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Swelling Equilibrium of a Binary Polymer Gel

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ABSTRACT: The swelling properties of a new class of biohybrid gels made of heparin and 4-arm star-poly(ethylene glycol) (PEG) were studied using mean-field concepts. Heparin acts as a multifunctional cross-linker and is highly charged in aqueous environments allowing for the functionalization of the gels with a plethora of biologically active molecules. The elasticity of the gels is provided by the flexible arms of the PEG. Varying the mole fraction of the heparin at preparation changes both the elasticity and the charge of the gel. We combine the classical Flory−Rehner model with a free energy contribution due to trapped counterions and derive a general equation for the equilibrium swelling of the gel as a function of the heparin-content at preparation, size of PEG molecules and salt concentration of the solvent. Varying the heparin content in the state of preparation results in opposite tendencies of the swelling behavior in the limit of low and high salt concentrations. At intermediate salt concentrations we find a regime where the heparin concentration at equilibrium swelling is almost independent of the heparin concentration at preparation. This provides access to novel gel-based biomaterials where physical stimuli (modulus) and biomolecular signals (modulated by the heparin concentration) can be varied independently. Our results are in good agreement with experimental data obtained at various gel compositions and salt concentrations of the aqueous environments.

I. Introduction

Swollen polymer networks, also called polymer gels received attention in biology and medicine as functional matrices to exogenously stimulate cells for both in vitro and in vivo applications. Gel materials offer the possibility to mimic characteristic features of cellular microenvironments. For that purpose, gel materials have to provide biomolecular and physical signals by incorporation of bioactive molecules within the gel (biofunctionalization) and by adjusting desired viscoelastic properties. A far going control over these parameters opens new options to trigger cell fate decisions and enables new concepts in the field of regenerative therapies.[1,2] The related applications require an aqueous environment at physiological salt concentration. Thus, the properties of gels at swelling equilibrium are of particular interest. In this work we consider a new class of binary hydrogels formed by the highly charged glycosaminoglycan heparin as cross-linker whereas the elastic material consist of the hydrophilic, intrinsically uncharged 4-arm star-poly(ethylene glycol) (PEG), see Figure 1.

The cross-linker heparin furthermore acts as anchor molecule for growth factors and adhesion ligands[3,4] and it has been shown that the material is a versatile platform to support cell based therapies in neurodegenerative diseases.[5] Therefore, the composition of the swollen gel materials is crucial for the biologic response. The material is formed by reaction of the EDC/sulpho-NHS activated carboxylic acid groups of heparin and the amino groups of the end-functionalized star-PEG. The design concept of the material allows to change the molar ratio of the PEG- to heparin molecules. As a result, the elastic properties of the resulting networks but also the charge content can be varied.

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The aim of the present study was to reveal the interplay of electrostatic effects, excluded volume interactions and entropic elasticity as a function of composition (mole fraction of both components) at swelling equilibrium. In order to perform this task, we will use a generalized Flory−Rehner approach which incorporates the charge effect due to the osmotic pressure of the counterions released by the heparin component.

The swelling of polymer networks can be described using a mean-field model for the mixing of polymer and solvent and considering a Gaussian model for network elasticity.[6] In terms of free energy, one can write

$$F = F_{el} + F_{mix} \tag{1}$$

where F_{el} denotes the free energy of the elastic component and F_{mix} is the contribution of solvent mixing. For large degrees of swelling, F_{mix} can be replaced by the contribution of the leading virial coefficient. For good solvents this is given by the contribution of the excluded volume interactions F_{ev}. Although the superposition principle of eq 1 is debated in the literature in particular for the case of low degree of swelling,[7−9] this assumption gives easy mathematical access to the equilibrium swelling behavior of uncharged polymer networks. The essential results of the FR-model can be reformulated using scaling concepts for F_{el} and F_{mix}.[10] Let us define the degree of (volume) swelling of a network by

$$Q = \frac{V}{V_0} \tag{2}$$

where V is the volume in the swollen state and V_0 denotes the volume in the dry (solvent-free) state of the network. The degree of equilibrium swelling, $\bar{Q}$, is defined by the minimum of the

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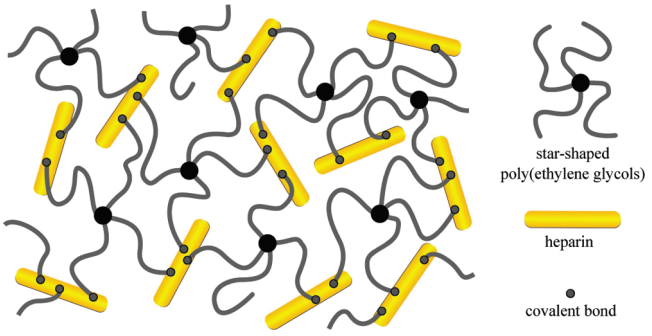

