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Synthesized negatively charged macromolecules (NCMs) for the surface modification of anticoagulant membrane biomaterials

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ABSTRACT

A series of negatively charged macromolecules (NCMs) including poly (sulfonated styrene-*co*-methyl methacrylate) (P(SS-*co*-MMA)), poly (acrylic acid-*co*-methyl methacrylate) (P(AA-*co*-MMA)) and poly (sulfonated styrene-*co*-acrylic acid-*co*-methyl methacrylate) (P(SS-*co*-AA-*co*-MMA)) are synthesized by reversible addition fragmentation chain transfer (RAFT) polymerization using carboxyl-terminated trithiocarbonate as a RAFT agent. Activated partial thromboplastin time (APTT) tests indicate that the NCMs can retard blood clotting due to the negatively charged groups. The synthesized NCMs can be blended with polyethersulfone (PES) in dimethylacetamide (DMAC) to prepare membranes by means of a liquid–liquid phase separation technique. The prepared membranes were regular and smooth, except P(AA-*co*-MMA) modified membranes which were crude and rough due to the poor miscibility of AA segment and PES. The NCM modified PES membranes exhibited good anticoagulant ability due to the existence of the large density of the negative charges on the membrane surface, which induced a strong electrostatic repulsion with the negatively charged blood constituents. Therefore, the P(SS-*co*-AA-*co*-MMA) was designed and prepared with appropriate proportions of SS, AA and MMA for better membrane performance. The results indicated that the P(SS-*co*-AA-*co*-MMA) had potential to improve the anticoagulant property of biomaterials and to be applied in blood purification.

1. Introduction

Anticoagulant membranes are extensively applied in biomedical fields as artificial organs, medical devices and disposable clinical instruments [1]. Contact of blood with foreign material surfaces may be accompanied by blood coagulation and platelet-activation [2]. So anticoagulant is needed in the practice application of medical devices made from these materials [3]. Actually, many polymeric biomaterials have been developed, but none of them are ideal in clinical situations, especially as blood contacting materials [4]. Thus, many studies have focused on the modification of conventional materials to improve the blood compatibility of blood-contacting materials [5].

The modification approaches include grafting negative charges or biological macromolecules (such as bovine serum albumin and heparin), grafting amphion polymers, designed microphase separation, and planting endothelial cell, and so on [6–12]. Many investigators have attempted to develop new synthetic anticoagulants or antithrombogenic materials [13–15]. Most of these materials are composed of ionic polymers containing sulfate, sulfamide and carboxylic acid groups, since it is believed that these ionic functional groups had a salutary effect on the anticoagulant activity [16–19]. Jozefonvicz et al. [20,21] reported that the modification of polystyrene and crosslinked dextrans by the incorporation of sulfonate and carboxylate moieties resulted in anticoagulant activity when these insoluble polymers contacted with blood. Magnani et al. [22–24] investigated the anticoagulant effect of modified hyaluronan (Hyal) by the insertion of sulfate to hydroxyl groups. The presence of a large density of negative charges in these macromolecules induces a strong electrostatic repulsion with the negatively charged blood constituents. The HyalSx derivatives showed a good anticoagulant activity and low platelet aggregation which increased with increasing the degree of sulfation [25]. However, few synthesized macromolecules with negative charges were developed and reported as a surface-modifying additive to modify conventional biomaterials.

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In our previous work, a heparin-like structure macromolecule (HLSM) containing the hydrophilic block–PVP and the functional groups —SO_3H, —COOH and —OH had been synthesized and blended with PES to prepare membrane, the anionic groups on the membrane surface may bind coagulation factors and thus improve anticoagulant ability [26]. However, it was found that the stability of the modified membrane is not very good due to the elution of the HLSM in water. Recently, a novel triblock copolymer of poly (styrene-co-acrylic acid)-b-poly (vinyl pyrrolidone)-b-poly(styrene-co-acrylic acid) (P(St-*co*-AA)-b-PVP-b-P(St-*co*-AA)) was synthesized for the modification polyethersulfone membrane with a hydrophilic and anionic surface to improve the membrane blood compatibility [27]. The elution of the additive from the membranes was avoided; however, the blended amount was only 5 wt% due to the microphase separation of PS chain.

In order to investigate the effects of functional groups on the properties of copolymers and membranes, in the present study, a series of negatively charged macromolecules (NCMs) including P(SS-*co*-MMA), P(AA-*co*-MMA) and P(SS-*co*-AA-*co*-MMA) were synthesized by reversible addition fragmentation chain transfer (RAFT) polymerization and were used as additives to improve the blood compatibility of conventional biomaterials. The MMA segment, which is often used as a biomaterial and medical material, was introduced as a hydrophobic block to the NCMs to prepare the novel hydrophiphilic block copolymers to avoid the elution of thee additives from membranes. In addition, there is good miscibility between PMMA and PES during membrane formation. As one of the most important polymeric materials widely used as blood contacting membrane in practice (e.g., hemodialysis) [28], PES was selected here as a substrate material to be modified. The activated partial thromboplastin times (APTTs) of the prepared NCMs and modified PES membranes were investigated.

