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Synthesis of boronate-containing copolymers of *N*,*N*-dimethylacrylamide, their interaction with poly(vinyl alcohol) and rheological behaviour of the gels

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Abstract

Cross-linking of polyvinylalcohol (PVA) by boronate-containing copolymer of *N*,*N*-dimethylacrylamide (DMAA, 1) was studied and compared to cross-linking of PVA by borate buffers in weakly alkaline solutions. The copolymer of $M_w = 19,000\ \mathrm{g\ mol^{-1}}$ containing 9 mol% *N*-acryloyl-*m*-aminophenylboronic acid (NAAPBA, 2) was prepared by free radical polymerization of the monomers, exhibiting copolymerization constants $r_1 = 0.84$ and $r_2 = 2.2$. Due to multipoint interaction of the copolymer with PVA via monodiols, the intermolecular cross-linking required for seven-fold and 10-fold lower boron concentrations as compared to borate buffers of pH 8.6 and 7.5, respectively. In rheological measurements, PVA–copolymer gels exhibited storage moduli ($G'_{\max}$) comparable to those of PVA–borate gels prepared at 7.5-fold higher boron concentration and the same pH 8.6, what testified to the similar concentration of cross-links in the gels. Therefore, DMAA–NAAPBA copolymer is a more effective cross-linker of PVA than borate. The PVA–copolymer gel exhibited much higher relaxation time (97 s) compared to PVA–borate gels (≤ 20 s) indicating a longer lifetime of junction zones. The 'shape stability' of the gel is suggested to originate in the structure of junctions, containing several boronate–diol complexes, between the macromolecules of PVA and the copolymer.

Keywords: Boronate; Poly(vinyl alcohol); Rheological behaviour

1. Introduction

Owing to specific and reversible interaction with diols, polyols, sugars and glycoproteins, phenylboronic acids are often employed as ligands in bioaffinity chromatography [1,2] and components of biocompatible, reversibly gelating systems [3–5]. In particular, copolymers of *N*-substituted acrylamides or *N*-vinylpyrrolidone, with *N*-acryloyl-*m*-aminophenylboronic acid (NAAPBA) were shown to form gels with poly(vinyl alcohol) (PVA) due to interaction of the pendant boronates with PVA diols [3,4]. These gels were studied as components of drug delivery systems sensitive to glucose: its high affinity to borates results in competitive binding and disintegration of the polymeric complexes. In such a way the gels were supposed to release insulin in response to increasing glucose concentration in human blood [4], i.e. to behave as smart biomaterials.

Recently, poly-L-lysine grafted with polyethylene glycol was studied as a soluble carrier for phenylboronic acid. The copolymers spontaneously assembled on red blood cell surfaces and sterically prevented their agglutination by lectins and by antibodies to blood groups. Being adsorbed to tissue surfaces they might prevent antibody binding to transplanted cells [6].

The above examples show potential applicability of water-soluble copolymers bearing phenylboronate functions to biomedical manipulations *in vivo*. Although, the prospects are wide, there are still many gaps in basic knowledge about boronate-containing monomers and copolymers, their gelation with PVA and properties of the resultant gels. Copolymerization constants of *N*-substituted acrylamides with NAAPBA, the most common co-monomer,

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Abbreviations: NAAPBA, *N*-acryloyl-*m*-phenylboronic acid; DMAA, *N*-dimethylacrylamide; PVA, poly(vinyl alcohol).

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are unknown. No comparison of PVA gelation with low and high molecular weight boronates has been done up to now, although complex formation between PVA and sodium tetraborate in alkaline media was widely studied and theoretically interpreted by many authors [7–12]. The obtained results were rarely employed to predict the aggregation states of PVA–borate systems at $pH < 9$ [13], where borate are present in their neutral inactive form. Similarly, viscoelastic properties of PVA–borate gels prepared in alkaline conditions were mostly studied [14]. It is worth to note that rheological behaviour of the gels at lower pH is more important for their applications as biomaterials. The tissues and cells contacting the gel should not be exposed to alkaline media.

In order to spread the existing models of PVA gelation to the fields of boronate-containing vinyl copolymers and lower pH, let us briefly consider fundamentals of PVA–borate complex formation. Sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$, borax) dissociates in water into boric acid $B(OH)_3$, monoborate $B(OH)_4^-$ and sodium ions [12]. The acid/base equilibrium between boric acid and its anion

$$B(OH)_3 + H_2O \rightleftarrows B(OH)_4^- + H^+ \quad (1)$$

has an equilibrium constant $pK_a = 9.0$ [9], i.e. the concentrations of the charged and neutral forms of boric acid are equal at pH 9. It is known that monoborate anions complex organic *cis*-diols and polyols, whereas neutral $B(OH)_3$ does not [12]. At $pH < pK_a$ the equilibrium (1) shifts to the left so that the complex formation with polyols becomes weaker due to the lowered amount of reactive monoborate. The portion of monoborate in the equilibrium mixture can be calculated according to the formula:

$$[B(OH)_3]/([B(OH)_4^-] + [B(OH)_3]) = 1/(1 + 10^{pK_a - pH}) \quad (2)$$

The complex formation of monoborate with *cis*-diols of PVA can be described by the following equations:

$$B + D \rightleftarrows BD;\ K_1 = [BD]/[B][D] \quad (3)$$

$$BD + D \rightleftarrows BD_2;\ K_2 = [BD_2]/[B][D] \quad (4)$$

where D is diol and B is $B(OH)_4^-$ [10]. Eqs. (3) and (4) describe the formation of monodiol (BD) and didiol (BD_2) complexes. Obviously, a PVA–borate gel can form if different chains of PVA become cross-linked by didiol complexes. In contrast, with polymeric boronate as a cross-linking reagent, the gel formation may proceed without formation of didiols, because one chain of the boronate-containing copolymer can interact with two or more chains of PVA via monodiols as shown in Fig. 1.

One can expect, therefore, two completely different phase diagrams of PVA complexes formed with low molecular weight borates and polymeric boronates. The present work aims at comparison of gel formation in these two systems brought to weakly alkaline or physiological pH and attempts to explain their behaviour from the viewpoint of existing equilibrium models. We also characterised the viscoelastic properties of the gels as they are supposed to exhibit certain mechanical stability and elasticity when used in biomedical applications.

