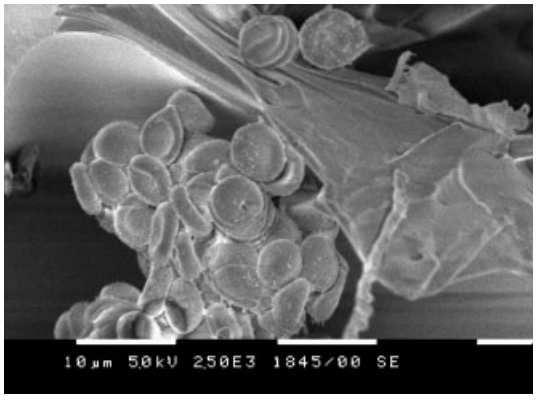
# Polyelectrolyte Complexes Based on (Quaternized) Poly[(2-dimethylamino)ethyl methacrylate]: Behavior in Contact with Blood

Elena Yancheva, Dilyana Paneva, Dobri Danchev, Laetitia Mespouille, Philippe Dubois, Nevena Manolova, Iliya Rashkov*

Polyelectrolyte complexes (PECs) between (quaternized) poly[2-(dimethylamino)ethyl methacrylate] (PDMAEMA) and (crosslinked) *N*-carboxyethylchitosan (CECh) or poly(2-acrylamido-2-methylpropane sodium sulfonate) (PAMPSNa) were prepared and characterized in terms of their stability, equilibrium water content, and surface morphology. The evaluation of the behavior of the studied PECs in contact with blood revealed that the (crosslinked) CECh/(quaternized) PDMAEMA complexes had lost the inherent PDMAEMA cytotoxicity but still preserved haemostatic activity. In contrast, the complex formation between (quaternized) PDMAEMA and PAMPSNa allowed the preparation of materials with improved blood compatibility.




## Introduction

A very attractive potential application of copolymers based on 2-(dimethylamino)ethyl methacrylate (DMAEMA) is their use in the preparation of non-viral vector gene delivery systems.[1–3] One of the major drawbacks of non-viral vectors is their cytotoxicity, especially to blood cells. Red blood cell agglutination or hemolysis occur on contact of DMAEMA-based copolymers with blood.[4] This is explained by electrostatic interactions between the positively charged DMAEMA-units and the negatively charged moieties on the surface of the red blood cells. The negative charges on the surface of the red blood cells are mainly due to the carboxyl groups of sialic acid,[5,6] which is located in the red blood cell glycocalix, but the acidic amino acid residues of the sialoglycoproteins of the cell membrane also contribute to the surface negative charge.[7] Agglutination occurs when the attractive interactions between the oppositely charged polyionic structures overcome the repulsive forces between the negatively charged surfaces of the red blood cells.[8] Hemolysis is associated with perturbation of the lipid bilayer[9] and depends on the concentration and on the molar mass of the polymer.[4] It is also known

E. Yancheva, D. Paneva, N. Manolova, I. Rashkov
Laboratory of Bioactive Polymers, Institute of Polymers, Bulgarian Academy of Sciences, Acad. G. Bonchev Street Bl. 103A, 1113 Sofia, Bulgaria
Fax: +35 92 870 0309; E-mail: rashkov@polymer.bas.bg
D. Danchev
Department of Haemostasis, Military Medical Academy, G. Sofijski Street 3, 1606 Sofia, Bulgaria
L. Mespouille, P. Dubois
Laboratory of Polymeric and Composite Materials, University of Mons-Hainaut, Place du Parc 20, B-7000 Mons, Belgium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that polycations, including poly[2-(dimethylamino)ethyl methacrylate] (PDMAEMA) can interact electrostatically with plasma proteins.[4] The presence of plasma proteins reduces both agglutination and hemolysis because the plasma proteins compete with the red blood cells of low negative charge for the polycation.[4] One of the strategies developed to improve the blood compatibility of DMAEMA-based copolymers is the introduction of a poly(ethylene oxide) block in their structure.[1,3] Another approach is the formation of polyelectrolyte complexes (PECs) with polyanions,[10,11] although in some cases increased toxicity has been reported.[12]

*N*-Carboxyethylchitosan (CECh) as a derivative of the natural polymer chitosan attracts attention because of the fact that it retains the valuable properties of chitosan, and, moreover, it is also soluble in neutral and alkaline media. It has been demonstrated that its effect on the haemostatic parameters is similar to that of chitosan.[13] The complex formation between CECh and synthetic polymers possessing anticoagulant activity, such as poly(2-acrylamido-2-methylpropanesulfonic acid),[14] leads to the preparation of polymer materials with improved blood compatibility.[13]

Recently, the complex formation between CECh and well-defined (quaternized) PDMAEMA synthesized by atom transfer radical polymerization (ATRP) was studied.[15] The DMAEMA-based copolymers prepared by ATRP attract interest because this synthetic method allows the preparation of polymers of desired molar mass and low polydispersity index, which can find potential applications in the biomedical field.[16–19] The CECh/PDMAEMA complex is formed in a narrow pH range at pH values close to 7. The quaternization of the tertiary amino groups of PDMAEMA enables complex formation with CECh not only in neutral media but also in alkaline media. A pH-sensitive polymer network has been obtained by crosslinking CECh with glutaraldehyde.[15] Crosslinked CECh is also capable of forming complexes with (quaternized) PDMAEMA. The complex formation in this case is accompanied by a network collapse. The studies of the antibacterial properties of the crosslinked CECh/(quaternized) PDMAEMA complexes have revealed that complex formation exerts a strong effect on the antibacterial properties of (quaternized) PDMAEMA. The complexes can be prepared in bulk [in the case of CECh/(quaternized) PDMAEMA complexes] or predominantly on the surface [in the case of crosslinked CECh/(quaternized) PDMAEMA complexes].

Taking into account our previous results, in the present study the approach of polyelectrolyte complex formation was applied to decrease the inherent toxicity of (quaternized) PDMAEMA against blood cells. CECh, which is the derivative of the natural polymer chitosan, or PAMPSNa were used as polymer partners. The (crosslinked) CECh/(quaternized) PDMAEMA complexes have been characterized in terms of their equilibrium water content and surface morphology. The interactions of (quaternized) PDMAEMA, (crosslinked) CECh, PAMPSNa, and their complexes with blood have been estimated *in vitro* with respect to their effect on some haemostatic parameters, blood cell counts, and morphology.

## Experimental Part

### Materials

Chitosan from crab shells (viscosity-average molar mass $5.4 \times 10^5$, determined as described elsewhere;[20] deacetylation degree 80% as determined by IR spectroscopy), acrylic acid (AA), and 2-acrylamido-2-methylpropanesulfonic acid (AMPS) were purchased from Fluka. AA was distilled under reduced pressure prior to use. 2-Ethylbromoisobutyrate (EB$^i$B, 98%), 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA, 97%), and copper(I) bromide (CuBr, 98%) were supplied by Aldrich and used as received. Tetrahydrofuran (THF, 99+%, from Chem-Lab) and 2-(dimethylamino)ethyl methacrylate (DMAEMA, from Aldrich) were passed through a column of basic alumina to remove the stabilizing agents. The monomer was stored under nitrogen at −20 °C. Triethylamine (99%, from Fluka) was dried over barium oxide for 45 h at room temperature and distilled under reduced pressure. All other reagents including the salts for preparation of the buffer solutions were of analytical grade and used without further purification. The following buffer solutions were prepared: pH = 4.8 ($CH_3COOH/NaOH$), pH = 7.0 ($KH_2PO_4/Na_2HPO_4$), and pH = 9.0 ($NaHCO_3/Na_2CO_3$). The concentrations of the reagents were chosen in such a way as to achieve a value of the ionic strength equal to 0.1. Blood was drawn by venipuncture from healthy volunteers who had not taken any medication for at least 10 d. The blood was collected in sodium citrate (3.13 wt.-%) at a blood/citrate ratio of 9/1 (v/v). Human pool plasma was obtained by centrifugation of citrated whole blood at 3 000 rpm for 10 min for separation of the blood cells. The reagents for determination of the haemostatic parameters (STA Neoplastin® Plus, STA Thrombin, STA APTT Kaolin, STA Fibrinogen, and STA Antithrombin III) were supplied by Roche Diagnostics (Germany).

### Preparation of *N*-Carboxyethylchitosan (CECh)

CECh was synthesized according to a previously reported procedure.[13,21] Briefly, chitosan (5 g, 0.03 mol) was dissolved in 250 mL of distilled water containing 4.3 g of acrylic acid (0.06 mol). The reaction mixture was heated for 24 h at 90 °C under constant stirring. After cooling, an aqueous solution of NaOH (1.25 mol · $L^{-1}$) was added to the reaction mixture until a pH of 10 was attained. The sodium salt of *N*-carboxyethylchitosan was purified by dialysis against distilled water for 2 d. The distilled water was changed repeatedly and the dialysis was conducted until the pH of the reaction mixture reached a value of 7 (yield: 90%).

$^1$H NMR (500 MHz, $D_2O$, 70 °C): $\delta = 2.06$ (s, *Glc*–NH–CO–$CH_3$), 2.42 (t, *Glc*–NH–$CH_2$–$CH_2$–COONa), 2.68 [t, *Glc*–N($CH_2$–$CH_2$–COONa)$_2$], 2.89 (t, H-2 of *Glc*–$NH_2$), 3.19 [t, H-2 of *N*-alkylated

aminoglucoside units (*Glc*–NH–$CH_2$–$CH_2$–COONa)], 3.45–4.15 [m, H-3, H-4, H-5, and H-6 of aminoglucoside (*Glc*–$NH_2$) and *N*-acetylated aminoglucoside units (*Glc*–NH–CO–$CH_3$), H-2 of *Glc*–NH–CO–$CH_3$ and *Glc*–NH–$CH_2$–$CH_2$–COONa], 4.52–5.09 (brs, H-1).

