Degradation of azo-containing polyurethane by the action of intestinal flora: its mechanism and application as a drug delivery system

Y. Kimura*, Y. Makita, T. Kumagai, H. Yamane and T. Kitao
Department of Polymer Science and Engineering, Kyoto Institute of Technology, Matugasaki, Kyoto 606, Japan

and H. Sasatani and S. I. Kim
Ono Pharmaceutical Co. Ltd, 3-1-1 Sakurai, Shimamoto-cho, Mishima-gun, Osaka 618, Japan
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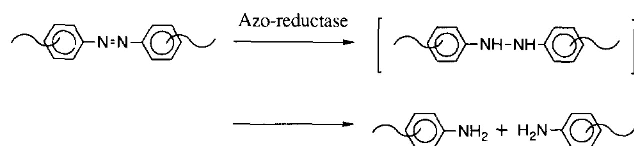
Several segmented polyurethanes comprising azo aromatic and hydrophilic groups were synthesized by reaction of *m*-xylylene diisocyanate with a mixture of *m,m'*-dihydroxyazobenzene, poly(ethylene glycol) (PEG, $M_n = 2000$) and 1,2-propanediol (propylene glycol, PG). Their polymer films were made by solution casting, and pellets of a hydrophilic drug (FOY-305) were coated with these polymers. Both the films and the coated pellets were incubated anaerobically in a culture of human intestinal flora. It was found that the azo groups in the polymer were reduced to hydrazo groups during incubation, which induced degradation of the films and the coatings without decreasing the molecular weight of the polymer. The rate of drug release from the pellets depended on both the azo and PEG compositions. Based on these findings, a new degradation mechanism of the polyurethane films and coatings is proposed.

(Keywords: azo reductase; intestinal flora; targeting large intestine; segmented polyurethane; large-intestine-degradable polymer; drug delivery)

INTRODUCTION

A new drug delivery system (DDS) targeting the large intestine has recently been proposed by Saffran *et al.*[1,2]. In their system a hydrophilic vinyl polymer crosslinked by an azo aromatic group was utilized as the coating material for drug pellets and capsules. When drug pellets and capsules coated with this polymer reached the large intestine, the azo groups were thought to be reduced to amines by the action of azo reductases which are liberated by intestinal floras[3–5]:



Scheme 1

By this reduction the crosslink was broken, with a part of the polymer coat solubilized in water, and the drug incorporated was released. However, the mechanism of degradation was not fully clarified, and it was noted that little weight loss of the polymer was found after it had been incubated in the flora culture. Furthermore, the preparation and coating of such a crosslinked polymer were found to be very difficult because of poor solubility in solvents. Consequently, development of soluble, large-intestine-degradable polymers as well as elucidation of their degradation mechanism is desired. In the present paper, we report on the segmented polyurethanes[6] comprising azo aromatic groups in the main chain, which can be degraded by the action of intestinal flora. The polyurethanes consist of three segments, i.e. (a) azo aromatic, (b) hydrophilic polyoxyethylene and (c) hydrophobic regular polyurethane segments (*Scheme 2*). Their degradability can be readily controlled by changing the segment ratios. The analysis of these polymers incubated in the flora culture indicated that the azo groups were reduced to hydrazo groups, not to amines, by the action of intestinal flora. This discovery is very useful in clarifying the degradation mechanism of the polymers, which was unknown[3], as well as clarifying the relationships between the polymer structure and the degradability.

EXPERIMENTAL

Materials

m,m'-Dihydroxyazobenzene (DHAB) was prepared according to the literature method[7]. *m*-Xylylene diisocyanate (XDI), propylene glycol (PG) and poly(ethylene glycol) with $M_n = 2000$ and $M_w/M_n = 1.2$ (PEG 2000) were commercial products from Nakarai Tesque (Kyoto, Japan). XDI and PG were purified by vacuum distillation. PEG was thoroughly dried *in vacuo*. The other compounds and the solvents were of reagent grade and were used without further purification.

