Redox- and pH-Responsive Cysteamine-Modified Poly(aspartic acid) Showing a Reversible Sol–Gel Transition[a]

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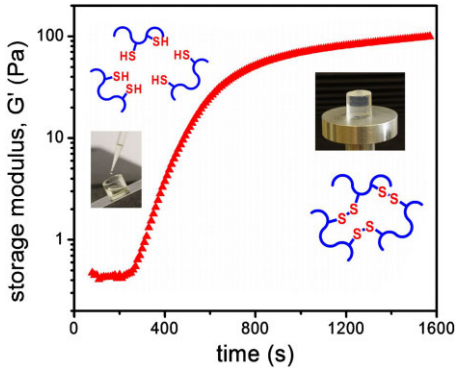
Synthesis and characterization of a pH- and redox-sensitive hydrogel of poly(aspartic acid) are reported. Reversible gelation and dissolution are achieved both in dimethylformamide and in aqueous medium via a thiol-disulphide interconversion in the side chain of the polymers. Structural changes are confirmed by Raman microscopy and rheological measurements. Injectable aqueous solutions of thiolated poly(aspartic acid) can be converted into mechanically stable gels by oxidation, which can be useful for drug encapsulation and targeted delivery. Reduction-facilitated release of an entrapped drug from disulphide cross-linked hydrogels is studied.



1. Introduction

In the past decades enormous effort has been invested in the development of responsive polymer hydrogels.[1–5] Their high water content, biocompatible and biodegradable features and their environmental sensitivity make them attractive for applications in various biomedical areas.[6–10] They can respond in a pre-determined fashion to different external stimuli, e.g., temperature,[11–16] light,[17–20] pH,[21–25] magnetic[26–29] or electric fields.[30,31]

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[a] **Supporting Information** is available from the Wiley Online Library or from the author.

Particular attention has been paid to multiresponsive polymers and hydrogels in order to achieve more specific answers to environmental triggers and to approach the complex behaviour of living systems.[32] A large number of studies focuses on dual-responsive, pH and temperature sensitive hydrogels with potential applications in targeted drug delivery.[25,33–36] However, selectivity of the proposed networks is in general unsatisfactory for accurate targeting. Future challenges in medical areas can be answered by multiresponsive systems that are triggered by bio-related stimuli, such as carbohydrate concentration (glucose-dependent insulin release), increased enzyme levels or altered redox conditions. For each biomedical stimulus, combined sensitivity to pH and temperature is beneficial for enhancing selectivity and reducing side effects in the body.

Here we focus on the exploitation of the changes in pH and redox potentials within the human body.[37] pH-sensitivity can be achieved by application of polyionic polymers. Redox-sensitivity can be obtained in the polymer network in two basic ways. A number of papers report the application of multivalent metal cations as redox-responsive moiety.[38–40] However, these studies lie outside

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the scope of our present paper. We discuss the use of thiol-disulphide interconversion in the polymer network. In this case, redox-sensitivity is designed by mimicking the redox processes in living cells. In view of the importance of thiol-disulphide exchange reactions in biological processes, this feature is of direct biological relevance.[41,42] In this respect, we recall the most widely known glutathione–glutathione disulphide redox couple, which plays an essential role in maintaining the redox potentials in the extracellular and intracellular medium.

Most studies use only the reduction-sensitive characteristic of disulphide linkages. Reduction of disulphide bonds by glutathione or other bio-relevant reducing agents is employed in protein and gene delivery or cellular imaging. This is a continuously developing field in current research, as testified by extensive reviews.[43,44]