## Synthesis of PDMAEMA by ATRP

PDMAEMA was synthesized by ATRP using $EB^iB$ and CuBr ligated by HMTETA as an initiator and a catalyst, respectively ([DMAEMA]/[$EB^iB$]/[CuBr]/[HMTETA] = 170/1/2). The mixture was purged with $N_2$ and the initiator $EB^iB$ (0.144 g, 0.73 mmol) was added under nitrogen flow. The reaction was carried out at 60 °C for 21 h. PDMAEMA was selectively isolated by precipitation in seven-fold volume excess of cold heptane, and was dried to constant weight (yield: 80%; conversion: 89%). The copper catalyst was removed by filtration of the PDMAEMA solution in THF through a basic alumina column. The residual copper content of purified PDMAEMA was less than 1.0 ppm, as determined by ICP-AES analysis using a Jobin Yvon 38-Plus inductively coupled plasma atomic emission spectrometer.

$^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.6$ [s, C($CH_3$) PDMAEMA], 1.4 (t, $CH_3$–$CH_2$–O initiator), 1.8 (s, $CH_2$ PDMAEMA), 2.0 (s, $CH_3$ initiator), 2.3 [s, N($CH_3$)$_2$ PDMAEMA], 2.65 [t, $CH_2$N($CH_3$)$_2$ PDMAEMA], 4.1 (t, O–$CH_2$ PDMAEMA; q, $CH_3$–$CH_2$–O initiator).

## Preparation of PDMAEMAQ50 and PDMAEMAQ100

5 g of PDMAEMA homopolymer ($31.8 \times 10^{-3}$ mol tertiary amine) was introduced in a round bottom flask and dissolved in THF (20 mL) under stirring at room temperature. Next, a defined amount of $CH_3I$ ($17.5 \times 10^{-3}$ mol for PDMAEMAQ50 and $38.2 \times 10^{-3}$ mol for PDMAEMAQ100) dissolved in 20 mL of THF was added. After 18 h, the quaternized homopolymer was recovered in the form of white powder by evaporating the solvent and residual $CH_3I$ and drying under reduced pressure until a constant weight was achieved (yield: 100%).

$^1$H NMR (300 MHz, $D_2O$): $\delta = 0.9$ [s, C($CH_3$) PDMAEMA], 1.2 (t, $CH_3$–$CH_2$–O initiator), 1.8 (s, $CH_2$ PDMAEMA), 2.0 (s, $CH_3$ initiator), 2.3 [s, N($CH_3$)$_2$ PDMAEMA], 2.55 [t, O–$CH_2$–$CH_2$–N($CH_3$)$_2$ PDMAEMA], 3.2 [s, N($CH_3$)$_3^+$I$^-$], 3.8 [t, $CH_2$N($CH_3$)$_3^+$I$^-$], 4.1 [t, O–$CH_2$–$CH_2$–N($CH_3$)$_2$ PDMAEMA; q, $CH_3$–$CH_2$–O initiator], 4.5 [t, O–$CH_2$–$CH_2$–N($CH_3$)$_3^+$I$^-$].

The degree of quaternization (QD) was calculated from the relative intensity of the $\beta$-methylene amino protons at 4.1 ppm [t, O–$CH_2$–$CH_2$–N($CH_3$)$_2$] and 4.4 ppm [t, O–$CH_2$–$CH_2$–N($CH_3$)$_3^+$I$^-$]. The same QD values were determined from the relative intensity of the $\alpha$-methylene amino protons at 2.55 ppm [t, O–$CH_2$–$CH_2$–N($CH_3$)$_2$] and 3.8 ppm [t, O–$CH_2$–$CH_2$–N($CH_3$)$_3^+$I$^-$].

## Synthesis of PAMPS by Conventional Radical Polymerization

PAMPS was synthesized by polymerization of AMPS initiated by the redox-initiating system $Na_2SO_3$, $(NH_4)_2S_2O_8$, and $Fe(NH_4)_2(SO_4)_2$ at 25 °C for 30 h. The unreacted monomer and the salts of the initiating system were removed by dialysis against deionized water for 3 days after repeated changes of the dialyzing water. The sodium salt of PAMPS (PAMPSNa) was prepared by dialysis against 0.05 mol·L$^{-1}$ NaOH aqueous solution for 7 h, followed by successive dialyses against deionized water until a pH of 7 was attained. PAMPSNa was finally dried under reduced pressure up to constant weight.

$^1$H NMR (300 MHz, $D_2O$): $\delta = 1.35$ [s, C($CH_3$)$_2$], 1.57 (d, $CH_2$–CH), 2.4 (t, $CH_2$–$CH$), 3.2 (s, $CH_2$–$SO_3^-Na^+$).

## Characterization of the Polymers

Size exclusion chromatography (SEC) analyses of PDMAEMA were performed in THF with 2 wt.-% triethylamine ($NEt_3$) at 35 °C using a Polymer Laboratories liquid chromatograph equipped with a PL-DG802 degasser, an isocratic HPLC pump LC 1120 (flow rate = 1 mL·min$^{-1}$), a Marathon autosampler (loop volume = 200 μL, solution concentration = 1 mg·mL$^{-1}$), a PL-DRI refractive index detector and three columns: a PL gel 10-μm guard column and two PL gel Mixed-B columns. The molar mass and molar mass distribution were calculated with reference to poly(methyl methacrylate) standards. SEC of PAMPSNa was carried out in water containing $LiNO_3$ (0.5 mol·L$^{-1}$) and $NaN_3$ ($6 \times 10^{-3}$ mol·L$^{-1}$) at 40 °C using a Waters apparatus equipped with three Shodex OH pak (SB 806M) columns and a double detector involving differential refractometry and viscometry (flow rate = 0.75 mL·min$^{-1}$). The molar mass and molar mass distribution were calculated with reference to pullulan ($\overline{M}_n$ = 22 800–788 000) and poly(oxyethylene) ($\overline{M}_n$ = 10 800–690 000) standards to build a universal calibration curve.

The potentiometric titration of CECh for determination of the p$K_a$ values of the carboxyl and amino groups in its structure was carried out according to the following procedure: 170 mg of CECh was dissolved in 100 mL of 0.083 N NaOH or 0.1 N HCl and titrated at 25 °C with 0.1 N HCl or 0.083 N NaOH, respectively. The pH values were recorded using a PHM201 pH meter with a combined electrode.[15] The reduced viscosity of CECh solutions was determined by a capillary Ubbelohde viscometer (capillary i.d. 0.45 mm) at 25 ± 0.1 °C.[15]

## Preparation of Crosslinked *N*-Carboxyethylchitosan

An aqueous solution of glutaraldehyde (0.1 wt.-%, 8 mL) was added to 1 wt.-% aqueous solution of CECh (50 mL) under constant stirring at room temperature ([$NH_2$]/[CHO] molar ratio = 1/1). The mixture was stirred for 30 min and subsequently poured into a Petri dish and dried at 40 °C to constant weight. The obtained film was purified from unreacted glutaraldehyde and CECh by successive washing with distilled water.

## Preparation and Characterization of PECs

The PECs between CECh and (quaternized) PDMAEMA were prepared at pH = 7.0 (ionic strength $I$ = 0.1).[15] Briefly, 0.2 wt.-% buffered solutions of CECh and (quaternized) PDMAEMA were


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mixed vigorously at 25 °C at the following molar fractions of DMAEMA units ($F_{DMAEMA}$): 0.64, 0.53, and 0.47 for the CECh/PDMAEMA, CECh/PDMAEMAQ50, and CECh/PDMAEMAQ100 complexes, respectively. The molar fraction of DMAEMA units ($F_{DMAEMA}$) was calculated using the following equation:

$$F_{DMAEMA} = n_{DMAEMA}/(n_{DMAEMA} + n_{CECh}) \quad (1)$$

where $n_{DMAEMA}$ and $n_{CECh}$ designate the number of moles of (quaternized) DMAEMA and CECh units, respectively, at a defined initial ratio of the polymer partners. The obtained complexes were recovered by centrifugation for 40 min at 4 000 rpm followed by three-fold washing with distilled water and subsequent drying. The yield of the complexes was determined gravimetrically.

Crosslinked CECh/(quaternized) PDMAEMA complexes were obtained according to the following procedure: pre-swollen films of crosslinked CECh (weight of dry sample: 50 mg) that had reached an equilibrium swelling degree in buffer solutions of pH = 7.0 ($I$ = 0.1) were immersed in 3 wt.-% buffered solutions (pH = 7.0, $I$ = 0.1) of (quaternized) PDMAEMA for 48 h. The molar ratio [CECh units]/[DMAEMA units] was 1/1. The obtained complexes were dried at 40 °C to constant weight.

The PECs between (quaternized) PDMAEMA and PAMPSNa were prepared as described elsewhere.[22] Briefly, 0.3 wt.-% buffered solutions of (quaternized) PDMAEMA and PAMPS (pH = 7.0, $I$ = 0.1) were mixed vigorously at 25 °C at the following molar fractions of DMAEMA units ($F_{DMAEMA}$): 0.52, 0.58, and 0.58 for the PAMPS/PDMAEMA, PAMPS/PDMAEMAQ50, and PAMPS/PDMAEMAQ100 complexes, respectively.

The equilibrium water content of the CECh/(quaternized) PDMAEMA complexes was evaluated in aqueous media of different pH values (4.0, 7.0, and 9.0) and constant ionic strength ($I$ = 0.1) at room temperature. Samples were withdrawn from the solutions at pre-determined time intervals, blotted, and weighed. The equilibrium water content ($\alpha_{eq}$) was determined using the following equation:

$$\alpha_{eq} = [(m_{eq} - m_0)/m_0] \times 100 \quad (2)$$

where $m_{eq}$ and $m_0$ denote the weights of the swollen and dry sample, respectively.

