Preparation and Characterization of Poly(Dimethyldiallyl Ammonium)Chloride and Antiglobulin Tests for Antibody Detection

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ABSTRACT: Poly(dimethyldiallyl ammonium)chloride [poly(DMDAAC)] was prepared in our laboratory and monomer-to-polymer structures were characterized by infrared (IR), proton, and carbon-13 nuclear magnetic resonance (NMR) as well as DEPT techniques. The polyelectrolytes were used to detect the antibodies of red blood cells (RBCs) in human sera and the results were compared with hexadimethrine bromide (polybrene), bromeline, and other methods. This article presents data supporting the use of the manual poly(DMDAAC) test; the 1-min procedure at room temperature provided a rapid and sensitive test. The states of agglutination of RBCs were investigated by transmission electron microscopy (TEM). The results of the morphology were in agreement with the manual poly(DMDAAC) test. J Appl Polym Sci 87: 1957–1961, 2003

INTRODUCTION

Quaternary ammonium polyelectrolytes have been shown to be useful for the detection of antibodies of red blood cells (RBCs) in human sera.[1–5] Here, we show the results of this application using the polymeric form of dimethyldiallylammonium chloride (DMDAAC) and the results were compared with those obtained from polybrene and other methods. The monomer (DMDAAC) was prepared by the reaction of allyl chloride, dimethylamine, and sodium hydride[6–8] and its conversion to the polymeric form was investigated by infrared and DEPT NMR techniques. The agglutination of red cells was determined by transmission electron microscopy (TEM).

EXPERIMENTAL

Reagents

The allyl chloride, dimethylamine, sodium hydrate, and other reagents used were of analytical grade, which were purchased from the Shanghai Chemical Reagent Co. (Shanghai, China) without further purification. The sodium ethylenediamine tetraacetic acid ($EDTA \cdot Na_2$), sodium citrate, normal saline, antibody sera, RBCs, and other biochemical reagents were from the Department of Transfusion, Nanjing Jinling Hospital (Nanjing, China). The manual poly(DMDAAC) test was performed in this hospital.

Instrumentation

Infrared spectra were recorded with a Bruker Vector 22 instrument (KBr pellets), NMR spectra were determined with a Bruker DRX-300 spectrometer in D_2O, and the transmission electron micrographs were taken with a Hitachi-800 microscope.

Preparation and methods

A flask equipped with a magnetic stirrer, thermometer, reflux, and addition funnel was filled with dimethylamine (80.0 mL); the flask was then immersed into an ice-cold water bath maintained usually at 10–15°C. During this time, a sodium hydride (20.4 g) aqueous solution (46–48%) and 50 mL of allyl chloride were added dropwise, separately, in 15 h. The mixture was stirred and heated at 50°C for 6–7 h, and then the reaction mixture was filtered using a Büchnet funnel removing the sodium chloride crystal, and the monomer DMDAAC was obtained. The monomer liquid was poured into a round flask, and 1.24 g of potassium persulfate and 50 mg of ferrous sulfate were placed under stirring and heating at 60°C for 6 h. An open beaker containing 250 mL of acetone/absolute alcohol (v/v 10:1) was kept at 50°C and the above reaction

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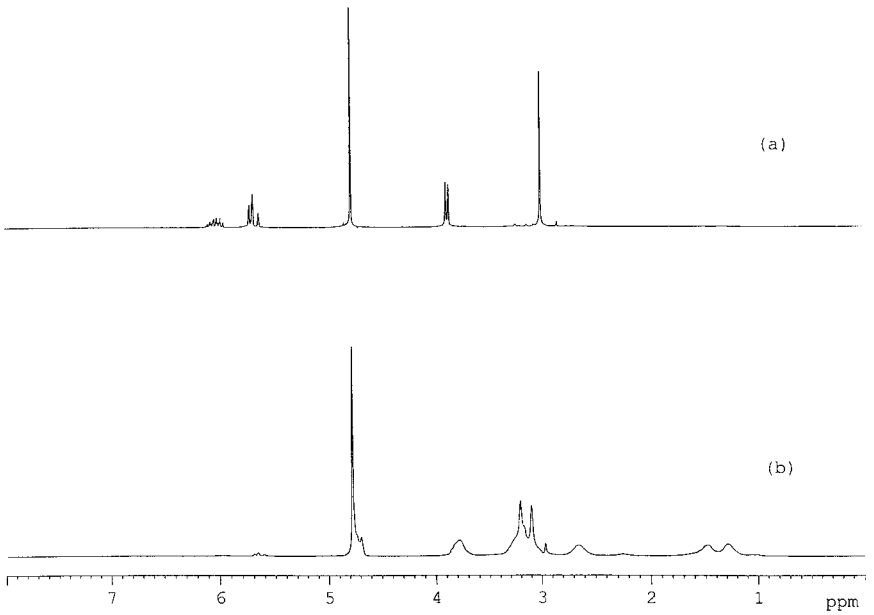

Figure 1 Spectra of (a) DMDAAC and (b) poly(DMDAAC).

solution was poured. At the end, the resulting solution was then set aside for 5 h, and the supernatant fluid was decanted. The white polymer was dried under a vacuum at 65°C until it reached a constant weight. Because of its hydroscopic nature, the polymer was stored in a desiccator.