The reverse direction, i.e., oxidation of thiols to disulphides, has been investigated much less frequently. Ravi and co-workers[45] reported a poly(acrylamide)-based polymer cross-linked by *N*,*N*′-bis(acryloyl)cystamine that exhibited a reversible sol–gel transition by oxidation and reduction and was applied in vitro as an injectable ocular lens filler. Hisano et al.[46] also reported a poly(acrylamide)-based polymer cross-linked by *N*,*N*′-bis(acryloyl)cystamine that was used to entrap islets. In the first step, both polymers were cross-linked via disulphide bond containing cross-linker to yield gels that facilitated removal of unreacted low molecular weight components after the synthesis. In order to prepare an injectable solution, the polymers were further dissolved by reduction, and then re-gelled by oxidation. The drawbacks of this strategy, such as slow and difficult removal of excess cross-linker and the large number of reaction steps, could be avoided by introducing thiol groups onto the polymer chains in the first step in the preparation of the pre-cursor solution of the polymer gel. Several natural and synthetic materials are used to synthesize thiol-grafted polymers, which can readily be converted into disulphide cross-linked networks to enhance structural stability or to develop fast gelling injectable materials in the case of sufficient thiol content.[47–52] The general benefit of this strategy is that the thiol content of the polymers could be controlled precisely during the synthesis, and gelation could be carried out from a polymer of well-defined composition. However, this strategy usually involves a difficult reaction step that utilizes water-soluble carbodiimide to establish amide bonds between the repeating units of the polymer and the thiol precursor. The use of carbodiimide requires further purification steps, usually dialysis and lyophilization.[49,53,54]

Poly(aspartic acid) is a biodegradable poly(amino acid) with biocompatible features due to its protein-like structure. Synthesis of reduction sensitive, disulphide cross-linked poly(aspartic acid)s was reported by Zrínyi et al.[24,55] However, the reversibility of the redox process was not investigated by these authors. Shu et al.[56] functionalized poly(aspartic acid) with cysteamine, but their synthetic method involved a carbodiimide mediated step and oxidation of thiols to disulphides was used only to improve stability of nanoparticles.

In the present work we introduce a simple strategy to prepare thiol-modified polymers with reversible redox-response based on poly(aspartic acid). We designed thiolated poly(aspartic acid)s that are applicable to the formation of bulk hydrogels with improved mechanical stability via oxidation of the injectable solution. pH-sensitivity and reversible redox-induced sol–gel transition of the developed poly(aspartic acid)s are also demonstrated.

2. Experimental Section

2.1. Materials

Imidazole (puriss p.a.) and Ellman's reagent (5,5′-dithiobis(2-nitrobenzoic acid)) were purchased from Sigma–Aldrich. S-aspartic acid (99%), dibutylamine (DBA, 99%), methanol (MeOH, 99.9%), cysteamine hydrochloride (97%), potassium chloride (99.5%), sodium tetraborate decahydrate (a. r.), sodium bromate (99%) and dithiothreitol (DTT, for biochem.) were purchased from Merck. Phosphoric acid (cc. 85%), hydrochloric acid (HCl, 35%) and dimethylformamide (DMF, pure) were purchased from Lach Ner. Citric acid monohydrate (99%) and sodium hydroxide (NaOH, a. r.) were purchased from Reanal (Hungary). Milli-Q reagent grade water ($\rho > 18.2$ MΩ · cm, Millipore) was used for aqueous solutions. All of the reagents and solvents were used without further purification. Synthesis and all the measurements were carried out at 25 °C.

2.2. Preparation

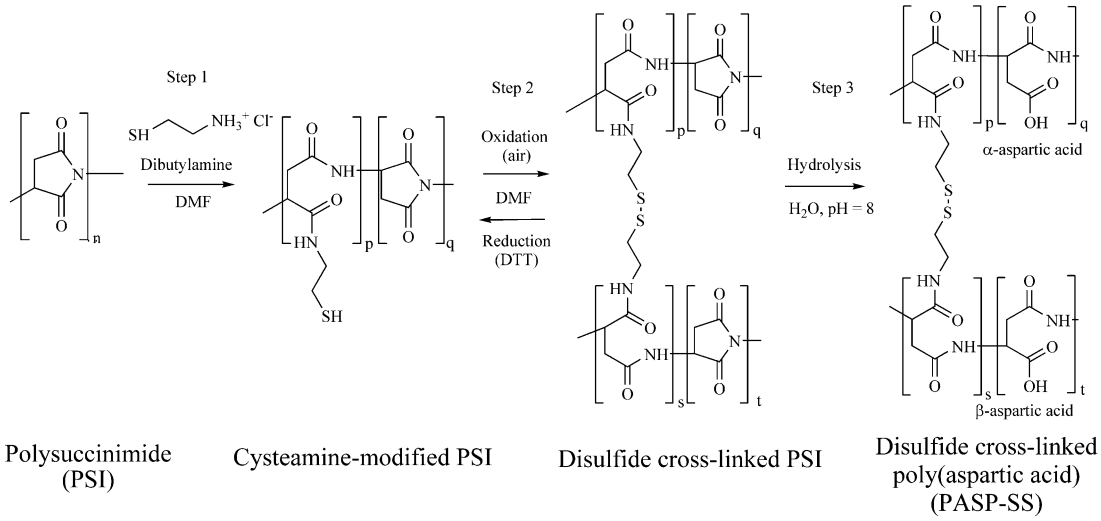
2.2.1. Synthesis of Polysuccinimide (PSI)