*To whom correspondence should be addressed

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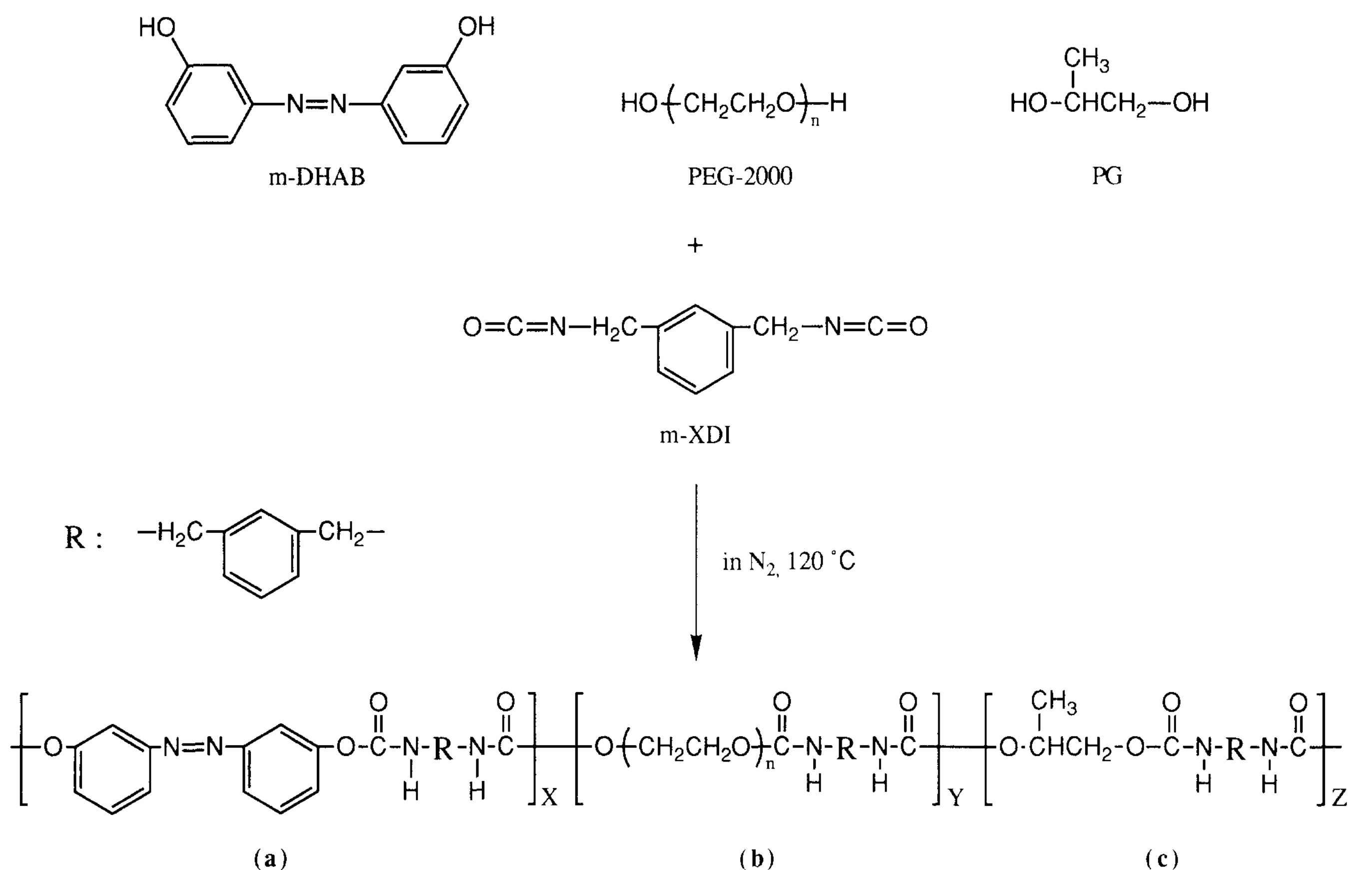


Scheme 2

The general anaerobic medium (GAM) was supplied from Kyowa Fermenting Co. Ltd (Japan). It is a serum-agar semifluid culture for growing anaerobic microorganisms, which comprises L-cysteine hydrochloride and sodium thioglycolate as the hydrogen donors. Dyes, Amaranth and CI Direct Blue, were provided by Tokyo Kasei Co. (Tokyo, Japan).