2. Materials and methods

2.1. Materials

Polyethersulfone (PES, Ultrason E6020P) was obtained from BASF, Germany. Methyl methacrylate, styrene and acrylic acid (MMA, St and AA; 99.0%) were purchased from UNI-CHEM, China. Tetrabutylammonium hydrogen sulfate was purchased from Alfa Aesar, USA. St, AA and MMA were pretreated by activated carbons before use. *N,N*-dimethylacetamide (DMAC; AR, 99.0%) and *tert*-butyl alcohol (*t*-BOH; 99.0%) were purchased from Chengdu Kelong Inc. (Chengdu, China) and used as the solvent. Azo-bis-isobutryonitrile (AIBN) was purchased from Chengdu Kelong Inc. (Chengdu, China), which was used as the initiator. All the other chemicals (analytical grade) were obtained from Chengdu Kelong Inc., China, and were used without further purification.

2.2. Synthesis and characterization of RAFT agent

According to the literature [29], carbon disulfide (27.4 g, 0.36 mol), chloroform (107.5 g, 0.9 mol), acetone (52.3 g, 0.9 mol), and tetrabutylammonium hydrogen sulfate (2.41 g, 7.1 mmol) were mixed with 120 mL of mineral spirits in a 1 L jacketed reactor cooled with tap water under nitrogen. Sodium hydroxide (50%) (201.6 g, 2.52 mol) was added dropwise over 90 min to keep the temperature below 25 °C. The reaction was carried out overnight. Then, 900 mL of water was added to dissolve the solid, followed by 120 mL of concentrated HCl to acidify the aqueous layer, and then stirred for 30 min under a nitrogen purge. After filtering and rinsing thoroughly with water, the solid was dried to constant weight and then 41.3 g of earth-colored product was collected.

Characterization: ^{1}H NMR (DMSO-*d*6, ppm from TMS): 1.59 (s, 12H, —CH_3), 12.91 (s, 2H, —COOH). FTIR (KBr, cm^{-1}): 1710 (⟩C=O), 1060 (⟩C=S).

2.3. Synthesis and characterization of poly (styrene-co-methyl methacrylate) (P(St-co-MMA))

Copolymerization of St and MMA was carried out in a sealed tube. The general procedure is as follows. St, MMA, RAFT agent, AIBN, and *t*-BOH were added into a tube. After bubbling for 30 min with nitrogen, the reaction mixture was allowed to warm under a nitrogen atmosphere to 80 °C, and the copolymerization was carried out for 5 h. After precipitating in ethyl ether, the product of P(St-*co*-MMA) was dried under vacuum at 50 °C overnight. The prepared products with the St/MMA/RAFT agent feed ratios of 1.0/4.0/0.05, 2.5/2.5/0.05, 4.0/1.0/0.05, 2.5/2.5/0.025 and 2.5/2.5/0.10 (wt%) were termed K1, K2, K3, K4 and K5, respectively.

Characterization: ^{1}H NMR (DMSO-*d*6, ppm from TMS): 12.91 (s, 2H), 1.59 (s, 12H) for RAFT agent-terminated segment, and 6.82–7.29 (s, 5H, Ar—H), 3.32–3.56 (s, 3H, CH_3—O—), 0.73–0.93 (s, 2H, CH_3—C—C=O) for P(St-*co*-MMA). FTIR (KBr, cm^{-1}): 1060 (⟩C=S) for RAFT agent-terminated segment, 758.5 and 702.9 (⟩C=O) for St segment, and 1731.9 (⟩C=O) for MMA segment.

2.4. Sulfonation of P(St-co-MMA)

The fresh prepared P(St-*co*-MMA) was sulfonated using concentrated sulfuric acid as the sulfonating agent. The weight ratio of the copolymer to the acid was 1:3. The copolymer was stirred at room temperature in the acid for 10 h, then the homogeneous solution was poured into ice-cold water, and sulfonated polymeric powders (poly(sulfonated styrene-*co*-methyl methacrylate), P(SS-*co*-MMA)) was precipitated, and then the powders were washed with cold water several times to remove the acid and then dried. The copolymers K1, K2, K3, K4 and K5 were sulfonated and termed K1-S, K2-S, K3-S, K4-S and K5-S, respectively.

Characterization: ^{1}H NMR (DMSO-*d*6, ppm from TMS): 1.59 (s, 12H) for RAFT agent-terminated segment, and 6.71–7.97 (s, 5H, SO_3H—Ar—H), 3.32–3.56 (s, 3H, CH_3—O—), 0.73–0.93 (s, 2H, CH_3—C—C=O) for P(St-*co*-MMA). FTIR (KBr, cm^{-1}): 1060 (⟩C=S) for RAFT agent-terminated segment, 1035.6 (⟩SO_3H) for SS segment, and 1731.9 (⟩C=O) for MMA segment.

2.5. Synthesis and characterization of poly (acrylic acid-co-methyl methacrylate) (P(AA-co-MMA))

Copolymerization of AA and MMA was carried out in a sealed tube. The general procedure is as follows. AA, MMA, RAFT agent, AIBN, and *t*-BOH were added into a tube. After bubbling for 30 min with nitrogen, the reaction mixture was allowed to warm under a nitrogen atmosphere to 80 °C, and the copolymerization was carried out for 5 h. After precipitation in ethyl ether, the product of P(AA-*co*-MMA) was dried under vacuum at 50 °C overnight. The prepared products with the AA/MMA/RAFT agent feed ratios of 0.72/4.0/0.05, 1.80/2.5/0.05, 2.88/1.0/0.05, 1.80/2.5/0.025 and 1.80/2.5/0.10 (wt%) were termed K6, K7, K8, K9 and K10, respectively.