Polysuccinimide (PSI) was synthesized by thermal polycondensation of aspartic acid in a mixture of mesitylene and sulpholane at 160 °C (7 h).[24] The reaction was catalysed by 16 mol% phosphoric acid. PSI was purified by precipitation with DMSO-MeOH and dried in vacuum at 25 °C. Its chemical structure was confirmed by ^{1}H NMR (300 MHz, DMSO-d_6, δ): 5.10 (d, 1H, CH); 3.20 and 2.75 (s,s, 2H, CH_2). After hydrolysis of the PSI, the average molecular weight of the resulting poly(aspartic acid) (PASP) was determined by HPLC size-exclusion chromatography (SEC). Nucleogel GFC-300 column was used (molecular weight range of 1–100 kDa) with PBS eluent. Average molecular weight of PASP was calculated to be $\overline{M}_w = 56.1$ kDa, PDI = 1.07.

2.2.2. Synthesis of Cysteamine-modified Poly(aspartic acid)

The preparation of cysteamine-modified poly(aspartic acid) is shown in Scheme 1.

Polysuccinimide (PSI) was reacted with cysteamine hydrochloride in DMF using DBA as deprotonating agent in equimolar



Scheme 1. Synthesis of cysteamine-modified PSI, disulphide cross-linked PSI and disulphide cross-linked poly(aspartic acid) (PASP-SS).

amount to cysteamine (step 1). DBA activated the amine group of cysteamine. Compositions of the prepared polymers are listed in Table 1. A typical procedure was as follows (sample code: B in Table 1): 0.485 g PSI (containing 5 mmol of succinimide repeating units) and 0.114 g cysteamine hydrochloride were dissolved in 9.272 g DMF under nitrogen atmosphere. DBA (170 μl, 0.129 g) was added dropwise to the solution.

Thiol side chains of the cysteamine-modified PSIs were oxidized into disulphide linkages by air (step 2). Thin (1.0 mm thick) disulphide cross-linked PSI gel films were cast.

Water-swellable poly(aspartic acid) hydrogels (PASP-SS) were prepared by the irreversible hydrolysis of the PSI gels in an aqueous buffer solution of pH = 8 (step 3). PASP-SS gels were stored in PBS after hydrolysis. For chemical analysis, PASP-SH polymers obtained from PASP-SS hydrogels were purified by dialysis and lyophilization, but it should be emphasized that responsive features of the synthesized hydrogels can be exploited with no further purification step, thereby simplifying the preparation with respect to other thiol-grafted polymers. Unless otherwise noted, all further experiments were carried out in aqueous medium.

2.3. Characterization Methods

2.3.1. Characterization of Cysteamine-Modified Poly(aspartic acid)s by NMR Spectroscopy

Disulphide cross-linked PASP (PASP-SS) gels were dissolved in PBS (pH = 7.4) containing 10 mM of DTT as reducing agent. The dissolution was complete after 15 min independently of the

Table 1. Composition and properties of thiol-modified polymers and disulphide cross-linked gels[a].

	Cysteamine modified polysuccinimide			Cysteamine modified poly(aspartic acid)s (PASP-SH)		Disulphide cross-linked poly(aspartic acid)s (PASP-SS)	
Sample code	c_{PSI} [wt%]	X_{prep} [mol%]	t_{gel} [min]	X_{SH} [mol%]	X_{CYS} [mol%]	Q_m [–]	G [kPa]
A	4.85	10	240	2.8	2.4	15	–[b]
B	4.85	20	150	3.0	2.6	12	–[b]
C	4.85	30	130	6.5	6.4	7	1.8 ± 0.1
D	9.70	10	53	2.9	2.6	9	–[b]
E	9.70	20	51	5.4	5.6	6	2.9 ± 0.2
F	9.70	30	25	2.0–3.5	1.5–3.5	Dissolved	–[b]

