Poly(vinyl alcohol)–heparin hydrogels as sensor catheter membranes

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Poly(vinyl alcohol)–heparin hydrogels with varying water content were synthesized for use as sensor catheter membranes. Films were cast from aqueous mixtures of poly(vinyl alcohol) (PVA), a photosensitive cross-linker *p*-diazonium diphenyl amine polymer (PA), glutaraldehyde (GA) and heparin. After drying, the films were cross-linked by successive UV irradiation and heat treatment. To get an indication about the cross-linking density of the networks, the water content of the hydrogels was measured after equilibration in water. Hydrogels from PVA, PA, GA and heparin, with a water content of 35–95%, could be obtained if the components were dissolved in saline instead of water. The release of heparin from PVA–heparin or PVA–PA–heparin hydrogels was studied using different receiving phases. The cumulative amount of released heparin appeared to be dependent on the initial water content of the hydrogels and the composition of the receiving phase. For the PVA–PA–heparin hydrogels as well as the PVA–heparin hydrogels the cumulative amount of released heparin in water was about six times higher than in a Tris buffer. Using Tris buffer as receiving phase PVA–PA–heparin hydrogels with water contents of 53, 61 or 71% released heparin for at least 3 wk. The cumulative amount of released heparin increased with initial water content of these hydrogels. Recalcification times (RCT) of plasma exposed to PVA–PA–heparin hydrogels (water content 53%), which released heparin at a low rate (2 μg/cm^2 per day), were markedly prolonged compared with the RCT values for PVA–PA hydrogels without heparin. This indicates that the activation of the coagulation of plasma exposed to these PVA–PA–heparin hydrogels is inhibited by the release of heparin from these hydrogels even at low rates. In addition, permeability studies showed that the PVA–PA–heparin hydrogels were impermeable for bovine serum albumin (BSA).

Keywords: Heparin, hydrogels, membranes, catheters

Sensor membranes must fulfil several requirements. A membrane for an oxygen sensor should have a good permeability for oxygen and electrolytes. Besides, when the sensors are used in biological systems such as blood, the membranes have to be blood compatible. Moreover, protein adsorption on to the membranes should be low, since this might result in fouling of the membranes. In this paper the synthesis and blood compatibility of poly(vinyl alcohol)–heparin hydrogels for use as sensor membranes is described. Miller and Peppas[1] observed a low protein adsorption on to poly(vinyl alcohol) hydrogels, whereas other investigators described the good blood contacting properties of poly(vinyl alcohol)–heparin hydrogels[2,3]. The aim of this investigation was to develop poly(vinyl alcohol)–heparin hydrogels which are impermeable for plasma proteins and release heparin for at least 1 wk.

For application as sensor membranes it is important that cross-linking for the formation of hydrogels can take place on the sensor *in situ* and therefore the possible use of a photosensitive cross-linker was investigated. The UV-induced cross-linking of poly(vinyl alcohol) (PVA) by tetrazonium salts has been reported by Tsunoda and Yamaoka[4,5]. It is assumed that after decomposition of these salts reaction occurs with hydroxyl groups of PVA, resulting in the formation of stable phenyl ether bonds (*Figure 1*). However, with tetrazonium salts only hydrogels with a low cross-linking density can be obtained. Thus, to obtain hydrogels with a higher cross-linking density the use of a second cross-linker is required, e.g. glutaraldehyde. (Cross-linking of PVA with glutaraldehyde has been studied previously[2,3,6–9].) The reaction of glutaraldehyde with hydroxyl groups of PVA, in the presence of a proton donor, leads to the formation of hemiacetals (*Figure 2*). Subsequently, these unstable hemiacetals can be converted into stable acetals by ring formation or by adding primary alcohols like methanol[6].

The system we used for the synthesis of PVA–heparin hydrogels was quite complex: PVA, diazonium salt (PA, Figure 3), glutaraldehyde and heparin. Formation of

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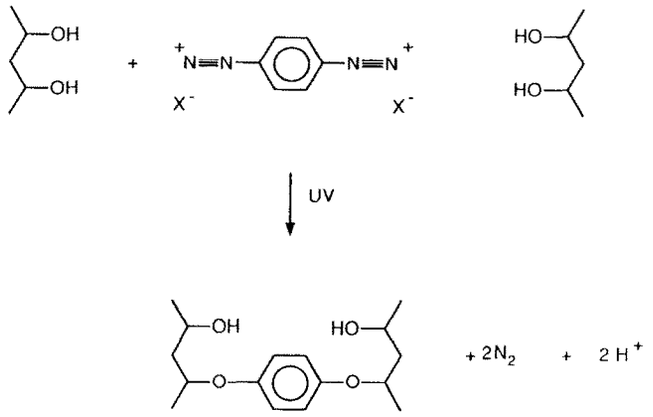