The surface morphology of the (crosslinked) CECh/(quaternized) PDMAEMA complexes was studied using scanning electron microscopy (SEM; Philips SEM 515). For this purpose, complexes swollen to equilibrium in buffer solution of pH = 7.0 and washed with distilled water were freeze-dried. The specimens were fixed onto sample holders by means of a double-sided adhesive tape, and were vacuum-coated with gold. The SEM images were taken on this surface of complexes that were in contact with vapors during the freeze-drying process.

## Clottability Assay of CECh and (Quaternized) PDMAEMA

The influence of CECh and (quaternized) PDMAEMA on blood coagulation was studied *in vitro* using human pool plasma and it was evaluated from the obtained data for prothrombin time (PT), thrombin time (TT), activated partial thromboplastin time (APTT), levels of fibrinogen, and antithrombin III. A coagulation analyzer STA Compact-Stago (France) was used. Solutions of the studied polymers in saline were added to human pool plasma to prepare a series of plasma samples containing the polymers in known concentrations. The time for clot formation was determined after mixing the plasma with the corresponding reagent (STA Neoplastin® Plus, STA Thrombin, STA APTT Kaolin, STA Fibrinogen, or STA Antithrombin III). The data obtained from the clottability assay was processed using the following equations in accordance with already reported studies:[11,23]

$$R_{PT} = \frac{PT_c}{PT_0}, \quad (3)$$

$$R_{TT} = \frac{TT_c}{TT_0}, \quad (4)$$

$$R_{APTT(kaolin)} = \frac{APTT(kaolin)_c}{APTT(kaolin)_0}, \quad (5)$$

$$R_{fibrinogen} = \frac{Fibrinogen_c}{Fibrinogen_0}, \quad (6)$$

$$R_{AIII} = \frac{ATIII_c}{ATIII_0} \quad (7)$$

where $PT_c$, $TT_c$, $APTT_c$, $Fibrinogen_c$, and $ATIII_c$ are the values of the haemostatic parameters in the presence of CECh or (quaternized) PDMAEMA and $PT_0$, $TT_0$, $APTT_0$, $Fibrinogen_0$, and $ATIII_0$ are those of the controls in the absence of polymers. The concentration of the polymers was in the range of 8–16 mg · $mL^{-1}$ in the total volume. In each case a control blank assay (without added polymer) was performed. Triplicate tests were performed and the haemostatic parameters are expressed with standard deviations (SD).

## Platelet and Erythrocyte Adhesion onto Materials Based on (Quaternized) PDMAEMA Complexes

The platelet and erythrocyte adhesion was studied by direct observation by SEM of the blood cells adhered onto the surface of the complexes. The studied (crosslinked) CECh/(quaternized) PDMAEMA and PAMPS/(quaternized) PDMAEMA complexes were prepared at a molar fraction of DMAEMA units required to obtain a maximum PEC yield at pH = 7.0 and $I$ = 0.1. Prior to the blood tests the PECs (dry weight ≈20 mg) were swollen in saline until reaching equilibrium water content and subsequently they were transferred into 1 000 μL of fresh venous whole citrated human blood obtained from different volunteers for 1 h at room temperature. After that the samples were washed carefully with saline to remove the non-adhered blood cells. The adhered blood cells were fixed by immersing the samples in 20 mL of 2.5 wt.-% solution of glutaraldehyde in saline at room temperature for 20 min. The samples were washed carefully with distilled water prior to freeze-drying in order to remove the NaCl from the saline. Before the SEM observations, the specimens were placed onto

sample holders and fixed by means of a double-sided adhesive tape, vacuum-coated with gold, and observed.

### Blood Cell Counting Test

A hematological analyzer (Sysmex-SF 3000, Japan) was used for blood cell counting. The investigations were carried out using fresh venous whole blood from healthy volunteers collected into EDTA tubes. Polymer solutions (polymer concentration in the total volume: 5 $mg \cdot mL^{-1}$) and complexes composed of polymers included in the same concentration were incubated in 500 μL of whole human blood at 25 °C for 30 min. The polymer solutions were prepared in saline and the (crosslinked) CECh/(quaternized) PDMAEMA and PAMPS/(quaternized) PDMAEMA complexes were obtained using a buffer solution of pH = 7.0 and $I = 0.1$ and prior to the test performance they were left in saline until they reached equilibrium water content. The influence of complex formation on the interaction of the polymers with blood was evaluated by counting the white blood cells, red blood cells, and platelets.

## Results and Discussion

### Properties of the Polymer Partners

The polymers used in the present study – (quaternized) PDMAEMA, the sodium salt of CECh, and PAMPSNa – are presented in Figure 1. The degree of mono- and disubstitution of CECh (subunits 1b and 1d, Figure 1.1) was determined by $^1$H NMR spectroscopy in $D_2O$ (70 °C, 500 MHz) from the relative intensity of the methylene protons from mono- and disubstituted CECh units [*Glc*–NH–$CH_2CH_2$COONa and *Glc*–N($CH_2$–$CH_2$–COONa)$_2$] at 2.4–2.7 ppm and the intensity of the signal for the proton from the methyne group in position 1 (H-1, subunit 1a, Figure 1.1) in the aminoglucoside CECh subunit at 4.4–4.8 ppm.[13,21] The total degree of substitution was 76%. The contents of mono- and disubstituted units, 51% and 25%, respectively, were calculated from the relative intensity of the protons from *Glc*–NH–$CH_2$–$CH_2$–COONa and *Glc*–N($CH_2$–$CH_2$–COONa)$_2$ at 2.42 ppm and 2.68 ppm, respectively. The degree of acetylation calculated from the $^1$H NMR spectrum was 17%. The content of non-substituted amino groups was 7%. As determined from the $^1$H NMR spectrum, CECh consists of the following monomer units: *N*-acetylglucosamine (1a), glucosamine (1c), mono- and disubstituted *N*-carboxyethylglucosamine units (1b, 1d) (Figure 1.1). The p$K_a$ values of the carboxylate anions and imino groups determined by potentiometric titration of CECh with HCl were 4.38 and 8.31, respectively, and the p$K_a$ values of the carboxyl groups and protonated imino groups determined by titration of CECh with NaOH were 8.87 and 10.68, respectively.

PDMAEMA (Figure 1, 2a) was synthesized by ATRP of DMAEMA in THF. The degree of quaternization (QD) of PDMAEMA was finely tuned by the initial tertiary amine/methyl iodide molar ratio.[16,19,24] Two quaternized PDMAEMA samples with QDs of 50 and 100% (Figure 1, 2b, and 2c), which will be further designated

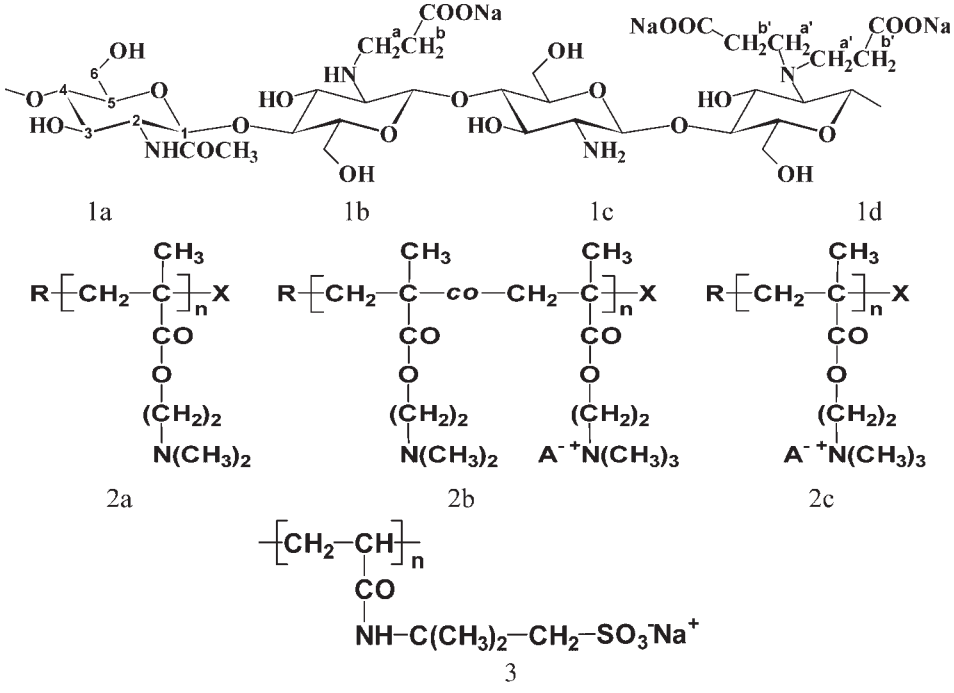


*Figure 1.* Fragments of the macrochains of the sodium salt of CECh (1), PDMAEMA (2a), PDMAEMAQ50 (2b) and PDMAEMAQ100 (2c); R = $C_2H_5OOC(CH_3)_2$; X = Br; A = I; and PAMPSNa (3).


