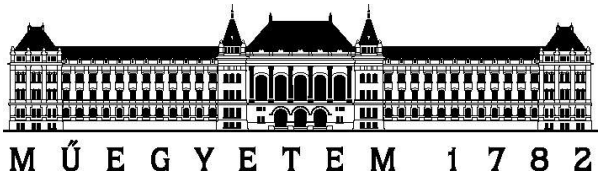

Tamás Gyenes

Preparation and swelling properties of amino acid-based gels

Extended Abstract

Thesis

for PhD Degree

Supervisor: Miklós Zrínyi
Consultant: Viktoria Torma

Department of Physical Chemistry and Materials Science, BME, Budapest

2007

Introduction

The importance of biodegradable polymers and hydrogel materials is being increasingly recognized and extensive studies have been conducted on their uses in various biomedical applications. Hydrogels based on both natural and synthetic polymers have continued to be of interest for encapsulation of drugs, and most recently, such hydrogels have become especially attractive to the new field of “tissue engineering” as matrices for repairing and regenerating a wide variety of biological tissues and organs.

Poly(amino acid)s with protein-like linkages are known to be biocompatible and biodegradable. The 20 proteinogenic amino acids in the peptide chain provide practically infinite variability of the polymers. The preparation of polymer gels with high swelling ability as well as good mechanical properties requires poly(amino acid) network chains with average polymerization degree exceeding 500. Therefore, amino acid-based hydrogels are usually synthesized by crosslinking biological or natural polypeptides, e.g. gelatin or alginates. An alternative approach for the preparation of high-molecular-mass artificial polyamides is the thermal polycondensation of amino acids.

The main purpose of the present work was to synthesize polymer gels from proteinogenic amino acids that are responsive to the alteration of chemical surroundings, such as pH or redox potential. As a first step, polymers with high polymerization degree were synthesized. Procedure for the modification and

cross-linking of poly(amino acids) by other biological molecules was then elaborated. This preparation procedure could be realized by the application of poly(succinimide), which can be prepared by the thermal polycondensation of aspartic acid (Figure 1.).

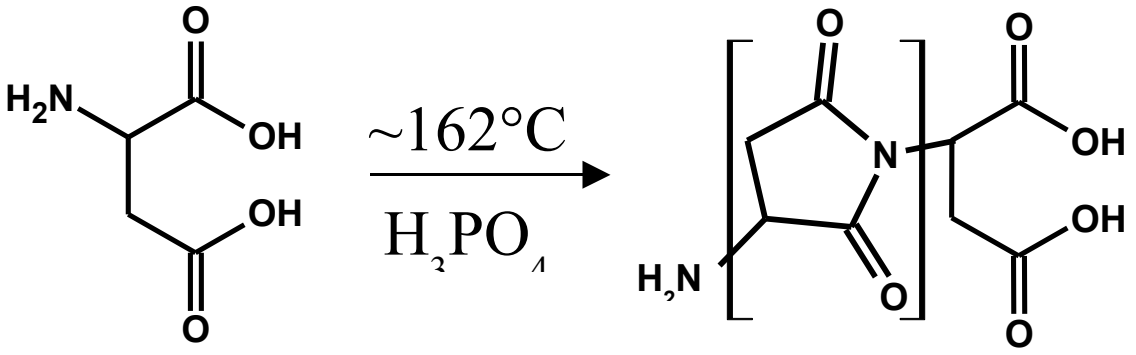

Figure 1. Thermal polycondensation of aspartic acid

Activated imide rings of poly(succinimide) can be opened by nucleophilic reagents, such as amine group containing reactants, forming amides (Figure 2.). Elaborating procedures for the modification and cross-linking of poly(succinimide) different kind of polymer gels containing exclusively amino acids can be synthesized.