[a] c_{PSI}: initial wt.-% of PSI in DMF; X_{prep}: feed ratio of cysteamine to succinimide repeating units during preparation; t_{gel}: gelation time of thiolated PSIs by air in DMF; X_{SH}: thiol-grafting density of poly(aspartic acid)s from Ellman's assay; X_{CYS}: molar ratio of side groups to repeating units from quantitative ^{1}H NMR spectroscopy; Q_m: swelling degree of hydrogels in aqueous solution at pH = 8; G: elastic modulus of the re-gelled PASP-SS in water; [b] not measurable.

composition of the hydrogels. For further analysis the reduced cysteamine-modified poly(aspartic acid) (PASP-SH) was purified by dialysis (cut-off weight: 10 kDa) against Milli-Q water. Solid polymers were obtained after lyophilization. Keeping the polymer in the solid state and applying low temperature (−80 °C) during lyophilization prevented the polymer from oxidation by air. To avoid oxidation, the dried polymer was stored at 10 °C.

The chemical structure was confirmed by ^{1}H NMR spectroscopy. The evaluated ^{1}H NMR spectroscopy signals were the following (500 MHz, D_2O, δ): 4.95 (s, CH in succinimide rings); 4.51 (s, CH in aspartic acid and cysteamine-modified succinimide rings); 3.54 and 3.38 (m, m, CH_2 in cysteamine side chains); 3.20 and 2.79 (s,s, 2H, CH_2 in repeating units). The molar ratio of cysteamine side chains to the repeating units of PASP-SH (X_{CYS}) was determined by quantitative ^{1}H NMR spectroscopy. X_{CYS} was calculated as the intensity ratio of methylene peaks of cysteamine side chains to the methylene peaks of the aspartic acid repeating units.

2.3.2. Ellman's Assay of Thiol-Modified Poly(aspartic acid)s

Ellman's assay[57] was used to determine the molar ratio of the thiol groups to the repeating units of PASP-SH (X_{SH}). Calibration was obtained by measuring thiol concentration of cysteamine solutions of different concentrations. The experimental parameters were the following: 1 800 μl of buffer solution (aqueous buffer solution at pH = 8:0.1 M imidazole, 1 mM EDTA; nitrogen was bubbled through the solution to remove oxygen), 180 μl aqueous solution of cysteamine hydrochloride and 20 μl of 10 mM aqueous solution of Ellman's reagent were measured into a 2 ml Eppendorf tube. The tube was incubated at 37 °C for 15 min to complete the reaction. UV-spectra (Analytic Jena Specord 200 spectrophotometer, Germany) were recorded immediately after the reaction. The peak at 405 nm was used for further evaluation (equation of calibration curve: $A = 1.161\ C_{SH} + 0.0447$, $R^2 = 0.9988$, $n = 5$).

Purification of the thiol-modified poly(aspartic acid)s was carried out in the same manner as for NMR spectroscopy. Thiol concentrations in the polymer solutions were determined according to the same experimental procedure as for the calibration of Ellman's assay. The concentration of thiol-modified poly(aspartic acid) was about 6 mM. Assuming that the polymers contained only aspartic acid and thiol modified aspartic acid units, the molar ratio of the thiol groups to the total number of repeating units (X_{SH}) was determined.

2.3.3. pH-Dependent Swelling

The swelling behaviour of the cross-linked PASP-SS hydrogels is pH dependent. The swelling degree (Q_m) was defined as the weight ratio of the swollen ($m_{\text{swollen gel}}$) and the dried polymer gel ($m_{\text{dried gel}}$):

$$Q_m = \frac{m_{\text{swollen gel}}}{m_{\text{dried gel}}} \quad (1)$$

The weight of the dried polymer gel was measured after the swollen gel had been carefully extracted in water and subsequently dried for 2 days in vacuum at ambient temperature.

PASP-SS hydrogels were swollen in aqueous buffer solutions of citric acid (from pH = 2 to 6), imidazole (from pH = 6 to 8) and sodium tetraborate (from pH = 8 to 12), pH values were adjusted by adding 1 M HCl or 1 M NaOH. The ionic strength of buffer solutions was adjusted to $I = 0.25$ M by adding KCl. The pH of the buffer solutions was controlled with a pH/ion analyser (Radelkis OP-271/1, Hungary).

