Controlling the Association of Adamantyl-Substituted Poly{*N*-[tris(hydroxymethyl)methyl]acrylamide} and a *β*-Cyclodextrin/Epichlorohydrin Polymer by a Small Drug Molecule – Naproxen

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Two polymeric substances, a poly{*N*-[tris(hydroxymethyl)methyl]acrylamide} (THMMA) substituted with adamantyl moieties and a *β*-cyclodextrin/epichlorohydrin polycondensate, formed a host–guest type complex, which resulted in the gel formation upon mixing of these two compounds at appropriate conditions. Introduction of a drug molecule, *i.e.*, naproxen, that was able to fill the *β*-cyclodextrin cavities, thus expulsing adamantyl moieties, led to disruption of such association and inhibition of gel formation. The conditions required for the association of the two polymeric components and formation of the gel, as well as the dynamics of its inhibition by addition of naproxen was established. The procedure of using solutions of two associating polymers and an appropriate drug competitor can be used at targeted viscosupplementation.

1. Introduction. – The adamantane molecule fits snugly into the internal cavity of *β*-cyclodextrin forming a host–guest associate (*cf. Fig. 1*). The stability constant (log *K*) of a complex between, *e.g.*, water-soluble *β*-cyclodextrin and hydrophobic adamantane-1-carboxylate in H_2O at pH 7.0 or 7.2 equals 4.60 ± 0.02 or 4.51 ± 0.02 [1]. Thus, cyclodextrin (CD) is often used as a mediator at 'dissolution' of small hydrophobic molecules in aqueous media [2].

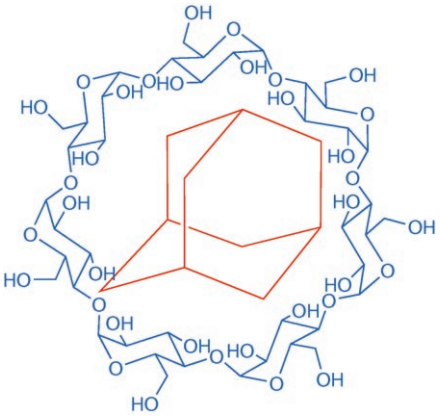

Fig. 1. *Host–Guest Associate of β-Cyclodextrin* (blue) *and Adamantane* (red) *Molecules*

Several manufacturers are currently supplying various cyclodextrin derivatives including those, in which, *e.g.*, β-CD is contained in the shorter or longer chains resulting in substances classifiable as CD oligomers or CD polymers (see, *e.g.*, [3]). Such 'polymerized' CDs have been utilized as polymers hosting various lipophilic substances. In the case when, *e.g.*, a water-soluble polymer is substituted with a hydrophobic moiety such as the adamantyl group, novel self-associating supramolecular structures can be produced. In general, supramolecular structures can be formed due to attractive non-covalent interactions, including ionic interactions, H-bonds, charge-transfer complex formation, stereo-complexation, hydrophobic interactions, as well as inclusion complexation [4–8].

Self-associating biopolymers become especially attractive due to a possibility to employ them at tissue engineering as matrices/scaffolds for the repair, regenerating, or replacement of a wide variety of tissues and/or organs. In general, by mixing two components, *i.e.*, one that bears the host and the second one bearing the guest, a supramolecular associate should be formed. One existing drawback is the kinetics of the self-associating process. The basic requirement to the property of a material used at viscosupplementation is *i*) to have an injectable low-viscous liquid, which *ii*) after its introduction into the given space would undergo self-association to form an artificial soft/hard tissue.

In the case of combining aqueous solutions of two (bio)polymers, the first carrying CD hosts and the second one bearing, *e.g.*, adamantyl moieties, the prerequisite *i* can hardly be fulfilled, since the bicomponent mixture may begin to associate already in the injection vessel. A possible solution how to eliminate this drawback has been recently patented [9]. The principal idea of the invention can be described as follows: '*The two aforementioned associating polymeric components premixed together with an appropriate drug serve as a (tricomponent) injection formula. Upon the injection of such a 'cocktail' into the site of application, the drug molecules initially should completely block the process of association. However, upon elimination (excretion) of the drug from the tissue environment, the desired (*in situ*) self-association of the polymer components will occur*' [10].