Characterization: ^{1}H NMR (DMSO-*d*6, ppm from TMS): 1.59 (s, 12H) for RAFT agent-terminated segment, and 12.52 (s, H, COO—H), 3.32–3.56 (s, 3H, CH_3—O—), 0.73–0.93 (s, 2H, CH_3—C—C=O) for SS and MMA segment). FTIR (KBr, cm^{-1}): 1060 (⟩C=S) for RAFT agent-terminated segment, 1618.8 (⟩COOH) for AA segment, and 1731.9 (⟩C=O) for MMA segment.

2.6. Synthesis and characterization of poly (styrene-co-acrylic acid-co-methyl methacrylate) (P(St-co-AA-co-MMA))

Copolymerization of St, AA and MMA was carried out in a sealed tube. The general procedure is as follows. St, AA, MMA, RAFT agent, AIBN, and *t*-BOH were added into a tube. After bubbling for 30 min with nitrogen, the reaction mixture was allowed to warm under a nitrogen atmosphere to 80 °C, and the copolymerization was carried out for 5 h. After precipitation in ethyl ether, the product of P(St -*co*-AA-*co*-MMA) was dried under vacuum at 50 °C overnight. The prepared products with the St/AA/MMA/RAFT agent feed ratios of 1.34/0.24/1.67/0.05, 1.67/1.20/0.05, 0.34/0.96/0.05, 1.67/1.20/0.025 and 1.67/1.20/0.10 (wt%) were termed K11, K12, K13, K14 and K15, respectively.

Characterization: ^{1}H NMR (DMSO-*d*6, ppm from TMS): 1.59 (s, 12H) for RAFT agent-terminated segment, and 12.52 (s, H, COO—H), 6.82–7.29 (s, 5H, Ar—H), 3.32–3.56 (s, 3H, CH_3—O—), 0.73–0.93 (s, 2H, CH_3—C—C=O) for P(St-*co*-MMA). FTIR (KBr, cm^{-1}): 1060 (⟩C=S) for RAFT agent-terminated segment, 1618.8 (⟩COOH) for AA segment, and 1731.9 (⟩C=O) for MMA segment.

2.7. Sulfonation of P(St-co-AA-co-MMA)

The fresh prepared P(St-*co*-AA-*co*-MMA) was sulfonated using concentrated sulfuric acid as the sulfonating agent. The weight ratio of the copolymer to the acid was 1:3. The copolymer was stirred at room temperature in the acid for 10 h, then the homogeneous solution was poured into ice-cold water, and sulfonated polymeric powders (poly(sulfonated styrene-*co*-acrylic acid-*co*-methyl methacrylate), P(SS -*co*-AA-*co*-MMA)) were precipitated, and then the powders were washed with cold water several times to remove the acid and then dried. The copolymers K11, K12, K13, K14 and K15 were sulfonated and termed K11-S, K12-S, K13-S, K14-S and K15-S, respectively.

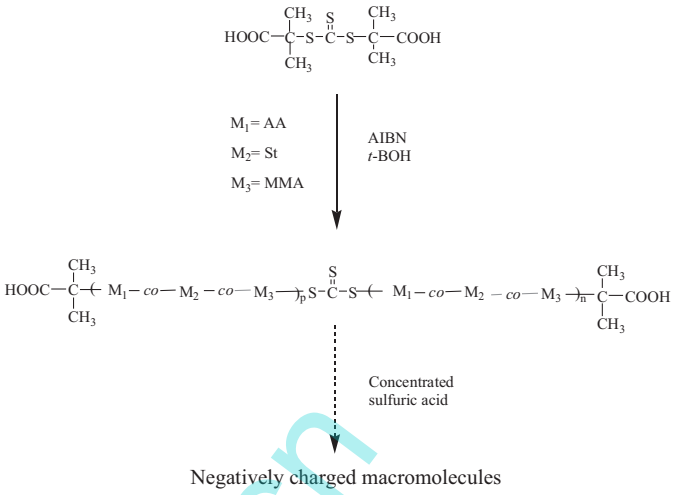
Characterization: ^{1}H NMR (DMSO-*d*6, ppm from TMS): 1.59 (s, 12H) for RAFT agent-terminated segment, and 12.52 (s, H, COO—H), 6.71–7.97 (s, 5H, SO_3H—Ar—H), 3.32–3.56 (s, 3H, CH_3—O—), 0.73–0.93 (s, 2H, CH_3—C—C=O) for SS, AA and MMA segments). FTIR (KBr, cm^{-1}): 1060 (⟩C=S) for RAFT agent-terminated segment, 1618.8 (⟩COOH) for AA segment, 1035.6 (⟩SO_3H) for SS segment, and 1731.9 (⟩C=O) for MMA segment.