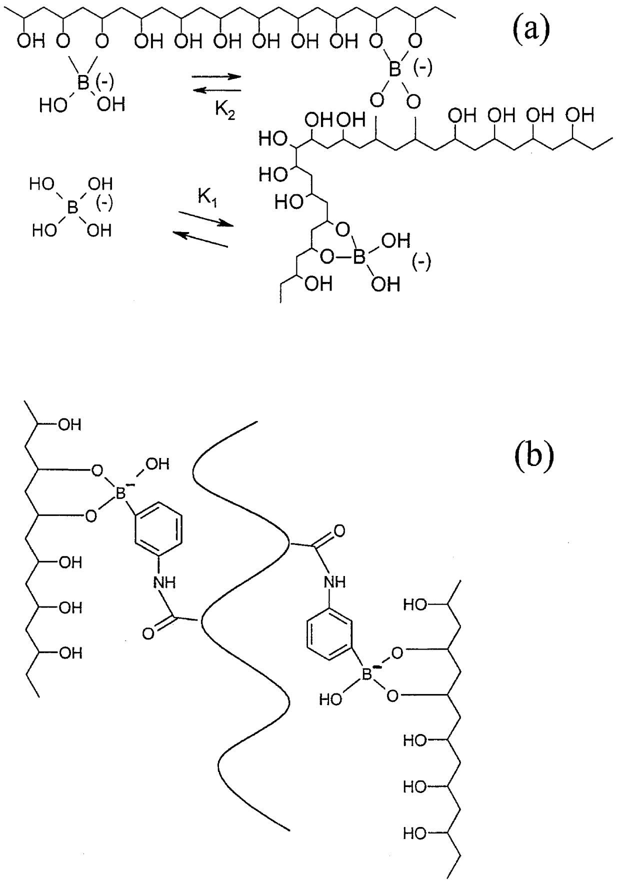

Fig. 1. Scheme of PVA cross-linking with borate ions through didiol complex formation (a) and with DMAA–NAAPBA copolymer through monodiol complex formation (b).

2. Materials and methods

2.1. Materials

1,4-Dioxane was dried over potassium hydroxide and distilled (101–102 °C). Diethyl ether, dried, was a product of Merck KGaA (Darmstadt, Germany). Acryloyl chloride was a product of Fluka Chemie AG (Buchs, Switzerland), 3-aminophenylboronic acid hydrochloride (3-APBA) and neutral aluminium oxide, type 507C, were products of Aldrich (Steinheim, Germany). 2,2'-Azobis(2-methylpropionitrile) (AMPN) was purchased from ACROS (Geel, Belgium). Polyvinylalcohol (PVA), Mowiol 20–98, $M_w = 125,000\ g\ mol^{-1}$, was purchased from Clariant GmbH (Frankfurt, Germany). Dimethylsulfoxid-D6 99,9 Atom%D was from Dr Glaser AG (Basel, Switzerland).

2.2. Synthesis of N-acryloyl-m-aminophenylboronic acid (NAAPBA)

3-APBA (1.72 g, 10 mmol) was dissolved in 20 ml 2 M NaOH (40 mmol) and cooled to 2 °C in an ice bath. Acryloyl chloride (1.6 ml, 20 mmol) was added dropwise to the solution of 3-APBA under intensive magnetic stirring for 10–15 min. Hydrochloric acid (2 M) was added dropwise to the reaction mixture in order to adjust its pH to ca. 1 and precipitate the product, which was separated by filtration on a sintered glass filter (Schott, Duran, No. 3) and washed on the filter by chilled distilled water (50 ml). The precipitate was dissolved in 40 ml distilled water on heating to ca. 60 °C and crystallized as light-violet needles for overnight at 8 °C. The substance was filtered off on Munktell No. 3 paper filter, washed with chilled distilled water and dried under vacuum in a desiccator over dry calcium chloride. The yield of AAPBA was 0.76 g (40%). ^{1}H NMR (CD_3SOCD_3): $\delta = 5.73$ (1H), 6.25 (1H), 6.45 (1H), 7.2–7.9 (4H), 8.0 (2H), 10.05 (1H).

2.3. Copolymerization of NAAPBA with N,N-dimethylacrylamide

2.3.1. Purification of DMAA

DMAA was separated from monomethyl ether of hydroquinone (polymerization inhibitor) by flash chromatography on a 0.9×2.5 cm column with dry activated aluminium oxide: 3 ml of the monomer was applied to the column and eluted fraction (1.9 ml) collected.

2.3.2. Preparative copolymerization

DMAA (1.86 ml, 18 mmol), NAAPBA (380 mg, 2 mmol) and AMPN (10 mg) were dissolved in 20 ml of ethanol. Free radical polymerization was started by heating the reaction mixture to 70 °C under nitrogen bubbling and carried out for 6 h. Thus, the obtained solution of copolymer was added dropwise to 200 ml diehtyl ether for precipitation of the copolymer and its separation from the monomers. The precipitate was collected by filtration on Munktell No. 3 filter paper, washed with diethyl ether and dried in air and under vacuum. The yield of the copolymer was 1.74 g (77%).

2.3.3. Polymerization of DMAA

DMAA (2.1 ml, 20 mmol), mercaptoethanol (7 μl, 0.1 mmol) and AMPN (40 mg) were dissolved in 20 ml of ethanol. Radical polymerization and isolation of the polymer was carried out as described in Section 2.3.2. The yield of the polymer was 1.2 g (55%). Molecular weight of 12,000 g mol^{-1} was calculated as described in Section 2.4 from the characteristic viscosity of PDMAA, which was found to be 0.10 dl g^{-1}.

2.3.4. Copolymerization at various molar ratios of the monomers

Copolymerization of DMAA and NAAPBA was carried out with 5, 10, 15 and 20 mol% of AAPBA in the reaction mixture, in the conditions described in Section 2.3.2. Samples (2 ml) were taken out from the reaction mixture in 5–7 min after the start and later on with intervals of few minutes at times. The samples were added to diethyl ether for precipitation of the copolymer, which was separated by filtration, washed with diethyl ether, dried on air and under vacuum. Conversion of monomers at given time was calculated as a ratio of the copolymer weight to the weight of monomers taken for polymerisation. Copolymerization constants r_1 and r_2 were calculated according to the method of Kelen and Tudos [15].