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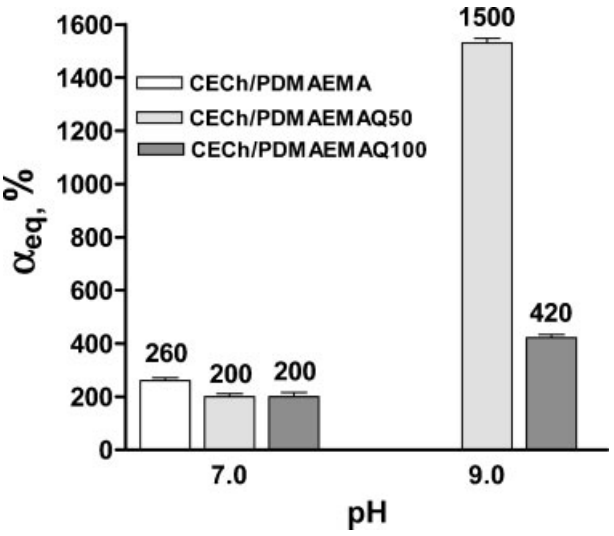


*Figure 2.* Dependence of the equilibrium water content ($\alpha_{eq}$, as determined from Equation (2)) of CECh/PDMAEMA ($F_{DMAEMA} = 0.64$), CECh/PDMAEMAQ50 ($F_{DMAEMA} = 0.53$), and CECh/PDMAEMAQ100 ($F_{DMAEMA} = 0.47$) complexes on the nature of DMAEMA-based polymers (pH = 7.0 and 9.0, $I = 0.1$, 25 °C).

as PDMAEMAQ50 and PDMAEMAQ100, were synthesized and isolated. The molecular characteristics of (quaternized) PDMAEMA are presented in Table 1. The QD value was determined by $^1$H NMR spectroscopy as previously reported.[16,24] The number-average molar mass ($\overline{M}_n$) of (quaternized) PDMAEMA was calculated from the degree of monomer conversion and from the initial monomer-to-initiator molar ratio. The apparent $\overline{M}_n$ was determined by SEC only in the case of PDMAEMA. Determination of the apparent $\overline{M}_n$ values of PDMAEMAQ50 and PDMAEMAQ100 was not carried out because the quaternization reaction does not affect the chain integrity. The molecular characteristics of PAMPSNa (Figure 1, 3a) are presented in Table 1.

## Characterization of Complexes Based on (Crosslinked) CECh and (Quaternized) PDMAEMA

Recently, investigations on the complex formation between (crosslinked) CECh and (quaternized) PDMAEMA have been performed.[15] In the present studies, the complexes were prepared according to the pre-determined optimal conditions[15] for preparation of maximum amounts of complexes at pH = 7.0 ($I = 0.1$). As previously found,[15] the $F_{DMAEMA}$ value (Equation (1)) required for the preparation of a maximum amount of CECh/(quaternized) PDMAEMA complex at pH = 7.0 depends on the nature of the polycation, and it is 0.64, 0.53, and 0.47 for the CECh/PDMAEMA, CECh/PDMAEMAQ50, and CECh/PDMAEMAQ100 complexes, respectively. The yields were 73, 90 and 90 wt.-% for the CECh/PDMAEMA, CECh/PDMAEMAQ50, and CECh/PDMAEMAQ100 complexes, respectively. The CECh/PDMAEMA complex is stable in neutral medium, whereas the CECh/quaternized PDMAEMA complexes are stable both in neutral and alkaline media. The complexes disintegrate in acidic medium and the individual polymer partners are released.

As physically crosslinked hydrogels, PECs are characterized by a pH-dependent swelling, which determines their behavior upon contact with body fluids. Therefore, the evaluation of the dependence of the equilibrium water content ($\alpha_{eq}$) on the medium pH value is of great importance for the determination of the behavior of these materials upon contact with body fluids, in particular with blood. In the present studies, the dependence of the $\alpha_{eq}$ value of the CECh/(quaternized) PDMAEMA complexes prepared at pH = 7.0 and $I = 0.1$ was evaluated using buffer solutions of pH = 7.0 and 9.0 ($I = 0.1$). In the case of the studied complexes, the time required for reaching equilibrium water content was 48 h. The dependence of the $\alpha_{eq}$ value on the nature of the DMAEMA-based polymer

*Table 1.* Molecular characteristics of DMAEMA-based (quaternized) polymers and PAMPSNa.

| Sample | Degree of quaternization[a)] | $\overline{M}_n$ (calcd)[b)] | $\overline{M}_n$ (SEC)[c)] | $\overline{M}_w/\overline{M}_n$ (SEC)[c)] |
|---|---|---|---|---|
| | % | | | |
| PDMAEMA | 0 | 24 000 | 23 900 | 1.32 |
| PDMAEMAQ50 | 52 | 35 000 | – | – |
| PDMAEMAQ100 | 100 | 45 000 | – | – |
| PAMPSNa | – | – | 526 000 | 2.60 |

[a)] As determined by $^1$H NMR spectroscopy in $D_2O$ from the relative intensity of aminomethylene protons at 4.1 ppm [O–$CH_2$–$CH_2$–N$(CH_3)_2$] and 4.4 ppm [O–$CH_2$–$CH_2$–N$(CH_3)_3^+I^-$]; [b)] As calculated using the equation $\overline{M}_n$ (calcd) = MW(EB$^i$B) + ([DMAEMA]$_0$/[EB$^i$B]$_0$) × conversion × {(QD/100) × [MW(DMAEMA) + MW($CH_3$I)] + (1 − QD/100) × MW(DMAEMA)}, where [DMAEMA]$_0$/[EB$^i$B]$_0$ is the initial monomer/initiator molar ratio, MW($CH_3$I) is the molecular weight of $CH_3$I, and QD is the degree of quaternization; [c)] As determined by SEC in THF + 2 wt.-% $NEt_3$ at 35 °C with reference to poly(methyl methacrylate) standards for PDMAEMA and in 0.5 M $LiNO_3$ aqueous solution at 40 °C according to a universal calibration for PAMPSNa.

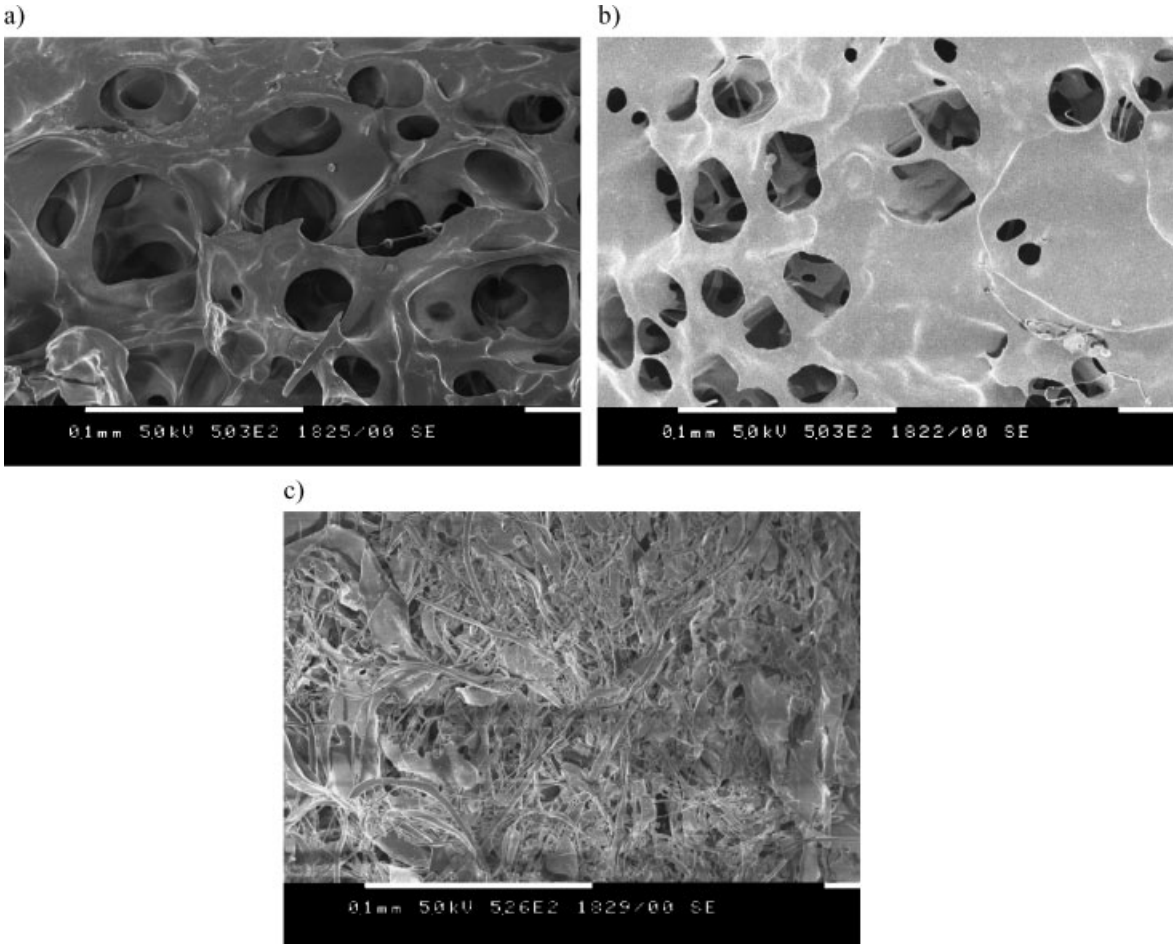


*Figure 3.* SEM micrographs of freeze-dried CECh/PDMAEMA (A, $F_{DMAEMA} = 0.64$), CECh/PDMAEMAQ50 (B, $F_{DMAEMA} = 0.53$), and CECh/PDMAEMAQ100 (C, $F_{DMAEMA} = 0.47$) complexes. Magnification: 503× (A, B), 526× (C).

at pH = 7.0 and 9.0 is shown in Figure 2. As seen from Figure 2, there is no significant difference between the $\alpha_{eq}$ values of the CECh/(quaternized) PDMAEMA complexes at pH = 7.0. This result was expected because at this pH value the amount of ionized free groups of both polymer partners is low; almost all of the carboxyl groups of CECh and the (quaternized) tertiary amino groups of PDMAEMA are engaged in the respective complexes. At pH = 9.0, the CECh/PDMAEMA complex dissolved completely and it was not possible to determine its $\alpha_{eq}$ value. The CECh/PDMAEMAQ50 complex is characterized by the highest $\alpha_{eq}$ value at pH = 9.0. This is explained by the occurrence of two processes. On the one hand, the non-quaternized tertiary amino groups of PDMAEMAQ50 at this pH value are in the non-protonated form, and the ionic bonds between them and the carboxylate anions of CECh are disrupted. On the other hand, at pH = 9.0 there are no betaine-type structures in CECh (the viscometrically determined isoelectric point of CECh is in the pH range from 5.4 to 5.8).[15] This leads to an increased amount of free hydrophilic groups and to increased swellability of the complex. The effect of the carboxyl groups liberated from the betaine-type structures of CECh on the swelling ability of the complexes is confirmed in the case of the CECh/PDMAEMAQ100 complex. The equilibrium water content of this complex is higher at pH = 9.0 than at pH = 7.0. A maximum yield of the CECh/PDMAEMAQ100 complex at pH = 7.0 is obtained at $F_{DMAEMA} = 0.47$.[15] The formation of a maximum amount of this complex at pH = 9.0 requires an excess of PDMAEMAQ100 ($F_{DMAEMA} = 0.57$). Thus, the complex obtained at pH = 7.0 and swollen at pH = 9.0 is characterized by an increased amount of carboxylate anions released from the betaine-type structures of CECh. This leads to enhanced hydrophilicity of the complex.

