Synthesis and Bacterial Degradation of Novel Azo-polymer for Colon-specific Drug Delivery *

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Abstract: Objective: To synthesize and characterize novel azo-polymers composed of 2-hydroxyethyl mathacrylate (HEMA), methyl methacrylate (MMA) and methacrylic acid (MAA) cross-linked with 4,4-divinyl azobenzene (DVAB) and to investigate the biodegradation of azo-polymer consisting of different quantities of comonomers *in vitro*. **Methods:** UV, IR ^{1}H, ^{13}C NMR spectra were applied to characterize the structure of copolymer. **Results:** The content of HEMA and MAA unit in the copolymer has a profound effect on the swelling equilibrium of azopolymer. SEM, DSC and GPC show the degree of bacterial degradation on the surface of copolymer was depended on the ratio of hydrophilic and hydrophobic unit content. **Conclusion:** This azopolymer could be expected to use as a colon-specific drug delivery carrier.

Key words: azo-polymer; biodegradation; colon-specific drug delivery; P(HEMA-MMA-MAA)
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1 Introduction

Several systems are currently being investigated as potential means for colonic drug delivery[1]. One factor exploited is the high concentration of intestinal microflora residing in the colon; It has been reported to be up to five-orders of magnitude greater than concentration in other parts of the gastrointestinal tract. Based on this knowledge there are several kinds of biodegradable materials as drug carriers for colon-specific drug delivery like dextran[2]. Chondroitin sulfate[3] and pectin[4] etc. One approach to colon-specific delivery involves the use of biodegradable azo-polymeric devices. Such devices remain intact in the upper part of the gastric intestinal tract but biodegrade on arrival to the colon.

In this paper we'll present the research on the synthesis, thermal properties, swelling behavior and biodegradation of azopolymer based on PH-independent novel three-components copolymers of 2-hydroxyethyl methacrylate (HEMA), methyl methacrylate (MMA) and methacryl acid (MAA) cross-linked with 4,4′-divinylazobenzene(DVAB).

2 Experimental

2.1 Materials

HEMA, MMA and MAA (all from sigma Co. USA) were purified by distillation methods under reduced pressure conditions.

2.2 Azobisisobutyronitrile

Azobisisobutyronitrile (AIBN) (from the center of chemical agent of Shanghai) was re- crystallized in the methanol solution.

2.3 Synthesis of DVAB

Synthesis of 4,4′-divinylazobenzene (DVAB): 10 g of 4-nitrostyrene were mixed with 100 mL of hot tert-butyl alcohol and the resulting solution was allowed to reflux. 8.7 g of freshly regenerated zinc dust were then added, followed by 34.3 mL of 8 mol/L aqueous NaOH solution, the formation of the azo-compound was signaled by the development of a red-brown color and then purified. The procedure of reaction as following was detected by chromatographic analysis.

2.4 Copolymerization of azopolymer

All copolymerization were carried out with HEMA, MMA, MAA, DVAB in different ratio in alcohol under

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the atmospheres of N_2.

The copolymers were formed via a free radical mechanism using AIBN as initiator at a concentration of $w = 1\%$ at 60 ℃ for 24 h. The copolymers were isolated and purified by repeated precipitation in purified ether without water for 3 times in order to remove any residual monomer and /or initiator.

2.5 The swelling test

The swelling parameter

$$Q = \frac{m_{wet}}{m_{dry}}$$

m_{wet} and m_{dry} presents the weight of azopolymer before and after drying respectively.

2.6 Bacterial degradation tests

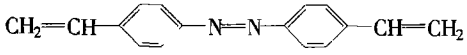
The isolated films were prepared with the 10% azopolymer alcohol solution by the casting methods. The film was incubated anaerobically (CO_2, H_2, N_2) in 30 mL saline dilution inoculated with 15 g freshly voided human faces at (37 ± 0.5)℃ those were collected from two healthy individuals on a normal Chinese diet. Seventy-two hours later the films were rinsed with distilled water, examined for degradation under Scanning Electron Microscopy and compared with films which were only incubated with saline dilution without human feces.