Measurements

^{1}H n.m.r. spectra were recorded on Varian XL-200 (at 200 MHz) and Hitachi R-24B (at 60 MHz) spectrometers with tetramethylsilane (TMS) as the internal standard. I.r. spectra were recorded on a Jasco IRA-1 spectrometer and a Fuji FIRIS-100 *FT*-i.r. spectrometer. U.v. spectra were measured on a Shimadzu UV-260 spectrophotometer. The molecular weight of the polymer was determined by gel permeation chromatography (g.p.c.), which was recorded on a Toso HLC-802A instrument fitted with a r.i. detector and a Toso CP-8000 data processor. A polystyrene gel column of Toso TSK gel G4000 H_8 (7.5 mm i.d. × 60 cm) was used with tetrahydrofuran (THF) as the eluent. The molecular weight was calibrated with polystyrene standards. The limited exclusion molecular weight was 4×10^5. Liquid chromatography (l.c.) was measured on a Shimadzu LC 6A chromatograph system fitted with a common ODA column (50 cm length) and a u.v. detector. Scanning electron micrographs (SEM) were taken on a Jasco JSM-T100 microscope. Tensile measurements were carried out on a Shinko Tsushin TOM 200D tensile tester.

Preparation of azo-containing polyurethanes

First 5.85 g (27.3 mmol) of DHAB and 18 g (90 mmol) of PEG were placed in a 200 ml round-bottomed flask equipped with a mechanical stirrer and a dropping funnel. The flask was evacuated by a vacuum pump for several hours in order to dry the contents, and was flushed with dry nitrogen gas. Then, 18 g (237 mmol) of PG was added to the flask. The flask was heated to 100°C with stirring under a nitrogen flow, and 51.4 g (273 mmol) of *m*-XDI was added dropwise over a period of 3 h from the dropping funnel. After the addition was over, the reaction mixture was kept stirred until its viscosity increased. At the end of the reaction a few grams of ethanol (EtOH) was added to the system to stabilize the terminal isocyanate groups. The product was then dissolved in 300 ml of 1,4-dioxane (DOX) and poured into 3000 ml of EtOH for reprecipitation. The precipitate was filtered, washed with ethanol, and dried in a vacuum oven at 70°C, to give a reddish brown powdery material. The polyurethanes with different compositions of the three components were also prepared likewise. The characterization of these polymers was made by ^{1}H n.m.r. spectroscopy. ^{1}H n.m.r. (in dimethylformamide-d_7): $\delta = 1.19$ (d, CH_3 for PG unit), 3.57 (s, CH_2O for PEG unit), 4.07 (d, CH_2 for PG unit), 4.28 (d, CH_2 for *m*-XDI unit), 4.96 (q, CH for PG unit), 7.16–7.26 and 7.51–7.68 ppm (m, C_6H_4 for *m*-XDI and DHAB units). By signal integration the segment ratios of the polymers were determined, which were almost equal to the feed ratios of the diol components. The results are summarized in *Table 1*.

Table 1 Segmented polyurethanes prepared

Polymer no.	PEG/PG/Azo (wt%)	Azo composition[a] (mol%)	M_n[b]	M_w/M_n[b]
1	50/50/6	3.5	9700	2.5
2	50/50/9	5	16 100	1.9
3	50/50/18	10	13 400	1.8
4	70/30/12	10	15 500	1.9

[a]Relative to the total glycol components
[b]By g.p.c.

Film preparation

A concentrated solution (ca. 20–40%) of the polyurethane in DOX was prepared. It was cast on a glass plate, solidified by atmospheric air drying and then thoroughly dried *in vacuo* (60°C, 0.1 mmHg). A yellow transparent film was formed on the glass substrate and was carefully taken off.