2.3.4. Measurement of Elastic Modulus

The elastic modulus of the PASP-SS hydrogels was determined from uniaxial stress–strain measurements using cylindrical samples (diameter ≈ height ≈ 1 cm) (Instron 5543 mechanical tester, USA). The modulus was calculated using the elasticity model of regular networks (for further details see S1 in the Supporting Information).

2.3.5. Raman Spectroscopy

Raman spectra were collected using a Horiba Jobin-Yvon LabRAM (France) system coupled to an external 633 nm He–Ne laser source in the spectral range 300–3 100 cm^{-1}. Lyophilized polymer samples were measured in the solid state and hydrogels measured in the swollen state without further sample preparation (for experimental details, see S2 in Supporting Information).

2.3.6. Oscillation Rheometry

All of the measurements were carried out with an oscillation rheometer (Anton Paar Physica MCR 301, Austria). Cone-plate geometry with a diameter of 25 mm was used (CP25-1). The sample gap was set to 0.049 mm.

The range of linear viscoelasticity was determined in the case of both polymer solutions and hydrogels (for further details see S3 in the Supporting Information). Oxidation-induced gelation was followed in time sweep mode with constant angular frequency ($\omega = 10\ s^{-1}$) and constant strain (1%) in the range of linear viscoelasticity. 100 μl polymer solution of PASP-SH-E (10 wt.-% polymer in PBS) was mixed with 100 μl of oxidizing agent (1 M $NaBrO_3$ in PBS). 80 μl of the solution was transferred onto the plate of the rheometer. Storage modulus was monitored as oxidation proceeded and finally, a cross-linked hydrogel was obtained between the plates of the rheometer. The gelation time was defined by the intersection of straight lines fitted to the increasing regime and the final plateau of the storage modulus. The hydrogel was re-dissolved with 0.020 g of solid DTT (reducing agent) to yield a polymer solution on the plate of the rheometer. A second oxidation step was carried out by adding 0.015 g of oxidizing agent (solid $NaBrO_3$) to the polymer solution.

2.3.7. Drug Release Measurements

The model drug molecule auramine dye was loaded into the hydrogel and its release kinetics was studied to demonstrate the potential of the system in reduction induced drug delivery. The drug was dissolved in an aqueous solution of PASP-SH-E (10 wt.-% polymer in PBS) at a concentration of 0.1 wt.-%. The drug-containing polymer solution was oxidized into hydrogel by adding an aqueous solution of oxidizing agent (1 M $NaBrO_3$ in PBS, volume ratio of polymer solution to bromate solution was 1:1). As the drug loaded gels were prepared by oxidation of the aqueous polymer solution in the presence of the dye we could avoid the difficult step of soaking the prepared gel in the concentrated solution of the drug. The amount of released drug was quantified by UV–vis spectroscopy (Analytic Jena Specord 200, Germany). Since auramine and

the reducing agent (DTT) have distinct non-overlapping absorption maxima in their UV–vis spectra, they could be simultaneously quantified and there was no need to separate the released drug from DTT.

Release kinetics was measured in PBS with DTT (1 mM) and without DTT to prove the redox-sensitivity of the prepared hydrogel. The rate of reduction was also monitored by UV–vis measurement of the oxidized form of DTT.

3. Results and Discussion

3.1. Characterization of Cysteamine-Modified Polysuccinimides

Cysteamine-modified PSIs were prepared in DMF with different polymer concentrations and molar ratios of cysteamine to repeating units (X_{prep}), as shown in Table 1. Cysteamine-modified PSIs were converted in air into disulphide cross-linked PSIs within 0.5–6 h, depending on the thiol content (Table 1). Cross-linked PSI gels could be dissolved into polymer solutions by adding the reducing agent (DTT) at a concentration of 1 mM. Dissolution was complete within 15 min. The reduced polymer solutions could be re-gelled by the air within 4–6 h. The process could be repeated several times in DMF, i.e., cysteamine-modified PSIs displayed a reversible response to the redox environment. To the best of our knowledge, this is the first report in the literature of a modified PSI with reversible redox sensitivity.