2.4. Molecular weight determination

Weight-average molecular weight (M_w) of PDMAA and DMAA–NAAPBA copolymer with boronate units molar percentage 0.09 was calculated from its intrinsic viscosity (0.14 dl g^{-1}) using the formula for PDMAA: $[\eta] = 17.5 \times 10^{-5} M_w^{0.68}$ [16]. An Ubbelohde viscosimeter was used to measure intrinsic viscosity of the copolymer in methanol at 25 °C.

2.5. Viscosity measurements in poly(vinyl alcohol)–borate-containing mixtures

Sodium tetraborate (0.1 M) was titrated with 0.4 M boric acid to prepare borate buffers with pH 7.5 or 8.6 and boron concentration of 400 mequiv. B l^{-1}. These buffers were diluted by distilled water to prepare a set of buffers with lower boron concentrations, their pH-values were adjusted, if necessary, to 7.5 or 8.6 by 0.2 M HCl or 0.2 M NaOH.

DMAA–NAAPBA copolymer (9 mol% NAAPBA) was dissolved in distilled water at a concentration of 20 mg ml^{-1}, pH of the solution was adjusted to 7.5 or 8.6 by adding 0.2 M NaOH. Boron concentration in the solutions was calculated as 16.8 mequiv. B l^{-1}. Copolymer solutions of lower boron concentrations were prepared by dilution of the first one with distilled water and pH adjustment to 7.5 or 8.6.

Thus, the prepared borate- and boronate-containing reagents (4 ml) were thermostated at 25 °C, quickly added to 5% w/v aqueous PVA (4 ml) thermostated at the same temperature and mixed by shaking. Kinematic viscosity of the mixtures (cSt) was measured in ca. 5 min after the mixing, in a calibrated Cannon–Fenske capillary viscosimeter at 25 °C. The collected data were then re-calculated to get dynamic viscosity of the mixtures (mPa s).

2.6. Preparation of gels

PVA gels cross-linked with borate buffers or DMAA–NAAPBA copolymer were prepared by mixing of the components in a way described in Section 2.5, allowed to equilibrate for 30 min, transferred into dry open vials and kept in a dessicator over water. The gels obtained with 150 and 400 mequiv. B l^{-1} borate buffer (pH 8.6 and 9.3) or 20 mequiv. B l^{-1} copolymer solution (24 mg ml^{-1}, pH 8.6) were studied using a rheometer.

2.7. Rheological measurements

The measurements were carried out on a controlled strain Bohlin VOR Rheometer (Metric Analysis, Stockholm, Sweden) using torque bars with ratings of 0.16 or 0.025 mN m. The full scale torque bar ratings of the instrument were from 0.025 to 30 mN m. The gels were squeezed in between stainless steel parallel plates (diameter 15 mm, gel thickness 1 mm) and subjected to oscillating rotational deformations with frequencies from 0.01 to 10 Hz at the strain of 0.6%. Afterwards a strain sweep from 0.01 to 10% at 2 Hz was performed to check that the frequency sweep was run in the linear viscoelastic region. All measurements were done at 25 °C, after an initial resting time of 5–10 min to relax residual stresses in the gel.

3. Results and discussion

3.1. Copolymerization of DMAA with NAAPBA

Molar ratios of monomer units (m_1/m_2) in a vinyl copolymer obtained at low conversions of the co-monomers can be expressed via their amounts in the feed and copolymerization constants r_1 and r_2:

$$m_1/m_2 = M_1(r_1M_1 + M_2)/M_2(r_2M_2 + M_1) \qquad (5)$$

where M_1 and M_2 are numbers of moles of the monomers in the feed, $r_1 = k_{11}/k_{12}$ and $r_2 = k_{22}/k_{21}$—ratios of elementary rate constants for addition of new monomers to the growing macromolecular radicals with the end-groups of types 1 and 2, respectively [15].

To calculate r_1 and r_2, copolymerization of DMAA (I) and NAAPBA (II) was carried out at low conversions of the monomers (production of the copolymer $\leq 15\%$), their molar ratio in the feed being varied. Molar percentage of NAAPBA $m_2/(m_1 + m_2)$ was calculated from the areas of resonance peaks in ^{1}H NMR spectra (see Fig. 2) as $(d + e)/(c + d + e)$ or $(d + e) : 6/a : 2$ and averaged. Molar percentage of DMAA $m_1/(m_1 + m_2)$ was calculated as $1 - m_2/(m_1 + m_2)$. Table 1 summarises feed and copolymer composition of DMAA–NAAPBA copolymers. Copolymerization constants $r_1 = 0.84 \pm 0.03$ and $r_2 = 2.2 \pm 0.3$ were calculated from these data by the method of Kelen and Tudos [15] using 95% probability limit.

It was reported earlier that copolymers of DMAA and NAAPBA obtained at 30–50% production by radical polymerization in ethanol were slightly enriched with boronate units compared to the feed composition [5]. In the copolymerization of DMAA (I) with *N*-phenylacrylamide (II), a monomer similar to NAAPBA, the copolymerization constants $r_1 = 0.63$ and $r_2 = 1.1$ indicated a higher reactivity of the second co-monomer [17]. Our data are in agreement with the referred findings.

Obviously, enrichment of the initially formed copolymer by NAAPBA units should result in their lower content in the lately synthesised chains. Fig. 3 shows production of DMAA–NAAPBA copolymer as a function of time and molar percentage of NAAPBA in the samples collected at different productions of the copolymer. Composition of a copolymer at production yields close to 100% should be equal to the composition of the feed. At typical yields of 75–87% obtained in this work the NAAPBA content in the copolymer was slightly lower than its content in the feed. A possible reason for that might be a higher NAAPBA content in the DMAA–NAAPBA oligomers soluble in diethyl ether-dioxane precipitation media and thus withdrawn from the copolymer samples.

Weight-average molecular weight of the copolymer prepared at $M_2/(M_1 + M_2) = 0.1$ and the yield of 77% equals to 19,000 g mol^{-1} as found by capillary viscosimetry. This corresponds to average polymerisation degree of ca. 180. One can evaluate, therefore, the number of NAAPBA-units per copolymer chain, which apparently varied from 10 to 20 for chains of the above length. About one half of these groups should be in the charged $PhB(OH)_3^-$ form at pH 8.6 and only 1 or 2 of them at pH 7.5 (pK_a of 3-(propionamido)phenylboronic acid is 8.6 [18]).