3 Results and discussion

To achieve successful bio-recognition of synthetic macromolecules in living systems a careful control of chemical physical and biological properties of synthesized macromolecules is necessary. We select three kinds of monomer, which has different mobile segment and hydrophilic properties in the backbone of copolymer.

3.1 Synthesis and characterization of DVAB

DVAB with the melting point about 137.7 ~ 138.6 ℃ was synthesized according to the synthetic procedure of John et al[5]. The yield of reaction is 30.38%. The infrared spectroscopy, ^1H NMR and ^{13}C NMR spectrum confirmed the structure of DVAB as following:



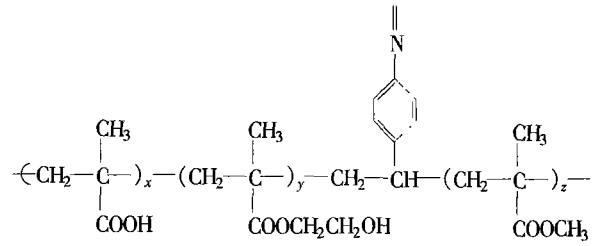
3.2 Synthesis and characterization of azo-polymers

In order to obtain soluble copolymers several copolymerization at different monomer/solvent ratio were carried out(Tab.1).

The results show that the soluble copolymers were obtained when the overall concentration of monomer was not higher than $w = 7\%$.

The UV spectrophotometry indicate that the largest absorption appeared at 342 nm for azopolymers, which is different from those for DVAB at 357 nm.

The infrared spectrum (Fig. 1) for azopolymers proves the formation of the copolymers, which are indicated by the disappearance of characteristic vinyl group absorption's (989.3, 902.5 cm^{-1} for DVAB) and 981.5, 925.6 cm^{-1} for MAA. The ^{13}C NMR spectrum (Fig. 2) further confirmed the chemical structures as following:



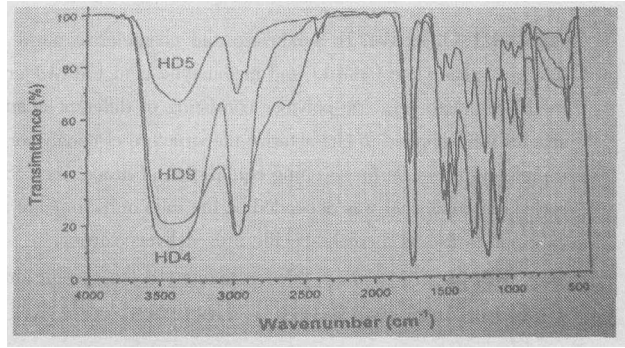


Fig.1 FTIR spectrogram of azo-polymer HD series

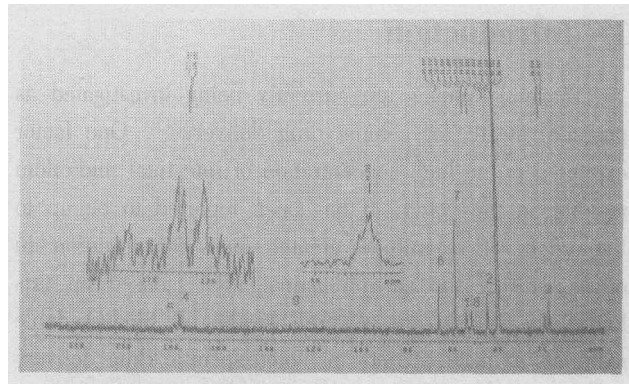


Fig.2 ^{13}C NMR spectrum of HD9

Tab.1 The feeding monomers in polymerization of azopolymer

Sample	HEMA	MMA	MAA	DVAB
HD 1	4.50	2.50	0.00	0.14
HD 2	5.00	2.00	0.00	0.14
HD 3	5.50	1.50	0.00	0.14
HD 4	6.00	1.00	0.00	0.14
HD 5	7.00	0.00	0.00	0.14
HD 6	5.50	1.00	0.50	0.14
HD 7	5.00	1.00	1.00	0.14
HD 8	4.50	1.00	1.50	0.14
HD 9	4.00	1.00	2.00	0.14

3.3 Swelling characteristic for azopolymers

The contents of HEMA and MAA in the copolymer have a profound effect on the factors that determine equilibrium swelling in these copolymers. Q value increased with increasing of MAA and HEMA contents.