Preparation of coated pellets

Spherical drug pellets containing FOY-305 (a hydrophilic medicine for pancreatitis made by Ono Pharmaceutical Co.[8]) were prepared by the powder coating method[9] using a centrifugal pellet moulding machine. On the nuclei of granular sugar a powder mixture of FOY-305 (50 wt%), lactose (30 wt%), microcrystalline cellulose (10 wt%) and 2-hydroxypropylcellulose acetate with low degree of substitution were coagulated with a 5 wt% aqueous solution of 2-hydroxypropylcellulose as the binder. The spherical pellets obtained were ca. 5 mm in average diameter.

The coating of the pellets was carried out by the pan-coating method[9]. A 5 wt% solution of the polyurethane in a chloroform/DOX mixture (1:1 in volume) was sprayed over the surface of the pellets and dried thoroughly to make a uniform coat of 10–20 μm in thickness.

Preparation of anaerobic culture of intestinal flora

It is known that more than 70% of human faeces comprises intestinal floras, both living and dead[10]. Therefore, human faeces collected without exposure to air as far as possible was utilized as the source of the flora[11]. Thus, under a nitrogen atmosphere ca. 1 g of human faeces was dispersed in 9 g of GAM, and the mixture was further diluted 10 times with GAM to obtain a standard solution of the flora. A portion of this solution was transferred to a culture tube, diluted 200 times with GAM, and cultured under anaerobic conditions at 37°C for 1 day. This culture was utilized for the incubation experiments.

The reductivity of the culture was assayed by incubating the blue azo dyes, Amaranth and CI Direct Blue. Since their chromophores were quenched by reduction of azo groups, the rate of reduction was traced by u.v. spectroscopy of the culture incubated. In the case of Amaranth (λ_{max} = 520 nm) 90% was reduced after incubation for 1 h, while in the case of CI Direct Blue (λ_{max} = 600 nm) 24 h was needed for its complete reduction. It was therefore admitted that Amaranth, a mono-azo dye, was more rapidly reduced than CI Direct Blue, a bis-azo dye. The reductivity of this culture was maintained for 4–5 days, but it rapidly decayed thereafter. These data indicated that the present culture of human faeces has a high reductivity for azo compounds.

Incubation of films

A prepared polyurethane film was dipped in the above culture of intestinal flora and incubated at 37°C under anaerobic conditions for 2–3 days. The film was then taken out from the culture, washed with water several times and dried thoroughly *in vacuo*. For the control experiment the same film was incubated in the GAM without human faeces under the same conditions, and was treated in a similar manner. Both films were analysed by spectroscopy and tensile tests.

Release study

The coated pellets obtained above were incubated in both the aforementioned culture of intestinal flora and GAM (control). The release of the drug FOY-305 from the pellets was assayed by l.c. of the cultures.

RESULTS AND DISCUSSION

Reduction of azo aromatic polyurethane in flora culture

The polymer films prepared from the segmented polyurethanes comprising azo aromatic groups and PEG blocks were incubated in the anaerobic culture of intestinal flora. As the control experiment the same films were incubated anaerobically in the GAM medium without intestinal flora added. *Figure 1* shows typical SEM photographs of the films incubated in the flora culture for 2 days, compared with those of the films incubated in the flora-free GAM medium. In the surface and interior of the former films many cracks and holes are noted, while in the control films the surface is as smooth as the original films. It is also known that the morphology change of the film was larger in the polymer with a larger azo composition. *Table 2* summarizes the results of tensile tests of these films having different segment compositions. The tensile elongation of the films incubated in the flora culture was much decreased in comparison with that of the corresponding control films. The tensile strength of the films with relatively higher azo aromatic composition was also lower than that of the control films. These data supported the conclusion that the polyurethanes containing azo aromatic groups are degraded by the action of intestinal flora.