Two drops of antibody serum were added to each tube and one drop of (2–5)% patient RBCs were also

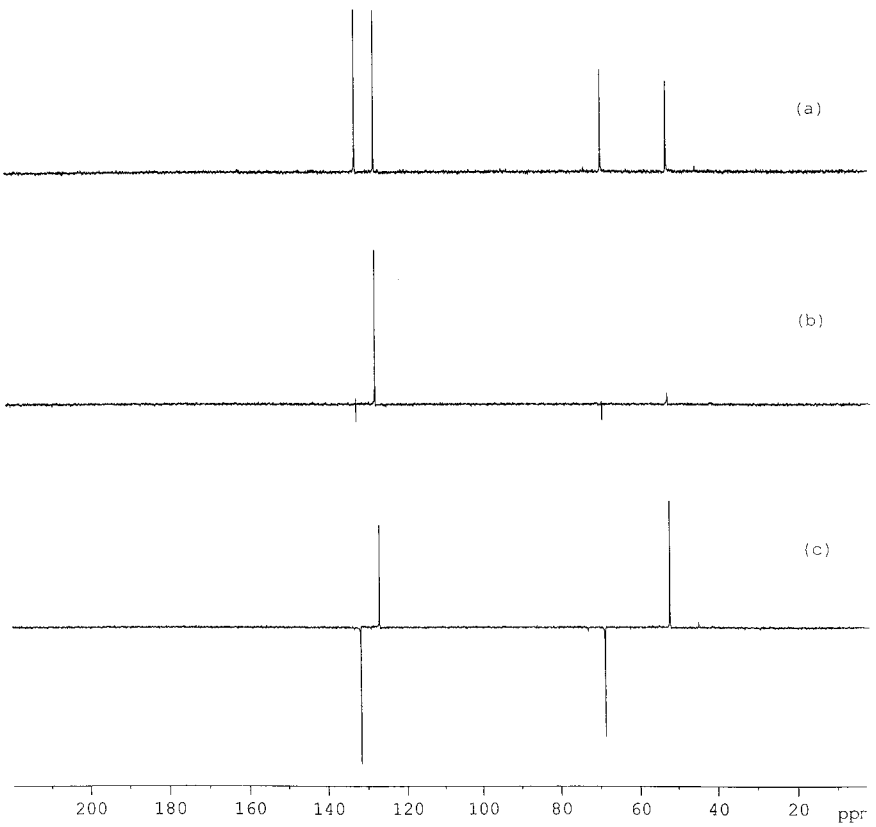

Figure 2 ^{13}C-NMR spectra of (a) DMDAAC, (b) DEPT-90, and (c) DEPT-135.

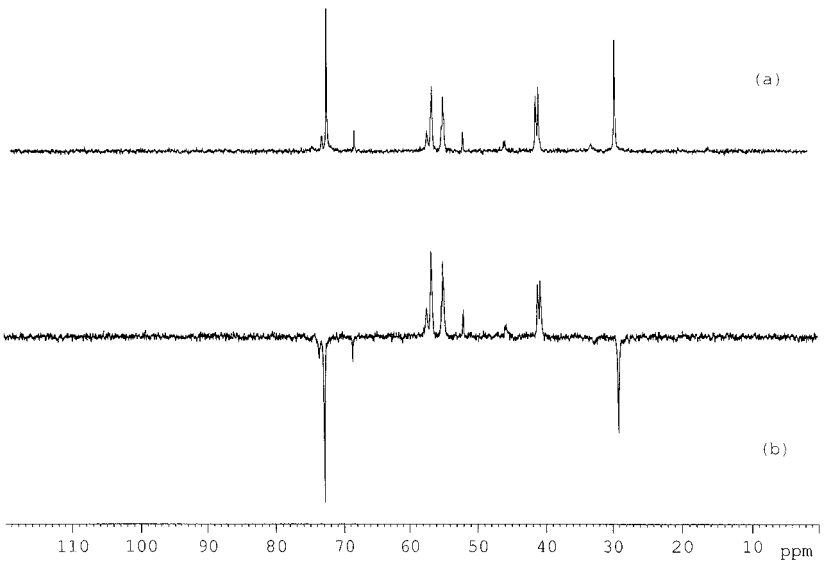

Figure 3 ^{13}C-NMR spectra of (a) poly(DMDAAC) and (b) DEPT-135.