Figure 1. Scheme of hydrogels made of heparin (yellow rods, color online), a highly charged, multifunctional cross-linker, and elastic, amine end-functionalized 4-arm poly(ethylene glycol) (4-functional star polymer) covalently cross-linked via amide bonds. Defects such as dangling ends, multiple star-heparin-cross-links, and unsaturated links on heparin lead to an imperfect network structure.

total free energy with respect to volume changes of the network which can be expressed by the balance of osmotic pressures of the components which contribute to the free energy. According to the FR-model it is given by $\bar{Q} \sim N^{3/5}$, where N denotes the number of Kuhn segments within the chain. An open question, however, concerns the nonaffinity of chain deformations in the network and the appearance of large heterogeneities in the swollen state, see ref 11. Typical degrees of swelling of noncharged polymer networks are in the range of $\bar{Q} \simeq 10$.

Synthetic polymers are usually hydrophobic and networks made of such polymers absorb only low amounts of water. Hydrophilic behavior of polymers is often caused by dissociation of ionizable groups. In those polyelectrolytes, the repeat units carry a certain charge and free ions of opposite charge, so-called counterions, are released to the solvent. A prominent exception is poly(ethylene glycol) (PEG) which displays hydrophilic behavior without carrying ionizable groups.

The effect of charge with respect to the swelling behavior can be taken into account by the osmotic pressure of the counterions released by the charged monomers in aqueous solution,[12,13] which gives rise to the generalized Flory−Rehner model according to

$$F = F_{el} + F_{mix} + F_{ch} \tag{3}$$

where F_{ch} accounts for the effect of trapped counterions and can be approximated by the contribution of the first virial coefficient. For low salt content of the solution the osmotic pressure caused by the gas of trapped counterions can dominate the contribution from the excluded volume and F_{mix} can be dropped in the calculations. This leads to $\bar{Q} \sim N^{3/2}$ and high stretching of the network strands. The strong osmotic pressure of the counterions of charged networks can explain the extremely high degrees of swelling which can reach values up to $\bar{Q} \simeq 1000$.

For salt containing solvents, exchange of counterions and salt ions between the network and the surrounding solvent is possible and the osmotic pressure due to charge effects is now given by the difference of the absolute concentration of ions in both subsystems. This results in an effectively lower counterion pressure which approaches zero for high salt concentrations. Thus, adding salt to the solution can lead to a crossover from charge-dominated to excluded-volume-dominated behavior.

From a conceptually point of view the heparin-cross-links are stiff filler-like units which contribute to the excluded volume of the network but not to its elasticity. On the other hand, heparin provides charge effects which act via the osmotic pressure of the counterions on the swelling behavior of the network and which can be controlled by the salt content of the buffer solution. Furthermore, the heparin units acts as targets for biofunctionalization. Therefore, this two-component (binary) system is quite different from hydrogels usually studied in literature.

The article is organized as follows: In section II, we define the properties of the gels and derive the osmotic pressures for the various contributions to the free energy given in eq 3. In sections III and IV, we derive the general solution for the equilibrium swelling taking into account a finite concentration of salt in the solvent. The heparin fraction at equilibrium swelling is discussed in section V. The numerical solution for the equilibrium swelling at various conditions is compared with experimental results in section VI. We conclude our findings in section VII.

II. Thermodynamic Properties of a Binary Gel in Good Solvent

We consider a binary gel composed of 4-functional star polymers (PEG) and rigid high-functional units (HEP) as displayed in Figure 1. Heparin acts as a multifunctional cross-linker for the PEG-molecules. As lower the mol-fraction of HEP as more end-monomers of PEG share the same cross-linker. The effective functionality of HEP is determined by the molar ratio of the two components before reaction. Let us denote the average functionality of HEP by f_{HEP}, we obtain

$$f_{HEP} = \frac{4n_{PEG}}{n_{HEP}} = 4\gamma \tag{4}$$

where n_{PEG} is the number of PEG and n_{HEP} the number of HEP in the final network structure, $\gamma = n_{PEG}/n_{HEP}$ denoting their molar ratio. Under ideal conditions, γ is given by the molar ratio of the components in the mixture before the reaction occurs. The dry volume of the two components is given by $V_0^{PEG} = n_{PEG}v_{PEG}$ and $V_0^{HEP} = n_{HEP}v_{HEP}$, respectively, and

$$\delta = \frac{v_{HEP}}{v_{PEG}} \tag{5}$$

denotes the molar volume fraction of the two components. The total volume of the dry network is therfore given by

$$V_0 = V_0^{PEG} + V_0^{HEP} = V_0^{HEP}\left(1+\frac{\delta}{\gamma}\right)\left(\frac{\gamma}{\delta}\right) \tag{6}$$