Figure 1 *Cross-linking of poly(vinyl alcohol) with tetrazonium salts[4, 5].*

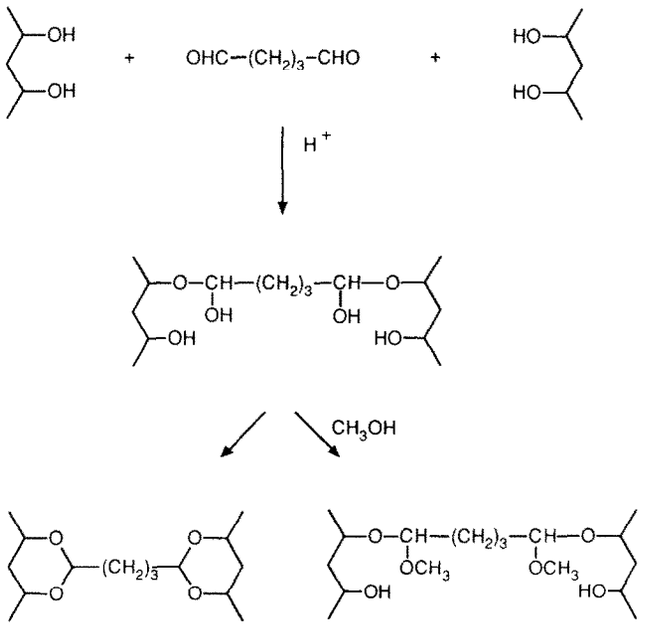

Figure 2 *Cross-linking of poly(vinyl alcohol) with glutaraldehyde.*

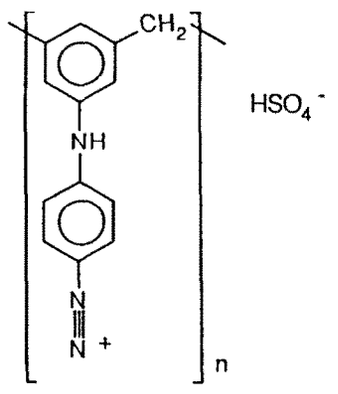

Figure 3 *p-Diazonium diphenylamine polymer (PA).*

hydrogels from PVA in the presence of heparin will probably lead to networks with physically trapped and/or covalently bound heparin. For instance, it is possible that the diazonium salt after decomposition reacts with hydroxyl groups from heparin, although no data have been reported in literature on this subject. On the other hand, glutaraldehyde can also react with heparin, but the reaction with the hydroxyl groups will only result in the formation of hemiacetal bonds. Stable

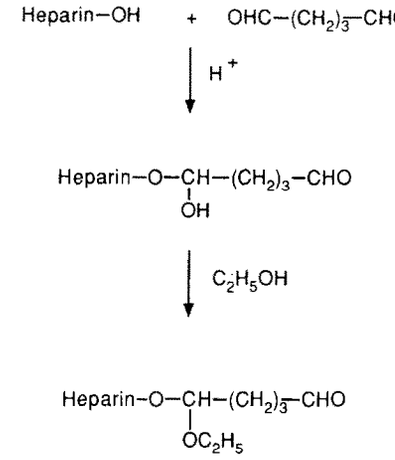

Figure 4 *Reaction of heparin with glutaraldehyde in the presence of ethanol[2].*

bonds can be formed by adding quenchers. Merrill *et al.*[2] used ethanol as a quencher to synthesize PVA hydrogels with covalently bound heparin (*Figure 4*). However, Sefton states in contrast to Merrill, that hydroxyl groups of heparin are not very reactive towards glutaraldehyde. According to Sefton[3, 10] covalently bound heparin can only be obtained, if in the heparin a reasonable amount of amino groups are present.

In our study heparin was used with a very small amount of amino groups. Because, in addition, no quenchers were added, it was assumed that after cross-linking the heparin molecules were physically trapped in the network. To control the release of heparin from the PVA hydrogels it was important to have information about the mesh size of the network, because permeability as well as release depend on the mesh size. However, calculation of M_c for these systems is quite complex and therefore the water content of the hydrogels was used as an indication for the cross-linking density.

Since various parameters were supposed to have an effect on the properties of PVA networks, the cross-linking process was studied in more detail to enable the synthesis of PVA-heparin hydrogels. The relation between the release of heparin from PVA-heparin hydrogels and the initial water content of the hydrogels was investigated, because for the PVA-heparin hydrogels the diffusion of heparin will occur through the water phase[6]. Release of heparin from films of PVA-heparin hydrogels and from PVA-PA-heparin hydrogels was investigated. After extraction of physically trapped heparin from coatings of hydrogels in glass tubes, the presence of covalently bound heparin was studied by measuring recalcification times. In view of a possible application of the PVA-heparin hydrogels as sensor membranes it is important to have information about the permeability for plasma proteins. Therefore the transport of bovine serum albumin through cross-linked PVA-PA014 hydrogels (with or without heparin) was studied in a diffusion cell.

EXPERIMENTAL

Materials

Poly(vinyl alcohol) (PVA, 88% hydrolysed, mol. wt 125 000 (as given by the supplier)), was purchased from Janssen Pharmaceutics (Beerse, Belgium). The photosensitive cross-linker, *p*-diazonium diphenylamine polymer (PA), was a

generous gift from PCAS (Longjumeau, France). Glutaraldehyde (GA) was purchased as a 25% aqueous solution (w/w) from Merck, Darmstadt, FRG (glutaraldehyde for electron microscopy according to P.J. Anderson). The ratio of the absorbance maxima at 235 nm (impurities) and at 280 nm (glutaraldehyde) was 0.23, which indicates a low degree of contamination[11-13]. Before use the 25% solution (w/w) was diluted with double-distilled water to a 10% solution (w/w). The diluted GA solution was stored at 4°C. Heparin (hep) from porcine intestinal mucosa was purchased from Diosynth (Oss, The Netherlands). The specific activity of the different heparin batches varied from 160 to 175 IU/mg (as indicated by the manufacturer). These heparins contained a low number of amino groups (approximately 1 glucosamine group per 200 glucose units)[14]. Bovine serum albumin (BSA) was purchased from Janssen Pharmaceutics (Beerse, Belgium). The reagents used in the heparin assays, PTT reagent and toluidine blue, were purchased from Boehringer (Mannheim, FRG) and Merck (Darmstadt, FRG) respectively.

Preparation of poly(vinyl alcohol) stock solutions

A PVA stock solution without PA was prepared by adding 10 g of PVA to 90 ml water. The solution was gently heated for a few minutes and stirred overnight. Subsequently, the viscous PVA solution was stored at room temperature. For PVA stock solutions with PA, PVA (10 g) was added to 90 ml water or saline. The PVA solution was heated and stirred as described above. PVA-PA stock solutions were prepared by adding PA to the PVA solution (0.2% and 0.3% (w/w) PA respectively). These solutions were stored in the dark at room temperature.