In order to investigate the relationship between the complex structure and the nature of the polycation, the surface morphology of the complexes was analyzed by SEM. For that purpose, CECh/(quaternized) PDMAEMA complexes prepared at pH = 7.0, $I = 0.1$ and at $F_{DMAEMA}$ required for obtaining a maximum PEC yield were swollen in a buffer solution of pH = 7.0, washed with distilled water, freeze-dried, and observed by SEM. The SEM


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micrographs of the surfaces of the complexes are shown in Figure 3. The surfaces of the CECh/PDMAEMA and CECh/PDMAEMAQ50 complexes are highly porous (Figure 3A, B). The CECh/PDMAEMA complex is characterized by a larger pore size than the CECh/PDMAEMAQ50 complex. The average length and the average width of the pores of the CECh/PDMAEMA complex are 39 and 27 μm, respectively, and in the case of the CECh/PDMAEMAQ50 complex they are 22 and 17 μm, respectively. These results are in good agreement with the results obtained from the determination of the equilibrium water content (Figure 2); the $\alpha_{eq}$ value of the CECh/PDMAEMA complex is higher than the $\alpha_{eq}$ value of the CECh/PDMAEMAQ50 complex. The surface of the CECh/PDMAEMAQ100 complex is fibrous and slightly porous (Figure 3C). This is due to the higher content of CECh. The observed fibrous surface is typical of natural polymers.

Previously it has been demonstrated that the crosslinking of CECh using glutaraldehyde (GA) does not interfere with the ability of CECh to form complexes with (quaternized) PDMAEMA.[7] Moreover, the crosslinking of CECh enables the preparation of novel PEC-based materials with different architectures. In the present study, a film of crosslinked CECh was prepared by a reaction with GA at a $[NH_2]/[CHO]$ molar ratio = 1/1 and it was characterized in terms of its swelling ability at different pH values of the medium. Crosslinked CECh is insoluble in aqueous medium over the entire pH range. The dependence of the equilibrium swelling degree of a film of CECh crosslinked with GA on the medium pH is shown in Figure 4. As seen from Figure 4, the lowest swelling degree was determined in acidic medium. At low pH values, the carboxyl groups of CECh are not ionized and part of the amino and imino groups that are capable of protonation and are responsible for the swelling of the film are engaged in chemical bonding (Schiff bases with GA). With the increase of the medium pH, the amount of the ionized carboxyl groups in the structure of CECh becomes higher, leading to enhanced hydrophilicity of the network (an increase in the swelling degree of the film from 500% in acidic medium to ca. 1 800% at pH = 7.0 is observed). The slightly higher value of the equilibrium swelling degree of the film at pH = 7.0 (1 800%) in comparison to pH = 9.0 (1 600%) is due to the fact that at pH = 7.0 there is still a certain amount (ca. 42%) of protonated imino and amino groups that contribute to the swelling of the film of crosslinked CECh.

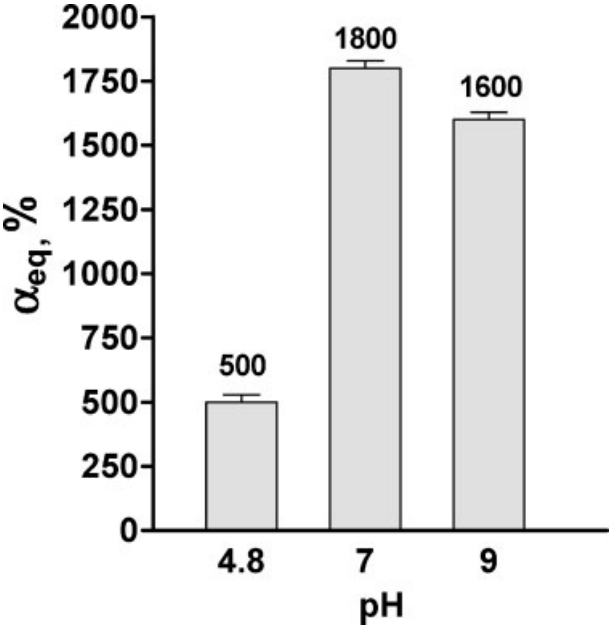


*Figure 4.* Dependence of the equilibrium swelling degree of a CECh film crosslinked with GA on the pH value of the medium ($I$ = 0.1, 25 °C).

As shown previously, the presence of carboxyl groups in the film of crosslinked CECh allows crosslinked CECh/(quaternized) PDMAEMA complexes to be formed. For this purpose, films of crosslinked CECh that had reached equilibrium swelling degree in a buffer solution of pH = 7.0 ($I$ = 0.1) were immersed in 3 wt.-% solutions of (quaternized) PDMAEMA in the same buffer ([CECh-units]/[DMAEMA-units] molar ratio = 1/1). The studies on the equilibrium water content of the crosslinked CECh/(quaternized) PDMAEMA complexes at pH = 7.0 revealed that the PEC formation does not lead to significant changes in the equilibrium swelling degree values compared to the film of crosslinked CECh. In a further set of experiments, the behavior of the obtained polymer materials in contact with blood was studied.

### Evaluation of the Behavior of (Quaternized) PDMAEMA, (Crosslinked) CECh, PAMPSNa, and their Complexes in Contact with Blood

During the first stage the effects of CECh and (quaternized) PDMAEMA on the haemostatic parameters, such as prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen, and antithrombin III (ATIII) levels, were evaluated. For this purpose, serial dilutions of CECh and (quaternized) PDMAEMA solutions were made in human pool plasma so that the concentration of the polymers was varied from 8 to 16 $mg \cdot mL^{-1}$. The dependence of the haemostatic parameters on the type of the studied polymer is presented in Figure 5. As seen, CECh exerts almost no effect on the haemostatic parameters and their values are close to those of the control sample. When conducting the experiments at higher concentrations of CECh (respectively 10 and 16 $mg \cdot mL^{-1}$), slight prolongation of PT, APTT, and TT was observed. The obtained results are in good agreement with

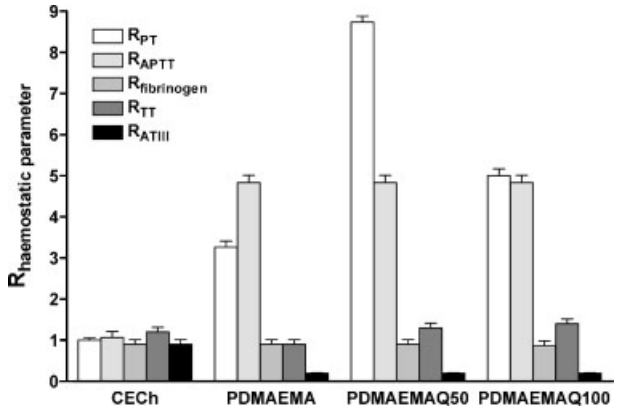


*Figure 5.* Dependence of $R_{PT}$, $R_{APTT}$, $R_{fibrinogen}$, $R_{TT}$, and $R_{ATIII}$ (as calculated from Equation (3)–(7) on the type of polymer (concentration 8 mg · mL$^{-1}$).

the data for CECh with a molar mass of ca. $4.6 \times 10^5$.[13] The behavior of CECh upon contact with blood does not differ considerably from that of its precursor chitosan, which does not exert any effect on the values of the haemostatic parameters.[25]

As seen from Figure 5, DMAEMA-based polymers cause significant prolongation of PT and APTT that is indicative of their effect both on the extrinsic and on the intrinsic coagulation pathway (Figure 5). The prolongation of PT is an indication of the influence of the DMAEMA-based polymers on the extrinsic (tissue factor dependent) coagulation pathway. On the basis of the obtained results it can be assumed that the DMAEMA-based polymers exert a strong inhibitory effect on factor VIIa or on its complex with tissue factor. On the other hand, the prolongation of APTT is indicative of the influence of the polymers on the intrinsic coagulation pathway. The prolongation of APTT in the presence of the polycation poly(ethylene imine) has been recently reported.[26] The prolongation was explained as interference of the polycation with the "activation" of the coagulation cascade by the thromboplastin reagent. Although the prolongation of PT and APTT is generally indicative of anticoagulant activity, it cannot be used independently as a measure of the capacity of the polymer materials to induce coagulation because the prolongation of these coagulation times can stem from interaction of the investigated polymers with some of the reagents used in the coagulation tests. Other important haemostatic parameters should also be taken into account in the overall evaluation of the effect of the studied polymers on the coagulation cascade, such as ATIII and fibrinogen levels and TT. It is important to note that DMAEMA-based polymers lead to a considerable decrease in the amount of ATIII in blood plasma, whereas the TT value and fibrinogen level remain unchanged. This shows that DMAEMA-based polymers have an ATIII binding affinity. The decrease of the level of the physiological anticoagulant ATIII in blood plasma can cause blood clotting.