added. After uniform mixing, six drops of a poly(DMDAAC) normal saline/EDTA · Na_2 solution were placed in each tube, and the tubes were agitated and set aside for 10 s at room temperature. The tubes were then centrifuged at 2000*g* for 15 s and the supernatant fluid decanted. The contents were examined for agglutination; if agglutination had occurred, two drops of a 0.2-mol/L trisodium citrate/glucose solution were added to the test tube and agitated. If agglutination appeared, it meant that the IgG antibody was examined as positive (+) or, on the contrary, as negative (−). Furthermore, the agglutination of RBCs was examined again by TEM.

RESULTS AND DISCUSSION

NMR spectroscopy

The ^{1}H-NMR spectra of the DMDAAC monomer and polymer are shown in Figure 1(a,b), respectively. In Figure 1(a), methyl protons give a single resonance peak at 3.0 ppm; the doublet peaks at 3.86 and 3.89 ppm are assigned to the methylene protons and are split (J = 7.4 Hz) by the olefin proton (—CH═). The triplet peaks at 5.7 and 6.1 ppm are attributed to the olefin protons of the —CH═ and CH_2═, respectively. From a comparison of Figure 1(a) and (b), it can be seen that the broad doublet peaks at about 1.5 ppm correspond to methylene protons and that methine gives a broad peak at about 2.64 ppm and that they are to coupled each other. The multiplet resonance peaks at 3.14 and 3.84 ppm are assigned to methyl and methylene protons bonded with quaternary nitrogen.

The ^{13}C-NMR spectra of the DMDAAC monomer gave four peaks at 44.6, 68.7, 127.0, and 131.7 ppm [see Fig. 2(a)] and the DEPT-90 [Fig. 2(b)] spectrum gave a single positive peak at 127.0 ppm and is assigned to the carbon of methine (═CH—). From the DEPT-135 [Fig. 2(c)] spectrum, the methylene carbons are observed as negative peaks at 68.8 and 131.7 ppm, while methyl and methine carbons are observed as positive peaks at 52.0 and 127.0 ppm. The ^{13}C spectra of poly(DMDAAC) are shown in Figure 3(a), and the chemical shifts are summarized in Table I. The strong and weak peaks arise from the *cis* or *trans* isomers, with respect to the carbon–methyl substitutions. For the poly(DMDAAC) compound, the N^+ methyls are nonequivalent in the *cis* isomer, but equivalent in the *trans* isomer. For our preparations, the *cis/trans* ratio was observed to be ~10/1. DEPT-135 [Fig. 3 (b)] experiments were carried out to confirm the assignment.

Infrared

The IR data of the DMDAAC monomer are shown as follows: The bands in the 1479 cm^{-1} region arise from methylene and methyl groups, methine exhibits an absorption band at 3018 and 961 cm^{-1}, and the band at 1638 cm^{-1} appears in the assignment of the ethylene linkage stretching vibration. A strong peak at 3440 cm^{-1} indicates OH absorption, owing to the hydroscopic nature of the monomer. From the IR data of

TABLE I
Carbon-13 Chemical Shifts of Poly(DMDAAC) (ppm)

Carbon	*cis*	*trans*
—CH—	40.4, 40.8	45.3, 45.5
—CH_2—CH_2—	28.6	32.2
N^+—CH_2—	72.5	73.2
N^+—CH_3	51.6, 54.6	56.9