Synthesis of poly(vinyl alcohol) hydrogels without photosensitive cross-linker

Syntheses of PVA hydrogels without PA were carried out according to the following general procedure. An aliquot of the 10% GA solution (0.1–1.0 ml) was diluted with 1 ml of water. Subsequently HCl (0.00–0.40 ml, 0.1 M) was added, which acts as a cross-linking catalyst[2,6,7-9]. In the last step water was added to a final volume of 3 ml. Heparin (50 mg), when used, was also dissolved in the GA (HCl) solution. The 3 ml GA (HCl) solution was mixed with 7 ml PVA stock solution and the mixture was stirred for 5 min. The homogeneous solution was cast on a perspex plate and the PVA film was dried overnight at room temperature. Finally, the dry PVA film was heat treated (0–24 h, 60°C) to complete the GA cross-linking.

Table 1 Composition of mixtures before casting

Code (% w/w)	[PVA] (% w/w)	HCl (ml)	[PA] (% w/w)	[GA] (% w/w)	[hep] (% w/w)
PVA	7	0–0.4[a]	0	0–1[b]	0
PVA	7	0–0.4[a]	0	0–1[b]	1
PVA-PA014	7	0	0.14	0–1[b]	0
PVA-PA014	7	0	0.14	0–1[b]	1
PVA-PA021	7	0	0.21	0–1[b]	0
PVA-PA021	7	0	0.21	0–1[b]	1

[a]Added volume of 0.1 M HCl from 0 to 0.4 ml.
[b][GA] from 0 to 1% (w/w).
PVA, poly(vinyl alcohol); PA, *p*-diazonium diphenylamine polymer; GA, glutaraldehyde; hep, heparin.

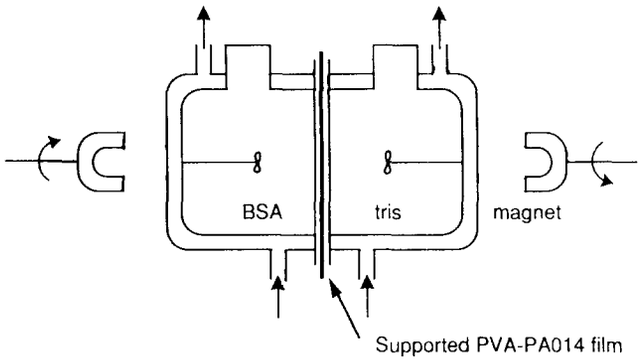

Figure 5 Schematic representation of the diffusion cell[15].

Synthesis of poly(vinyl alcohol) hydrogels with photosensitive cross-linker

For these hydrogels almost the same procedure was used as described for the PVA hydrogels without PA. Therefore only the deviations from this procedure will be mentioned. HCl was not added to the GA/heparin solution and instead of the PVA stock solution PVA-PA solutions were used. The dry PVA films were irradiated with UV (Heraeus TQ 150 Z3) at room temperature for 0–5 min before the heat treatment. The concentrations of the components (PVA, HCl, PA, GA and hep) used in the synthesis of the hydrogels are summarized in *Table 1*.

Swelling behaviour

The water content of the hydrated hydrogels was determined after 24 h equilibration in water or phosphate buffered saline (PBS). The water content was calculated using the relationship: Water content (%) = $(1 - W_{dry}/W_{eq}) \times 100$ (%).

(W_{dry} = initial dry weight of the films; W_{eq} = wet weight after equilibration in water or PBS.)

Transport of bovine serum albumin

BSA transport through cross-linked PVA-PA014 films was measured in a diffusion cell[15], consisting of two cylindrical compartments (*Figure 5*, half-cell volume 45 ml; effective membrane area 9.8 cm^2). The compartments were double-walled for thermostating using a thermostated water bath (T = 37°C). A flat-bladed turbine positioned at the centre was driven by a magnet outside the compartment at a stirring rate of 1000 rev min^{-1}.

Cross-linked films were positioned between the two cylindrical compartments. Macroporous filters made of poly(ethylene) (Spectra Medical Industries Inc., Los Angeles, USA) were used to support the films and to prevent flexing and tearing due to their low mechanical strength. These filters had a mesh size of 1240 μm and a thickness of 760 μm.

The donor compartment was filled with a solution of BSA (1 g/l) in Tris buffer (25 mM, 0.9% NaCl (w/w), pH 7.4 (HCl)), while the receptor part of the cell was filled with Tris buffer. The progress of the BSA diffusion was followed by taking 1 ml samples from the receptor compartment and analysis in a UV spectrophotometer at 280 nm. After removal of each sample 1 ml of Tris buffer (T = 37°C) was added to maintain the volume constant.

Heparin assay: activated partial thromboplastin times

Activated partial thromboplastin times (APTT) were determined for a qualitative estimation of heparin in washing

solutions. Fresh frozen human CPD plasma (Bloodbank Twente and Achterhoek, The Netherlands) was thawed at 37°C and subsequently stored at 0°C. An aliquot of plasma (50 μl) was mixed with 50 μl washing solution. (Plasma 50 μl) mixed with 50 μl water or Tris buffer was used as reference.) To this mixture a kaoline-cephaline suspension (PTT reagent from Boehringer, 50 μl) was added and the solution was incubated at 37°C. After exactly 2 min 50 μl $CaCl_2$ solution (25 mM) was added and the APTT value was registered. A prolonged APTT value compared with the value for water indicated the presence of heparin in the washing solution.

