Chinese Chemical Letters Vol. 15, No. 4, pp 449-452, 2004
http://www.imm.ac.cn/journal/ccl.html

Preparation and Adsorption Properties of PAM Based Adsorbents for Plasma Lipoproteins

Hai Tao LI[1,2]*, Zhi YUAN[1], Xin Fu CHEN[1], Bin LIU[3], Bin SHEN[3], Bing Lin HE[1]

[1]The State Key Laboratory of Functional Polymer Materials for Adsorption and Separation, Institute of Polymer Chemistry, Nankai University, Tianjin 300071
[2]Department of Chemistry, Hunan Normal University, Changsha 410081
[3]Tianjin Medical College, Tianjin 300052

Abstract: Crosslinked macroporous polyacrylamide (PAM) was prepared with inverse phase suspension polymerization technique. After treatment with hydrazine, the polymer was functionalized with chloroacetic acid, trifluoroacetic acid diethylenetriaminepentaacetic acid (DEPAA), and maleic acid, respectively, and PAM based adsorbents bearing carboxyl functional groups for low density lipoprotein (LDL) apheresis use were obtained. The blood compatibility and the adsorption properties for plasma lipoproteins of PAM based adsorbents were investigated.

Keywords: Macroporous polyacrylamide, blood compatibility, adsorption, LDL apheresis.

Low density lipoprotein (LDL) apheresis provides a safe and effective treatment for the patients with homozygous familial hypercholesterolaemia. Established methods involve either adsorption of apolipoprotein B containing lipoproteins by affinity columns containing anti-apolipoprotein B antibodies or dextran sulphate, or their precipitation at low pH by heparin, in each instance after first separating plasma from blood cells with a cell separator[1]. Polyacrylamide based adsorbents promise a practical clinic use for direct whole blood perfusion[2]. The aim of this work is to clarify the basic aspects of the adsorbents for LDL apheresis, such as the building of porous structure of the polymer matrix, the blood compatibility and the effect of the type of functional groups on the blood compatibility and adsorption properties.

Crosslinked microporous polyacrylamide (PAM) matrix was synthesized using free-radical inverse phase suspension polymerization technique. N, N′-methylenebisacryl-amide (Bis) was used as a crosslinker, ammonium persulfate and N, N, N′, N′-tetra-methylethylenediamine (TMEDA) were used as an initiator and an assistant initiator, respectively. In a typical experiment, 9.6 g acrylamide, 2.4 g Bis, 0.1 g ammonium persulfate, 2.0 g sodium chloride and drops of TMEDA were dissolved in 60 mL H_2O below 10°C. 500 mL four-necked round bottom flask was loaded with 200 mL mixture of liquid paraffin and carbon tetrachloride containing 0.2% span-80 (V/V=2). Nitrogen

* E-mail: haitao-li@hunnu.edu.cn

was introduced into the reaction vessel and the solution stirred under nitrogen atmosphere. The temperature of the organic mixture was sustained below 10°C before the premix solution of monomers was added. After the monomer solution was added into the vessel with stirring, the temperature was raised to 60°C in 3 hours and sustained for another 3 hours. The resultant beads were filtrated and washed with hot water, acetone and dried at 45°C under reduced pressure. Figure 1 shows the morphology of the resultant PAMs. Figure 1 indicates that the crosslinking degree needs to be equal or higher than 15% if macroporous PAM is to be prepared, otherwise no porous structure exists in resultant PAM. The specific surface areas and mean pore diameters of the dried resultant PAM beads were measured on a BET nitrogen adsorption apparatus (Micromeritics, ASAP 2010) and listed in Table 1. The results suggested that the specific surface areas and the pore structure of the resultant polymer beads could be adjusted by changing the crosslinking degree[3].