The aim of this communication is to present the results obtained when adamantyl-substituted poly{*N*-[tris(hydroxymethyl)methyl]acrylamide} (THMMA-Ad4) and a β-CD/epichlorohydrin polycondensate (ER 921A) were used as the two self-associating H_2O-soluble polymers, while a drug, naproxen (*Fig. 2*), was selected as a blocker of association/aggregation of the two polymers.

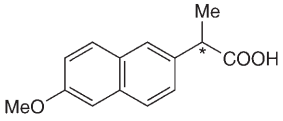

Fig. 2. *Naproxen* (*: the stereogenic C-atom)

2. Results. – 2.1. *Determination of Intrinsic Viscosity of the Polymers.* Dependencies of the solution-specific viscosity from the polymer concentrations were almost linear, and the following intrinsic viscosity values were estimated:

$[\eta] = 0.236$ dl g^{-1} for the THMMA-Ad4 polymer in H_2O at 25°;

$[\eta] = 0.108$ dl g^{-1} for the ER 921A polymer in H_2O at 25°.

As can be seen, the value of the intrinsic viscosity of THMMA-Ad4 is more than two times higher than that of the ER 921A polymer. However, the ratio of the polymer M_w values is much higher (> 5.6).

2.2. *Assessment of Gel Formation at Mixing of THMMA-Ad4 with the ER 921A Polymer.* Three concentrations of both polymers (described in the *Exper. Part*, *Sect. 2.2.*) were investigated in the experiments involving gel formation. When two samples of higher concentrations of THMMA-Ad4 (0.9 and 1.8 g dl^{-1}) were mixed with the samples containing two higher concentrations of the ER 921A polymer (1.75 and 3.5 g dl^{-1}), the gel was formed, if the content of the polymer added reached *ca.* 20% (*w*/*v*) in the mixture of the polymers. Solutions containing lower concentrations of THMMA-Ad4 (0.45 g dl^{-1}) and of the ER 921A polymer (0.875 g dl^{-1}) did not form the gel even when more than 30% of THMMA-Ad4 was added to the ER 921A polymer, or *vice versa*.

For the subsequent experiments, H_2O solutions containing 1.75 g dl^{-1} of the ER 921A polymer and 0.9 g dl^{-1} of THMMA-Ad4 mixed in the ratio of 2:1 were used. Formation of the gel at these conditions was positively established.

2.3. *The Effect of Naproxen on the Gel Formation.* Naproxen as a blocker of association of the two polymers was used to establish the dissociation conditions of the gel formed by THMMA-Ad4 and ER 921A polymers. The *Table* shows the results of blocking experiments, in which various concentrations of naproxen sodium salt were used to disrupt the association of the two used polymers.

Table. *Dependence of Gel Formation in the H_2O Solutions of THMMA-Ad4 and the ER 921A Polymers on the Concentration of Naproxen Sodium Salt Present in the Mixture*

Solution of ER 921A (1.75 g dl^{-1}) [μl]	Solution of THMMA-Ad4 (0.9 g dl^{-1}) [μl]	Naproxen concentration [μM]	Formation of gel[a])
200	100	33	+++
200	100	66	++
200	100	99	+
200	100	131	–
200	100	164	–

[a]) As can be seen, 33-μM concentration of naproxen sodium salt does not inhibit the formation of gel in solution containing 1.16% (*w*/*v*) ER 921A and 0.3% (*w*/*v*) THMMA-Ad4. On the other hand, 131 μM concentration of naproxen sodium salt is a threshold value, above which no gel formation is observed.

2.4. *Removal of Naproxen from the Mixture of Polymers.* The ability of naproxen to block the association of the two polymers can be applicable when the formation of the gel directly within a certain system (at a definite site) is desirable. In such a system, a solution of two associating polymers containing a blocker can be introduced, and the formation of the gel will be enabled by removal of the blocker. This hypothesis was corroborated by the results of an experiment involving elimination of naproxen sodium salt from the H_2O solution containing THMMA-Ad4 and ER 921A polymers. Using

the ultrafiltration through the membrane with the 10 kDa cut-off, naproxen was gradually removed, and, as a result, formation of the gel was observed. The results are shown in *Fig. 3*.