It can be assumed that the observed changes in the haemostatic parameters are due to electrostatic interactions with certain plasma proteins. It is known that polycations interact selectively with one or several defined proteins that are present simultaneously in a protein mixture, such as human plasma.[4] The interactions with plasma proteins depend on numerous factors, which include the properties of polymers (hydrophilic–lipophilic balance, available chemical functional groups carrying positive and negative charges, etc.) as well as the properties of proteins (electrical charge, available chemical functional groups, stability of the protein structure, etc.). Because of the presence of positive charges in the macromolecule of (quaternized) PDMAEMA it can be expected that electrostatic interactions will occur preferentially with proteins that have isoelectric points in the acidic pH range and possess a negative net charge (factor VIIa, antithrombin III) at the physiological pH of plasma (pH = 7.4), while the electrostatic interactions with proteins with a positive net charge (thrombin, fibrinogen) should be weaker. Indisputably, however, the interactions that occur upon contact of the polycations with plasma are much more complicated and further investigations are needed to get an insight into their mechanisms.

In a further set of experiments the behavior of (cross-linked) CECh and (quaternized) PDMAEMA was studied using blood cell counting tests. These tests allow quantitative evaluation of the changes that occur in the blood cell counts after contact of the polymers with whole blood.[3] Fresh human blood from different volunteers was collected in EDTA tubes and the polymers were incubated in whole blood at 37 °C for 30 min. The studies were performed using saline solutions of the polymers. The dependence of the blood cell counts (white blood cells, red blood cells, and platelets) on the nature of the polycation after 30 min of incubation in whole blood is presented in

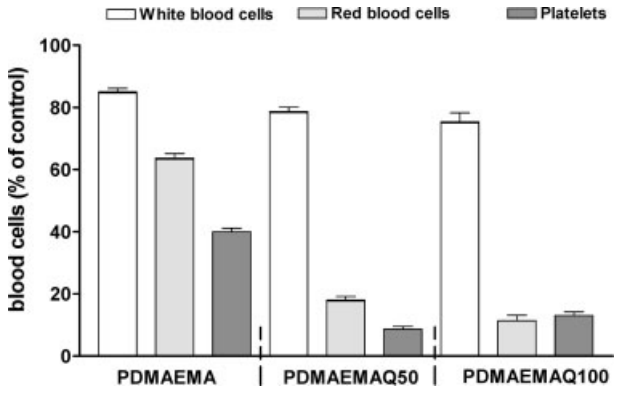


*Figure 6.* Dependence of the blood cell counts on the nature of the polycation (polymer concentration 5 mg · mL$^{-1}$).


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Figure 6. As seen, (quaternized) PDMAEMA caused a considerable decrease in the blood cells counts. The obtained results are in good agreement with the data for the ability of PDMAEMA to cause hemagglutination and its weak hemolytic activity.[4] As seen from Figure 6, the quaternization of PDMAEMA leads to enhanced toxicity against the blood cells, which increases in the following order: PDMAEMA < PDAEMAQ50 ≤ PDMAEMAQ100. Bearing in mind that the hemagglutination caused by the polycations is due to their interaction with negatively charged sialic acid residues on the red blood cell membranes, the enhanced toxicity of quaternized PDMAEMA as compared to PDMAEMA can be explained by the different amount of positive charge along the (quaternized) PDMAEMA chains at the physiological pH of blood (pH = 7.4). At pH = 7.4, only 30% of the DMAEMA units are positively charged. Due to the presence of quaternized units, in the case of PDMAEMAQ50 and PDMAEMAQ100 the amount of the positive charge responsible for the hemotoxicity is 80 and 100%, respectively. The red blood cell and platelet counts in the presence of PDMAEMA are reduced up to ca. 60 and 40%, respectively. The net charge density of quaternized PDMAEMA is independent of the pH of the medium. This accounts for the more substantial decrease in the red blood cell and platelet counts in the presence of quaternized PDMAEMA as compared to the counts determined in the presence of non-quaternized PDMAEMA (Figure 6). Figure 7 shows the change in the white blood cell, red blood cell, and platelet counts after 30-min incubation of CECh and crosslinked CECh in whole blood. As seen, the blood cell counts decrease significantly in the presence of CECh. This result reveals that, similarly to chitosan, CECh induces alterations in the blood cells due to electrostatic interactions of the positively charged amino and imino groups in its structure with the negatively charged moieties on the blood cell surfaces.[25,27] The engagement of the amino and imino groups of CECh in covalent bonds by crosslinking with glutaraldehyde results in a polymer material that is characterized by considerable compatibility with the blood cells. As seen from Figure 7, the cell counts are almost unaffected by the presence of crosslinked CECh. It is worth noting that in this case the polymer is in a swollen state and not in solution, whereas the behavior of non-crosslinked CECh upon contact with blood was evaluated in solution. This also accounts for the difference in the behavior of CECh and crosslinked CECh upon contact with whole blood. It might be assumed that the contact between crosslinked CECh and the blood cells occurs predominantly on the film surface. A schematic presentation of the interactions between the blood cells and (crosslinked) CECh is shown in Figure 8. As seen from Figure 8, in the case of a CECh solution, all of the amino and imino groups (which are responsible for the low compatibility of CECh with the blood cells) are accessible for electrostatic interaction with the cells. In the case of the film of crosslinked CECh, the blood cells have to penetrate into the film in order to come in contact with the free (i.e., non-engaged in covalent bonds) amino and imino groups located in the bulk of the film. In this case, the porosity of the material that determines the ability of the blood cells to penetrate into the bulk of the film is of major importance.

In order to evaluate the effect of complex formation between (crosslinked) CECh and (quaternized) PDMAEMA,

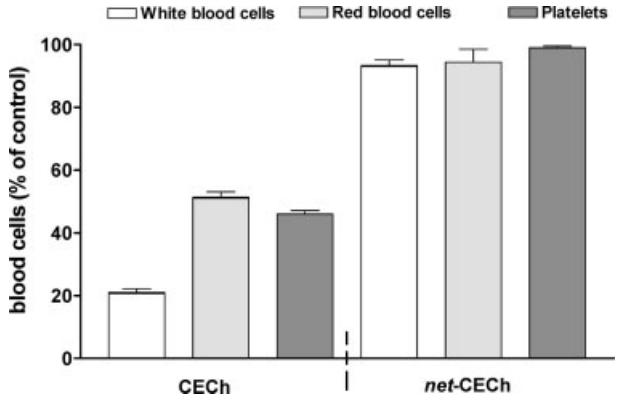


*Figure 7.* Dependence of the blood cell counts on the crosslinking of CECh (polymer concentration 5 mg · mL$^{-1}$). Crosslinked CECh is designated as *net*-CECh.

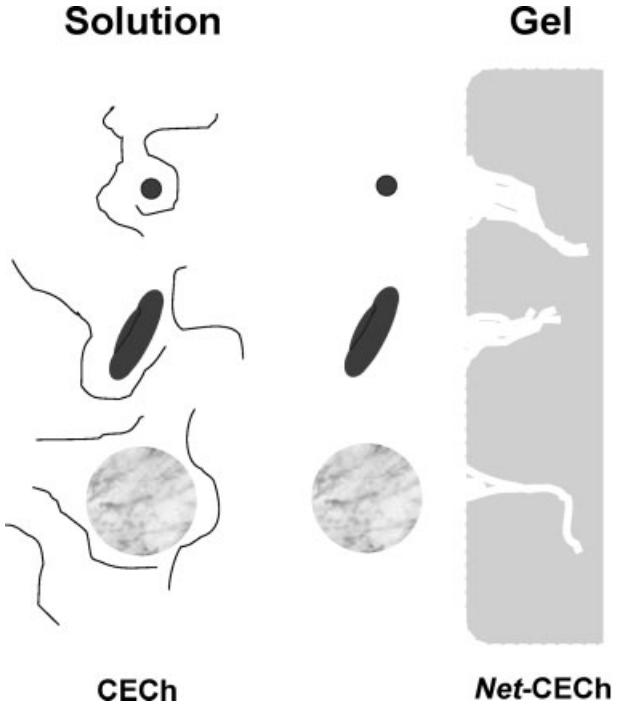


*Figure 8.* Schematic representation of the interaction of the blood cells with a CECh solution and a film of crosslinked CECh (designated as *net*-CECh).

their complexes prepared at pH = 7.0 at a molar fraction of DMAEMA units required to obtain the highest yield were tested in whole blood using the blood cell counting test. The results obtained for the complexes as well as for the starting polymers are presented in Figure 9. As seen, the effect of the polymer materials on the blood cell counts depends significantly on the type of CECh – crosslinked or non-crosslinked – as well as on the nature of the polycation. The complex formation between (crosslinked) CECh and (quaternized) PDMAEMA results in a significant decrease of the toxicity of the polymer partners towards the white and red blood cells; the counts of the latter remain almost unchanged after 30-min contact with the complexes. The platelet counts, however, are considerably low. An explanation of this result might be found in the structure of the complexes. The CECh/PDMAEMA and CECh/PDMAEMAQ50 complexes are highly porous, as

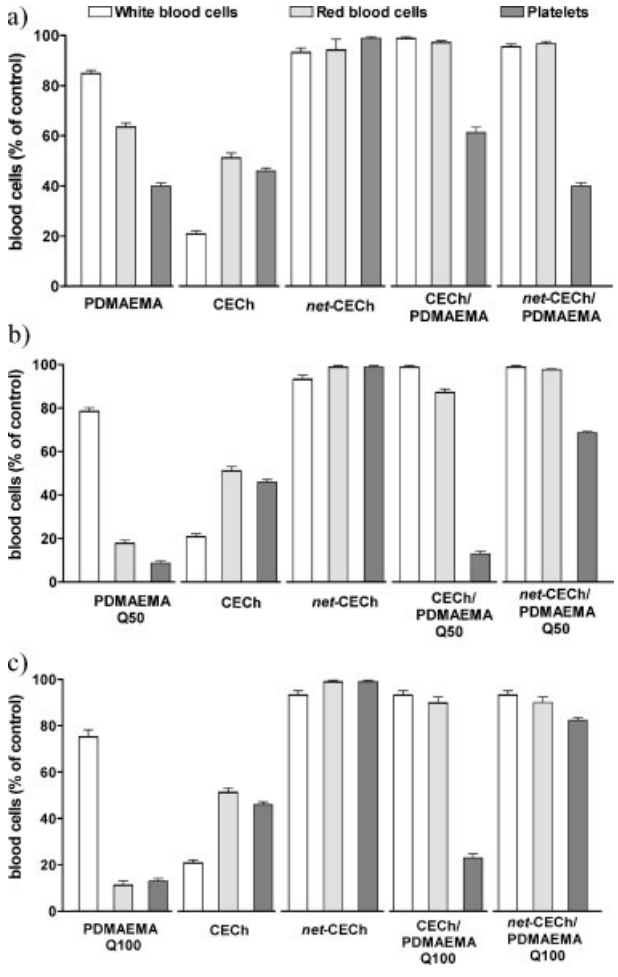


*Figure 9.* Effect of PDMAEMA (A), PDMAEMAQ50 (B), PDMAEMAQ100 (C), (crosslinked) CECh, and their complexes (A–C) on the counts of the white blood cells, red blood cells and platelets; concentration of the polymers 5 $mg \cdot mL^{-1}$, concentration of the (crosslinked) CECh/(quaternized) PDMAEMA complexes 10 $mg \cdot mL^{-1}$; duration of the contact with whole blood 30 min.

