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A novel potentiometric method for the determination of real crosslinking ratio of poly(aspartic acid) gels

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ABSTRACT

In order to obtain nontoxic functional polymer gels for biomedical applications, chemically crosslinked poly(aspartic acid) gels have been prepared using 1,4-diaminobutane as crosslinker. The presence of COOH and amino groups on the network chains renders these gels pH sensitive. Due to the specific hydrophobic–hydrophilic balance, these gels show a significant volume transition at a well-defined pH close to the p*K* value of uncrosslinked poly(aspartic acid). Since the magnitude of volume change critically depends on the degree of crosslinking, it is an important task to determine the topological characteristics of these networks. A novel method based on potentiometric acid–base titration has been developed to assess the crosslinking ratio, excluding physical crosslinks and entanglements. It turned out that only 25% of all crosslinker molecules forms real crosslinks between the poly(aspartic acid) chains; the rest react with one of its functional groups and forms short pendant side chains. At a nominal crosslinking ratio of 0.1, the number average molecular mass between crosslinks is found to be $M_c = 2300$.

1. Introduction

Development of new drug-delivery systems involves a multidisciplinary approach based on polymer science, pharmaceutics, molecular biology and nanotechnology. The main aim is to minimize drug degradation and loss by targeted delivery and release, as well as prevent harmful side effects. The drug carrier plays an important role in increasing the availability of the drug molecules at the disease site. pH- and temperature-responsive smart polymer hydrogels, as a new type of biomaterial, show great promise as carriers due to their local environmental sensitivity and to their swelling–deswelling controlled-release kinetics [1,2]. In order to develop pH-sensitive drug delivery matrices, polyelectrolyte gels with abrupt volume changes are required. In addition to this stimuli responsive attribute, the biocompatibility and biodegradability of the drug carrier is also important. As opposed to many synthetic polymers, the degradation products of poly(amino acid)-based biomaterials are nontoxic small-molecule nutrients, which are excreted by physiological processes in the body [3]. Besides these advantages, the virtually unlimited structural diversity of the poly(amino acid)-based hydrogels offers the possibility to realize the well-defined balance between hydrophobic and hydrophilic interactions which is the necessary condition of abrupt volume change. The magnitude of volume change strongly depends on the degree of crosslinking [4], and therefore the knowledge of crosslink concentration is crucial for the development of poly(amino acid)-based biomaterials for targeted drug delivery and release.

The mechanical and swelling properties of swollen networks (gels) strongly depend on how they are formed. Preparation of polymeric networks may be achieved in two different ways: simultaneous polymerization and crosslinking of monomers. Another possibility is crosslinking of linear or polymer chains using a small amount of crosslinking agent. These reactions may be carried out either in solution or in solid state. The points of linking, called junctions, are usually randomly distributed along the chains. The network chains between two junction points exhibit a distribution of molecular mass. The topological structure of a perfect network may be described by various parameters: the average mass of network chains, the M_C, functionality of the junctions, the number of network chains, and number of junctions. Although a perfect network can never be obtained in reality, these quantities are used to characterize the network structure. Deviation from the perfect structure is due to the presence of dangling chains, loops and entanglements. A theoretical estimate of the most important network parameters for all types of structural imperfections has not yet been established.

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Another useful parameter is the crosslinking ratio, X_n. This is defined as the molar ratio of crosslinking agent to the monomer units building up the polymeric network:

$$X_n = \frac{n_c}{n_m} \quad (1)$$

where n_c is the total number of crosslinker molecules and n_m is the total number of monomer units in the gel. It often happens that not every crosslinker molecule forms such linkages that interconnect two chains. Therefore X_n in Eq. (1) represents a nominal crosslinking ratio. A distinction can be made between crosslinker molecules according to their reacted functionality (see Fig. 1).

Let n_2 denote the number of crosslinker molecules (A-type in Fig. 1) that have reacted with both of their functional groups interconnecting two primary polymer chains, forming junctions. Crosslinker molecules that have reacted with only one of their functional groups, forming dangling side chains, may remain in the system (B-type in Fig. 1). The number of such molecules is denoted by n_1. They do not form real crosslinks. It cannot be excluded that crosslinker molecules exist that do not react at all (C-type in Fig. 1). The number of such free molecules is denoted by n_0. These unreacted molecules can be washed out from the gel together with the sol fraction.