It is interesting to note that a lower HEP fraction leads to a stronger network with higher-functional cross-links and a higher fraction of the elastic component (PEG). The number of reactive sites of HEP limits γ to a maximal value γ_{max}.

In the following we consider a simplified model of swelling where the elastic free energy of the (active) network structure is balanced with the effect of excluded volume (second virial coefficient) and with the osmotic pressure exerted by counterions in the case of charging, according to eq 3. The change of the elastic part of the free energy due to isotrope swelling of the network structure (elastic contribution) is given by

$$F_{el} = \frac{3}{2} g_f n_{PEG}\left[\left(\frac{V^{PEG}}{V_0^{PEG}}\right)^{2/3} - 1\right] \tag{7}$$

where V^{PEG} and V_0^{PEG} denote the volume of the elastic component (PEG) in the swollen and in the dry state, respectively. Here and in the following we use thermodynamic energy units: $k_BT = 1$. The prefactor g_f depends of the network model (affine: no cross-link fluctuations, phantom: cross-link fluctuations are taken into account[14,15]):

$$g_f = \begin{cases} 1 & \text{affine} \\ \dfrac{f-2}{f} = g_0 g_x = g_0(1-\gamma_c/\gamma) & \text{phantom} \end{cases} \tag{8}$$

where f denotes the average functionality of the cross-links. If we assume an ideal network structure where all PEG-units also form

additional 4-functional cross-linkers, we obtain $g_0 = 3$ and $\gamma_c = {}^1/_3$. In practice, γ_c will be higher because of defects in the network structure. The corresponding osmotic pressure as derived from eq 7 is given by

$$\Pi_{el} = g_f \frac{n_{PEG}}{V_0^{PEG}} \left(\frac{V^{PEG}}{V_0^{PEG}} \right)^{-1/3}$$

where we suppress signs for simplicity. In the following we will always assume $Q \gg 1$ which allows us to set $V^{PEG} \simeq V$ by ignoring the contribution of the volume fraction V_0^{HEP}/V in the swollen state. Using eq 6 we obtain

$$\Pi_{el} = g_f \frac{1}{v_{PEG}} Q^{-1/3} \left(1 + \frac{\delta}{\gamma} \right)^{-1/3} \tag{9}$$

The osmotic pressure due to excluded volume interactions in the semidilute state, being the relevant state at swelling equilibrium, is given by

$$\Pi_{ev} = v \left(\frac{V_0}{V} \right)^2 = v Q^{-2} \tag{10}$$

Here, all components (PEG and HEP) are assumed to contribute to the pair interaction which dominates the semidilute state, and v denotes the strength of effective excluded volume interactions. We note that a more accurate description for the osmotic pressure due to excluded volume effects is given by the Des Cloizeaux law, see refs 10 and 16. In the osmotic equilibrium, however, this is usually compensated by the corresponding correction of the elasticity in the semidiluted state.[16]

We assume, that only HEP is charged in aqueous solution and neglect any ion adsorption effects to PEG. The number of counterions released by the dissociation of one HEP molecule is denoted by q_{HEP}. Because of charge neutrality of the macroscopic network the effect of electrostatic interactions on swelling can be expressed by the osmotic pressure of the mobile counterions.[12,13] The osmotic pressure of the counterions which are trapped within the network structure (due to the overall effect of the charged HEP) is given by

$$\Pi_{ch}^0 = \frac{q_{HEP} n_{HEP}}{V} = \kappa \left(\frac{\delta}{\gamma} \right) \frac{1}{1 + \frac{\delta}{\gamma}} Q^{-1} \tag{11}$$

where we have introduced the number of charged groups per volume of the HEP molecule

$$\kappa = q_{HEP}/v_{HEP} \tag{12}$$

III. Equilibrium Swelling

At very low concentration of salt, the osmotic pressure caused by the trapped counterions dominates the swelling equilibrium. In this case, we have

$$\Pi_{el} = \Pi_{ch} \tag{13}$$

resulting in the following expression for the swelling equilibrium by using eqs 9 and 11:

$$\overline{Q}_{ch} = \left(\frac{\kappa v_{PEG}}{g_f} \right)^{3/2} \left(\frac{\delta}{\gamma} \right)^{3/2} \left(1 + \frac{\delta}{\gamma} \right)^{-1} \tag{14}$$