3.2. Gelation of PVA with DMAA–NAAPBA copolymers and low molecular weight borates

Intended to function as biomaterials, gelated PVA–borate systems are most preferable in form of stable, homogeneous gels. According to the literature, such gels can be produced from concentrated aqueous solution of PVA and sodium tetraborate (borax), taken at concentration

Table 1
Molar percentage of NAAPBA in the feed and in the copolymers

Feed, $M_2/(M_1 + M_2) \times 100\%$	Copolymers, $m_2/(m_1 + m_2) \times 100\%$	Conversion of monomers (%)
5	6.2 ± 0.1	15
10	12.4 ± 0.1	8
15	19.5 ± 0.5	2
20	26.1 ± 0.5	2

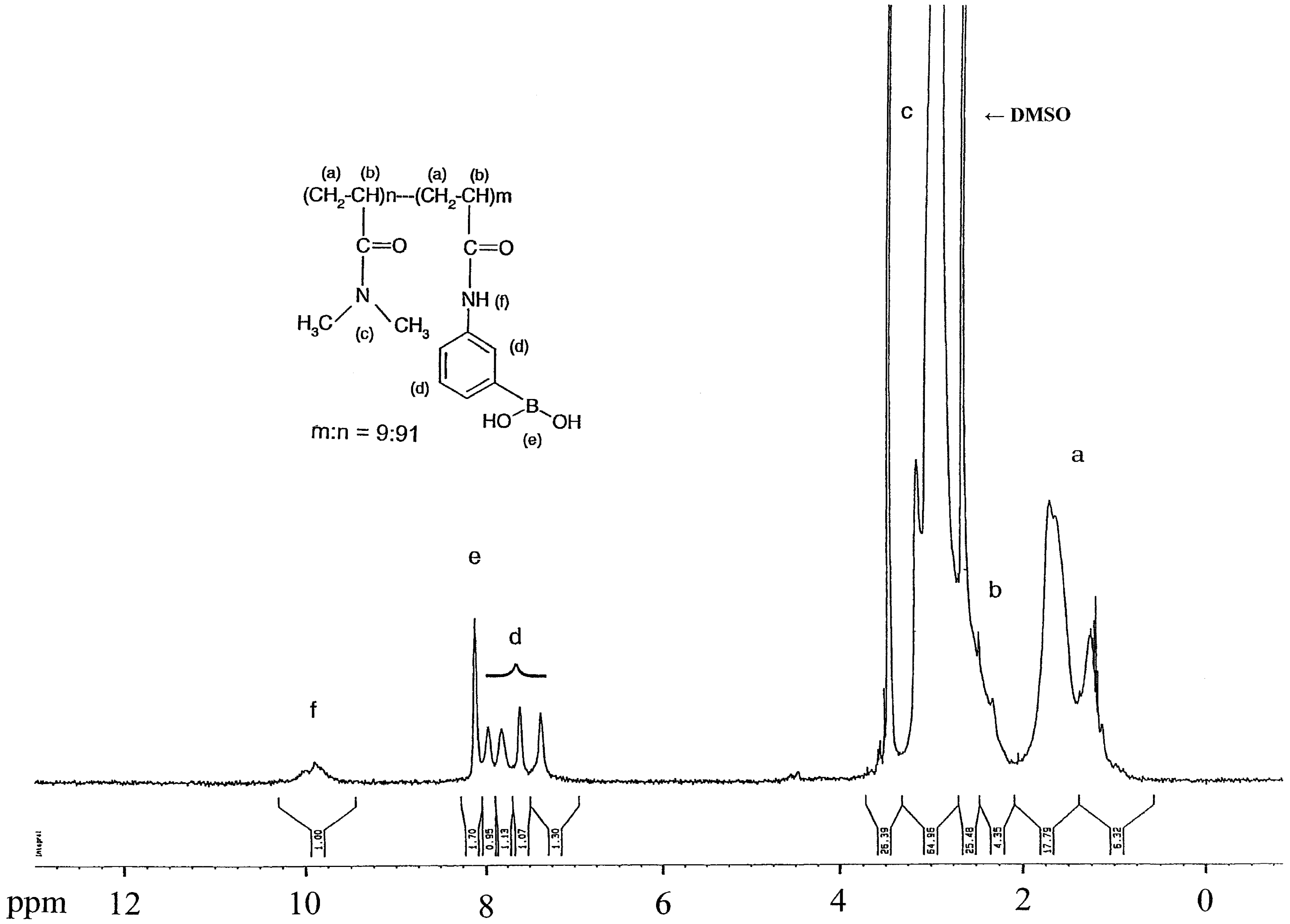

Fig. 2. ^{1}H NMR spectrum of DMAA–NAAPBA copolymer was recorded on a Bruker DRX400 spectrometer operating at 400.1 MHz for ^{1}H at 21 °C. The dry copolymer was dissolved in dimethylsulfoxide-D6 at 5 mg ml^{-1}. Chemical shifts are reported as ppm downfield from tetramethylsilane.

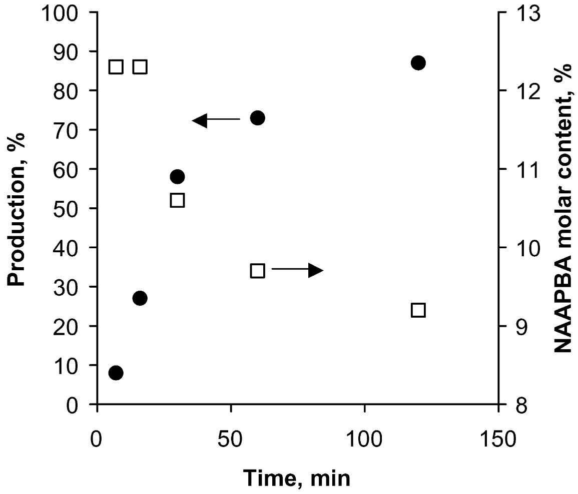

Fig. 3. Production of DMAA–NAAPBA copolymer, $M_2/(M_1 + M_2) = 0.1$, as a function of time (●); percentage of boronate-containing units in the copolymer prepared at different productions (☐).