2.8. Preparation of modified polyethersulfone membranes

The membranes of PES/NCMs were prepared by a phase inversion technique. PES and the synthesized NCM were dissolved in the solvent DMAC by vigorous stirring until a clear homogeneous solution was obtained. The concentration of PES and NCM was 16% and 4% (wt%), respectively. After vacuum degassing, the casting solutions were prepared into membranes by spin coating coupled with a liquid–liquid phase separation technique at room temperature. The membranes were rinsed with distilled water thoroughly to remove the residual solvent. All the prepared membranes were in a uniform thickness of about 60–70 μm. The K1-S, K2-S, K3-S, K4-S, K5-S, K6, K7, K8, K9, K10, K11-S, K12-S, K13-S, K14-S and K15-S modified PES membranes were termed $M_{K1\text{-}S}$, $M_{K2\text{-}S}$, $M_{K3\text{-}S}$, $M_{K4\text{-}S}$, M_{K5}, M_{K6}, M_{K7}, M_{K8}, M_{K9}, M_{K10}, $M_{K11\text{-}S}$, $M_{K12\text{-}S}$, $M_{K13\text{-}S}$, $M_{K14\text{-}S}$ and $M_{K15\text{-}S}$, respectively.

2.9. Characterization

FTIR spectra were measured with FT-IR Nicolet 560 (Nicol American) instrument. To prepare FTIR sample, the copolymer was dissolved in DMAC and cast on a potassium bromide (KBr) disc with the thickness of about 0.8 mm. The ^{1}H NMR spectra were recorded on a Varian Unity Plus 300/54 NMR spectrometer using



Scheme 1. Reaction pathway for synthesis of NCMs.

DMSO-*d* as the solvent at room temperature. The morphologies of the membranes were observed with an XL 30ESME scanning microscope. The structures and the elements of the membrane surfaces were investigated by reflected FTIR. Atomic force microscopy (AFM) studies were conducted using CSPM400 (Benyuan Nano-Instruments Ltd., China).

The number-average molecular weight of the copolymer was determined by gel permeation chromatography (GPC) on an HP1100 using two PL gel columns (10 μm, 104 Å; 10 μm, 500 Å), and using monodisperse polystyrene as the standard. The mobile phase was tetrahydrofuran (THF). The sample concentration was 1.0 g/L. The detector was RID, and the flow rate was 1.0 mL/min.

2.10. Clotting time of the NCMs and the modified membranes

To evaluate the antithrombogenicity of the NCMs and the modified membranes, activated partial thromboplastin time (APTT) was measured by an automated blood coagulation analyzer CA-50 (Sysmex Corporation, Kobe, Japan), and the test method was described as follows: At the beginning of the APTT test, healthy human fresh blood (Dr. F. Ran, healthy, man, Chinese, 32 years old) was collected in vacuum tubes containing sodium citrate as an anticoagulant (anticoagulant to blood ratio, 1:9 V/V), and the platelet-poor plasma (PPP) was obtained after centrifuging at 4000 rpm for 15 min. Synchronously, the copolymer or the membrane (0.5 cm × 0.5 cm, three pieces) was immersed in 0.2 mL PBS (pH = 7.4) for 1 h. The PBS was removed and then 0.1 mL of fresh PPP was introduced. After incubating at 37 °C for 30 min, 50 μL of the incubated PPP was added into a test cup, followed by the addition of 50 μL APTT agent (Dade Actin Activated Cephaloplastin Reagent from SIEMENS) (incubated 10 min before use), and incubated at 37 °C for 3 min. Thereafter, 50 μL of 0.025 M $CaCl_2$ solution was added, and then the APTT was measured. At least three measurements were averaged to get a reliable value, and the data were analyzed by statistical method.

3. Results and discussion

3.1. Preparation of negatively charged macromolecules (NCMs)

The schematic procedures for the synthesis of NCMs (P(SS-*co*-AA-*co*-MMA) was taken as example) are shown in Scheme 1. For facile copolymerization of monomers, trithiocarbonate (S,S′-Bis(α,α′-dimethyl-α″-acetic acid)-trithiocarbonate) was selected as the RAFT agent. The trithiocarbonate has extremely high chain-transfer efficiency and control over the radical polymerization

Table 1
The synthesis conditions and characteristic data of P(St-*co*-MMA), P(AA-*co*-MMA) and P(St-*co*-AA-*co*-MMA).[a]

No.	CTA (g)	AA (g)	St (g)	MMA (g)	M_n ($\times 10^3$)[b]	M_w/M_n[b]	*Conv* (%)[c]
K1	0.050	–	1.00	4.00	27.3	1.69	66.5
K2	0.050	–	2.50	2.50	29.9	1.72	70.2
K3	0.050	–	4.00	1.00	31.0	1.75	74.0
K4	0.025	–	2.50	2.50	40.4	1.82	65.8
K5	0.100	–	2.50	2.50	31.7	1.61	78.2
K6	0.050	0.72	–	4.00	16.5	1.68	71.1
K7	0.050	1.80	–	2.50	19.7	1.54	74.7
K8	0.050	2.88	–	1.00	18.0	1.50	78.6
K9	0.025	1.80	–	2.50	21.0	1.73	69.2
K10	0.100	1.80	–	2.50	10.2	1.49	79.0
K11	0.050	0.24	3.00	1.67	14.3	1.70	69.2
K12	0.050	1.20	1.67	1.67	21.0	1.60	75.2
K13	0.050	2.16	0.34	1.67	15.0	1.68	76.0
K14	0.025	1.20	1.67	1.67	29.7	1.73	70.1
K15	0.100	1.20	1.67	1.67	9.5	1.44	76.8