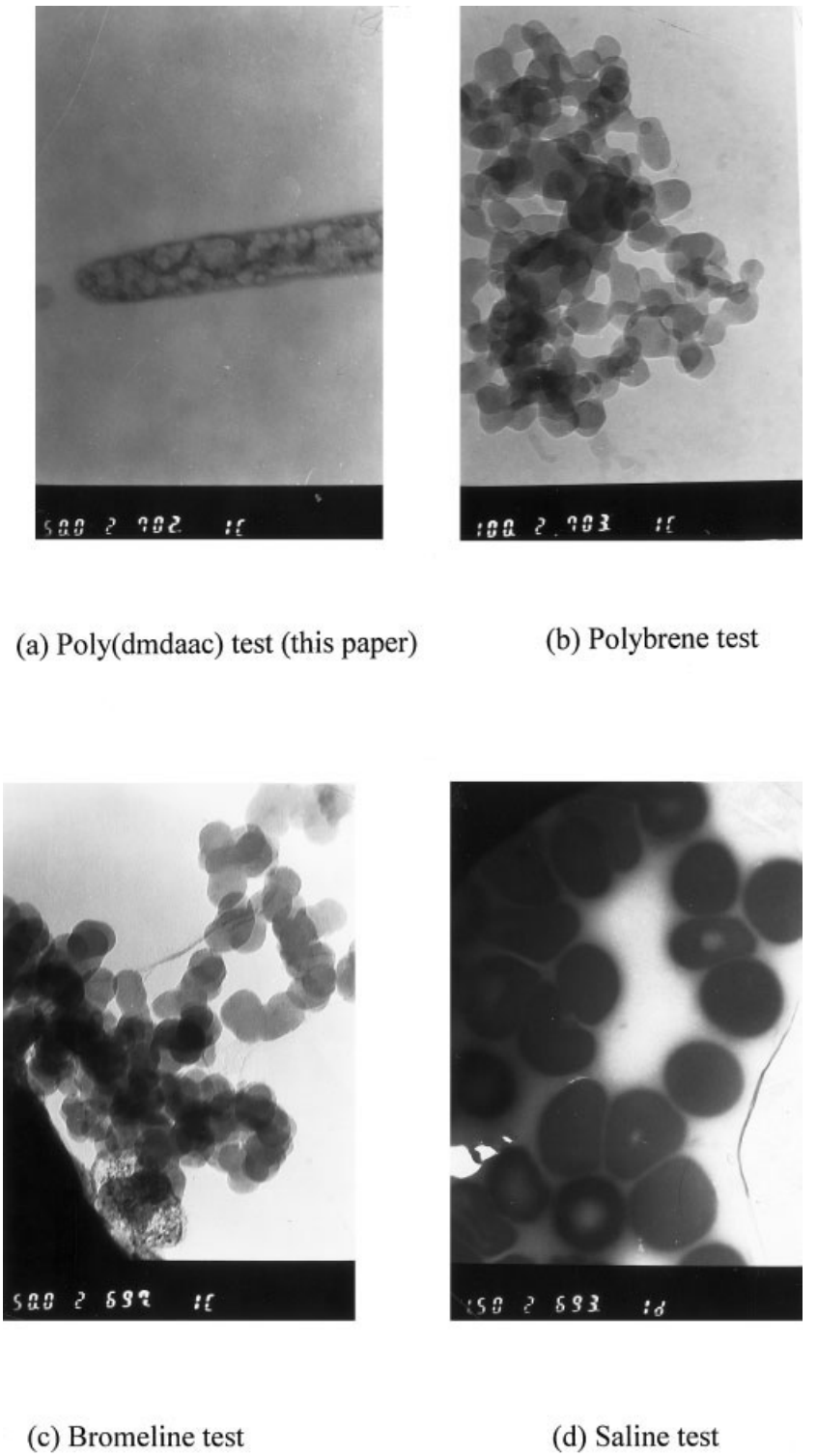

Figure 4 TEM photographs of agglutination of RBCs.

poly(DMDAAC), it can be seen that the characteristic absorption bands at 1640 and 947 cm^{-1} became weak, indicating that the monomer performed the polymerization. The results are in agreement with the NMR analysis.

TEM

In one case, the detections of anti-D in manual poly(DMDAAC), polybrene, bromeline, and saline methods were carried out, and the results of agglutination were determined by TEM. The TEM photographs of

TABLE II
Comparison of Several Methods

	Testing methods				
Anti-	Saline	Bromeline	AGT[a]	Polybrene	Poly(DMDAAC) (this article)
D	0	3+	3+	3+	3+
C	0	1+	2+	2+	2+
E	0	2+	1+	1+	1+
c	0	1+	2+	1+	1+
e	0	1+	1+	1+	1+

[a] Antiglobulin test.

the test results are shown in Figure 4 (a–d). It can be seen from Figure 4(a–c) that the agglutinations of red cells took place, but the saline technique failed [Fig. 4(d)].

Detection of incomplete antibodies

Parallel tests were performed in poly(DMDAAC), polybrerne, antiglobulin test (AGT), bromeline, and saline for IgG (anti-D, C, E, c, and e). The results are shown in Table II. The poly(DMDAAC) method is in good agreement with those reported for polybrene and other techniques and was sensitive for testing antibodies in RBCs in human sera. In addition to its sensitivity, the poly(DMDAAC) method can be completed within 1 min at room temperature.

Antigen was detected in 890 patients during crossmatching tests. Table III shows that two of 890 were nonreactive and 888 of 890 were reactive. The results show that the poly(DMDAAC) method was consistent with polybrene and bromeline.

TABLE III
Results of Antigen Test

	Methods		
Activity	Poly(DMDAAC) (this article)	Polybrene	Bromeline
Nonreactive	2	2	2
Reactive	888	888	888

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