>0.07 M, i.e. close to its solubility in water at room temperature [7]. That was a reason why we have chosen 5% w/v aqueous solution of PVA ($M_w = 125{,}000$) for the present study. Characteristic viscosity, $[\eta]$, of the PVA sample was found to be 1.05 dl g^{-1} (25 °C) so that the chosen concentration exceeded $1/[\eta]$ by several times and the prepared PVA solution could be certainly regarded as a concentrated one [19].

A good indicator of intermolecular cross-linking in the PVA–borate system is its viscosity [4,10]. The viscosity was studied as a function of boron concentration either in borax-H_3BO_3 buffer solution or in the solution of DMAA–NAAPBA copolymer mixed with 5% w/v aqueous PVA in equal volumes, and presented in Fig. 4. On increasing boron concentration the viscosity gives a sharp rise indicating approach of the system to the state of gel. Mechanism of the gel formation in PVA–borax system has been proposed earlier [7] and can be summarised as follows. In concentrated solution of PVA, there exist multiple contacts between macromolecules. Gelation starts if concentration of $B(OH)_4^-$ ions is high enough to ensure at least one intermolecular cross-link per chain. In 2% w/v PVA solution ($M_w = 145{,}000$ g mol^{-1}) such threshold concentration of sodium tetraborate was ca. 0.01 M or 40 mequiv. B l^{-1} (pH 9.1) [7]. At lower pH, more acceptable from the viewpoint of biomedical applications, the threshold concentration of borate should be higher because the portion of charged $B(OH)_4^-$ ions is lower ($pK_a = 9.0$ [9], see formula (2)). Indeed, intermolecular cross-linking of PVA ($M_w = 125{,}000$ g mol^{-1}) was well expressed at ca. 60 mequiv. B l^{-1} in the borate buffer (pH 8.6), as shown in Fig. 4. PVA formed homogeneous, mechanically handlable gels with borax–H_3BO_3 buffers in the range of boron concentration from 100 to the maximal 400 mequiv. B l^{-1} at pH 8.6.

In this paper, we define the threshold boron concentration as that, at which viscosity of the system exceeds by one order of magnitude the viscosity of PVA solution (3.7 mPa s at 2.5% w/v PVA). The former is close to the maximal viscosity (ca. 100 mPa s) still measurable by the capillary viscosimeter in real time. The threshold boron concentration was ca. seven-fold lower if the polymeric boronate was used as a cross-linker of PVA instead of borate buffer at pH 8.6 (see Fig. 4). The difference between pK_a values of *N*-acyl-*m*-phenylboronic acid (8.6 [18]) and boric acid (9.0 [9]) resulting in ca. 1.8-fold higher concentration of anionic boronate over anionic borate at pH 8.6 cannot completely explain the observed effect.

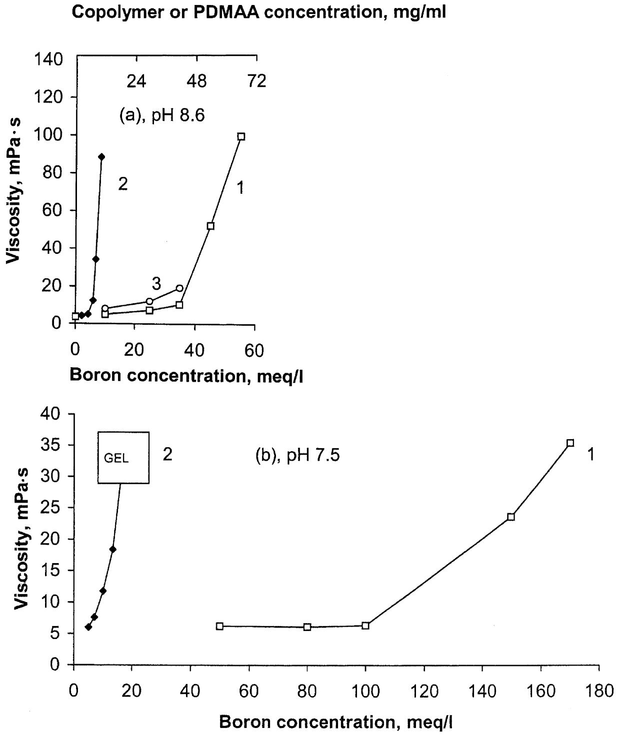

Fig. 4. Viscosity of PVA (5%w/v) mixtures with DMAA–NAAPBA copolymer (◆), borate buffers (☐) or PDMAA dissolved in borate buffers (○) as a function of boron and polymer concentration 8.6 (a) and pH 7.5 (b). DMAA–NAAPBA copolymer contains 9 mol% NAAPBA units.

One might suppose that the easier gelation of PVA with the polymeric boronate comes from mixing of two polymers resulting in higher viscosity of the system. To check this, control experiments on PVA–borate complex formation were done in the presence of poly-*N*,*N*-dimethylacrylamide (PDMAA). PDMAA was dissolved at concentrations of 12, 30 and 42 mg ml^{-1} in borate buffers containing 10, 25 and 35 mequiv. B l^{-1} (pH 8.6). By their boron and PDMAA contents the mixtures corresponded to DMAA–NAAPBA solutions of concentrations 12, 30 and 42 mg ml^{-1}. The copolymer solution used for preparation of the gel with PVA contained 20 mequiv. B l^{-1} and 24 mg copolymer/ml, see Section 2.6 and Fig. 4. Addition of the model mixtures to 5% w/v PVA gave homogeneous systems with viscosities comparable to those obtained by addition of borate buffers of the same boron concentration (pH 8.6) to 5% w/v PVA in the absence of PDMAA (see Fig. 4). These data show that the simultaneous presence of borate, PDMAA and PVA in the mixture does not ensure the gel formation, but attachment of multiple boronate groups to the polymeric chain is sufficient for that at much lower boron concentration.

To characterize the cross-linking reaction quantitatively let us evaluate the equilibrium constant of monodiol formation between PVA diols and $PhB(OH)_3^-$ and compare it to the constants of association between PVA diols and $B(OH)_4^-$ known from the literature [10,11]. Due to exclusion volume effects combined with mutual penetration of interacting chains, an adequate model of reversible interaction between two polymers with different structures and dimensions [20] might be difficult to analyse mathematically, especially if one of the polymers exists in a concentrated solution. We consider, therefore, a rough approximation presuming a uniform distribution of the reactive functions through the volume and evaluate apparent association constants $K_{1,app}$ as if the equilibria were established between low molecular weight reagents.