Figure 2 shows the u.v. spectrum of the polyurethane taken from a film incubated in the flora culture, compared with that of the original polymer (sample: polymer no. 3

Table 2 Changes in molecular weight and mechanical properties of the polyurethane films after incubation in flora culture[a]

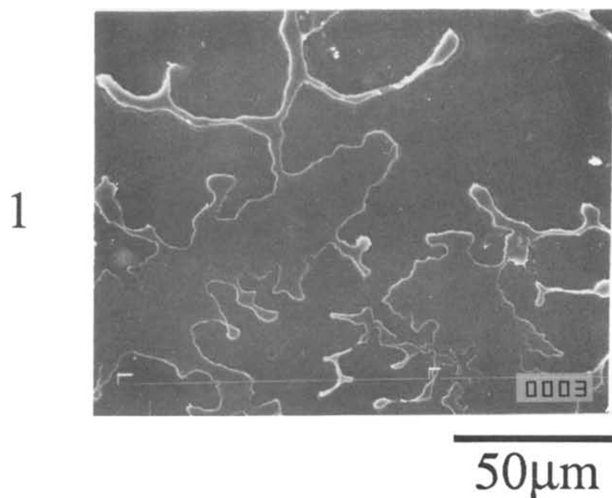
Polymer no.	Azo composition (mol%)		Tensile strength (MPa)	Tensile elongation (%)	M_w[b]
1	3.5	Control	33	73	9700
		Treated	43	53	9700
2	5.0	Control	122	540	16 100
		Treated	31	17	17 600
3	10.0	Control	28	400	13 400
		Treated	9	45	12 800

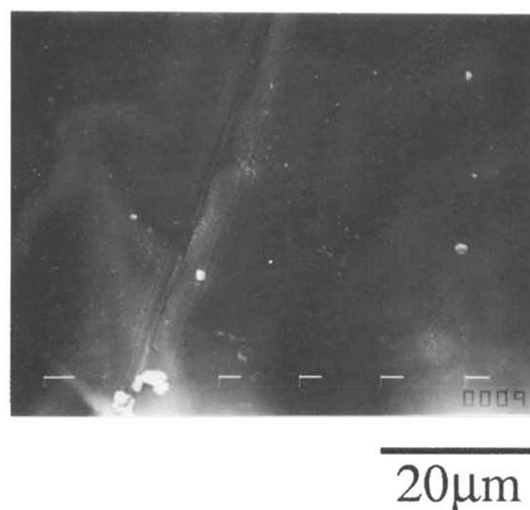
[a]After incubation in the flora culture (treated) and the GAM medium (control) for 2 days
[b]By g.p.c.

Treated
(a)

Control
(b)

1





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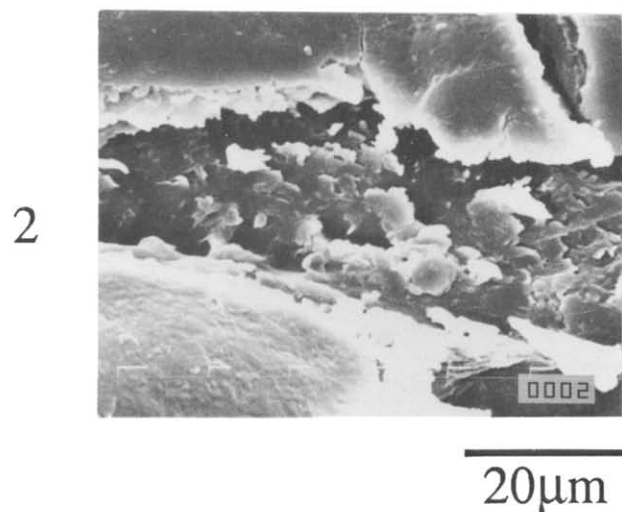


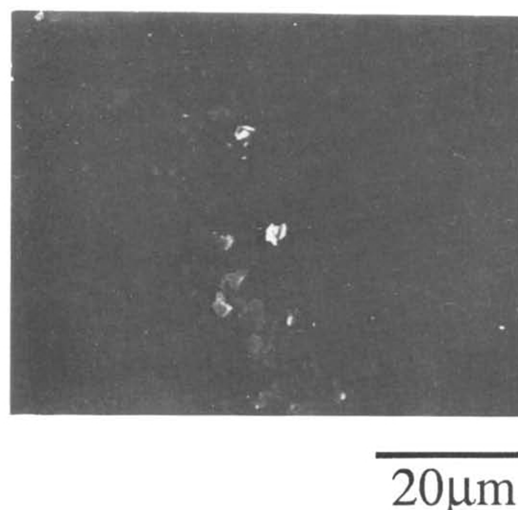


Figure 1 SEM photographs of the polyurethane films incubated (a) in the flora culture and (b) in the GAM medium (control) for the polyurethanes (polymer no. 1 and no. 2) with different compositions