Figure 1 SEM micrographs of crosslinked polyacrylamide beads

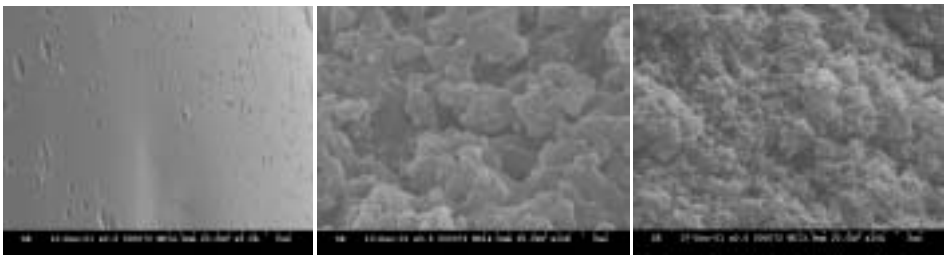
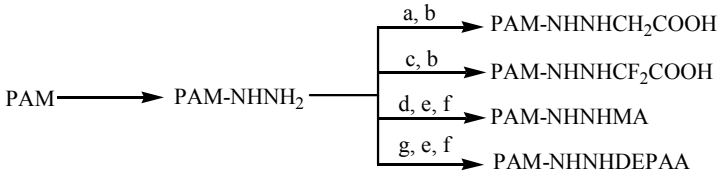


Table 1 Preparation and properties of crosslinked polyacrylamide beads

Sample	Crooslinking degree (%)	Specific area (m^2/g)	Mean pore diameter (nm)
PAM_5	5	-	-
PAM_{15}	15	14.2	22.8
PAM_{20}	20	18.3	20.7
PAM_{25}	25	17.9	21.1
PAM_{30}	30	19.9	20.4

Protocol of carboxylic acids being immobilized on PAMs was as following scheme:



a: $ClCH_2COOH$, b: NaOH, c: F_3CCOOH, d: maleic anhydride, e: DMF, f: 4-dimethylaminopyridine, g: Diethylenetriaminepentaacetic bisanhydride

The effects of reaction time, temperature and the concentration of hydrazine on the reaction of PAM with hydrazine were investigated in detail. The weak base and acid

exchange capacities of the hydrazine modified PAMs were measured with the method described in ref. 4 and listed in Table 2. From the test A in Table 2, one can conclude that the reaction time no longer affected the exchange capacity of the modified PAMs when it was longer than 4 hours. Therefor, 5 hours reaction time was adopted in the later experiments. The temperature dependence of the weak base exchange capacity of the modified PAMs indicated that hydrolysis of amide might occur when the temperature was as high as 100°C. This deduction was ascertained by the fact that no weak acid exchange ability was detected for modified PAMs as the reaction temperature were lower than 90°C and 0.40 mmol/g weak acid exchange capacity was measured for the modified PAM_{25}, when the temperature was 100°C. The results of test C showed that the concentration of hydrazine did not affect the exchange capacity very much. So that 42.5% hydrazine hydrate solution was selected in our later experiments.

Carboxyl groups were immobilized onto hydrazine modified PAMs. The weak acid exchange capacities of the adsorbents were measured and the results were listed in **Table 3**. The results indicated that the carboxyl groups could be conveniently immobilized onto hydrazine modified PAMs by covalent binding. Both haloacetic acid and anhydride could be the starting reactant. The existence of carboxyl groups on the PAM based adsorbents were further ascertained by infrared spectroscopy of the adsorbents. The exchange capacity of PAM based adsorbents showed that the smaller crossling degree of the polymeric matrix was, the more functional groups were immobilized on it.

Table 2 Effects of reaction conditions on the modification of polyacrylamide with hydrazine

Test A		Test B		Test C	
Reaction time (h)	Exchange capacity (mmol/g)	Temperature (°C)	Exchange capacity (mmol/g)	Hydrazine (wt%)	Exchange capacity (mmol/g)
2	2.85	65	1.90	12.1	3.80
3	3.26	75	3.50	18.2	4.13
4	3.50	80	4.02	24.3	4.48
5	3.54	85	4.44	36.4	4.85
6	3.58	100	4.17	48.6	4.74

Other reaction conditions: starting polymer: A: PAM_{30}, B: PAM_{25}, C: PAM_{20}, reaction temperature: 75°C for A and C, hydrazine concentration: 42.5% (wt%) for A and B.