Heparin assay: metachromic shift of toluidine blue

The metachromic assay was used to measure the amount of heparin released from cross-linked PVA-heparin or PVA-PA014-heparin films. Toluidine blue (35 mg) was dissolved in 1 l water and the solution was filtered before use. To obtain calibration curves aliquots of 50 or 100 μl heparin solution (concentration ranging from 0 to 0.50 mg/ml) were added to 1.5 ml of the filtered toluidine blue solution. After mixing, the absorbance at 600 nm was measured against air. Samples (50 or 100 μl) were taken during the release experiments and subsequently mixed with 1.5 ml toluidine blue solution. Using the calibration curve, heparin concentrations in the samples were determined.

Heparin release from cross-linked PVA-heparin films

Heparin release from PVA-heparin or PVA-PA014-heparin films (2 × 2 cm) was studied using water, saline or Tris buffer (V = 5 or 10 ml) as the receiving phase. The solutions with the films were placed in a shaking bath (37°C). Samples were taken and analysed on heparin with the metachromic toluidine blue assay.

Plasma recalcification times

Recalcification times of plasma were determined in contact with hydrogel coatings after extraction of heparin. Glass tubes were coated with PVA-PA014 hydrogels as follows. The inside of the tubes (Boro silicate, ID 12 mm, length 55 mm, Renes, Zeist, The Netherlands) was coated with an aqueous solution of PVA (7% w/w), PA (0.14% w/w) and when used GA and hep. The tubes were dried, UV irradiated and heat treated as described for PVA-PA014 films. Glass tubes coated with PVA-PA014-heparin hydrogels were filled with water to remove unbound heparin and incubated at room temperature. After 24 h the washing solution was analysed on the presence of heparin with the APTT test. The washing procedure was repeated until no more heparin could be detected in the washing solution. Finally, the tubes were washed with Tris buffer (4 cycles of 24 h).

Glass tubes were coated with silicone rubber (SR), reference material for determination of recalcification times, according to the following procedure. The inside of the tubes was covered with a 5% (w/w) solution of SR (General Electric, Bergen op Zoom, The Netherlands) in THF. The tubes were dried overnight in a vacuum oven (60°C). To remove acetic acid the coated tubes were thoroughly washed with water.

Human citrated plasma was thawed and stored as described above. Glass tubes coated with PVA-PA014-heparin, PVA-PA014 and SR and non-coated glass tubes were filled with Tris buffer and equilibrated for 24 h at room temperature. 500 μl plasma was pipetted into a test-tube and incubated at 37°C. After exactly 2 min 50 μl $CaCl_2$ solution (200 mM) was added. The time interval between the $CaCl_2$ addition and detection of the first fibrin threads with a stainless steel hook was taken as the recalcification time (RCT).

RESULTS AND DISCUSSION

Cross-linking without PA

Effect of HCl concentration. To study the effect of the HCl concentration on the degree of cross-linking, PVA hydrogels resulting from reactions in the presence of GA but without heparin were synthesized. As expected, no cross-linking occurred without HCl addition. The cross-linking density of the PVA hydrogels initially increases using increasing amounts of HCl, but at higher HCl concentrations the water content becomes independent on the HCl concentration. In addition, cracks had been formed in the PVA films after heat treatment. Therefore 0.2 ml HCl was used in further experiments.

Effect of heat treatment. Although without heat treatment hydrogels were formed, a considerable increase of the cross-linking density was observed after a heat treatment at 60°C for 90 min. Longer heat treatment did not cause a further increase in cross-linking density and therefore heat treatment during 90 min was used in subsequent experiments.

Effect of GA concentration. The influence of the GA concentration on the water content of hydrated PVA hydrogels ([hep] = 0%) is shown in *Figure 6*. As expected, the water content of these gels decreased with increasing GA concentration. There is especially a strong reduction in water content, when the GA concentration is raised from 0.05 to 0.5% (w/w).

Effect of the presence of heparin. PVA hydrogels synthesized in the presence of heparin had a slightly higher water content than the hydrogels prepared without heparin (*Figure 6*). In this respect it should be noted that the swelling behaviour of cross-linked PVA was also studied by Sefton and co-workers although another synthesis was used[10]. They also found a decreasing water content with an increasing GA concentra-

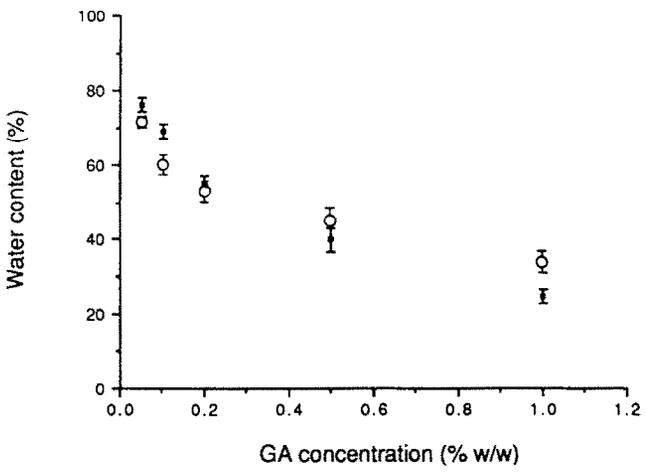

Figure 6 Water content of hydrated PVA hydrogels as a function of the glutaraldehyde (GA) concentration [PA] = 0, [hep] = 0%, ■; [PA] = 0, [hep] = 1% (w/w), ○; HCl: 0.2 ml 0.1 M, heat treatment: 90 min at 60°C). PA, p-diazonium diphenylamine polymer; hep, heparin.

Table 2 Effect of UV irradiation and heat treatment on water content of PVA-PA014 hydrogels ([GA] = 0.2% w/w), [hep] = 0% (w/w))

UV irradiation	Heat treatment (90 min at 60°C)	Water content (% (w/w))
–	–	a
+	–	95
+	+	40
–	+	a

a: PVA–PA film dissolves.
PVA, poly(vinyl alcohol); PA, *p*-diazonium diphenylamine polymer; GA, glutaraldehyde; hep, heparin.

tion. Addition of heparin had a very small effect on the water content of the hydrated hydrogels.