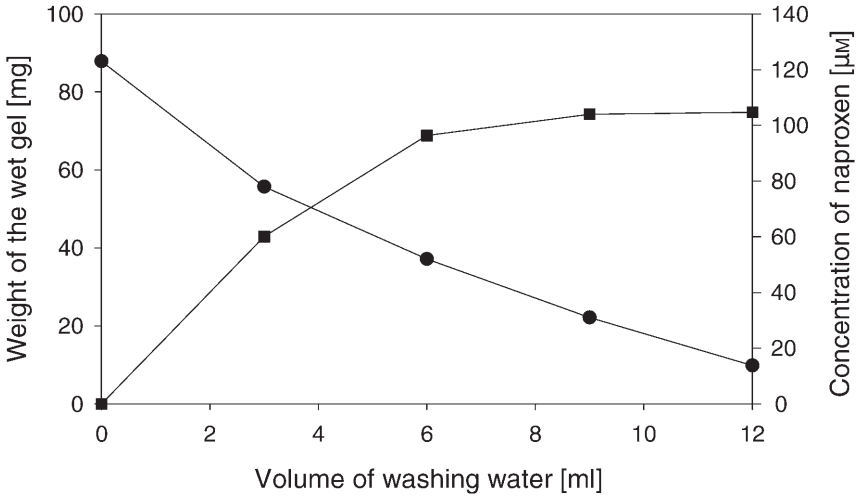

Fig. 3. *Elimination of naproxen from the solution of two polymers.* A solution containing 1.16% ER 921A, 0.3% THMMA-Ad4, and 130 μM naproxen was ultrafiltrated and washed with H_2O using 10 YM membrane. The weight of the gel (–■–) rest on the membrane, and concentration of naproxen (–●–) in the filtrate were determined.

The increase of gel amount observed during the removal of naproxen stopped when its concentration decreased to 31 μM. This value is in a good agreement with the results presented in the *Table*.

3. Discussion. – Cyclodextrins (α, β, or γ) belong to a family of torus-shaped cyclic oligosaccharides composed of six, seven, or eight α-1,4-linked D-glucopyranosyl units. While the outer surface of the molecule is hydrophilic, its inner cavity is hydrophobic. Thus, CDs can form inclusion complexes (host–guest complexes) with the hydrophobic molecules/drugs or with the hydrophilic ones, which carry hydrophobic moieties. The equilibrium between the complexed and free drug can be exploited to govern the availability of the non-complexed drug during the treatment of the patient. Simplifying, it can be speculated that the lesser is water solubility of a given drug molecule, the slower is its release from the CD cavity.

In our experiments on the investigation of the association of adamantyl-substituted poly{*N*-[tris(hydroxymethyl)methyl]acrylamide} with a β-CD/epichlorohydrin polymer, adamantane-1-ethanol as well as 1-(2-bromoethyl)adamantane [11] were substances of choice in the function of the blocker. However, our experiments regarding association of the polymers THMMA-Ad4 and ER 921A with these two blockers revealed an impossibility to 'rinse' these blockers either from a separate polymer ER 921A or from its associate with THMMA-Ad4.

One of the possible solutions, as has been demonstrated by our experiments, could be replacement of a blocker of the adamantyl type by a blocker bearing naphthyl

moieties. As has been reported [1], β-cyclodextrin readily forms a complex with naphthalene itself, the $\log K$ value of the formed complex in H_2O being 2.83. In aqueous solution at pH 2, naproxen easily associates with the molecules of β-CD with the complex stability constant $\log K = 3.17$. Taking into account its low toxicity and good tolerance, naproxen appears to be a substance of choice for blocking/filling of the β-cyclodextrin cavities with adamantyl moieties bound to certain (bio)polymers. Other suitable candidates are represented by, *e.g.*, flurbiprofen or piroxicam with reported $\log K$ values of 3.29 (3.65) or 1.97, respectively [1].

The grants VEGA 2/4143/26, 2/5002/5, and 2/7028/27 from the *Grant Agency for Sciences of the Ministry of Education of Slovak Republic* and *Slovak Academy of Sciences*, and the grant APVV-51-017905 from the *Agency for Research and Development, Bratislava*, are gratefully acknowledged. The authors are indebted to Dr. *Š. Stankovský*, Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava, for the synthesis of 1-(2-bromoethyl)adamantane.