3.2. Characterization of Cysteamine-Modified Poly(aspartic acid)s

Disulphide cross-linked poly(aspartic acid) hydrogels (PASP-SS) were yielded by the hydrolysis of cystamine cross-linked PSIs. The swelling degree (Q_m) of the prepared PASP-SS hydrogels in aqueous medium at pH = 8 showed a strong dependence on composition (Table 1). The swelling degree decreased with increasing initial polymer concentration and increasing modification ratio. After dissolution of PASP-SS hydrogels with DTT the molar ratio of thiol groups to aspartic acid repeating units in PASP-SH polymers determined by Ellman's assay (X_{SH}) and the molar ratio of cysteamine side-chains to the aspartic acid repeating units obtained from ^{1}H NMR spectroscopy (X_{CYS}) were in good agreement in each case. This proved that oxidation of thiols to disulphides could be avoided during lyophilization and the two independent methods were applicable for characterizing the final composition of the polymer network. However, it should be noted that because the real grafting density is significantly smaller than the applied feed ratio, it is essential to determine the actual modification ratio of thiolated polymers. For further studies, we chose the polymer with sample code PASP-SH-E because hydrogels emanating from this polymer (PASP-SS-E) showed the highest mechanical stability in aqueous medium. Although the PSI gel with sample code F was prepared with the largest cysteamine and PSI content, it dissolved during the hydrolysis. This behaviour may be explained by the fast gelation in step 1 which hindered the modification reaction and led to a heterogeneous structure. It is confirmed by the large standard deviations of X_{CYS} and X_{SH}.

3.3. pH-Dependent Swelling of Disulphide Cross-linked Poly(aspartic acid) Gels

Disulphide cross-linked poly(aspartic acid) hydrogels consist of polyelectrolyte networks with carboxylic acid groups in the repeating units. The pH-dependent swelling in aqueous medium of PASP-SS-E gel is illustrated in Figure 1.

The swelling degree exhibits a sudden increase at pH = 4.1 This value was determined from the 2nd derivative of the modified Peppas–Brannon-Peppas model.[24] pH-dependent morphology is illustrated by the scanning electron micrographs of lyophilized samples. The inset b in Figure 1 exhibits a more porous structure at pH = 8 than the compact structure observed in acidic medium (Figure 1, inset a). At pH = 8, carboxylic groups are deprotonated, resulting in a larger swelling degree and a bigger average pore size. At acidic pH values, the repeating units are in their protonated form and, owing to the non-ionized PASP chains, the volume and the pore size of the hydrogels decrease. This behaviour may be beneficial in pH-induced controlled drug delivery employing swelling-controlled release.

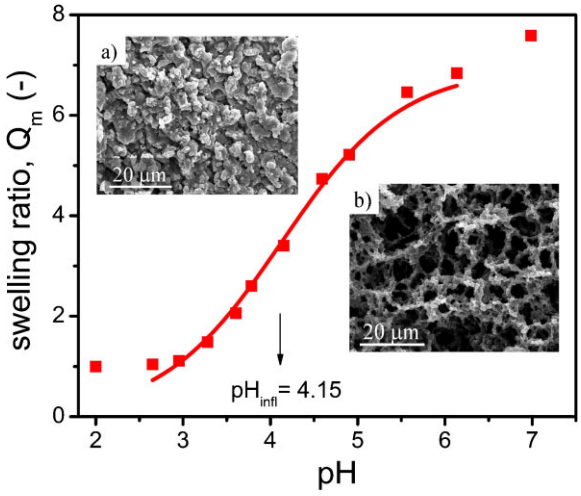


Figure 1. pH-dependent swelling of disulphide cross-linked PASP (PASP-SS) gels in aqueous medium; red squares are measured data while continuous line is a model fit using the modified Peppas–Brannon-Peppas model.[24] Insets show SEM micrographs of lyophilized gels from (a) acidic and (b) alkaline media, respectively.