observed by SEM (Figure 3). The platelets, due to their small size (2–4 μm), can penetrate into the pores of these complexes and be physically retained in the bulk of the complexes (Figure 8). After their penetration the platelets may come into contact with the free positively charged nitrogen-containing groups located in the bulk of the complexes. This can lead to platelet activation and aggregation resulting in a decrease of the platelet count in the blood sample.

For further evaluation of the behavior of the complexes upon contact with blood, the red blood cell and platelet adhesion onto the surface of CECh/(quaternized) PDMAEMA complexes was studied using SEM. For this purpose, the complexes were kept in whole blood for 1 h. The SEM micrographs of the surfaces of the complexes after contact with whole blood are shown in Figure 10. Aggregates of red blood cells with unchanged morphology were observed on the surface of the complexes, mainly in their pores (Figure 10). This confirms the assumption that the porous structure of the complexes is one of the major factors that favor adhesion and the formation of aggregates of red blood cells on the surface of the complexes. This result is in good agreement with the results obtained from the blood cell counting tests in the presence of the complexes, which showed a decrease in the blood cell counts (Figure 9). The presence of free positive charges of (quaternized) PDMAEMA or CECh located on the surface of the complexes increases the possibility of agglutination of the red blood cells. Moreover, the possibility for occurrence of hydrophobic interactions between the polycationic fragments and the lipid bilayer of the red blood cell membranes is an additional factor influencing the adhesion and agglutination of the red blood cells.[28] Although the data obtained from the blood cell counting test (Figure 9) showed that the platelet counts were significantly decreased, no adhered platelets or platelet aggregates were observed on the surface of the complexes by SEM. The possibility of platelet penetration into the bulk of the complexes, thus not allowing their observation, is not excluded.

The unchanged morphology of the red blood cells implies that the complexes do not exhibit hemolytic activity, which is characteristic of the polymer partners in solution. However, they have the ability to cause agglutination of the red blood cells, as well as to decrease the platelet counts, which is most probably associated with their ability to cause platelet activation and aggregation. These properties of the polymer materials make them potential candidates for application as haemostatic agents.

The complex formation between crosslinked CECh and (quaternized) PDMAEMA allows the preparation of polymer materials with a smoother, non-porous surface. Even at high magnifications, no pores in the structure of the


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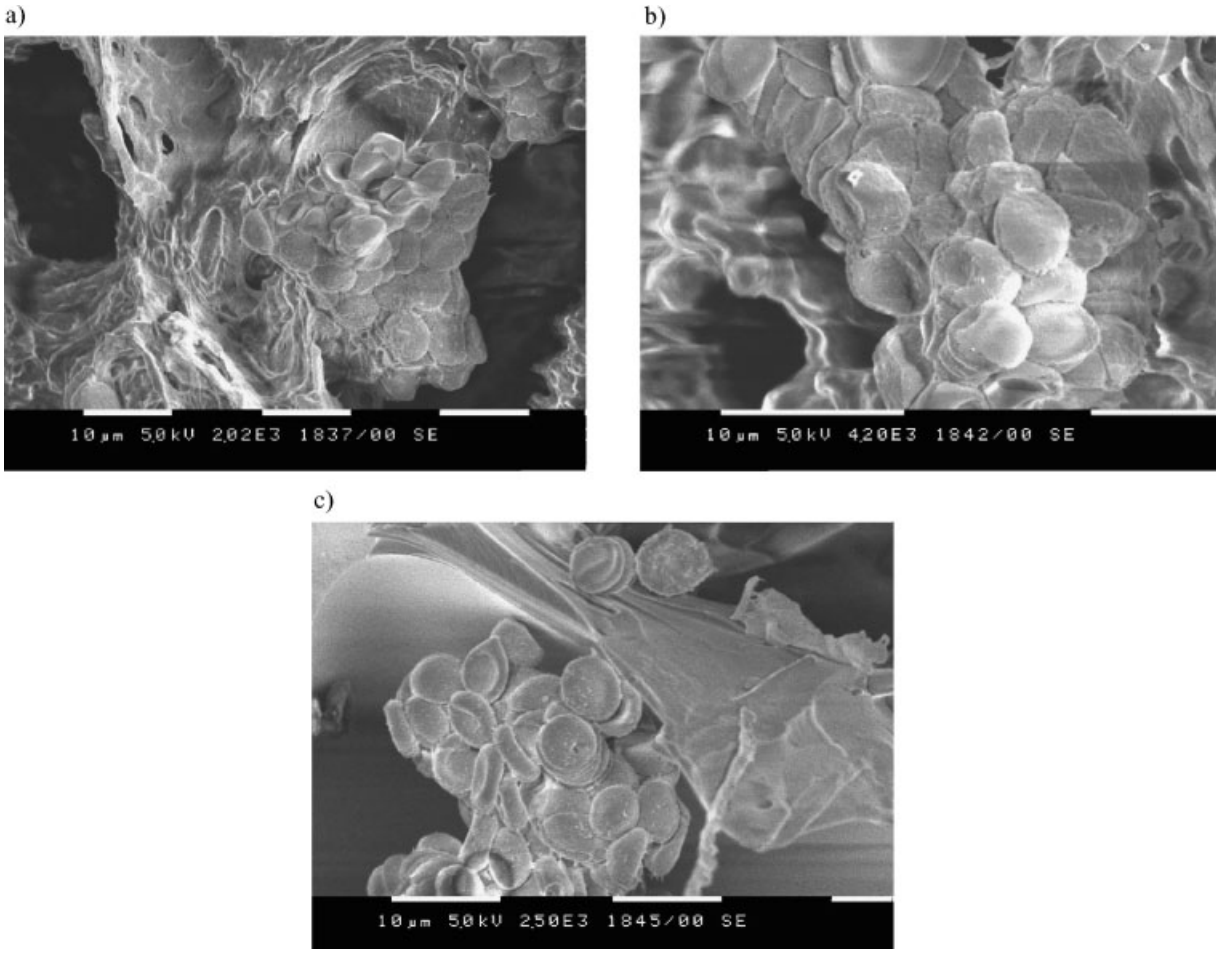


*Figure 10.* SEM micrographs of freeze-dried complexes after 1-h contact with whole blood: CECh/PDMAEMA (A, $F_{DMAEMA} = 0.64$), CECh/PDMAEMAQ50 (B, $F_{DMAEMA} = 0.53$), CECh/PDMAEMAQ100 (C, $F_{DMAEMA} = 0.47$). Magnification: 2 020× (A), 4 200× (B), 2 500× (C).

crosslinked CECh/(quaternized) PDMAEMA complexes were observed (Figure 11). The results from the blood cell counting tests showed that these materials are characterized by lower toxicity towards the red and white blood cells (Figure 9). The decrease of the platelet count in the presence of crosslinked CECh/(quaternized) PDMAEMA complexes is lower as compared to the decrease observed in the presence of complexes based on non-crosslinked CECh. This is explained by the lack of porosity of the surface in the case of crosslinked CECh, as seen from Figure 11. No adhesion of blood cells is observed on the surface of crosslinked CECh (Figure 11A). The SEM studies reveal that the crosslinked CECh/PDMAEMA complex induces slight red blood cell agglutination and small aggregates are observed on the surface (Figure 11B). These results are in good agreement with those obtained from the blood cell counting tests (Figure 9A), which show that the red blood cell count in this case is almost unchanged indicating that hemolysis does not occur. As already mentioned, a significant decrease in the platelet count was observed. This fact is explained by the partial protonation of the tertiary amino groups of PDMAEMA at pH = 7.4 (the pH value of blood). As seen from Figure 9, the increase in the stability of the complexes at this pH value in the following order: crosslinked CECh/PDMAEMA - < crosslinked CECh/PDMAEMAQ50 ≤ crosslinked CECh/PDMAEMAQ100, leads to a significant decrease of the interaction of the complexes with the platelets. A small number of red blood cells with unchanged morphology are observed by SEM on the surface of the crosslinked CECh/PDMAEMAQ50 and crosslinked CECh/PDMAEMAQ100 complexes (Figure 11C, D). The lack of porosity of these complexes does not allow penetration of the blood cells in depth and the charges that are located in the bulk of the complexes are not accessible to the cells. Therefore, it can be concluded that the crosslinked CECh/(quaternized) PDMAEMA complexes are characterized by lower haemostatic activity as compared to the complexes based on non-crosslinked CECh.