In the limit of large functionality, $\gamma \gg 1$, this results in $\bar{Q}_{ch} \sim (v_{PEG}/\gamma)^{3/2}$ independent of the network model. The chain length of PEG-arms in units of statistical monomers, N, is given by $N \sim v_{PEG}$. Thus, we obtain $\bar{Q} \sim N^{3/2}$ which leads to strong stretching of network chains with the end-to-end distance scaling according to $R \sim N$. This is a known result for charged polymer gels, see for a recent study of Mann et al.[13] On the other hand, $\bar{Q}$ decreases with increasing γ (lower HEP-fraction). Although this is expected because the number of counterions decreases with increasing PEG fraction, this behavior is clearly limited by $\bar{Q} = 1$.

For highly salted solutions the effects of charges is weak and the excluded volume forces dominate the swelling equilibrium. In this case, we have

$$\Pi_{el} = \Pi_{ev} \tag{15}$$

Using eqs 9 and 10, we obtain for the equilibrium degree of swelling

$$\overline{Q}_{ev} = (v v_{PEG})^{3/5} g_f^{-3/5} \left(1 + \frac{\delta}{\gamma} \right)^{1/5} \tag{16}$$

In the limit of large functionality this results in $\bar{Q} \sim N^{3/5}$ independent of γ. Again, scaling with respect to the chain length correspond to the known result,[6] see also.[15] In contrast to the case of counterion dominated swelling, the equilibrium degree of swelling does not decrease with γ for $\gamma \gg 1$. This is clearly because the elastic force saturates at high values of γ (which corresponds to the affine network model). Thus, the minimal degree of swelling due to excluded volume effects is given by

$$Q_0 = \left(\frac{v v_{PEG}}{g_0} \right)^{3/5} \sim (vN)^{3/5} \tag{17}$$

Well above the Θ-point, for $v \gg 1/N$, we obtain $Q_0 \gg 1$. In case of charge effects Q_0 sets the lower limit for $\bar{Q}$ and we obtain: $\gamma_{max}^{ch} \sim \delta \kappa N^{3/5}/v^{2/5}$ for the maximum value of γ at which charge effects can play a role.

To study the combined effect of charges and excluded volume without salt effects we have

$$\Pi_{el} = \Pi_{ev} + \Pi_{ch}^0 \tag{18}$$

This results in the following nonlinear equation

$$Q^{5/3} = \frac{v_{PEG}}{g_f} v \left(1 + \frac{\delta}{\gamma} \right)^{1/3} + \frac{v_{PEG}}{g_f} \kappa \frac{\delta}{\gamma} \left(1 + \frac{\delta}{\gamma} \right)^{-2/3} Q \tag{19}$$

which can be rewritten in dimensionless form as

$$\omega^{5/3} = f(x) + \eta_0 h(x) \omega \tag{20}$$

with a relative degree of swelling of

$$\omega = \frac{Q}{Q_0} \tag{21}$$

and the parameter

$$\eta_0 = Q_0 \frac{\kappa}{v} \sim N^{3/5} \tag{22}$$

as well as the functions $f(x) = (1 + x)^{1/3}/g_x$, $h(x) = x(1 + x)^{-2/3}/g_x$. The composition of the gel is controlled by the molar volume fraction of the HEP given by

$$x = \frac{\delta}{\gamma} \tag{23}$$

The parameter η_0 controls the relative strength of the counterion effect as compared to excluded volume. Note that η_0 varies with the chain length of the PEG-arms. Therefore, changing the molecular weight of the PEG provides control about the relative strength of both forces.

