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Effect of polymerization conditions on the network properties of dex-HEMA microspheres and macro-hydrogels

J.T. Chung[a], K.D.F. Vlugt-Wensink[b,c], W.E. Hennink[b], Z. Zhang[a,*]

[a] *Centre for Formulation Engineering, Chemical Engineering, School of Engineering, The University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK*
[b] *Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, P.O. Box 80082, 3508 TB Utrecht, The Netherlands*
[c] *Octoplus Technologies, OctoPlus b.v. Zernikedreef 12, 2333 CL, Leiden, The Netherlands*

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Abstract

Dextran-hydroxy-ethyl-methacrylate (dex-HEMA) hydrogels in the form of microspheres are an attractive system for the controlled delivery of protein drugs. In this work, the microspheres were prepared by a water-in-water emulsion polymerization process. The polymerization reaction was initiated by potassium peroxodisulfate (KPS) and catalyzed by *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED). The effect of the initiator concentration, reaction temperature and pH on the mechanical and network properties of the microspheres were investigated. The size and size distribution of the microspheres, equilibrium water content, and methacrylate conversion were also determined. The mechanical properties of single microspheres were measured by a micromanipulation technique and the rheological characteristics of the same material in the form of macroscopic hydrogel slabs were determined by a controlled stress rheometer. The results showed that the Young's moduli of the microspheres and of macroscopic slabs measured by these two methods were in good agreement. Higher KPS initiator concentrations resulted in a more rapid polymerization with a shorter gelation and lag time, and a higher Young's modulus of the gels. An increase in temperature also resulted in a more rapid polymerization with a shorter gelation and lag time. However, the Young's modulus of the gels decreased with an increase in polymerization temperature. The pH had no significant effect on the mechanical properties of the microspheres. This study demonstrates that the network properties of dex-HEMA hydrogels can be tailored by the polymerization conditions, which opens the possibility to modulate the release rate of entrapped compounds.

Keywords: Dex-HEMA hydrogel; Microsphere; Radical polymerization; Mechanical property; Network property; Micromanipulation.

1. Introduction

The progressive development in biotechnology has resulted in the availability of a great variety of pro-

* Corresponding author. Tel.: +44 121 414 5334; fax: +44 121 414 5324.
E-mail address: Z.Zhang@bham.ac.uk (Z. Zhang).

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tein drugs for therapeutic purposes. However, in general protein drugs have a short half-life after parenteral administration and they have a low bioavailability after oral administration. Therefore, there is an urgent need for suitable delivery systems for these relatively new therapeutic agents. Biodegradable hydrogels, hydrophilic polymeric networks that swell in water, but do not dissolve, are promising delivery systems for pharmaceutically active proteins (Kamath and Park, 1993; Peppas et al., 2000; Hennink and Van Nostrum, 2002). Because of their high water content, hydrogels are well compatible with protein drugs and, importantly, they possess a good biocompatibility with living tissues.

Dextran is a water-soluble bacterial exopolysaccharide consisting mainly of α-1,6-linked D-glucopyranose residues, and is an ideal polymer to form hydrogels due to its absent toxicity (Mehvar, 2000). In recent years, different dextran hydrogels have been developed. Dextran hydrogels can be formed by the coupling of (metha)acrylic acid to dextran, followed by radical polymerization (Edman et al., 1980; Van Dijk-Wolthuis et al., 1995). However, these hydrogels are not degradable under physiological conditions, and can only be degraded by dextranase (Edman et al., 1980; Franssen et al., 1999b). Therefore, a next generation of hydrogels with hydrolytically sensitive carbonate ester groups was developed: dex-HEMA, dextran derivatized with hydroxyethyl methacrylate (Van Dijk-Wolthuis et al., 1997a,b). It has been shown that by varying the DS (degree of substitution, the number of methacrylates per 100 glycopyranose residues) and the initial water content of the gel, the degradation rate of the hydrogels could be tailored from days to months (Van Dijk-Wolthuis et al., 1997c). Moreover, the feasibility to load injectable dex-HEMA microspheres with protein drugs was demonstrated. The preparation of these protein-loaded microspheres was achieved in an all aqueous environment (Franssen et al., 1999a; Stenekes et al., 2000b; De Groot et al., 2002). Dex-HEMA microspheres are formed by a polymerization reaction initiated by potassium peroxodisulfate (KPS) and catalyzed by *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED). It is therefore likely that the polymerization rate, which is in turn dependent on the polymerization conditions (initiator and catalyst concentration, temperature and pH) (Stenekes and Hennink, 2000a), may have a significant effect on network properties (crosslink density, pore size) of the microspheres. This can affect both the degradation rate of the microspheres and the release rate of an entrapped protein.