[a] Synthesis conditions: $V_{t\text{-BOH}} = 20$ mL, $m_{AIBN} = 0.01$ g; $T_p = 80\,^{\circ}C$; $t_p = 6$ h.
[b] M_n and M_w/M_n were determined by gel permeation chromatography (GPC).
[c] Conversions were determined gravimetrically.

because the carbon attached to the labile sulfur atom is tertiary and bears a radical-stabilizing carboxyl group [29]. Telechelic carboxyl-terminated polymers could be easily obtained due to the dicarboxyl trithiocarbonate. These series of products are the random copolymers because of the addition of monomers simultaneously before starting the reaction. The synthesized copolymers contained St units, and (P(St-*co*-MMA) and P(St-*co*-AA-*co*-MMA)) can be further sulfonated by concentrated sulfuric acid [30]. For the extraction of the homopolymers of PSS, PAA, and P(SS-*co*-AA), the prepared products were ground into fine powders and immersed in H_2O for one week. The remaining products were the NCMs of P(SS-*co*-MMA), P(AA-*co*-MMA) and P(SS-*co*-AA-*co*-MMA).

The synthesis conditions and characteristic data of P(St-*co*-MMA), P(AA-*co*-MMA) and P(St-*co*-AA-*co*-MMA) are shown in Table 1. As shown in Table 1, the monomer units of the compositions in the macromolecules' chains were successfully adjusted by the feed molar ratios, and the differences in the molecular weight are primarily attributable to controlling the amounts of the RAFT agent and monomers. The molecular weight distribution of the prepared products ranged from 1.44 to 1.82. The conversion ratios of the monomers were about 70%. P(St-*co*-MMA) and P(St-*co*-AA-*co*-MMA) were further sulfonated with concentrated sulfuric acid, and P(AA-*co*-MMA) were remained as prepared.

The color and water-solubility of the NCMs are shown in Table 2. It could be found that all the prepared NCMs containing SS units appear yellow or yellow-based colors, and the color darkened for the macromolecules with large amounts of SS units. The water-solubility of the copolymers depended on the MMA and SS contents in the macromolecules' chains. For example, K1-S was insoluble in water (less SS and more MMA), K3-S was soluble in water (less MMA and more SS), and K2-S showed enormous swelling in water (equal amounts of SS and MMA), as shown in Fig. 1. In addition, the effect of the molecular weight of the NCMs on water-solubility was found to be insignificant.

Table 2
Color and water-solubility of NCMs.

No.	Color[a]	Water-solubility[b]
K1-S	Light yellow	Insoluble
K2-S	Yellow	Turbid
K3-S	Dark yellow	Soluble
K4-S	Yellow	Turbid
K5-S	Yellow	Turbid
K6	White	Insoluble
K7	White	Insoluble
K8	White	Turbid
K9	White	Insoluble
K10	White	Insoluble
K11-S	Dark yellow	Soluble
K12-S	Yellow	Turbid
K13-S	Light yellow	Insoluble
K14-S	Yellow	Turbid
K15-S	Yellow	Turbid

[a] Based on nake-eyes observation.
[b] Preparation conditions: $m_{H_2O} = 20$ g, $m_{NCMs} = 0.8$ g; temperature = 20 °C, stirring for 12 h.

3.2. Anticoagulation performance of NCMs

APTT was used to examine the anticoagulative activities for NCMs with different concentrations (see Fig. 2). As a whole, the blood clotting time with a small amount of NCMs (0.5 mg/0.10 mL) was longer than that of the pure plasma, and the plasma was even incoagulable when the concentration of the NCMs was over 2 mg/0.10 mL. It suggests that adding NCMs to blood may inhibit the blood clotting. Furthermore, with the SS or AA contents in the chains of the NCMs increasing, the anticoagulant properties of the NCMs improved obviously. Holding to the premise with monomer

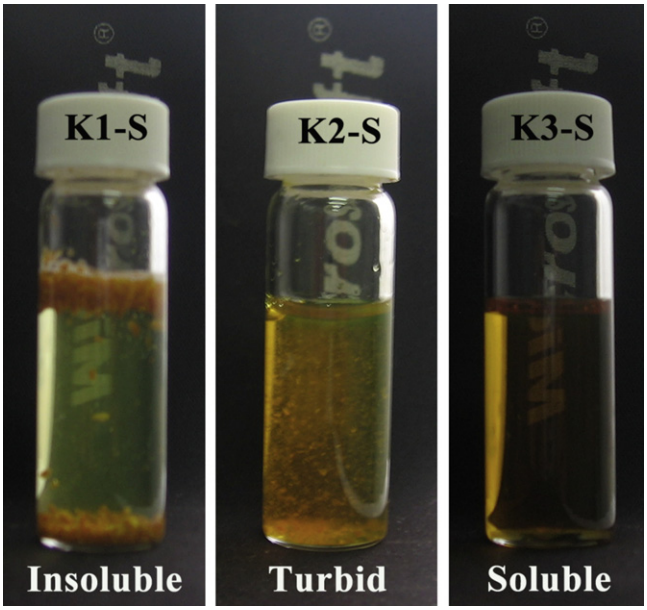


Fig. 1. Water-solubility of NCMs (0.3 g copolymer/5 mL water, stire and let stand for 30 min).