IV. Effect of Salt

In a solution containing salt with the concentration c_s, the osmotic pressure caused by the ions is given by

$$\Pi_{ch} = \Delta c \tag{24}$$

where Δc denotes the difference between the ion concentration inside and outside of the gel. If we consider charge neutrality in both subsystems we obtain

$$\Pi_{ch} = \Pi_{ch}^0 \eta(y) \tag{25}$$

Here, we have introduced the function

$$\eta(y) = (1+y^2)^{1/2} - y \tag{26}$$

and the variable

$$y = \frac{2c_s}{q_{HEP}c_{HEP}} = 2c_s \frac{Q_0}{\kappa}\omega\frac{(1+x)}{x} \tag{27}$$

For details of the derivation see Appendix A. The variable y denotes the relative amount of salt-ions and counterions. For salt-free solution, $y = 0$, we obtain $\Pi_{ch} = \Pi_{ch}^0$ and in the limit of high salt concentration, $y \gg 1$, we obtain $\Pi_{ch} \to 0$. Substitution of eq 25 into eq 18 leads to

$$\omega^{5/3} = f(x) + \eta_0 \eta(y) h(x) \omega \tag{28}$$

Thus, as a result of the Donnan-equilibrium condition, we can calculate the relative strength of electrostatic and excluded volume forces as a function of the salt concentration. Equation 28 is suitable for a numerical solution of $\omega(x,c_s)$ with only two fit parameters Q_0 and v. Other parameters can be obtained from experiments. The parameter Q_0 depends on details of the model for network elasticity (defects, topological disorder etc.) and v depends on the mapping of the real excluded volume parameter onto the Flory−Huggins-lattice. In addition, γ_c should be chosen according to the apparent gel-point of the experimental system. Returning to original units the numerical solution yields the equilibrium degree of swelling $\bar{Q}(\gamma,c_s) = \omega Q_0$ as a function of the mole fraction of PEG and the salt concentration.

V. Heparin Fraction under Equilibrium Conditions

Heparin is the basis for any subsequent biofunctionalization of the gel matrix. It allows the attachment of adhesive ligands and loading of growth factors.[5] Thus, the volume fraction of heparin in the sample at equilibrium swelling is an important parameter for biomedical applications, as it controls the degree of biofunctionalization (biomolecular stimulus) of the gel material. It is defined by

$$\bar{c}_{HEP} = \frac{V_0^{HEP}}{V} = \frac{1}{Q_0\omega}x(1+x)^{-1} \tag{29}$$

with the solution ω of eq 28.

For the case of counterion dominated (electrostatic) swelling ($\eta(y) = 0$) by using either eq 28 or eq 14 we obtain

$$\bar{c}_{HEP} = \frac{1}{Q_0{\eta_0}^{3/2}}{g_x}^{3/2}x^{-1/2} = {g_0}^{3/2}(\kappa v_{PEG})^{-3/2}\left(\frac{\gamma}{\delta}\right)^{1/2}\left(1-\frac{\gamma_c}{\gamma}\right)^{3/2} \tag{30}$$

where the latter relation corresponds to the phantom network, see eq 8. The HEP content is a monotonously increasing function of γ. For the phantom network $\bar{c}_{HEP}$ approaches zero for $\gamma \to \gamma_c$ which sets the lower limit for the functionality which is necessary to obtain a mechanically stable network. In the following we will consider only the phantom case noting that for higher values of γ there is no difference between both models. In the limit of $\gamma \gg \gamma_c$ we obtain

$$\bar{c}_{HEP} \sim \gamma^{1/2} \tag{31}$$

In this limit, the network elasticity is independent of the effective functionality of cross-links and hence of γ; see eq 8. Decreasing the HEP-fraction (increasing γ) leads to less counterions and thus decreasing driving force for swelling. Since the equilibrium degree of swelling decreases more than proportional as $\bar{Q} \sim \gamma^{-3/2}$, see eq 14, the HEP-concentration at equilibrium swelling increases.

For excluded volume dominated swelling ($\eta(y) = 1$), using either eq 28 or eq 16, we obtain

$$\bar{c}_{HEP} = \frac{1}{Q_0}{g_x}^{3/5}x(1+x)^{-6/5} = \frac{1}{Q_0}\frac{\delta}{\gamma}\left(1+\frac{\delta}{\gamma}\right)^{-6/5}\left(1-\frac{\gamma_c}{\gamma}\right)^{3/5} \tag{32}$$

The volume fraction of HEP is decreasing for $\gamma \gg \gamma_c$ with a limiting behavior of

$$\bar{c}_{HEP} \sim 1/\gamma \tag{33}$$

As noted above for larger values of γ elasticity does not depend on γ and equilibrium swelling becomes independent of γ for excluded volume driven swelling, see eq 16. Thus, the decrease of $\bar{c}_{HEP}$ is a simple consequence of the reduced mol-fraction of HEP by increasing γ. Because the phantom network is unstable for $\gamma = \gamma_c$ and the degree of equilibrium swelling is diverging, we obtain $c_{HEP} \to 0$ also in the case of excluded volume swelling. As a consequence, a maximum of $\bar{c}_{HEP}(\gamma)$ is predicted for this model. The position of the maximum depends on δ and γ_c.