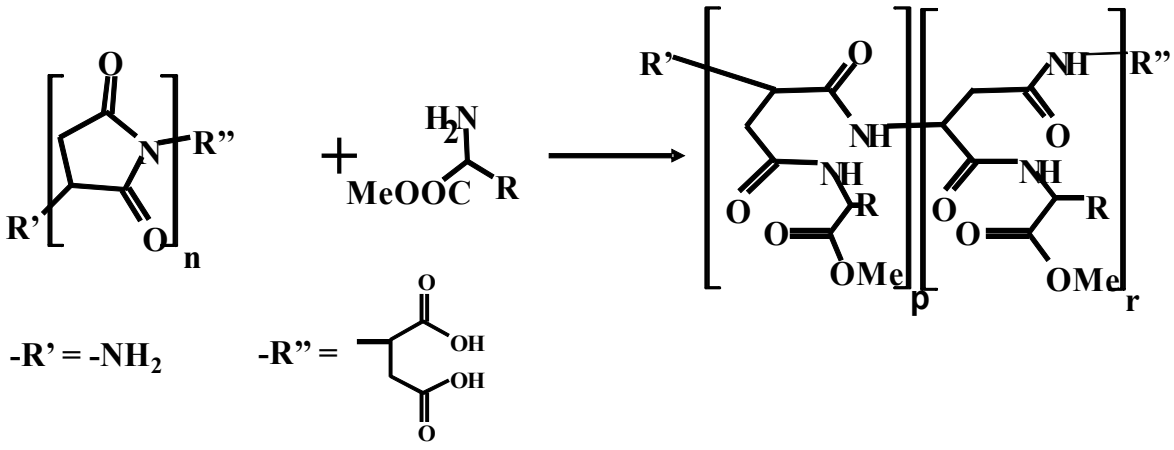

Figure 2. Modification of poly(succinimide) by amino acids

Owing to the biocompatibility of the constituent monomers, the resulting polymer gels may be promising candidates for pharmaceutical applications such as artificial muscle or controlled drug delivery systems. Conducted in the Department of Physical Chemistry and Material Sciences at the Budapest University of Technology and Economics, my PhD work opens a new field: artificial poly(amino acid) based gels as smart materials.

Experimental

The synthesized polymers have been characterized by NMR and dynamic light scattering methods. Physical and chemical properties of the prepared gels have been studied by equilibrium swelling, elastic modulus and swelling kinetics measurements and by potentiometric titration.

New scientific results

1. Poly(amino acid) has been synthesised by the thermal polycondensation of aspartic acid for the preparation of transparent monolith gels. NMR measurements have proved the dependency of the polymer's branching ratio from the catalyst molar ratio used in the polymerisation process.

2. Entirely amino acid-based polymers were prepared by side-chain attachment of serine, glycine and phenylalanine to poly(succinimide). Molar fraction of the introduced amino acid side chains has been determined by NMR measurements.

3. Amino acid based monolith gels has been synthesised based on poly(succinimide). Putrescin, spermidine and spermine have been used as cross-linkers.

4. Procedure has been elaborated for cross-linking polysuccinimide by lysine. As a result monolith gels with entirely amino acid-based network have been synthesised. The gelling process could be accelerated by certain amount of phosphoric or acetic acid.

5. Procedure has been elaborated for cross-linking polysuccinimide by cystamine. As a result poly(amino acid) gels containing disulfide bonds have been synthesised. The reaction of cystamine with poly(succinimide) could be catalysed by phosphoric or acetic acid.

6. Procedure has been elaborated for cross-linking of poly(succinimide) with two different cross-linkers: the reducable cystamine and the non-reducable diaminobutane at different ratios.

7. Poly(amino acid) network has been synthesised from poly(succinimide) and gelatine, in which the free amino groups of gelatine chemically reacted with the succinimide rings.

8. A novel method based on potentiometric titration has been developed to determine the cross-linking density of polymer gels. The method was used to determine crosslink density of poly(succinimide) gels chemically cross-linked by 1,4-diaminobutane. The results show that only 25±5 percent of the applied diaminobutane acts as true chemical net point.

9. The consecutive reactions of hydrolysis and swelling kinetics of polysuccinimide- and poly(aspartic acid)-based gels were studied at different pH values. Two distinct swelling steps were proposed. The cooperative diffusion coefficient has been found to be three orders of magnitude higher in pH 14 solution than at pH 8.

10. It has been concluded, that the poly(succinimide) gels have abrupt volume change around pH 7.3 because of the chemical opening of succinimide rings. In case of cystamine cross-linked gels increasing of swelling degree until complete dissolution of the gels can be observed over pH 8.5.

11. New theory has been developed based on the Brannon-Peppas – Peppas model, leading to an equation that – in contrast to the original theory – can be applied in wider ranges of pH and salt concentration. The new equation was succesfully used to evaulate quantitatively the equilibrium swelling measurements of the gels cross-linked by cystamine and diaminobutane. Cross-linking density and polymer – solvent interaction parameter were determined.