The total number of crosslinker molecules, n_c is the sum of n_0, n_1 and n_2:

$$n_c = n_0 + n_1 + n_2 \quad (2)$$

Only the crosslinker molecules of A-type represent real junctions; therefore, the real crosslinking ratio, X_r is defined as follows:

$$X_r = \frac{n_2}{n_m} \quad (3)$$

It is obvious that $X_r \leqslant X_n$. In order to determine the real crosslinking ratio, it is necessary to know not only n_c, but also the number of such molecules that have not reacted with both of their functionalities: n_1 and n_0. It is reasonable to assume that long after the gelation took place, no free crosslinker molecule of C-type can be found in the gel ($n_0 \approx 0$); therefore one may write that:

$$n_2 = n_c - n_1 \quad (4)$$

In case of bifunctional crosslinking molecules, the junctions have a functionality of four. The number average molecular mass of the network chains between the crosslinks, M_C, can be calculated by a simple relation:

$$M_c = \frac{M_m}{2X_r} = \frac{n_m}{2(n_c - n_1)} M_m \quad (5)$$

where M_m represents the molecular mass of the repeating monomer units (M_m = 115 for poly(aspartic acid)).

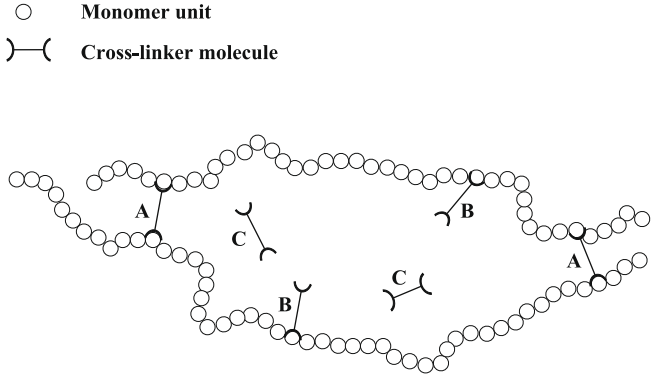

Fig. 1. Schematic representation of different types of crosslinker molecules. Open circles denote the monomer units of the polymer chains.

In the present work, the pH sensitivity of polymer networks composed entirely of amino acids has been studied. The main purpose was to study the efficiency of crosslinking reaction of poly(aspartic acid) chains by 1,4-diaminobutane (DAB). Based on aqueous acid–base potentiometric titration, a novel method is developed to determine the crosslinking ratio in these gels. This approach relies upon the H^+ consumption of monomer unit carboxylates as well as that of the crosslinking molecules.

2. Materials and methods

2.1. Materials

L-Aspartic acid (puriss, 99.0%), phosphoric acid (a.r., 85%), methanol (p.a., 99.8%), citric acid monohydrate (a.r., 99.5%), sodium chloride (p.a.) were obtained from Reanal Ltd. (Hungary). Mesitylene (Fluka, purum, 98%), sulfolane (Aldrich, 99%), dimethyl sulfoxide (DMSO, Fluka, purum, 99%), DAB (Sigma, purum, 98%) and dibutylamine (DBA, Riedel-de Haën, 99%) from Sigma–Aldrich were used. All reagents and solvents were used without further purification. Bidistilled Millipore water (specific resistance: 18.2 MΩ ;cm) was used throughout this study.

2.1.1. Synthesis of poly(succinimide) (PSI) chains

PSI was synthesized by thermal polycondensation of aspartic acid with phosphoric acid, using a solvent mixture of mesitylene and sulfolane [5,6]. The average molecular mass of PSI chains was determined in DMSO solution by static light scattering to be 73,000.

2.1.2. Crosslinking of PSI chains by DAB

In order to crosslink the precursor polymer chains, 0.97 g of PSI (corresponding to 0.01 mol of succinimide monomer units) was dissolved in 9 ml DMSO and 0.088 g (0.001 mol) DAB was added under continuous stirring. This corresponds to a nominal crosslinking ratio of 1/10. The reactive mixture was poured into special molds to yield cylindrical gels. Gelation was completed in 30 min, then the gels were removed from the molds. The volume of the gel swollen in DMSO was 10.4 cm^3.

2.1.3. Formation of poly(aspartate) (PASP) gels

The transparent PSI gels (0.2 g) obtained in the previous step was placed into 50 ml of 0.1 M NaOH solution at room temperature for 3 h. The gel first became opaque due to the diffusion of water into the polymer network and then the turbidity changed slowly to transparent, again starting from the surface. Upon alkaline hydrolysis, PASP chains are formed, containing both α- and β-peptide linkages [6,7] as depicted in Fig. 2.

Fig. 3 shows the structure of the hydrolyzed polymer network. One can recognize two kinds of crosslinker molecules bound to the network chains: two real crosslinks and two dangling side chains.

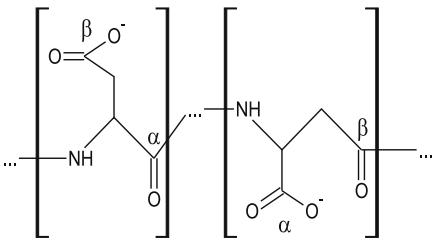

Fig. 2. Structure of the two possible monomer units in hydrolyzed poly(aspartic) chains, containing α- or β-peptide bonds and β- or α-carboxylate side chains, respectively.