Based upon Eqs. (3) and (4), equations of material balance $[B]_0 = [B] + [BD]$ (for PVA–copolymer system), $[B]_0 = [B] + [BD] + [BD_2]$ (for PVA–borate system), and taking into account a large molar excess of PVA diols over borate/boronate in both the studied systems ($[D] \approx [D]_0$), one can develop the following expressions for concentrations of phenylboronate monodiols [BD] and borate didiols $[BD_2]$:

$$[BD] = K_{1,app}[B]_0[D]_0/(1 + K_{1,app}[D]_0) \tag{6}$$

$$[BD_2] = K_1K_2[B]_0[D]_0^2/(1 + K_1[D]_0 + K_1K_2[D]_0^2) \tag{7}$$

Assuming that at least two $PhB(OH)_3^-$ functions per copolymer chain should react with PVA for gel formation, one can equal the doubled concentration of monodiols, 2[BD], to the molar concentration of DMAA–NAAPBA chains as the condition for formation of a gel. The apparent equilibrium constant $K_{1,app}$ evaluated in this way equals to $0.5\ l\ mol^{-1}$, which is less than the values of K_1 reported for PVA–$B(OH)_4^-$ system: $4\ l\ mol^{-1}$ [10] and $2.8\ l\ mol^{-1}$ [11]. The lower value of $K_{1,app}$ probably originates in the sterically limited interaction between PVA and DMAA–NAAPBA copolymer: boronate–diol complex formation takes place in the zones of mutual penetration of macromolecules (junction zones) [20]. As far as $K_1 > K_{1,app}$, the low boron concentration required for PVA cross-linking with the copolymer may result only from multipoint interaction of the copolymer with PVA via monodiols, as shown in Fig. 1. DMAA–NAAPBA copolymer formed homogeneous gels with PVA at boron concentrations above 15 mequiv. B l^{-1}, instead of 100 mequiv. B l^{-1} found with borate buffers of pH 8.6. Therefore, DMAA–NAAPBA copolymer is more effective cross-linker of PVA than borate.

For gelation of PVA with $B(OH)_4^-$ formation of didiols is ultimately needed, while their concentration ought to be equal to or more than molar concentration of PVA chains. One can equate the latter concentration to $[BD_2]$ from Eq. (7) and calculate $K_2 = 0.5\ l\ mol^{-1}$ using K_1 value of $4\ l\ mol^{-1}$ reported in the work [10] for a wide range of ionic strengths. Formation of didiols seems to proceed to a lower extent as compared to formation of monodiols. This can be a consequence of electrostatic repellence between PVA chains carrying some negatively charged monodiols [10] and one of the reasons why the low molecular weight borate is a less efficient cross-linker compared to DMAA–NAAPBA copolymer.

Although the chains of DMAA–NAAPBA copolymer contain very few charged boronate functions at pH 7.5 (see Section 2.1), they still cross-linked PVA (see Fig. 4(b)) yielding viscous liquids at low boron concentration. At higher concentrations the reaction mixture segregated into a gel and liquid phase. Apparently, the gel was formed by PVA cross-linking with the fraction of longer chains of the DMAA–NAAPBA copolymer sample. Segregation of the reaction mixture and formation of non-homogeneous gels was earlier described in the literature for PVA-borax system [7,8]. The ratio between the threshold boron concentrations displayed by borate buffer and polymeric cross-linker at pH 7.5 becomes ca. 10. Borate buffers of pH 7.5 and boron concentration lower than 200 mequiv. B l^{-1} formed with PVA uniform viscous liquids. At concentration of 400 mequiv. B l^{-1} a non-homogeneous PVA gel was formed as only 3% of overall borate existed in the reactive anion form.

3.3. Viscoelastic properties of PVA–borate and PVA–copolymer gels

Owing to the different mechanisms of PVA cross-linking with borate and boronate-containing copolymer, the resultant gels strongly differ in their viscoelastic properties. We compared these two types of gels prepared at boron concentrations exceeding the threshold values (see Fig. 4(a)) by 2.4-fold: 150 mequiv. B l^{-1} for borate gel and 20 mequiv. B l^{-1} for the gel cross-linked by DMAA–NAAPBA copolymer. Due to the similar dependence of [BD] and $[BD_2]$ from $[B]_0$ (see Eqs. (6) and (7)), and the same pH 8.6, one may expect similar amounts of the chemical cross-links in the gels at given conditions. PVA gel cross-linked by borax at saturating concentration (400 mequiv. B l^{-1}, pH 9.3), a system well described in the literature [14], was also studied for comparison.

The storage G' and loss modulus G'' were measured as functions of frequency (f) for the above samples kept in saturated water vapour at room temperature (22 °C) for several days. $G'(f)$ and $G''(f)$ did not show large variations with time, although the first measurements made in 1–2 h after the mixing of the components yielded somewhat lower values of the moduli. Fig. 5 shows the frequency sweeps of the storage and the loss moduli and the phase angle ($G'(f)$ and $G''(f)$ and delta, respectively, open symbols); the averages of 8 or 9 measurements were calculated at some frequencies and given as closed symbols with 95% probability limits. Phase angle, a measure of ratio between viscous and elastic properties of gels, was calculated as arctan G''/G'. The gels exhibited constant G' and G'' as functions of strain up to 10%, at frequency of 2 Hz, which agrees well with the data reported in Ref. [14].