Cross-linking with PA

Effect of UV irradiation and PA concentration. To determine the decomposition rate of PA during the formation of the PVA–PA hydrogels, the UV absorption of dry PVA–PA014 and PVA–PA021 films was studied. It turned out that the absorption peak of PA at 376 nm had completely disappeared after 2 min irradiation. The effect of PA concentration on the cross-linking density of PVA–PA hydrogels was studied by measuring the water content of PVA–PA014, PVA–PA021 and PVA–PA050 films after UV irradiation (no heat treatment). The hydrogels from these PVA–PA films had comparable water contents (90–95%), which indicates that the degree of cross-linking does not depend on the PA concentration.

Effect of heat treatment. As mentioned PVA–PA hydrogels obtained by cross-linking with UV irradiation had a high water content. Application of heat increased the cross-linking density considerably (*Table 2*), which was also observed for the hydrogels without PA.

Effect of GA concentration. To get information about the influence of the GA concentration on the properties of hydrogels, resulting from PVA with PA (0.14% (w/w)), cross-linking was studied with different GA concentrations. Hydrogels were obtained with a decreasing water content when increasing the GA concentration (*Figure 7*). The strong effect of the GA concentration on the cross-linking of PVA–PA014 hydrogels is remarkable since no acid catalyst was added during the synthesis of these networks. As was previously described, cross-linking with GA in the absence of PA was not observed when the acid catalyst was omitted. However, protons will be formed in the reaction of PVA with PA (*Figure 1*), which obviously catalysed the cross-linking by GA. Comparison of the water content of PVA hydrogels (*Figure 6*) and of PVA–PA014 hydrogels (*Figure 7*) clearly shows that for all GA concentrations addition of PA resulted in higher cross-linking densities.

Effect of the presence of heparin. Figure 8 shows that with water as a solvent PVA–PA014 hydrogels were obtained with the same water content for all GA concentrations. This phenomenon can be explained by the fact that the negatively charged heparin complexes with the positively charged PA and as a consequence the GA cross-linking of PVA cannot be catalysed by PA. It should be noted that addition of a GA/heparin solution to the PVA–PA014 stock solution resulted in a turbid solution.

To prevent the formation of a complex between heparin and PA the synthesis of the hydrogels was also performed using saline as a solvent. In this case the electrostatic interaction between PA and heparin is hindered by NaCl and therefore PA can promote the cross-linking of PVA by GA. *Figure 8* shows that the water content of the hydrogels is substantially lower than when using water as a solvent. However, the water content of these hydrogels varies only in a small GA concentration range. Besides, in this range the water content of these hydrogels is significantly higher than for the PVA–PA014 hydrogels without heparin (*Figure 7*). This difference might be attributed to a partial complexation of PA with heparin. Consequently networks consisting of PVA and heparin might be formed with a higher water content.

The water content of PVA–PA014 hydrogels with heparin remains constant at GA concentrations higher than 0.4% (*Figure 8*). Obviously the PA concentration is too low to realise a complete GA cross-linking at GA concentrations above 0.4%. This explanation is supported by the results obtained with gels, for which a higher concentration of PA was used (*Figure 9*). Again the synthesis of the hydrogels was performed using saline as a solvent. For GA concentrations lower than 0.4% a similar decrease of the water content with increasing GA concentration as for PVA–

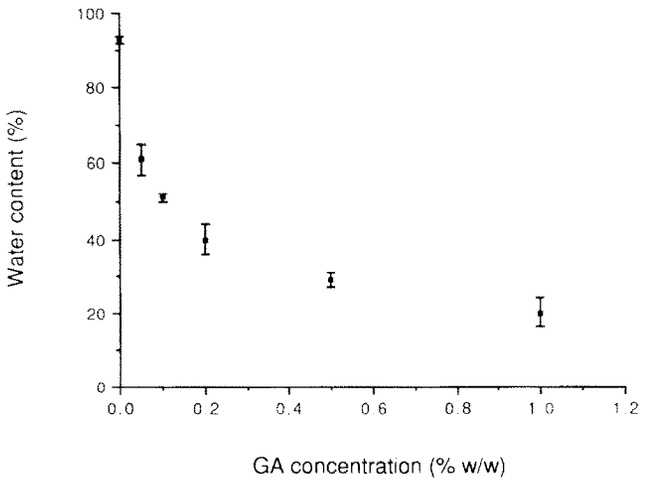

Figure 7 Water content of hydrated PVA–PA014 hydrogels as a function of the glutaraldehyde (GA) concentration ([heparin] = 0% (w/w), heat treatment: 90 min at 60°C).

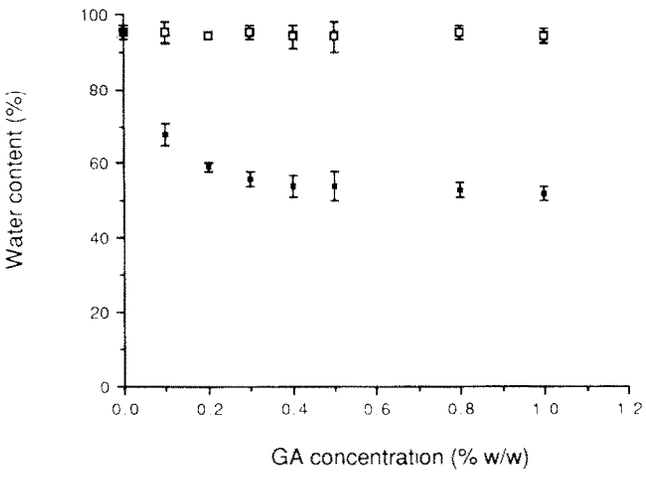

Figure 8 Water content of hydrated PVA–PA014 hydrogels as a function of the glutaraldehyde (GA) concentration ([heparin] = 1% (w/w), heat treatment: 90 min at 60°C). Solvent: □, water; ■, saline.