VI. Combined Effect of Good Solvent and Charges and Comparison with Experiments

The opposing behavior of $\bar{c}_{HEP}(\gamma)$ given by eqs 31 and 33 will lead to some sort of compensation if both swelling forces act together. Thus, at intermediate values of η a nearly constant behavior of $\bar{c}_{HEP}(\gamma)$ can be expected. Numerical solution of eq 28 yields the equilibrium degree of swelling and, using eq 29, also the volume fraction of HEP at equilibrium swelling.

We compare our results with experimental data obtained for gels made of heparin (HEP) and amine-terminated star-poly(ethylene glycol) (PEG) covalently cross-linked via amide.[5] After gel formation equilibrium swelling measurements were performed in solutions of various salt concentrations, e.g. deionizied water (Milli-Q) ($c_s \simeq 10^{-6}$ M), PBS ($c_s \simeq 0.15$ M), and 1 M $CaCl_2$ ($c_s \simeq 1$ M) to obtain the corresponding degree of volume swelling Q.

Experimental data for all values of γ and all salt concentrations have to be fitted with only two model parameters Q_0 and v using substance specific data (see Table 1). In addition we estimated the value of γ_c from experiments. Here, we selected the lowest possible value of γ, where a stable gel can be formed. Defects in the network structure such as multiple bond of a single PEG to the same HEP, dangling ends of PEG and other nontrivial effects of topological disorder will have an effect both on γ_c and Q_0. The best fit parameters for all curves are given as $Q_0 = 24.3$ and $v = 22.8$ mol/dm^3 at $\gamma_c = 1.0$ (see Tab. 1).

In Figure 2, we display the volume swelling ratio as a function of γ. Clearly, the swelling ratio is a monotonous decreasing function with increasing γ, i.e., with lower molar fraction of heparin as predicted by theory. For high salt concentrations (excluded

Table 1. Substance Specific Data and Best Fit Parameters for All Experimentally Obtained Data in Different Solutions at $\gamma_c = 1.0$[a]

δ	q_{HEP}[17]	κ [mol/dm^3]	γ_{max}	M_{HEP} [g/mol]	ρ_{HEP} [g/cm^3]	M_{PEG} [g/mol]	ρ_{PEG} [g/cm^3]	Q_0	v [mol/dm^3]	γ_c
0.885	76	10.665	7.0	14 000	1.975	10 000	1.249	24.3	22.8	1.0

[a] M_{HEP}, ρ_{HEP}, M_{PEG} and ρ_{PEG} corresponds to molar mass and density of heparin and amine-terminated poly(ethylene glycol), respectively.

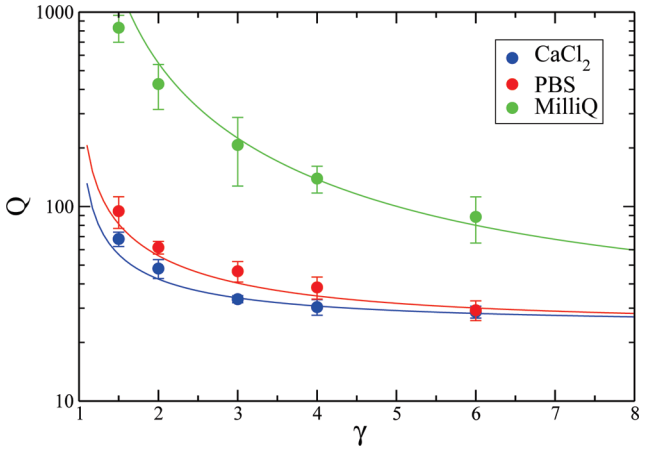

Figure 2. Swelling ratio Q as a function of the molar PEG/HEP fraction γ with $\gamma_c = 1.0$. The molar volume ratio has been set to $\delta = 0.885$ and the best fitting parameters are given by $Q_0 = 24.3$ and $v = 22.8$ for all curves according to eq 28. The upper (green) line corresponds to the counterion effect dominating case, the lower line (blue) corresponds to the excluded volume effect dominating case. The middle line (red) corresponds to the intermediate regime of combined effects. The experimental data (with standard deviation) in same order: deionized water (Milli-Q), 1 M $CaCl_2$ solution, PBS-solution.