Table 3
Preparation of NCM modified membranes.[a]

No.	DMAC-solubility	Names of membranes	Characteristic of membranes[b]
K1-S	Soluble	$M_{K1\text{-}S}$	Light yellow; smooth
K2-S	Soluble	$M_{K2\text{-}S}$	Yellow; smooth
K3-S	Soluble	$M_{K3\text{-}S}$	Yellow; smooth
K4-S	Soluble	$M_{K4\text{-}S}$	Yellow; smooth
K5-S	Soluble	$M_{K5\text{-}S}$	Yellow; smooth
K6	Turbid	M_{K6}	White; smooth
K7	Turbid	M_{K7}	White; irregular
K8	Turbid	M_{K8}	White; irregular
K9	Turbid	M_{K9}	White; irregular
K10	Turbid	M_{K10}	White; irregular
K11-S	Soluble	$M_{K11\text{-}S}$	Yellow; smooth
K12-S	Soluble	$M_{K12\text{-}S}$	Light yellow; smooth
K13-S	Turbid	$M_{K13\text{-}S}$	Light yellow; irregular
K14-S	Soluble	$M_{K14\text{-}S}$	Light yellow; smooth
K15-S	Soluble	$M_{K15\text{-}S}$	Light yellow; smooth

[a] Preparation conditions: m_{DMAC} = 20 g, m_{PES} = 0.32 g, m_{NCMs} = 0.8 g; temperature = 20 °C, stirring for 24 h.
[b] Based on nake-eyes observation.

units of compositions, the molecular weights have a little influence on the clotting time.

3.3. Preparation of NCM modified membranes

In order to prepare NCM modified PES membranes, the synthesized NCMs and PES were added to DMAC and stirred for several hours. The miscibility of NCMs with PES in DMAC was investigated. As presented in Table 3, the mixtures of NCMs and PES were observed showing different affinity to DMAC. P(SS-*co*-MMA) and P(AA-*co*-SS-*co*-MMA) could be dissolved in DMAC. However, the mixtures of P(AA-*co*-MMA) and PES showed a poor dispersion in DMAC due to the poor miscibility of P(AA-*co*-MMA) and PES, although the MMA segment and PES can obtain good dispersion effect [31]. In addition, less AA content in the P(AA-*co*-SS-*co*-MMA) chains led to a better miscibility of P(AA-*co*-SS-*co*-MMA) and PES. NCM modified PES membranes were further prepared by a liquid–liquid phase separation technique at room temperature. The color and the morphology of the modified membranes were observed. P(SS-*co*-MMA) and P(AA-*co*-SS-*co*-MMA) modified PES membranes were yellow and smooth. Rather, P(AA-*co*-MMA) modified PES membranes were white and look irregular, in which there were a lot of holes. The pictures of the unmodified and modified PES membranes are shown in Fig. 3.

Fig. 3 also presents the AFM images of the unmodified and modified PES membranes. From the figure, it could be found that the surfaces of the membranes were altered on several levels after blending with the NCMs. There are lots of tiny grooves and fine holes on P(SS-*co*-MMA) and P(AA-*co*-SS-*co*-MMA) modified membranes. In addition, phase separation of P(AA-*co*-MMA) and PES was observed on the modified PES membranes after adding P(AA-*co*-MMA).

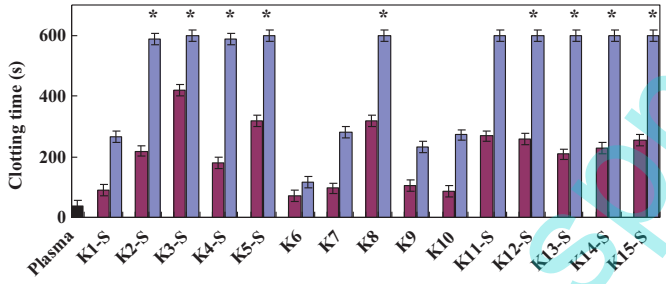


Fig. 2. APTTs for the NCMs (left sides: 0.5 mg/0.10 mL; right sides: 2.0 mg/0.10 mL). The stars indicate that the clotting times were beyond the scope of the present range of measurement.