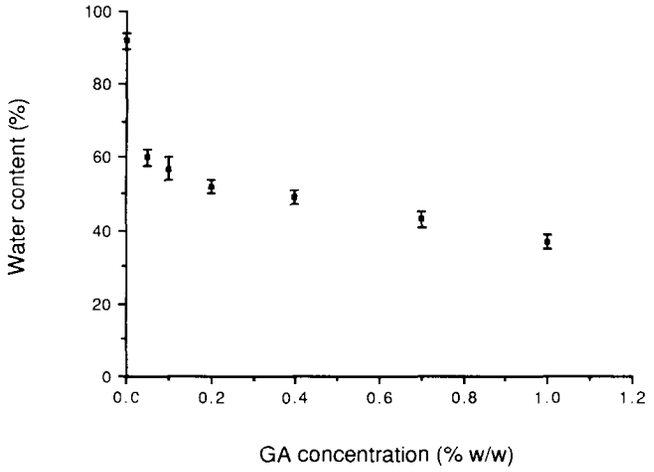

Figure 9 Water content of hydrated PVA-PA021 hydrogels as a function of the glutaraldehyde (GA) concentration ([heparin] = 1% (w/w), heat treatment: 90 min at 60°C). Solvent: saline.

Table 3 Results of diffusion experiments with bovine serum albumin (BSA) for different cross-linked PVA-PA014 membranes

[PVA] (% w/w)	[PA] (% w/w)	[GA] (% w/w)	[hep] (% w/w)	Water content (%)	Result
7	0.14	0.00	0.00	95	Equilibration within 24 h
7	0.14	0.05	0.00	60	No BSA transport after 72 h
7	0.14	0.40	1.00	53	No BSA transport after 72 h

PVA, poly(vinyl alcohol); PA, *p*-diazonium diphenylamine polymer; GA, glutaraldehyde; hep, heparin.

PA014 gels with heparin was observed. However, in contrast with the PVA-PA014 gels, for PVA-PA021 gels a further decrease in water content was found when the GA concentration was raised above 0.4%. It seems that for these gels the PA concentration is high enough to catalyse the GA cross-linking even at higher GA concentrations.

Transport of BSA

The results of the diffusion experiments are shown in *Table 3*. With PVA-PA014 membranes without GA and heparin (water content 95%), equal BSA concentrations in both compartments of the diffusion cell were reached within 24 h. For PVA-PA014 films with a lower water content (60%, [GA] = 0.05 (w/w)), no BSA transport through the membrane could be observed after 72 h. PVA-PA014-heparin membranes with a somewhat lower water content (53%, [GA] = 0.4% (w/w)) also showed no permeation of BSA.

Reinhart *et al.*[6] also studied diffusion of BSA through PVA hydrogels cross-linked by GA. Using an initial GA concentration of 0.2% (w/w) in the formation of the hydrogels, the BSA transport was obstructed. The discrepancy between the minimal GA value reported by Reinhart *et al.*[6] and the minimal GA concentration found in this study might be attributed to the additional cross-linking effect of PA. We found for PVA hydrogels without PA a water content of 55% using 0.2% GA (w/w) (see *Figure 6*). Reinhart *et al.* used a quencher (methanol), which may have lowered the cross-linking density of their hydrogels.

Table 4 Data for synthesis and water content of PVA-heparin and PVA-PA014-heparin hydrogels used in release experiments ([PVA] = 7% (w/w) and [hep] = 1% w/w))

[GA] (% w/w)	HCl (ml 0.1 M)	[PA] (% w/w)	Water content (%)	Code of hydrogel
0.05	0.20	0.00	71	A
0.20	0.20	0.00	56	B
0.60	0.20	0.00	37	C
0.05	0.00	0.14	71	D
0.10	0.00	0.14	61	E
0.40	0.00	0.14	53	F

PVA, poly(vinyl alcohol); PA, *p*-diazonium diphenylamine polymer; GA, glutaraldehyde; hep, heparin.

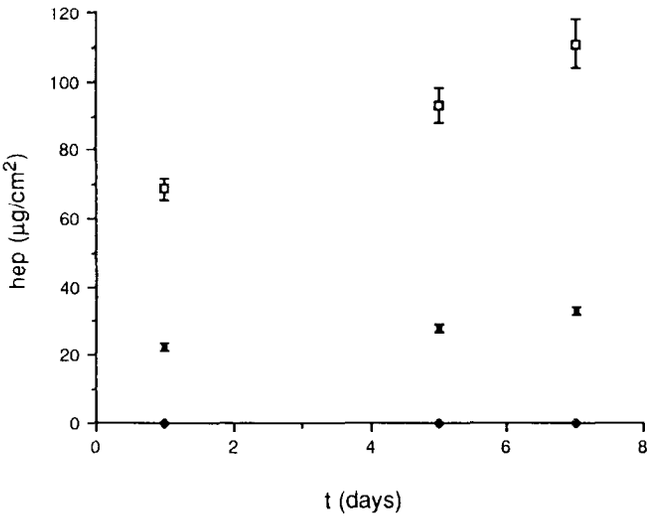

Figure 10 Cumulative amount of heparin released from PVA-heparin hydrogels with different water content (see Table 4*) (□, A 71%; ■, B 56%' ♦, C 37%) (receiving phase: Tris buffer).*