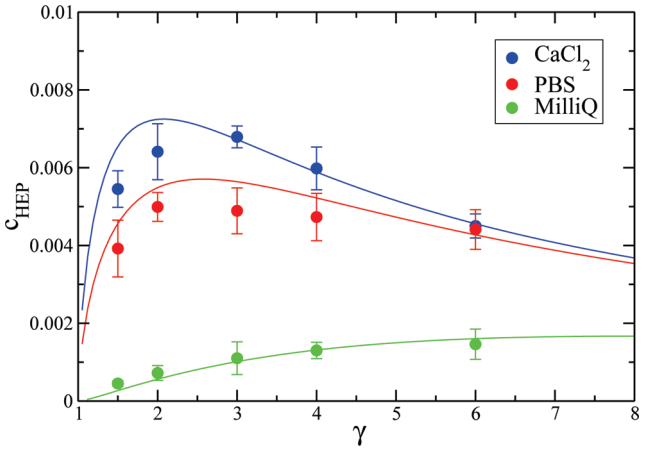

Figure 3. Concentration of HEP in equilibrium swollen state (in ml/ml) fitted with the theoretical prediction according to eqs 29 and 28. Lines and data points correspond to those in Figure 2.

volume effect dominates), the equilibrium degree of swelling saturates into a plateau for high values of γ, see eq 16, which is also displayed in the experimental data. In the opposite case of low salt concentration (counterion osmotic pressure effect dominates), the degree of equilibrium swelling is monotonously decreasing with increasing γ because the density of counterions is decreasing. The critical value of $\gamma_c = 1$ is reflected by a divergency of the degree of swelling. Deviation from the theoretical predictions are most obvious for very high degrees of swelling at low salt concentration. Since chains are expected to be overstretched in this regime, Gaussian elasticity overestimates the degree of swelling.

In Figure 3, we compare the prediction of the mean-field theory with the experimental data for the heparin content at equilibrium swelling as a function of γ. Most noticeable is the maximum of the heparin content at low values of γ, which is a direct consequence of the phantom network model. Also note that the salt effect is nicely reflected by the Donnan equilibrium and there was no freedom to fit the various curves independently. Deviations of the experimental data in particular for low values of γ might be attributed to the simplicity of the network model. At intermediate salt concentrations (PBS), a nearly constant heparin concentration for values of $\gamma > 2$ is found as a consequence of the opposing tendencies of the heparin concentration in the limiting cases of high and low salt concentrations. This crossover behavior can be tuned by the molecular weight of the PEG-chains, which makes it possible to adjust the plateau-like behavior to the applied salt-concentration.

VII. Conclusions

Mean field theory considering both excluded volume and charge effects (trapped counterions) can be successfully applied to describe the swelling behavior of hydrogels composed of charged and uncharged components. Using only two fit parameters our predictions for the equilibrium degree of swelling as well as for the heparin content at swelling equilibrium were demonstrated to be in good agreement with experimental data over a wide range of compositions of the gel and salt concentrations of the ambient medium.

In our model of network elasticity network defects (i.e., dangling ends of PEG, multiple bonds of PEG with the same heparin molecule) were taken into account by a shift in the critical functionality of the phantom network. This rather crude approximation might account for deviations of the experimental data in the range of small values of the functionality. A prominent feature of the phantom model is the maximum in the heparin content as a function of the functionality for excluded volume driven swelling. The occurrence of the maximum was clearly confirmed in the experiments. Thus, the affine network model can be excluded. A more detailled investigation of network defects and their effect on network swelling can be obtained by computer simulations which are in progress.

A particularly interesting result for the application of the investigated gels is the nearly independent variability of the heparin concentration and the mechanical properties of the gels at physiological conditions. The latter depend strongly on the equilibrium degree of swelling (degree of cross-linking) whereas the heparin concentration is nearly invariant. This surprising effect is a result of the superposition of electrostatic and excluded volume interactions with respect to the heparin concentration at swelling equilibrium. The relative contribution of both effects is controlled by the salt concentration of the solvent and can be tuned from a charge dominated behavior (low salt content) to an excluded volume dominated behavior (high salt content). At high salt content, decreasing the molar fraction of heparin does not change the swelling pressure caused by excluded volume. Thus, the heparin concentration at equilibrium swelling decreases with the decreasing molar fraction of heparin. In the opposite case of low salt content, decreasing the molar fraction of heparin leads to a lower counterion induced swelling pressure and thus to a lower degree of swelling. As a result the heparin concentration at equilibrium swelling is increasing with decreasing molar fraction of heparin.