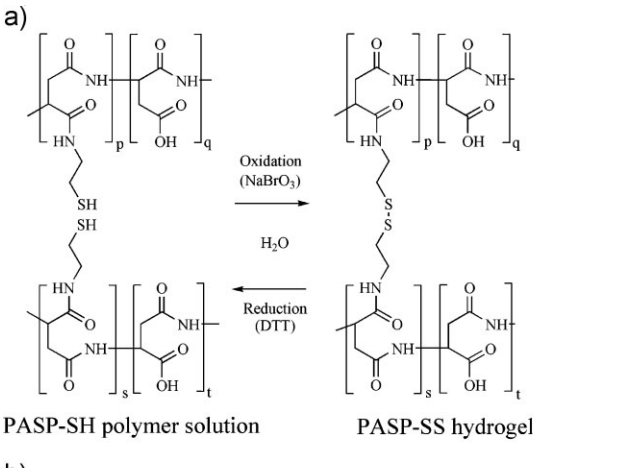


Figure 2. (a) Reaction scheme of reversible thiol-disulphide exchange in aqueous medium; (b) oxidation induced sol–gel transition of cysteamine modified poly(aspartic acid) (PASP-SH-E, left) in PBS resulting in bulk hydrogel (PASP-SS-E, right).

3.4. Reversible Redox Sensitivity of Cysteamine-Modified Poly(aspartic acid)s

Disulphide cross-linked PASP-SS-E gels were dissolved in PBS by adding DTT at a concentration of 1 mM. Dissolution completed in 10 min. Re-gelation of the reduced PASP polymers (PASP-SH) was studied in PBS (pH = 7.4) using 1 M sodium bromate as oxidizing agent in a volume ratio 1:1 with respect to the reduced polymer solution (Figure 2).

Re-gelation of the polymers occurred for sufficiently large thiol concentrations of the polymer chains (Table 1). As shown in Figure 2, the low viscosity polymer solution could be converted into a transparent bulk hydrogel by oxidation. The elasticity and mechanical stability of the hydrogels indicated the formation of a chemically cross-linked structure. Elastic modulus of PASP-SS hydrogels yielded from PASP-SH-C and PASP-SH-E polymers could be determined (Table 1).

Redox-induced dissolution of PASP-SS-E hydrogels and re-gelation of aqueous PASP-SH-E polymer solutions were also monitored by Raman spectroscopy (Figure 3). The disulphide peak, which corresponds to the oxidized state (PASP-SS-E hydrogel), appeared at 510 cm^{-1}, and its intensity decreased in the reduced state (PASP-SH-E polymer), while the thiol peak at 2 573 cm^{-1} appeared only in the reduced state. The other characteristic thiol peak at 661 cm^{-1}, which could also distinguish the redox states, partially overlapped a characteristic peak of PASP at 640 cm^{-1}. It could be concluded that the sol–gel transition of thiol/disulphide modified PASP networks was caused by redox reaction in aqueous medium.

Oxidation induced gelation was also observed by oscillation rheometry (Figure 4). The storage modulus (G') was measured as a function of time after adding oxidizing agent (1 M $NaBrO_3$ in PBS) to the aqueous polymer solution of PASP-SH-E. The gelation time was calculated to be 120 ± 5 s. The hydrogel (PASP-SS-E) was dissolved into polymer solution via reduction by solid DTT. In the second oxidation step the gelation time was similar to that in the first case (110 ± 7 s) (i.e., the sol–gel transition is reversible for at least two cycles). The results of Raman microscopy and rheological characterization confirmed the reversible redox response of the modified poly(aspartic acid)s in aqueous medium.

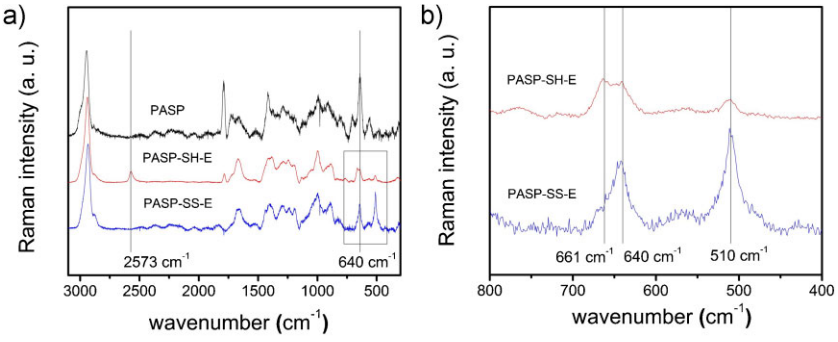


Figure 3. (a) Raman spectra of non-modified poly(aspartic acid) (PASP), cysteamine-modified polyaspartic acid (reduced state, PASP-SH-E) and water-swollen disulphide cross-linked poly(aspartic acid) (oxidized state, PASP-SS-E); (b) detailed spectra of reduced (PASP-SH-E) and oxidized (PASP-SS-E) state in the range 400–800 cm^{-1}.