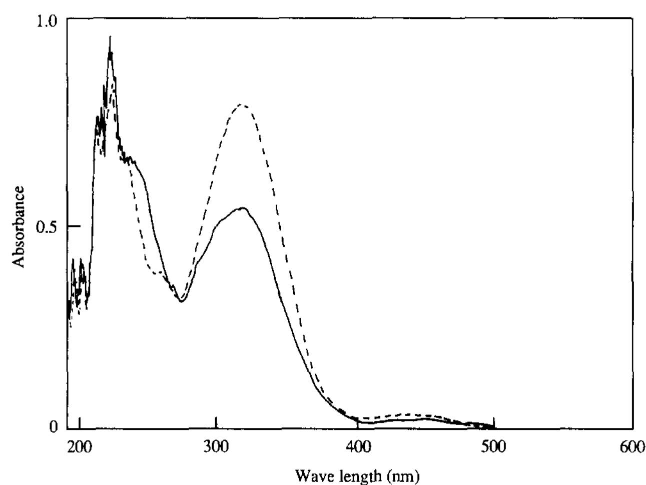


Figure 2 U.v. spectrum of the polyurethane after incubation in the flora culture (——) compared with that of the original (-----)

of *Table 1*). The absorbance at λ_{max} of 320 nm, which was ascribed to the characteristic absorption of the azo aromatic chromophore, was almost 40% lower for the film incubated in the flora culture than that of the original film. The film incubated in the flora-free culture did not exhibit such a decrease in the absorbance of the same band. It was therefore confirmed that a considerable amount of azo groups were reduced by the intestinal flora.

The molecular weights of the polymers taken from the control film and the film incubated in the flora culture were almost the same as each other in each experiment, as shown in the last column of *Table 2*. The polyurethanes with the azo composition of 5.0 and 10 mol% showed slight changes in molecular weight after incubation in flora culture, probably due to their difference in hydrodynamic volume. It was therefore known that the polymer backbones of the polyurethanes were not broken

during incubation, even though the azo groups were reduced by the action of flora. From these results it is reasonably considered that the azo aromatic groups were not reduced to amino groups but to hydrazo groups without breaking the polymer backbone. This fact was strongly supported by the ^{1}H n.m.r. spectrum of the polymer taken from the film incubated in the flora culture, in which the complex multiplet signals due to the aromatic protons of the hydrazo-substituted phenyl groups appeared at $\delta = 6.6-6.8$ ppm.

When the films incubated in the flora culture were exposed to air, the absorption band at 320 nm due to the azo aromatic chromophore gradually increased again. Its absorbance became almost the same as that of the original polyurethane (same as the broken curve of *Figure 2*) on exposure for a week. This was due to the oxidative dehydrogenation of the hydrazo group to the azo group. This backward reaction also supported the reduction of the azo groups to hydrazo groups in the bacterial treatment of the polyurethanes.

Drug release from polymer-coated pellets

Spherical pellets comprising a hydrophilic drug FOY-305 were coated with the above azo aromatic polyurethanes by the pan-coating method. The average thickness of the coats was approximately 10–20 μm. The coated pellets obtained were incubated in the anaerobic culture of intestinal flora in order to investigate the drug release from them. As control experiments the same coated pellets were incubated anaerobically in the GAM medium. The results are shown in *Figure 3*. With the polyurethane having the azo composition of 5 mol%, the release rate of the drug was very slow even in the flora culture. Only 20% of the drug incorporated had been released after incubation for 6 h, although it was twice as much as that obtained in the flora-free GAM medium. With the polyurethane having a higher azo composition of 10 mol% under the same PEG/PG ratio, the drug release was much accelerated in the flora culture, and more than 90% of the drug had been released after incubation for 6 h. In the flora-free media, in turn, the release was found to be less than 20%. These findings indicated that the drug release was activated by the action of intestinal flora. It was also known that the release was initiated after 2 h of dipping in the flora culture. This time lag may be due to the time necessary for the coating film to absorb water in its hydrophilic region. Since drug release from the control pellets incubated in the GAM medium was initiated after 4 h of dipping, the drug is considered to leak spontaneously through the polymer coats by diffusion if the coats are fully water-absorbed. In the presence of intestinal flora the water-absorbed polymer coats can be reduced more easily by the enzymes liberated by the flora so that degradation occurs more rapidly.