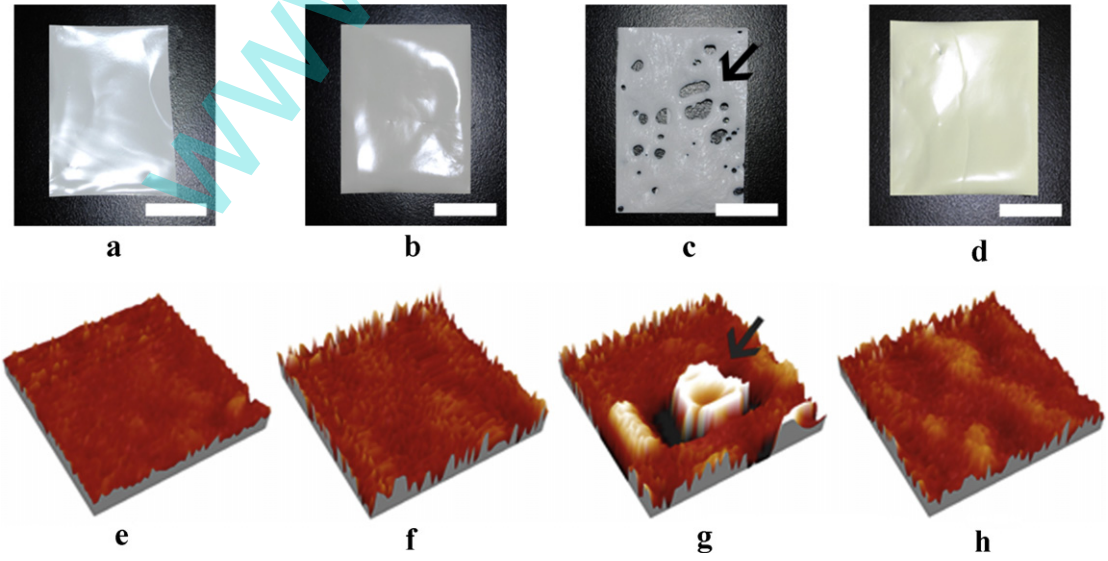


Fig. 3. Images of unmodified (a) and P(SS-*co*-MMA), P(AA-*co*-MMA) and P(AA-*co*-SS-*co*-MMA) modified PES membranes (b–d), scale bars correspond to 2 cm; AFM images of unmodified (e) and P(SS-*co*-MMA), P(AA-*co*-MMA) and P(AA-*co*-SS-*co*-MMA) modified PES membranes (f–d), the scan size of these AFM images is 1 μm × 1 μm.

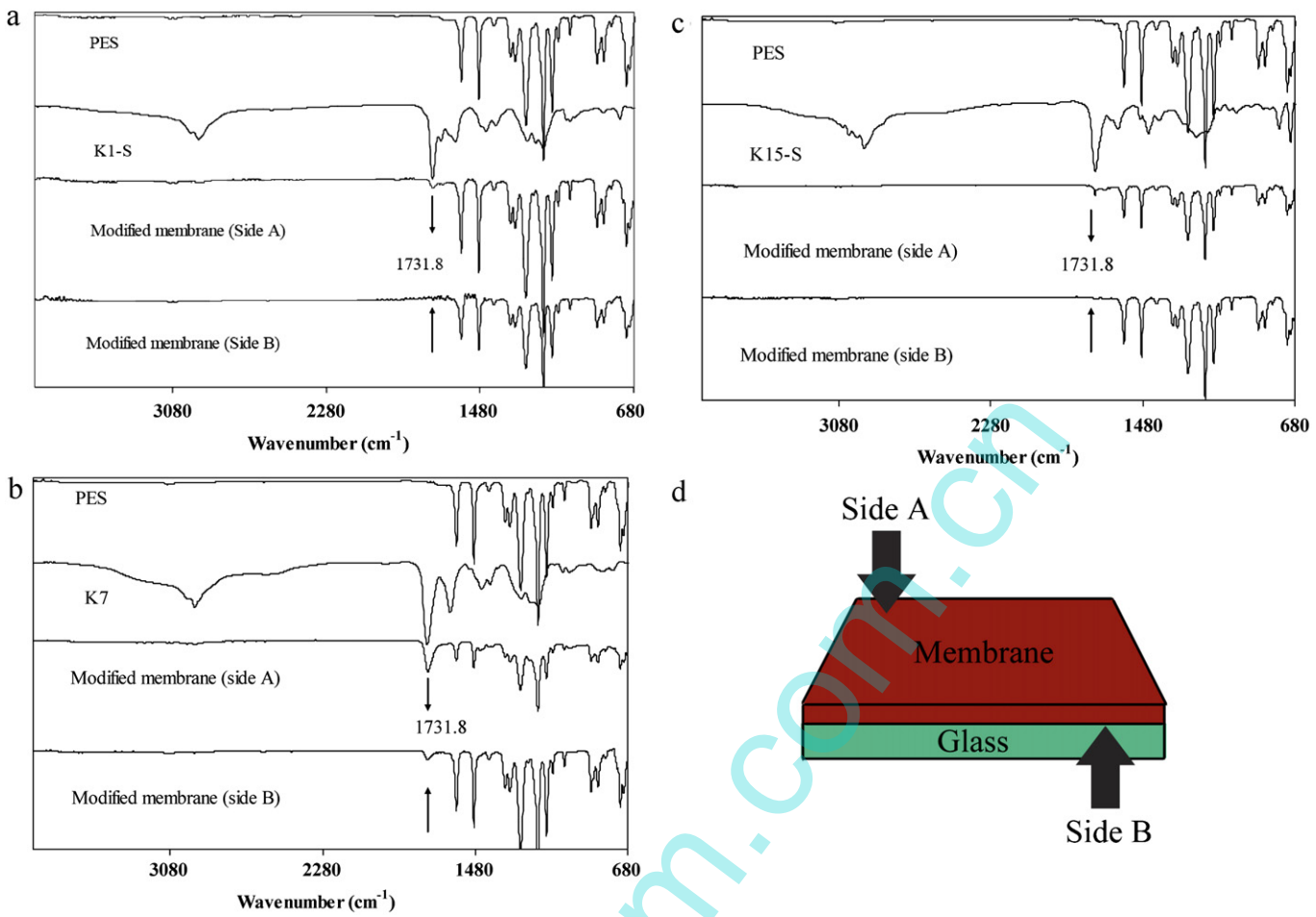


Fig. 4. FTIR spectra for PES, NCMs and modified PES membrane surfaces.