3.5. Reduction Induced Release of Entrapped Model Drug

Results of reduction-induced drug release experiments are shown in Figure 5. In the first case release of model drug from PASP-SS-E hydrogels was examined without reducing agent. The observed release of the model drug was slow, with only 20% of the molecules being released over the whole time of measurement. After a reductive stimulus – addition of DTT – a burst release occurred and the total

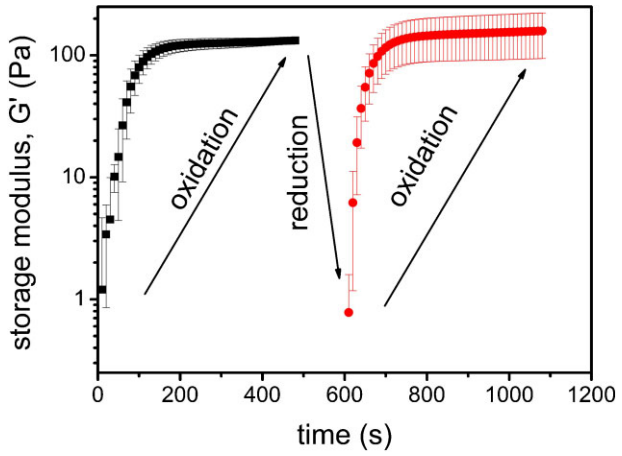


Figure 4. Reversible redox-induced sol–gel transition of PASP-SH-E in PBS followed by oscillation rheometry.

amount of released dye was five times more than without DTT, practically the whole amount of the entrapped drug being released during the measurement time. Due to the sudden increase in swelling a large amount of the drug was released even before the reduction-induced dissolution of the hydrogels was complete. These results proved that the redox environment controlled the release profile. Further characteristics of release kinetics are still to be investigated in order to monitor the mechanism of drug delivery more precisely.

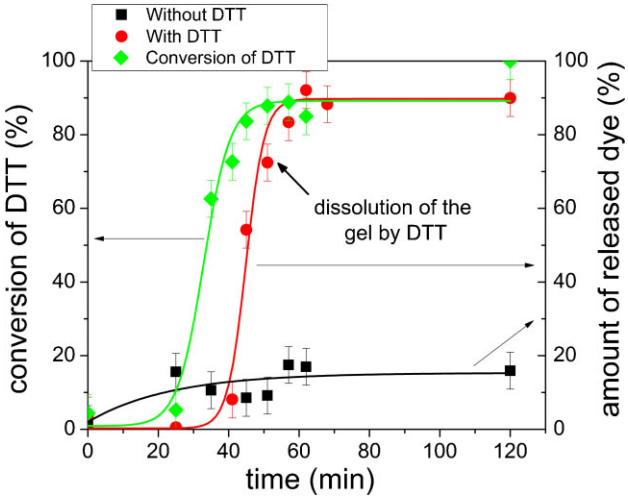


Figure 5. Reduction-triggered release of model dye from PASP-SS-E hydrogels in PBS; drug release with and without reducing agent (DTT) and conversion of DTT as a function of time. A large amount of the drug was released even before the complete reduction-induced dissolution of the hydrogels.

4. Conclusion

We report the synthesis of new cysteamine-modified polysuccinimide (PSI) and poly(aspartic acid) polymers. Cysteamine-modified PSIs showed reversible redox-response in organic medium (DMF), while cysteamine-modified poly(aspartic acid)s had multiresponsive character: reversible pH- and redox-sensitivity in aqueous medium. The thiol-disulphide interconversion was confirmed by Raman spectroscopy while the macroscopic changes in the mechanical properties were monitored by rheometry. The stimuli sensitive properties along with the high water content and good mechanical stability make disulphide cross-linked PASP hydrogels good candidates for human biological applications such as drug delivery systems and implants, as well as for further applications in which in situ gelation is beneficial. However, their drug release characteristics must be studied in more detail for future exploitation. Beside the proposed polymers, the synthetic method can be applied to various redox-sensitive gels for use as redox actuators in general applications.

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