In the case of the pellets coated with the polyurethane having a higher PEG composition (PEG/PG = 70/30) at the azo composition of 10 mol%, the release rate of drug was fast even in the flora-free GAM medium, because that polymer coat was more hygroscopic. In the flora culture the release rate of drug became even faster, because the interaction between the azo aromatic groups in the polymer chain and the intestinal flora became easier in such a water-absorbed state.

These results indicated that the release pattern of the drug can be controlled by changing the compositions of both azo and PEG units. For sustained release of drug, for example, polyurethanes with less azo compositions are useful. For delivering enough drug to allow its absorption from the large intestine, the polyurethanes with relatively high compositions of azo and PEG units should be used.

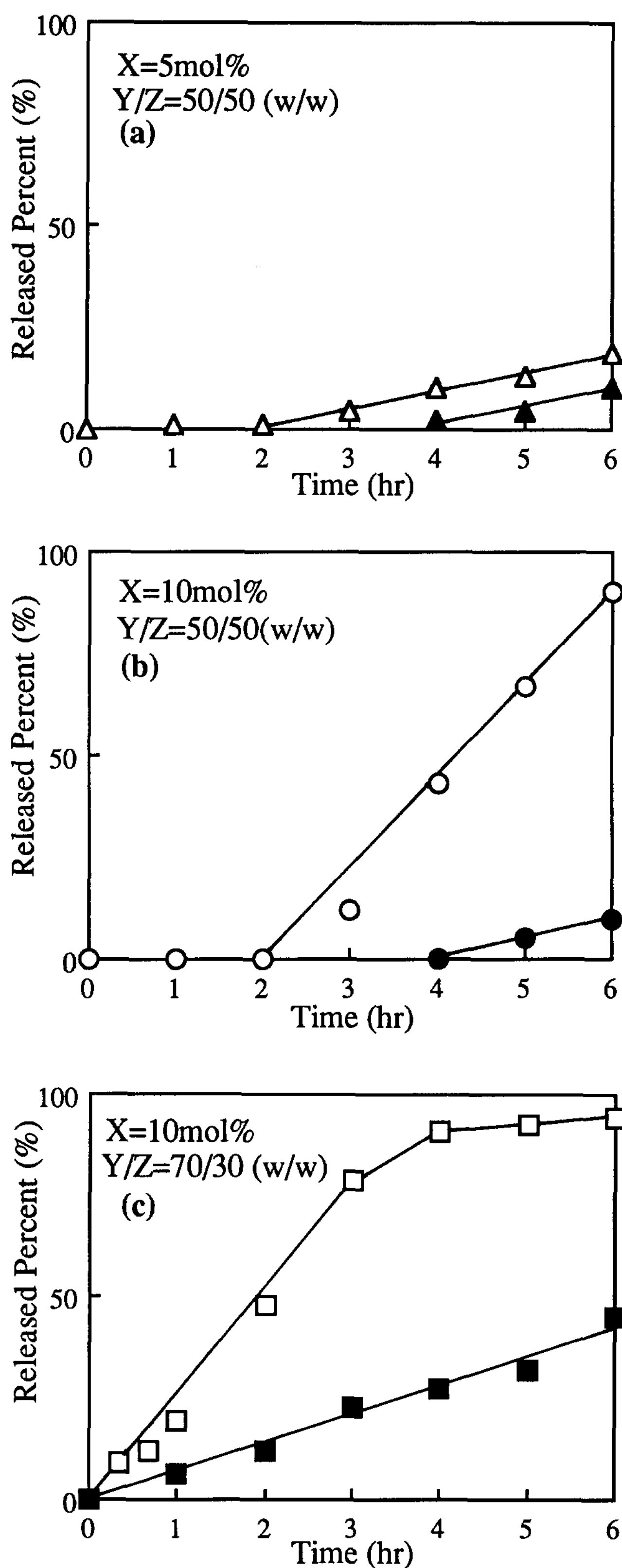
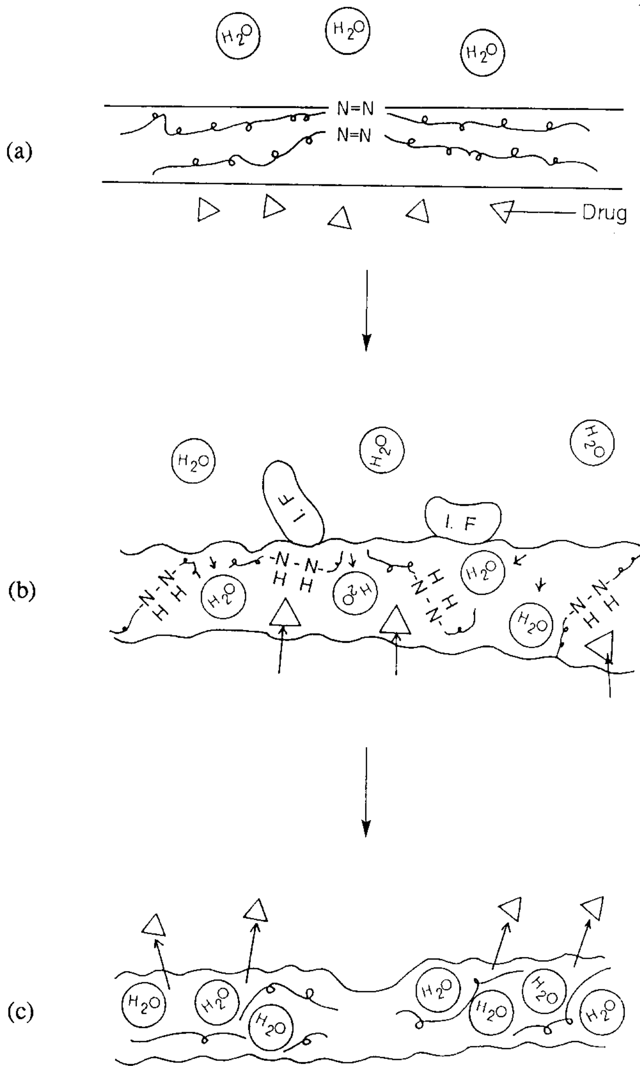


Figure 3 Release rate of drug from the drug pellets coated with the polyurethanes of (a) no. 2, (b) no. 3 and (c) no. 4. The open and closed symbols show the data in the flora culture and in the GAM medium (control), respectively



Scheme 3 Mechanism of the degradation of the polyurethane film and the drug release in the flora culture

Mechanism of drug release

A plausible mechanism for drug release is shown in *Scheme 3*. In the first stage, the polymer consisting of both hydrophobic and hydrophilic segments absorbs considerable water in the aqueous environment, and forms a hydrophobic/hydrophilic phase-separated structure similar to that of the so-called hydrogel. The azo aromatic segments are involved in the hydrophobic region by which the polymer is crosslinked (*Scheme 3a*). The intestinal flora would have access to these groups, especially to those on the surface, and would release the azo reductases, by which the azo groups are reduced to hydrazo groups. By this reduction the polymer conformation is changed, and the cohesion of the azo segments is broken, leading to the collapse of the film structure. Then, water uptake is allowed inside the film (*Scheme 3b*). As this reaction proceeds the drug incorporated is released outside the pellets (*Scheme 3c*). In this mechanism the observation of film degradation without decrease in molecular weight is clearly explained. We are now studying the structural effects of the azo aromatic and isocyanate groups on the degradability.

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