Recently, some of us have shown that PAMPSNa possesses anticoagulant activity comparable to that of the natural anticoagulant heparin[14] and its complex

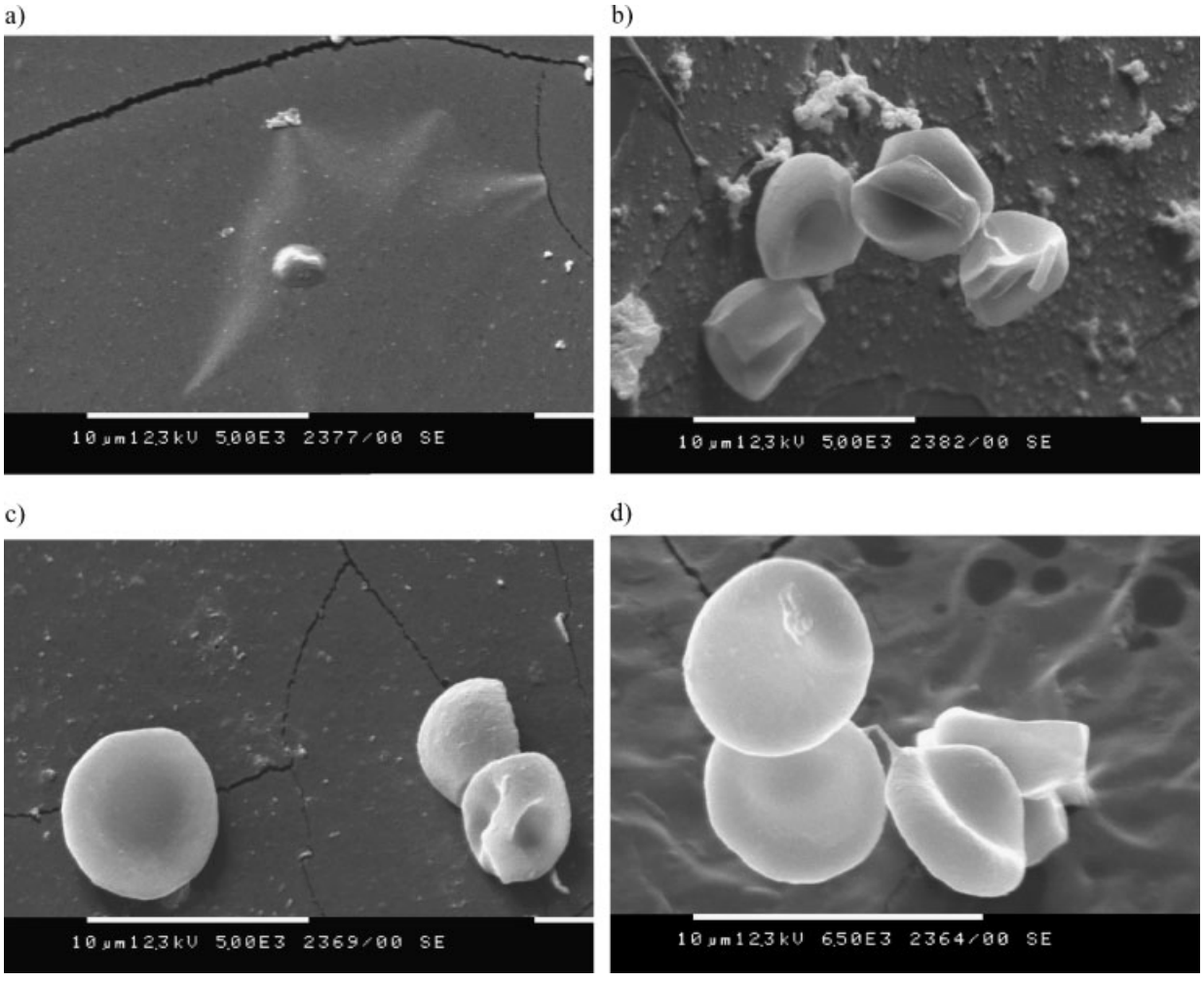


*Figure 11.* SEM micrographs of a film of crosslinked CECh (a), and its complexes with PDMAEMA (b), PDMAEMAQ100 (c), and PDMAEMAQ50 (d) after 1-h contact with whole blood. Magnification: 5 000× (a–c), 6 500× (d).

formation with haemostatic polymers leads to polymer materials with improved blood compatibility.[11] We have studied the complex formation between (quaternized) PDMAEMA and PAMPSNa.[22] The PECs stability depended on the QD of PDMAEMA and the medium pH. At pH = 7.0 ($I = 0.1$) a complex with the highest yield is prepared at $F_{DMAEMA} = 0.52$, 0.58, and 0.58 for the PAMPS/PDMAEMA, PAMPS/PDMAEMAQ50, and PAMPS/PDMAEMAQ100 complexes, respectively. In order to evaluate the behavior of these complexes in contact with blood, the blood cell counting test and observation by SEM were employed. The results obtained from the blood cell counting tests revealed that PAMPSNa did not affect the blood cell count, which is in accordance with our previous results concerning its anticoagulant activity.[14] The PAMPS/(quaternized) PDMAEMA complexes did not change the blood cell counts, an indication of improved blood compatibility of these polymer materials as compared to their polycationic precursors. The SEM micrographs of the PAMPS/(quaternized) PDMAEMA complexes after 1-h contact with whole blood are shown in Figure 12. As seen, few non-agglutinated red blood cells with unchanged morphology were observed on the surface of the complexes. No platelet adhesion was observed. These results are in good agreement with the results from the blood cell counting tests, as well as with our previous results concerning the blood compatibility of chitosan/PAMPS and CECh/PAMPS complexes.[5,16]

## Conclusion

In the present study, polymer materials based on complex formation between (quaternized) PDMAEMA and (crosslinked) CECh or PAMPSNa were prepared. Their behavior in contact with blood depended strongly on the degree of quaternization of PDMAEMA. Although the inherent hemolytic activity of the DMAEMA-based polymers was


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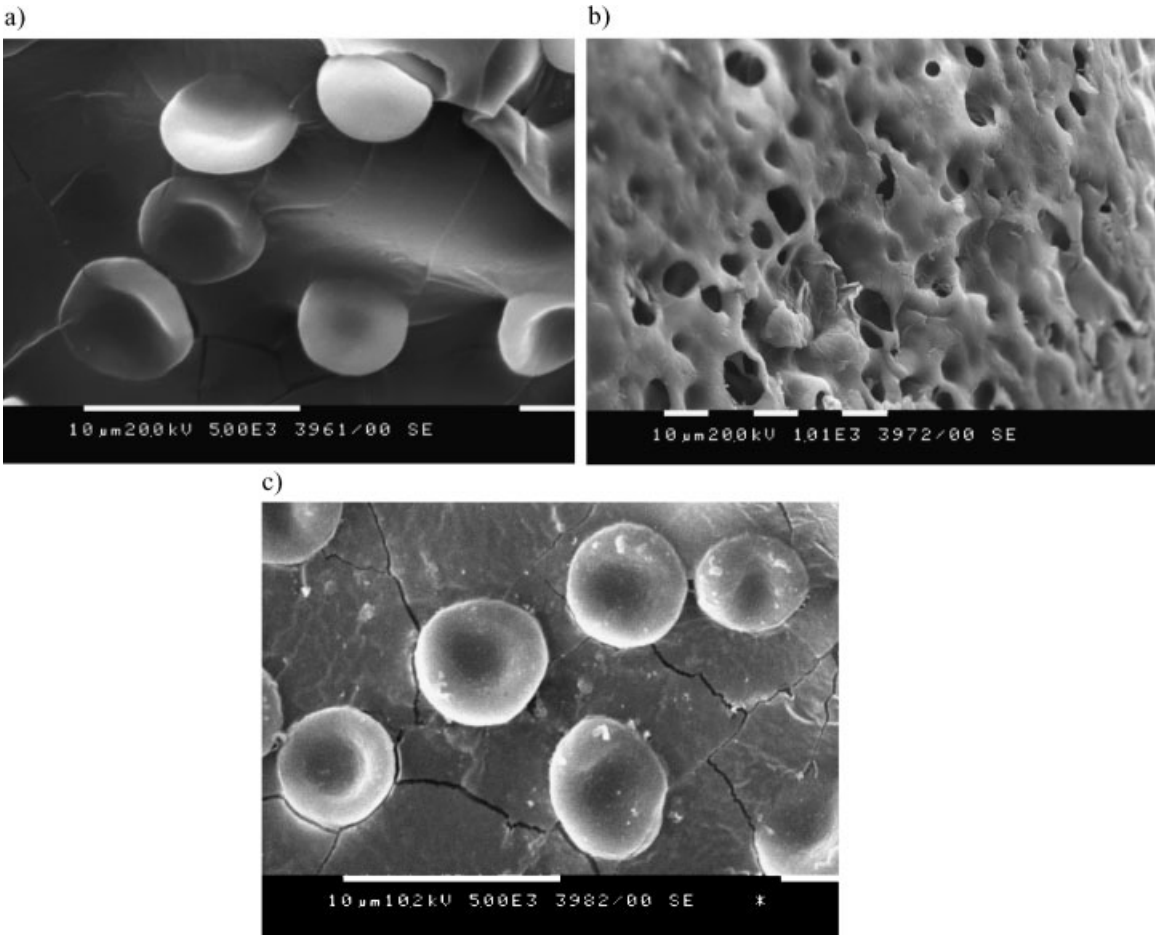


*Figure 12.* SEM micrographs of freeze-dried complexes after 1-h contact with whole blood: PAMPS/PDMAEMA (a, $F_{DMAEMA} = 0.52$), PAMPS/PDMAEMAQ50 (b, $F_{DMAEMA} = 0.58$), and PAMPS/PDMAEMAQ100 (c, $F_{DMAEMA} = 0.58$). Magnification: 5 000× (a, c), 1 010× (b).

decreased significantly by the complex formation, the complexes still possessed haemostatic activity. The cross-linking of CECh led to complexes with lower haemostatic activity. The cytotoxicity of (quaternized) PDMAEMA was remarkably decreased by its incorporation in complexes with a synthetic polymer with heparin-like activity – PAMPSNa. The obtained results reveal that the behavior of (quaternized) PDMAEMA in respect to blood cells can be modulated by complex formation with an appropriate polymer partner.

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