Tab.2 The effect of the feeding monomer HEMA and MAA on the value Q in HD samples

HEMA[1)]	m/g	4.5	5.0	5.5	6.0	7.0
	Q	1.21	1.37	1.50	1.56	1.60
MAA	m/g	0.5	1.0	1.5	2.0	
	Q	1.59	1.61	1.64	1.70	

1) HEMA: MAA = 0; MAA: HEMA + MAA = 6 g

3.4 Bacterial degradation of the azopolymer

SEM photograph shows that the results of bacterial degradation were divided into three cases(Fig.3). At the first case no degradation was observed for the film with more contents of MMA. At the second case the morphology of the film shows roughly on the surface without hole for the sample of more contents of HEMA. At the third case the film shows a large number of irregularly shaped holes being strongly influenced by incubation due to more content of hydrophilic segment of MAA in copolymer chains.

DSC shows that T_g decreased from 132.7 to 113.7 after incubation of 72 h for copolymer. This means that some chains of copolymer were more flexible after degradation and some molecular with lower molecular weight after degradation takes as plasticizer in the mobile chains. GPC also shows that the molecular weight of copolymer decreases and distribution increase after incubation of films (Tab.3).

Tab.3 The change of molecular weight and its distribution before and after the incubation

HD 9	$M_n/10^5$	$M_w/10^5$	$M_z/10^6$	D
Before	1.1289	3.7786	1.0401	3.3471
After	0.9461	3.5345	0.9290	3.7359

4 Conclusions

The synthesis characterization, swelling behavior and azo-degradation test show novel azo-copolymer P(HEMA-co-MMA-co-MAA) may be potentially utilized as carriers for colon-specific drug delivery systems, if reasonable monomer ratio in the backbone of the copolymer was optimally selected.

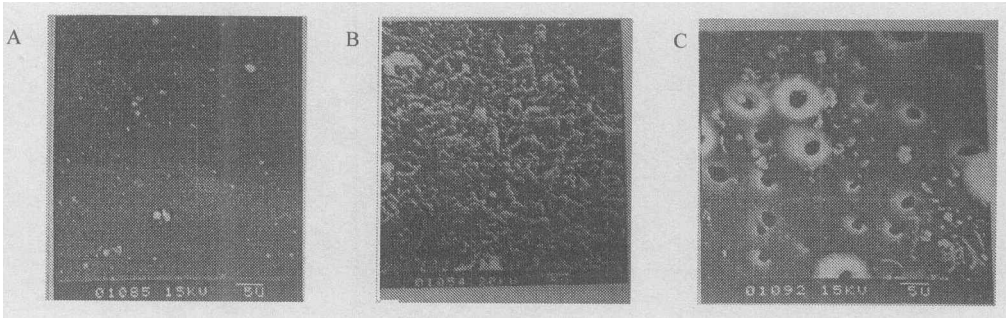


Fig.3 No degradation

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新型结肠定位给药系统偶氮聚合物的合成与细菌降解

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摘 要:合成与表征了用二乙基偶氮苯(DVAB)偶联的甲基丙烯酸羟乙酯(HEMA) - 甲基丙烯酸甲酯(MMA) - 甲基丙烯酸(MAA)偶氮共聚物,用紫外、红外、核磁共振表征该共聚物结构。结果表明共聚物中 HEMA 与 MAA 含量对偶氮共聚物溶涨性能有很大影响。SEM、DSC、GPC 测试表明,偶氮聚合物的菌解程度取决于共聚物中亲疏水成分含量。偶氮共聚物有望成为结肠靶向药物的载体。

关键词:偶氮聚合物; 生物降解; 结肠定位给药

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