Three different PVA-gels prepared with borate buffers of pH 8.6 (a), pH 9.3 (b) and the boronate-containing copolymer (pH 8.6) are shown in Fig. 5. All three samples show a region at low frequencies where liquid behaviour is dominating ($G'' > G'$), followed by a 'cross-over frequency' where $G' = G''$ and a region at high frequencies where elastic properties are dominating ($G' > G''$). Hence, the three PVA-gels can be characterised by the frequency where $G' = G''$, which when approximated to a model with a single Maxwell element, gives the relaxation time ($t_{\text{relax}} = 2\pi/f$) of the system, and the plateau value of G' at high frequencies ($G'_{\max}$) (see for example Ref. [21]). The estimated values of t_{relax} and $G'_{\max}$ for the three PVA-gels in Fig. 5(a)–(c) are given in Table 2. The relaxation time is taken at the frequency where $G' = G''$ and $G'_{\max}$ is the maximum value of G' at around 10 Hz. Although a single Maxwell element, and hence a single relaxation time cannot describe the rheological behaviour of the samples perfectly (especially not the gel prepared with the boronate-containing polymer) it clearly outlines the great differences between the gels. The frequency dependence of G' and G''

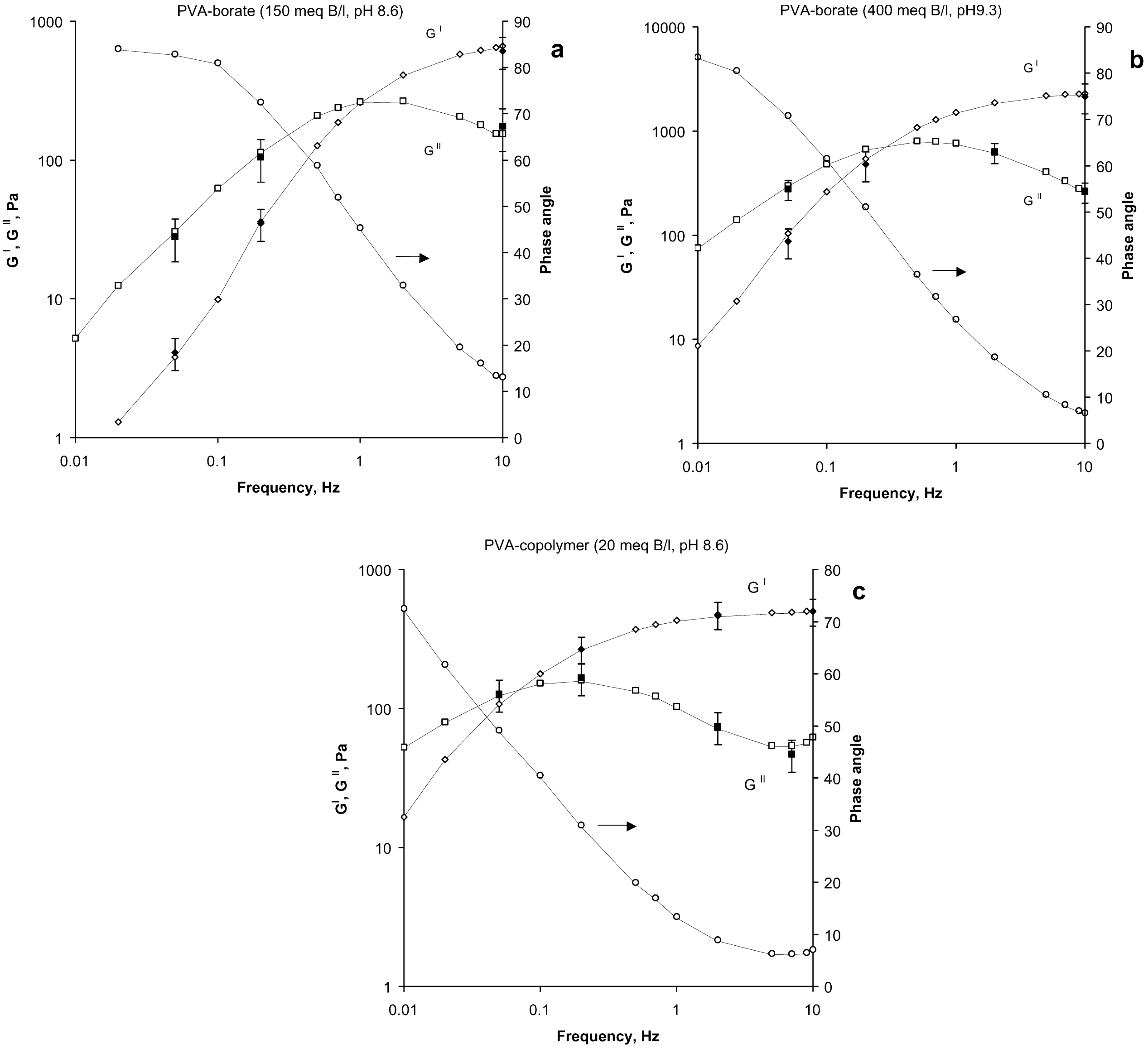

Fig. 5. Frequency sweeps observed with 5% w/v PVA gels cross-linked by borate buffers of pH 8.6 (a, 150 mequiv. B l^{-1}), pH 9.3 (b, 400 mequiv. B l^{-1}) and by DMAA–NAAPBA copolymer (c, 20 mequiv. B l^{-1}, pH 8.6). Storage modulus G' (◇), loss modulus G''(□) and phase angle (○) are plotted as functions of oscillation frequency. Averages of 8 or 9 measurements are given as closed symbols (◆, ■) with 95% probability limits.

Table 2
Values of t_{relax}, G'_{max}, n' and n'' for the three PVA-gels in Fig. 5(a)–(c)

Cross-linker	t_{relax} (s)	G'_{max} (Pa)	n'	n''
Borate, 150 mequiv.uiv. B l^{-1}, pH = 8.6	6.3	670	1.4	1.0
Borate, 400 mequiv. B l^{-1}, pH = 9.3	20	2300	1.5	0.8
NAAPBA–DMAA, 20 mequiv. B l^{-1}, pH = 8.6	97	500	1.0	0.5

at low frequencies where $G'' > G'$ are summarised in the power-law constants n' and n'' (G' prop $\omega^{n'}$ and G'' prop $\omega^{n''}$, respectively). The values of n' and n'', given in Table 2, may illustrate the deviation from the Maxwell model. For a single Maxwell element n' and n'' are 2 and 1, respectively. Our gels produced with borate buffers resulted in n-values not far from what the model predicts. However, for the gel produced with the copolymer as a cross-linker a single Maxwell element is no longer enough to describe the behaviour of the gel (Table 2).