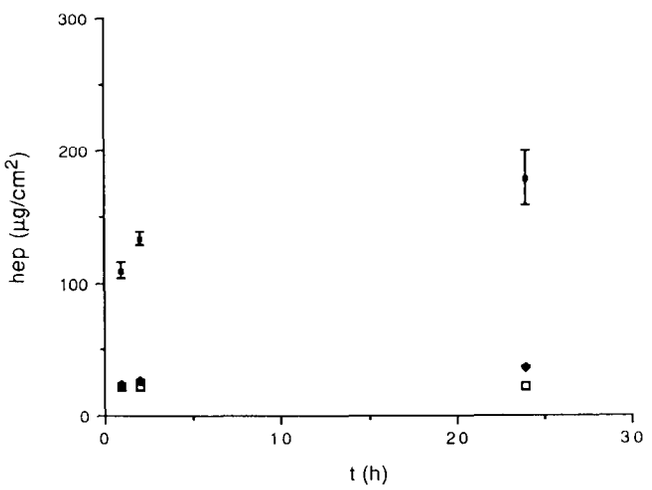

Figure 11 Cumulative amount of heparin released from PVA-heparin hydrogel B using different receiving phases (water content 56%, see Table 4*). □, Tris; ♦, saline; ■, water.*

Release of heparin

Release of heparin was studied with hydrogels, synthesized as indicated in *Table 4*. Heparin release from cross-linked PVA-heparin films without PA was investigated to get information about the optimal water content for heparin release from PVA-PA-heparin films. Using Tris buffer as a receiving phase, the amount of released heparin depended

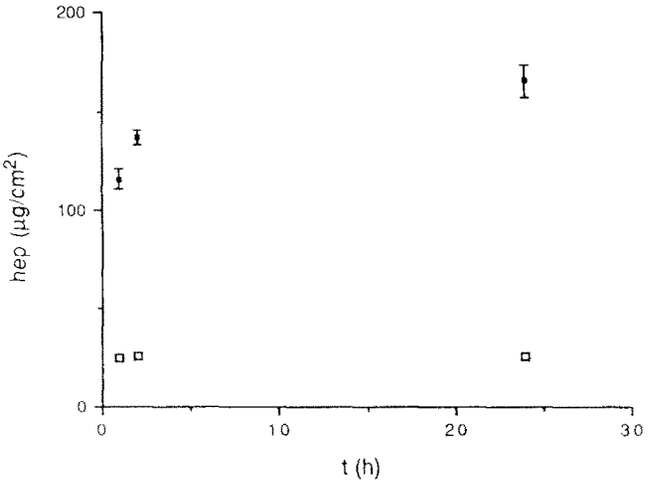

Figure 12 Cumulative amount of heparin released from PVA-PA014-heparin hydrogel F using different receiving phases (water content 53%, see Table 4*). □, Tris; ■, water.*

on the water content of PVA-heparin hydrogels (*Figure 10*). The amount of heparin released from PVA-heparin hydrogel A with a water content of 71% was higher than from hydrogel B with a water content of 56%. For the latter hydrogel, the increase in cumulative amount of released heparin was very low and close to the detection limit of the heparin assay. From PVA-heparin hydrogel C with a water content of 37% no release of heparin was observed. The heparin release from PVA-heparin hydrogel B (water content 56%) in different receiving phases is given in *Figure 11*. Using water as a receiving phase the cumulative amount of heparin released after 24 h was about six times higher than the amount of heparin released in Tris buffer or saline.

When heparin release was studied from PVA-PA014-heparin films with a water content of 53% (hydrogel F), the amount of released heparin also depended on the receiving phase. After 24 h the heparin released in water was about seven times higher than in Tris buffer (*Figure 12*). Heparin release from PVA-PA014-heparin hydrogels (D, E and F) with different water contents (71, 61 and 53%), was measured in Tris buffer (*Figure 13*). It was found that the cumulative amount of released heparin increased with increasing initial water content of the hydrogels. Release from a hydrogel with an initial water content of 53% was again low. After correction for the increase in surface area, there were still substantial differences between the cumulative amounts of heparin released from the three hydrogels (*Table 5*).

Comparison of the results of *Figure 10* with those of *Figure 11* and of *Figure 12* with those of *Figure 13* shows that the type of the receiving phase has more influence on the heparin release rate from heparinized PVA hydrogels (with or without PA) than the mesh size of the networks. Because we found that the swelling in water and in phosphate buffered saline (PBS) was the same, these results indicate a lower release rate of heparin in the buffer solution. A higher release rate of heparin in water than in buffer (PBS) was also observed by Kim and Kim[16] for a silicone rubber/poly(ethylene oxide)/heparin blend. Contrary to our results, they found a higher water content for these blends in water than in PBS.

Table 5 Cumulative amounts of heparin released from PVA-PA014-heparin hydrogels after 7 d[a]

Code of hydrogel	Water content (%)	Hydrated surface area[b] (cm^2)	Correction factor	Released heparin ($\mu g/cm^2$)
D	71	6.76	0.59	101.5
E	61	6.00	0.67	56.3
F	53	5.29	0.76	26.6

[a]Corrected for increase of surface area.
[b]Dry surface area was 4.00 cm^2 for all films.