Information on the network properties of these particles may be inferred from their mechanical properties. However, there is a lack of direct mechanical characterization of such small particles (several micros in diameter) due to technical difficulties. Conventionally, an indirect method is to determine the mechanical properties of macroscopic hydrogel slabs by a rheometer (De Smedt et al., 1995; Meyvis et al., 2002). The method, however, assumes that the mechanical properties of the macroscopic hydrogel slabs are the same as those of microspheres and that the material is elastic. Recently, a novel micromanipulation technique has been developed, which is capable of directly measuring the mechanical properties of single particles ranging from 1 mm up to millimeter in diameter (Zhang et al., 1999; Sun and Zhang, 2001; Thomas et al., 2001). The feasibility of using this novel method to evaluate the mechanical properties of single dextran microspheres was demonstrated (Stenekes et al., 2000b). The objective of this work is to get insight into the network properties of dex-HEMA microspheres prepared under different conditions. The network properties (crosslink density, pore size) were derived from the mechanical properties as obtained by the micromanipulation technique. For comparison and verification, the rheological characteristics of dex-HEMA macroscopic hydrogel slabs were also determined.

2. Materials and methods

2.1. Materials

PEG 10,000, dextran T40, TEMED and KPS were obtained from Fluka (Buchs, Switzerland). Hydroxyethyl methacrylate derivatized dextrans (dex-HEMA) were synthesized and characterized according to Van Dijk-Wolthuis et al. (1997a). The DS (degree of substitution; the number of HEMA group per 100 glucopyranose units) was determined by proton nuclear magnetic resonance.

2.2. Preparation of microspheres

Dex-HEMA microspheres were prepared by a water-in-water emulsion method with a PEG10,000/

Table 1
Preparation conditions and the equilibrium water content, volume mean diameter and methacrylate conversion of dex-HEMA microspheres

Formulation	T (°C)	pH	$[KPS]_{dex}$ (mmol/l)	$[TEMED]_{dex}$ (mmol/l)	Equilibrium water-content (%)	Volume mean diameter (μm)	Methacrylate conversion (%) ± 1%
1	37	7	4.9	25.4	74.1 ± 0.1	6.2 ± 0.1	91
2	37	7	2.5	25.4	70.4 ± 1.0	6.4 ± 0.2	89
3	37	7	1.0	25.4	75.4 ± 2.5	5.4 ± 0.2	90
4	37	7	0.2	25.4	80.2 ± 1.2	5.3 ± 0.2	31
5	37	7	2.5	12.7	69.8 ± 0.1	7.0 ± 0.6	94
6	37	7	1.0	6.3	73.3 ± 0.7	11.0 ± 1.0	88
7	21	7	4.9	25.4	73.6 ± 1.0	5.0 ± 0.1	92
8	50	7	4.9	25.4	78.4 ± 2.0	6.2 ± 0.3	95
9	37	6	4.9	25.4	75.0 ± 0.7	4.5 ± 0.1	93
10	37	5	4.9	25.4	74.0 ± 0.6	4.8 ± 0.1	92
11	37	7	0.5	25.4	75.0 ± 0.6	5.2 ± 0.2	90

The values after the sign (±) in all tables represent two times of the standard error.

dextran phase volume ratio of 40/1 (Franssen and Hennink, 1998; Stenekes et al., 1998) using varying KPS and TEMED concentrations, polymerization temperature and pH (Table 1). The dex-HEMA used had a degree of substitution (DS) of 7. Different concentrations of KPS and TEMED in the dextran-enriched phase were prepared in 0.01 M phosphate buffer. Formulation 1 represents microspheres prepared under standard conditions (Stenekes et al., 1998). The concentration of KPS was varied in Formulations 2–4 and 11. Both the KPS and TEMED concentrations were varied in Formulations 5 and 6. The temperature (T) was varied in Formulations 7 and 8, and the pH in Formulations 9 and 10. For polymerization at 37 and 50 °C, a water and an oil bath were used, respectively. The polymerization at 21 °C was done at room temperature. Formulations 9 and 10 were prepared at pH 6 and 5, respectively (0.01 M acetate buffer). For each formulation, three batches of approximately 5 g (dextran and PEG phase) were independently prepared. The microspheres were freeze–dried in order to maintain their stability during storage and were rehydrated in 0.1 M acetate buffer pH 5 for 1 h before their mechanical properties were measured.

Dex-HEMA macroscopic hydrogel slabs were prepared by radical polymerization of aqueous solutions of dex-HEMA (Van Dijk-Wolthuis et al., 1997b).