The different properties in dynamic shear of the three gels are evident from Table 2 and Fig. 5. For the two gels prepared with PVA and borate buffer (150 mequiv. B l^{-1}, pH 8.6, Fig. 5(a) or 400 mequiv. B l^{-1}, pH 9.3, Fig. 5(b)) the most striking difference was observed in G'_{max}, which increased approximately three times at the higher pH (Table 2), probably due to the higher concentration of $B(OH)_4^-$ in the reaction mixture. Interestingly, an increase of the buffer concentration to 400 mequiv. B l^{-1} at pH 8.6 did not influence the frequency sweeps of G' and G'' significantly (data not shown). Hence, the increase in G'_{max} can be ascribed to an increase in the concentration of chemical cross-links, and therefore to a lower molecular weight of the chain segment [22] between the boron–didiol complexes obtained at pH 9.3 compared with the situation at pH 8.6. The higher G'_{max} may also originate in a larger amount of anionic charges held by monodiols in the PVA-borate network at pH 9.3, thus resulting in a stronger repellence of the polymeric chains.

Consistent with the increase in G'_{max}, the relaxation of the gel was slowed down (t_{relax} increased) approximately three times at pH 9.3 (Table 2). In general, an increase in t_{relax} can be ascribed to a longer lifetime of the cross-links, for example through physical forces or to polymer entanglements, a higher cross-link density, or a combination of these. For the gels prepared with borate buffer in the present study the increase in relaxation time (t_{relax}) seems likely to be a consequence of low concentration of protons, slower dissociation of cross-links, and, therefore, their longer lifetime at the higher pH. Strong pH-dependence of t_{relax} in galactomannan–borax gels was earlier found by Pezron et al. [23]. As mentioned earlier, no effect on the frequency dependence of G' and G'' was observed when the borate buffer concentration was increased from 150 to 400 mequiv. B l^{-1} at pH 8.6. This is also in accordance with the study reported by Robb and Smeulders (Ref. [14]), where it was concluded that neither borate nor PVA concentration influenced the distribution of relaxation times.

The effect of PVA cross-linking with DMAA–NAAPBA copolymer was investigated on a gel where the boron concentration was 7.5 times lower than in the gel prepared with the borate buffer of the same pH 8.6. Under these conditions only a moderate decrease in G'_{max} was observed (Fig. 5 and Table 2). Although the boron concentration was reduced, the cross-link density (G'_{max}) was more or less on the same level as was supposed in the beginning of this section. The difference in G'_{max} may come from the larger amount of anionic charges held by monodiols in the PVA–borate network.

On the other hand a large increase in the relaxation time (t_{relax}) was observed (Table 2). It was obvious that PVA cross-linked with DMAA–NAAPBA copolymer instead of borate buffer increased the lifetime of the cross-links considerably. According to the frequently sited definition of a gel by Almdal and co-workers [24], the storage modulus $G'(\omega)$ of a gel should exhibit a pronounced plateau with $G''(\omega) \ll G'(\omega)$ to times at least of the order of seconds. It may be argued that our samples are not 'true gels' as the PVA–borate gels behaved more like concentrated liquids than gels. On the other hand, the PVA–copolymer gel is about to comply with the definition. Still, solid-like materials obtained from aqueous solutions of PVA and borate (or polymeric boronate) are usually called 'gels' in the literature [5,8,12–14,23].

The presence of two sorts of interacting polymer chains slowed down the relaxation process in the gel, either by causing entanglements or due to the different strength of chemical cross-links. The cross-linking may involve binding of several PVA chains to a copolymer molecule and vice versa, where each chain-to-chain interaction might involve several boronate–diol complexes. The lifetime of a group of cross-links can be expected to be much longer than that of a single boronate–diol complex. One can conceive, therefore, an entangled and elastic gel network, where dissociation of a boronate–diol complex leads to smaller structural changes compared to those of the PVA gel cross-linked by borate. As the average concentration of cross-links in the copolymer gel and the borate gel of pH 8.6 was similar, it seems likely that the difference originated in different spatial arrangement of cross-links. They could be more uniformly distributed in the borate gel and more organised in clusters in the copolymer gel. Its higher 'shape stability' (longer relaxation time) originates in the higher local concentration of the cross-links in the junction zones between the boronate-containing copolymer and PVA.

4. Conclusions

We have compared cross-linking of PVA by borate buffers and boronate-containing, water-soluble copolymers under weakly alkaline conditions, i.e. in the media where either borate or boronate to large extent is present in their neutral, inactive form. The polymeric cross-linker enables the gel formation at much lower concentrations of boron, due to multipoint interaction of the copolymer with PVA via monodiols. One can obtain transparent, homogeneous gels with both the cross-linking reagents at pH 8.6 in a wide range of boron concentration. The borate- and copolymer-based PVA gels with similar concentrations of cross-links exhibit very different relaxation times, which are much higher for the second gel, probably due to multiple complex formation in the intermolecular junctions The rheological behaviour of the PVA-copolymer gel also agrees better with general definitions of a gel, while the PVA-borate samples behaved more like concentrated liquids. By varying pH, chemical structure and concentration of the cross-linker one can prepare PVA gels with different viscoelastic properties.

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Appendix A. Evaluation of apparent equilibrium constants at pH 8.6

For evaluation of $K_{1,\mathrm{app}}$, the molar concentration of DMAA–NAAPBA chains in the reaction mixture exhibiting the highest registered viscosity was calculated as 2.6×10^{-4} M, presuming the copolymer molecular weight $1.9 \times 10^{4}\ \mathrm{g\ mol^{-1}}$. Overall concentration of boron in the mixture with PVA was calculated as 4.2 mequiv. l^{-1}, the concentration of its anionic form being 50% at $\mathrm{pH} = \mathrm{pK_a} = 8.6$, i.e. $[\mathrm{B}]_0 = 2.1$ mequiv. l^{-1}.

Aqueous PVA solution (5% w/v) contains 0.57 M diols; approximately half of them were presumed to be in isotactic configuration appropriate for binding to borate or boronate (Mowiol PVA was reported to be atactic by the manufacturer). Mixing with DMAA–AAPBA copolymer in equal volumes decreases the concentration of active diols twofold to $[\mathrm{D}]_0 = 0.14$ M.

Analogously, when calculating $\mathrm{K_2}$ for borate–didiol interaction, $[\mathrm{D}]_0 = 0.14$ M, molar concentration of PVA chains in the mixture with borate buffer was 2×10^{-4} M, and concentration of active borate was 8×10^{-3} M, i.e. ca. seven-fold lower than the overall boron concentration in the buffer displayed the highest registered viscosity (see Fig. 4a; the concentration of active monoborate anions is ca. 3.5-fold less than boron concentration; two-fold dilution takes place on mixing).

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