The decoupling of heparin concentration at equilibrium swelling from the physical properties of the gel at intermediate (physiological) salt concentrations offers valuable options for the

use of the gels as adaptable artificial matrix systems for in vitro and in vivo applications. Loading the heparin with biologically active molecules permits to present various biochemical stimuli to cells grown in contact with the gel matrix. Varying the elastic properties of the gel provides control over additional cues synergistically acting on cells, and both stimuli can be controlled independently.

In sum, our model provides a rational base for the directed adjustment of polymer networks through variation of the size and the concentration ratio of the incorporated components. The novel approach allows for a far-reaching modulation of biochemical and physical characteristics of the obtained gel materials being decisive for their applicability in cell based therapies.

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Appendix A: Donnan Equilibrium and Swelling Pressure

We consider the charges located on the HEP as immobilized. The concentration of mobile charges inside the network are denoted by $c_{\pm}$ and the charges outside the network are denoted by $C_{\pm}$. Charge neutrality requires

$$c_+ = c_- + q_{HEP}c_{HEP} \qquad (34)$$

$$C_+ = C_-$$

The salt concentration of the solution is kept constant $C_{\pm} = c_s$, and $c_{HEP} = n_{HEP}/V$. Assuming a constant electrostatic potential caused by the charges in both subsystems we obtain the Donnan equilibrium condition according to

$$c_+ c_- = {c_s}^2 \qquad (35)$$

Using eq 34 we obtain for the total concentration of mobile ions inside the gel:

$$c_+ + c_- = q_{HEP}c_{HEP}\left[1 + \left(\frac{2c_s}{q_{HEP}c_{HEP}}\right)^2\right]^{1/2} \qquad (36)$$

The total osmotic pressure is defined by the difference of the total ion concentration between both subsystems. Let us introduce the variable

$$y = \frac{2c_s}{q_{HEP}c_{HEP}} = 2c_s Q_0 \omega \frac{1}{\kappa}\frac{1+x}{x} \qquad (37)$$

Then, we obtain

$$\Pi_{ch} = c_+ + c_- - C_+ - C_- = \Pi^0_{ch}[(1+y^2)^{1/2} - y]$$

$$= \Pi^0_{ch} \cdot \eta(y)$$

where $\Pi^0_{ch} = q_{HEP}c_{HEP}$ denotes the maximum ion pressure for $c_s = 0$. It corresponds exactly to eq 11. For $c_s \gg q_{HEP}c_{HEP}$, the ion pressure is vanishing and excluded volume effects are dominating. Substitution of these results into the osmotic equilibrium yields to eq 28.

Appendix B: Experimental Details

Gel Preparation. Heparin (MW 14 000, Calbiochem (Merck, Darmstadt, Germany), amine end-functionalized 4-arm starPEG (MW 10 000, Polymer Source, Inc., Dorval, Canada), EDC (Sigma−Aldrich, Mïnchen, Germany) and sulpho-NHS (Sigma−Aldrich) (2:1 ratio of EDC:sulpho-NHS) were dissolved in deionized, decarbonized water (Milli-Q-water) on ice, respectively. A 2-fold molar excess of EDC to amine groups of PEG was used. The heparin, EDC and sulpho-NHS solutions were mixed and kept on ice (approx. 2−4 °C) for 15 min to activate the heparin carboxylic acid groups, before the PEG solution was added. After mixing, the reaction mixture was kept at room temperature for 14 h to form a gel. The molar ratio of PEG to heparin was varied from 1.5 to 6. For swelling experiments gel disks were used. A drop of reaction mixture was sandwiched between two hydrophobic coverslips. After gel formation the coverslips were removed.

Volume Swelling Measurements. The initial diameters of the gel disks were measured using callipers, before the gels were washed and swollen in deionized, decarbonized water (Milli-Q-water), PBS (Sigma−Aldrich) and 1 M $CaCl_2$ (Sigma−Aldrich) solution at room temperature, respectively. The aqueous solutions were changed four times, once per hour and once again after storage overnight. At least after 24 h swelling equilibrium was reached. The final diameter of the swollen disks was measured. Experiments were carried out at least four times. The degree of volume swelling Q was calculated as follows: $Q = V/V_0 = (d/d_{reac})^3 \cdot V_{reac}/V$ where d is the diameter of the swollen gel disk, d_{reac} the diameter of the unswollen gel disk (cured reaction mixture), V_{reac} the volume of the cured reaction mixture and $V_0 = n_{PEG}v_{PEG} + n_{HEP}v_{HEP}$ the volume of the dry gel. The molar volumes of heparin and PEG were calculated from the densities, which were determined by means of helium pycnometry (Ultrapycnometer 1000T, Quantachrome Instruments).

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