Table 3 Immobilization of caboxylic acid on hydrazine modified PAMs

Starting polymer	Exchange capacity of the resultant polymers (mmol/g)			
	PAM-$NHNHCH_2COOH$[a]	PAM-$NHNHCF_2COOH$[b]	PAM-NHNH-MA[c]	PAM-NHNH-DEPAA[d]
PAM_5	5.05	6.02	5.11	4.01
PAM_{15}	4.06	4.84	4.06	3.35
PAM_{20}	3.82	4.54	3.89	2.90
PAM_{25}	3.34	4.21	3.25	2.65
PAM_{30}	3.05	3.83	3.08	2.33

Reaction conditions: temperature 75°C, reaction time: 8 hr for a and b, 6hr for c and d.

In the developing of polymeric adsorbents for hemoperfusion, the blood compatibility of the adsorbents is a major concerned factor [1,2,5]. It is well known that polyacrylamide hydrophilic gel is of excellent bio-compatibility. But the blood compatibility of chemically modified crosslinked polyacrylamide was not clear. The changes in rabbit blood contents were measured after being cycled in a PAM based adsorbent column. The results were listed in **Table 4**. PAM based adsorbents showed tolerable blood compatibility, except for the adsorbent bearing maleic acid as functional group (a significant reduction of blood platelet was observed on this adsorbent).

The adsorption properties of PAM based polyanion adsorbents were characterized by static adsorption approach. The adsorption method was described in ref. 6. The results listed in **Table 5** suggested that PAM based polyanion adsorbents could selectively adsorb LDL from human plasma. And the adsorption capacity increased with the increasing of acidic strength of the functional groups.

Table 4 Blood compatibility of polyacrylamide based adsorbents

Adsorbents	Changes in blood contents after hemoperfusion for 1.5hour (%)			
	White blood cell	Red blood cell	hemoglobin	Blood platelet
PAM	-8.4	-6.0	-2.8	-8.3
PAM-NHNH-CH_2COOH	-9.5	-6.9	-3.5	-13.5
PAM-NHNH-CF_2COOH	-8.0	-14.0	-1.2	-11.8
PAM-NHNH DEPAA	-9.6	3.7	3.1	-18.5
PAM-NHNH MA	-6.5	15.1	13.2	-46.4

Table 5 Adsorption properties of PAM based adsorbents for human plasma lipoproteins

Adsorbents	Adsorption capacity				
	TG (μmol/g)	Total Cholesterol (mg/g)	HDLc (mg/g)	LDLc + VLDLc (mg/g)	Selectivity (LDLc + VLDLc)/HDLc
PAM	0.26	0.50	0.15	0.35	2.33
PAM-NHNH-CH_2COOH	0.30	1.54	0.40	1.14	2.85
PAM-NHNH-CF_2COOH	0.20	2.31	0.60	1.71	2.85
PAM-NHNH DEPAA	0.70	2.38	0.62	1.76	3.03

Acknowledgment

This work was supported by the National Key Project of Fundamental Research (G1999064707)

References

1. R. T. Gilbert, *Atherosclerosis*, **2003**, *167*(2003), 1.
2. J. Draeger, U. Julius, K. Kraenzle, *et al.*, *Eur. J. Clin. Invest.*, **1998**, *28*(12), 994.
3. H. T. Li, K. Y. Shi, Z. Yuan, B. L. He, *Chin. Chem. Lett.*, **2003**, *14*(3), 267.
4. B. L. He, W. Q. Huang, *Ion Exchange and Adsorption resins*, Shanghai Sci. & Tech. Edu. Press, **1995,** pp. 126-127.
5. S. S. Kim, H. W. Kim, S. H. Yuk, *et al.*, *Biomaterials*, **1995**, *17*(8), 813.
6. Y. H. Yu, H. Q. Gu, B. L. He, *et al.*, *Acta Polymeric Sinica,* **1997**, 5, 606.

Received 14 April, 2003