Experimental Part

1. *Materials.* The molecular characteristics of poly{*N*-[tris(hydroxymethyl)methyl]acrylamide}, the sample named THMMA, were $M_w = 190.3$ and $M_n = 51.5$ kDa. The chemical structure of THMMA sample, substituted with 2% (molar) of adamantyl moieties (THMMA-Ad4), has been recently published [12]. The molar mass of the used β-CD/epichlorohydrin polycondensate, namely the sample ER 921A, was *ca.* 33.5 kDa [13]. The β-CD/epichlorohydrin polymer prepared by the polycondensation reaction contained, on average, 42–67% (*w*/*w*) of β-cyclodextrin [12][14–17].

Naproxen sodium salt ((+)-(*S*)-enantiomer purity $\geq 98\%$) was a generous gift of Mr. *Mallikarjun Chityala* from the company *Matrix Laboratories Ltd.*, Secunderabad, India. Used H_2O was of redistilled de-ionized quality grade.

2. *Methods.* 2.1. *Capillary Viscometry.* The viscosity measurements were carried at $25 \pm 0.2°$ using an *Ubbelohde*-type viscometer. The diameter of the viscometer capillary was 0.4 mm. The solvent/diluent applied was H_2O of redistilled de-ionized quality. The flow-through capillary time of the pure diluent was 162.0 s.

The THMMA-Ad4 starting concentration used was 4 g dl^{-1}, while the starting concentration of the ER 921A sample was 8.75 g dl^{-1}. Dilutions of the polymer solns. were performed directly in the viscometer reservoir. The lowest concentrations of THMMA-Ad4 and ER 921A samples were 0.2 and 1.5 g dl^{-1}, resp.

2.2. *Gel Formation at Mixing THMMA-Ad4 and ER 921A Polymers.* Aq. solns. of the tested polymers of definite concentrations were prepared and mixed together. Gel formation in the mixtures was inspected visually with a help of a magnifying glass. Three concentrations of each polymer were examined, namely THMMA-Ad4 concentrations were 1.8 (*A*), 0.9 (*B*), and 0.45 g dl^{-1} (*C*), while those of ER 921A were 3.5 (*I*), 1.75 (*II*), and 0.875 g dl^{-1} (*III*). The exper. protocol was as follows:

a) 8 ml of THMMA-Ad4 soln. of a given concentration (*A*, *B*, or *C*) was 'titrated' with the given soln. of the ER 921A solution (*I*, *II*, or *III*), until the gel was formed;

b) 8 ml of ER 921A soln. of a given concentration (*I*, *II*, or *III*) was titrated with the given soln. of the THMMA-Ad4 solution (*A*, *B*, or *C*), until the gel was formed.

In experiments *a* as well as *b*, *A* was mixed with *I*, *B* with *II*, and *C* with *III*, resp.

2.3. *The Effect of Naproxen on the Gel Formation.* Naproxen was determined spectrophotometrically at 262 nm. Drug concentrations were calculated on applying the naproxen extinction coefficient predetermined in the H_2O soln. ($\xi = 5.209 \times 10^3$ l mol^{-1} cm^{-1}; unpublished data).

The soln. of ER 921A (concentration *II*) containing various amounts of naproxen (within the range 33–164 μM) was mixed with THMMA-Ad4 soln. (concentration *B*) in the ratio of 2 : 1, and the formation of the gel was checked.

2.4. *Naproxen Release from the Mixture of Polymers.* The solns. of the two polymers investigated, *i.e.*, with identical concentrations as described in *Sect. 2.3.*, with added naproxen (131 μM) upon mixing

formed clear transparent solutions. These solns. were then subjected to ultrafiltration through a *YM 10* membrane (*Millipore Corporation*, Bedford, MA, USA) with a 10-kDa cut-off. After the filtration, 3 ml of dist. water (same volume as that of the initial soln.) was added to the gel-like material rest on the membrane in the ultrafiltration vessel to dissolve residual naproxen and those portions of the two polymers that did not create gel. Subsequently, concentration of naproxen in the soln. and weight of the wet gel were determined. Both components (soln. and gel) were placed back into the ultrafiltration vessel and the ultrafiltration was performed again. The steps: *i*) addition of H_2O, *ii*) assessment of naproxen and gel content, and *iii*) ultrafiltration were repeated until the amount of the gel ceased to increase.

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Received October 2, 2006