The NCM modified PES membranes were prepared and there may be abundant NCMs concentrated on the surface of the membranes, which were further confirmed by FTIR characterizations. Fig. 4 shows the FTIR spectra of PES, NCMs and modified PES membranes, respectively. It could be seen that the most significant changes were the appearance of the peaks at 1731.8 cm^{-1}, which were the characteristic peaks of the NCMs. From Fig. 4, it was also found that there are abundant NCMs in the outer surface (getting in touch with water during the liquid–liquid phase separation) than that in the inner part of the membrane (contacting with the glass).

3.4. Anticoagulation performance of modified membranes

The APTT tests for the PES and the modified PES membranes are presented in Fig. 5. It was found that the APTTs of the modified membranes increased obviously compared with that of the PES membrane; and with the increase of the SS or AA contents in the chains of the NCMs, the APTTs of the modified membranes increased significantly. K3-S-based membrane showed lower APTT due to the high hydrophilicity, which was caused by the elution of P(SS-*co*-MMA) from the membrane. In addition, the decrease of the molecular weight makes it easier for the NCMs to be eluted from the membranes during the liquid–liquid phase separation process. K8-based membrane showed the highest APTT due to the high AA content and the relatively less elution of P(AA-*co*-MMA) from the membrane.

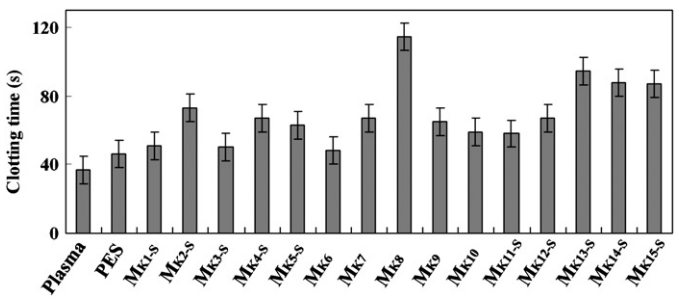


Fig. 5. APTTs for the NCM modified PES membranes. $n = 3$.

The blending of NCMs improved the anticoagulation of the PES membranes because of the functional groups of —COOH and —SO_3H. When the groups contacted with blood, it might combine or react with the coagulation factors, especially antithrombin III (ATIII), which is the natural antagonist for thrombin, the protein which enzymatically cleaves fibrinogen to form the fibrin clot [32,33], and the APTT could be prolonged. As mentioned above, P(AA-*co*-MMA) modified membrane showed the highest APTT, however, the PAA-based polymers have poor miscibility with PES. That is to say, it is difficult to prepare PAA-containing NCM modified membrane. Therefore, the NCMs with less —COOH and more —SO_3H groups (for instance, K12-S, K14-S and K15-S) have better application prospect for the surface modification of anticoagulant biomaterials.

3.5. The relationship between the structure and property of modified membranes

One possible anticoagulant mechanism of NCM modified membrane can be summarized as Fig. 6. The NCM modified membrane

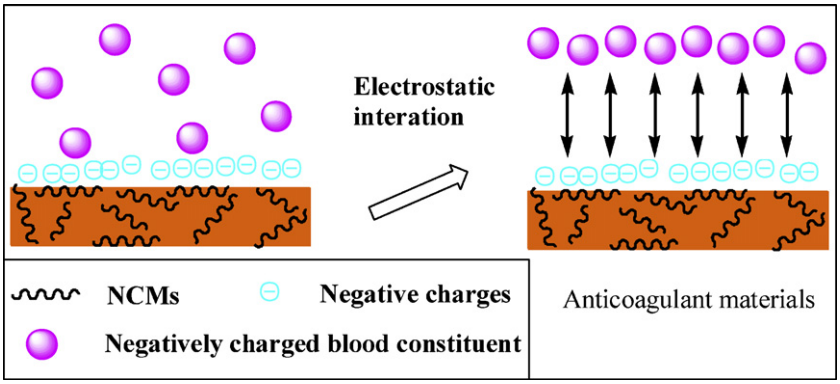


Fig. 6. The schematic illustration for the interaction between negatively charged blood constituents and the anticoagulant biomaterial surface.

contains negative charges on its surface. It is expected that NCM modified membranes have good anticoagulant properties because the anticoagulant activity is associated with the presence of these ionic functional groups [34]. It is well known that there is an distribution of negative charges on cells' surface of blood constituent including red blood cell, white blood cell, platelet, plasma protein, and so on [25]. The electrostatic interaction of the negatively charged membrane surface and negatively charged blood constituents helps prevent blood clotting.

4. Conclusions

In summary, a series of negatively charged macromolecules were synthesized by RAFT polymerization using carboxyl-terminated trithiocarbonate as the RAFT agent. The synthesized NCMs retarded the blood clotting and could be used as surface modifying additives to improve the blood compatibility of conventional anticoagulant biomaterials. P(AA-*co*-MMA)s modified PES membranes were crude and rough due to the poor miscibility of AA segments and PES. P(SS-*co*-AA-*co*-MMA) with appropriate proportions of SS, AA and MMA were designed and prepared. The P(SS-*co*-AA-*co*-MMA) modified membrane exhibited good anticoagulant ability due to the existence of the negative charges on its surfaces. The electrostatic interaction of negatively charged membrane surfaces and negatively charged blood constituents helps prevent blood clotting. The results indicated that the NCMs had potential to improve the anticoagulant property of biomaterials and to be applied in blood purification.

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