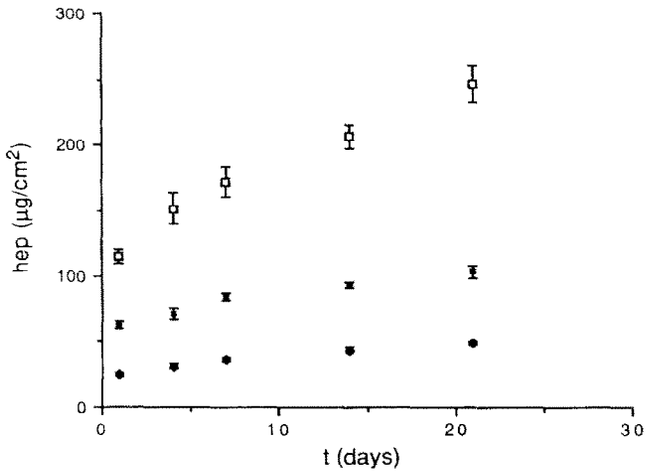

Figure 13 Cumulative amount of heparin released from PVA-PA014-heparin hydrogels (D, E and F) with different water content (see Table 4*) (□, D 71% water; ■, E 61% water; ♦, F 53% water) (receiving phase: Tris buffer).*

Plasma recalcification times

For test-tubes coated with PVA-PA014-heparin hydrogels without GA no heparin could be detected in the solution after two washing cycles of 24 h. Test-tubes coated with PVA-PA014-heparin hydrogels with GA (0.4% w/w) had to be washed for at least 14 d to remove the heparin. After extraction of the physically trapped heparin with water, RCT values for PVA-PA014-heparin coatings were slightly prolonged compared with the RCT values for PVA hydrogels without heparin (*Figure 14*). On the other hand, markedly prolonged RCT values (> 20 min) were observed for coatings from PVA-PA014-heparin hydrogels (water content 53%), after 7 d washing with Tris buffer instead of water. These prolonged RCT values correspond with relatively low heparin release rates, assuming that the release rate of heparin from films and coatings is about the same (*Figure 13*, hydrogel F). The results of these *in vitro* experiments indicate that the activation of the coagulation in plasma exposed to PVA-heparin hydrogels might be inhibited even at considerably low release rates of heparin. However, *in vivo* studies are required to determine the minimal release rate from PVA-heparin hydrogels for a good thromboresistance.

In studies of heparin-releasing polymeric materials different values for a minimal heparin release rate have been reported. Tanzawa *et al.*[17,18] described a hydrophilic heparinized polymer in which heparin is ionically bound via quaternary ammonium groups. From *in vitro* and *in vivo* studies it was concluded that the minimal heparin release rate was 4×10^{-2} $\mu g\ cm^{-2}\ min^{-1}$ ($57.6\ \mu g\ cm^{-2}\ day^{-1}$). However, Lin *et al.*[19] reported a ten times lower minimal value ($5.7\ \mu g\ cm^{-2}\ day^{-1}$) from hydrophilic copolyether-urethane-heparin hydrogels with poly(ethylene oxide) and poly(tetramethylene oxide) as soft segments. Lin *et al.*[19] supposed that differences in the polymeric matrices might

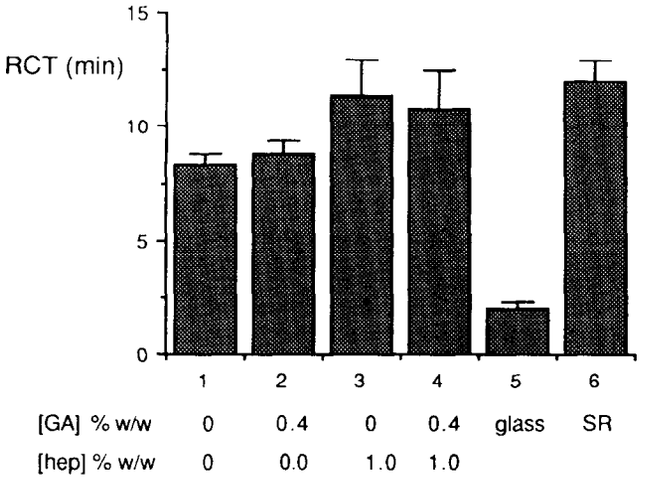

Figure 14 Recalcification times of plasma exposed to various PVA-PA014 coatings after extraction with water (glass and silicone rubber (SR) as references).

be one of the factors which could explain the discrepancy in minimal release rates. Polymeric materials with cationic groups, such as Tanzawa's material, are generally thrombogenic, whereas poly(ethylene oxide) containing copolyether-urethanes are relatively blood compatible.

With the PVA-heparin hydrogels described in this study a minimal heparin release rate of approximately 2 $\mu g\ cm^{-2}\ day^{-1}$ was found. Therefore our results also indicate that with non-ionic hydrophilic polymeric systems only a low release rate of heparin is needed for thrombo-resistance.

CONCLUSIONS

PVA hydrogels were obtained by cross-linking PVA with GA in the presence of an acid catalyst. The water content of these hydrogels could be varied by the GA concentration and by heat treatment. In the synthesis of PVA hydrogels with a photosensitive cross-linker PA, the cross-linking of PVA by GA is promoted by PA as well as by heat treatment. Water content of these hydrogels was about the same as for the hydrogels, obtained without PA.

For the PVA as well as the PVA-PA hydrogels heat treatment of the films resulted in a 50-60% reduction of the water content of the hydrogels. The equilibration water content of all these hydrogels varied between 25 and 75%. Hydrogels from PVA, PA, GA and heparin, with a water content of 35-95% were obtained if the components were dissolved in saline instead of water.

The permeability studies showed that PVA-PA014-heparin hydrogels with an initial water content of 53% are impermeable for albumin (BSA). The release rate of heparin from PVA-heparin and PVA-PA014-heparin hydrogels depends on the initial water content of the hydrogels as well as the receiving phase. For PVA-heparin and PVA-PA014-heparin hydrogels with about the same water content (56 and 53%), the cumulative amount of released heparin after 24 h was about six times lower in Tris buffer than in water. Using a Tris buffer as the receiving phase, the cumulative amount of heparin released from both types of heparinized PVA hydrogels increased with increasing initial water content. Heparinized PVA-PA014 hydrogels varying in initial water content from 53 to 71% released heparin for at least 3 wk.

Activation of the coagulation of plasma exposed to PVA-PA-heparin hydrogels, as measured by recalcification times, was markedly inhibited by release of heparin even at low release rates (2 $\mu g\ cm^{-2}\ day^{-1}$).

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