 28% of the drug was released in the RCCRM and PB, respectively. The capsules coated with PEE2 solution with 10% PEG 400 exhibited an even higher release, of about 39 and 29% in RCCRM and PB, respectively, after 24 h (Fig. 2J).

Increasing PEG 400 to 15% in the coating solution further enhanced the release of the drug. Up to 49% of the drug was released from the capsules coated with PEE1 after 24 h in the RCCRM and 28% in PB. The release from the capsules coated with PEE2 released 59% and 35% in RCCRM and PB respectively after 24 h (Fig. 2K and 2L).

An attempt to increase the amount of PEG 400 in the coating solution to 20% resulted in a coat which was unable to dry completely. The coating remained sticky for a prolonged period of time. Thus no release experiments were possible with these capsules.

The results show that the two-layer capsules are unsuitable for use in colonic drug delivery systems. The drug is released too early. Taking into account the gastrointestinal transit time as reviewed by Gruber et al. (1987), the drug will be released in the stomach or proximal ileum.

The three-layer capsules without PEG 400 also proved to be less amenable for colon-specific drug delivery systems. Although it appears that most of the drug will be released in the large intestine, it is only a small percentage of the total amount of the drug that is released. In order to reach a sufficient plasma level, a large amount of the drug will be required to be incorporated in the capsules. This will inevitably lead to a danger of overdose in case the capsule bursts, accidentally or otherwise and releases the drug not in the manner originally intended.

The enhancement of the drug release after addition of PEG 400 to the coating solution is attributed to increased hydrophilicity of the coating, which in turn facilitates the interaction of azoreductase with the polymeric coating. This supports an earlier observation whereby polysorbate 80 enhanced biodegradation of poly(ether-ester) azopolymers (Samyn et al., 1995). It should be noted that there was no significant difference in the release of the drug between the capsules placed in PB with and without PEG 400.

The three-layer capsules with PEG 400 with 15% PEG 400 showed promising results as a potential preparation for use in colon-targeting. Since the transit time of the drug in the colon ranges between 20 and 30 h (Hardy, 1989), it is plausible that a sufficient amount will be released after a longer period.

The difference in the rate of release of ibuprofen from the capsules in RCCRM and those in PB can only be explained by the effect of azoreductase on azobonds of the poly(ether-ester) azopolymers. The enzyme catalyses the reduction of the bonds leading to amines via amide intermediates. The mechanism of this biodegradation is discussed in detail elsewhere (Samyn et al., 1995). Soluble flavoproteins which are dependent on nicotinamide adenine dinucleotide phosphate (NADPH) are involved. Soluble flavins can act under anaerobic conditions as electron shuttles between NADPH-linked flavoproteins and other electron acceptors. The azo compounds, being electron acceptors are thus reduced to primary amines. The breakdown of the azo bonds is thought to create weak areas where the drug can pass through. The graphs show a slow release at first, but the rate increases as more 'pores' are created when more bonds are broken.

In the three-layer capsules, it was noticed that PEE2 coated capsules released the drug faster and in higher amounts than those coated with PEE1. This again can be explained by the difference in the hydrophilicity between the polymers. Whereas PEE1 has 16-hydroxyhexadecane in its structure, PEE2 has 12-hydroxydodecane. The former, being higher in the homologous series is more hydrophobic compared to the latter. PEE2 is therefore more accessible to azoreductase than PEE1. Thus capsules coated with PEE2 released the drug faster than those coated with PEE1.

When the coating is thicker, as in the four-layer capsules, the enzyme fails to penetrate up to the

innermost layers, and hence fails to break all the bonds. Thus the four-layer capsules did not release the drug in 24 h. But, with more time, the enzyme may reach those layers to effect biodegradation with subsequent release of the drug. However, from the practical point of view, very slow release of drug may not be acceptable. The capsule may already be out of the GI when the drug is released. Therefore also these capsules are considered not suitable for colon-specific drug delivery systems.

4. Conclusion

The capsules coated with azopolymer/Eudragit film released more drug when placed in the RCCRM than in PB. The increase in the drug release was due to degradation of azo bonds by azoreductase in the RCCRM. The release was a function of the thickness of the film, being higher the thinner the film. However, the capsules coated with the film devoid of polyethylene glycol (PEG) did not release satisfactory amounts of drug after 24 h. Therefore the capsules are not recommended for use as colon-specific drug delivery systems.

Addition of PEG in the coating solution enhanced the drug release. 5% PEG increased the release only in the two-layer capsules, while in case of the three-layer capsules, drug release was affected by 10–15% PEG. Increasing PEG to 20% resulted in a weaker film.

The two-layer capsules released the drug too early to be of any use in colon-targeting. The four-layer capsules did not release the drug at all in 24 h, which also disqualified their use in colon-specific drug delivery. The three-layer capsules with 15% PEG showed satisfactory release of the drug, and these might be useful in colon-specific drug delivery.

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