12. The equilibrium swelling degree of polymer gels cross-linked by both cystamine and diaminobutane was shown to be easily modulated by solution circumstances (pH 10 alkali or ditiotreithol solution). The increase in gel volume ranged in a predictable manner from 1 to 5 in a 0.25 M salt solution and from 1 to 100 in distilled water.

Applications

The synthesized gels may find practical applications in various field of biomedicine such as artificial muscle, implants or controlled drug delivery systems.

Publications

Book Chapter:

1. Filipcsei G., Fehér J., Szilágyi A., **Gyenes T.** és Zrínyi M: Intelligent Soft Materials; Közgyűlési előadások 2000, 3. kötet, pp. 1065-1078, Magyar Tudományos Akadémia, Budapest 2001

2. **Gyenes T.**, Filipcsei G., Zrínyi M.: Polimergélek mint hatóanyag hordozók, A gyógyszerészeti reológiai kutatás 40 éve Szegeden (1964-2004), Kedvessy Emlékkönyv, p119-127, ed.: Erőss István, JatePress 2004

Related papers:

3. **Gyenes T.**, Zrínyi M.: Controlled Drug Delivery Systems (Paradigm change in pharmacology)
   Acta Pharmaceutica Hungarica, 2001. *71,(4)* pp. 405-421

4. Torma V., **Gyenes T.**, Szakács Z., Noszál B., Némethy A., Zrínyi M.: Novel amino acid-based polymers for pharmaceutical applications
   Polymer Bulletin 2007, 59, 311-318

5. **Gyenes T**., Torma V., Zrínyi M.: Swelling Properties of Aspartic Acid-Based Hydrogels
   Colloids and Surfaces A doi:10.1016/j.colsurfa. 2007.06.016

6. **Gyenes T**., Torma V., Gyarmati B., Zrínyi M.: Synthesis and swelling properties of novel pH sensitive poly(aspartic acid) gels.
   Acta Biomaterialia, közlésre elfogadva (minor revision)

Patent:

7. Zrínyi M., **Gyenes T**., Torma V., Némethy Á., Gyarmati B.: Preparation of poly(succinimide) gels

Conferences:

Oral presentations:

8. **Gyenes T**.: Aszparaginsav alapú hidrogélek. BME VBK 4. Doktoráns konferencia, 2007.

9. **Gyenes T.**, Torma V., Gyarmati B., Némethy Á., Zrínyi M.: Responsive Properties of Smart Poly(amino acid) Based Gels. GelSympo2007 Tokió

10. **Gyenes T.,** Torma V., Némethy Á., Gyarmati B., Zrínyi M.: Responsive Properties of Smart Poly(amino acid) Based Gels. 9CCC Siófok

11. Gyarmati B., **Gyenes T**., Némethy Á., Torma V., Szilágyi A., Zrínyi M.: A környezet pH-jára és redox-potenciáljára érzékenyen reagáló poliaminosav-gélek. XXX. Kémiai Előadói Napok, Szeged, 2007

12. Némethy Á., **Gyenes T**., Gyarmati B., Torma V., Szilágyi A., Zrínyi M.: Poliaminosav-gél alapú hatóanyag-leadó rendszerek szintézise és tulajdonságai. XXX. Kémiai Előadói Napok, Szeged, 2007

Posters:

13. **Gyenes T.**, Szabó D., Zrínyi M.: Electric and Magnetic Field Controlled Drug Delivery Systems Realised by Intelligent Hydrogels. 6th International Symposium on Polymers for Advanced Technologies. Eilat, Israel, 2001

14. **Gyenes T.**, Torma V., Szakacs Z., Gyarmati B., Solt H., Némethy Á., Zrínyi M.: Swelling properties of aspartic acid-based hydrogels, poszter, ECIS konferencia, Budapest, 2006

15. **Gyenes T.**, Torma V., Szakacs Z., Gyarmati B., Solt H., Némethy Á., Zrínyi M.: Swelling properties of aspartic acid-based hydrogels. poszter, Summer School at the Institut d'Etudes Scientifiques de Cargese (Corsica, Fr), 2007

16. Némethy Á., **Gyenes T**.,Torma V., Gyarmati B., Zrínyi M.: Amino Acid Based Hydrogels for PH Induced Drug Delivery. Poszter, 9CCC Siófok, 2007

17. Gyarmati B., **Gyenes T**., Torma V., Némethy Á., Zrínyi M.: Aspartic Acid Based Hydrogels. Poszter, 9CCC Siófok, 2007