2.3. Characterization of microspheres and macroscopic slabs

Each measurement was carried out in triplicate unless otherwise stated. The size and size distribution of dex-HEMA microspheres were measured by a laser light blocking technique (Accusizer™, model 770, Particle Sizing Systems, Santa Barbara, CA, USA). The microspheres were dispersed in reversed osmosis water. The equilibrium water content of the dextran microspheres was quantified by the blue dextran assay (Stenekes and Hennink, 1999). The methacrylate conversion was determined by HPLC analysis (Stenekes and Hennink, 2000a). In brief, 100 μl of the suspension of freshly prepared microspheres was added to 4.9 ml of 0.02 M NaOH. Next, 1 ml of 2 M acetic acid solution was added to convert the methacrylate anion into methacrylic acid. Hundred microlitres of this sample was injected onto a RP-18 column (Lichrosphere, Merck, Darmstadt, Germany). A degassed 90/10 reverse osmosis water/acetonitrile mixture that was adjusted to pH 2 with perchloric acid was used as the mobile phase with a flow rate of 1 ml/min. The chromatograms obtained were analyzed to quantify methacrylic acid in the sample.

The polymerization kinetics and rheological characteristics of macroscopic hydrogel slabs were quantified from a time-based polymerization or gelation profile obtained using an AR 1000-N controlled stress rheometer (TA Instruments, Inc., Ghent, Belgium), as described in Meyvis et al. (2002).

The mechanical properties of single microspheres were measured by a micromanipulation technique. The principle of this technique is to compress a single microsphere between two parallel surfaces to a given deformation. Simultaneously the force being imposed on the particle is measured by a force transducer. The details of this technique are described elsewhere

(Zhang et al., 1999; Sun and Zhang, 2001; Thomas et al., 2001). The size of each microsphere was measured from its microscope image. A probe with a flat end of 20 μm in diameter was connected to the force transducer (0.5 g, 403 Aurora Scientific Inc, Ont., Canada), which was set to travel at 2 μm/s. Thirty microspheres from each formulation were measured. The experiments were conducted at room temperature (21 °C).

3. Results and discussion

3.1. Size and equilibrium water content of microspheres

A typical volume-weighted size distribution of microspheres is presented in Fig. 1; the mean diameter is 6.2 μm (standard deviation 3.2 μm). The size distribution is Gaussian (chi-square test, 95% confidence). Table 1 shows that the volume mean diameter (D[3,0]) of the microspheres prepared under different conditions varied from 4.5 to 7 μm. Microspheres with such an average size can be administered to patients by injection. The microspheres made from Formulation 6 (mean diameter 11 μm) were significantly bigger than the others. This might result from coalescence of particles during the post-emulsion stabilization stage (Stenekes et al., 1998).

The equilibrium water content of the microspheres prepared under the different conditions varied between 70 and 75% (Table 1). However, the microspheres prepared at relatively low KPS initiator concentration (Formulation 4) had a higher equilibrium water content (∼80%) than the others. This is likely due to the low methacrylate conversion, which results in a relatively low hydrogel crosslink density and thus in a high swelling capacity.

3.2. Methacrylate conversion

It has been reported that an excessive amount of the initiator (KPS) may cause undesired oxidation of an entrapped protein (Cadée et al., 2001). Therefore, it is worth to establish the minimum amount of initiator that can result in a high degree of methacrylate conversion.

The methacrylate conversion after 30 min polymerization was approximately 90%, except for Formulation 4 (conversion 31%) which was prepared with a relatively low KPS concentration (Table 1). Obviously, at this low concentration, KPS is the limiting factor of the reaction. This is expected and coincides with the previous work of Stenekes and Hennink (2000a).

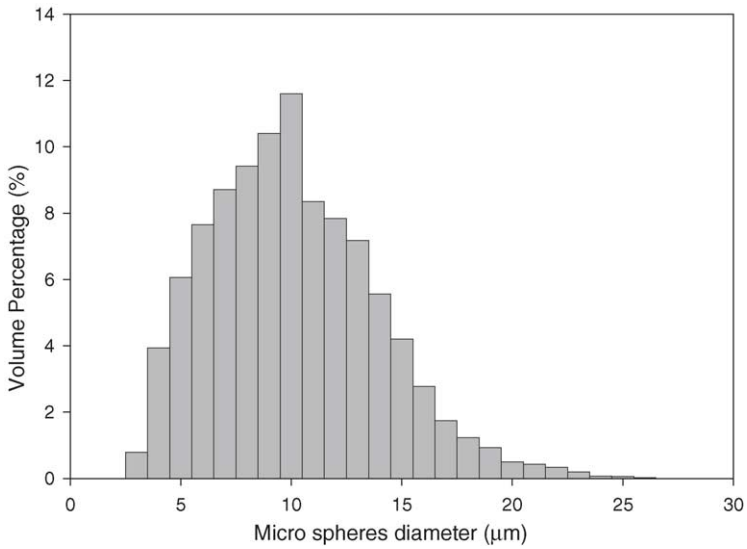


Fig. 1. A typical size distribution of dex-HEMA microspheres (Formulation 1).

3.3. Polymerization kinetics and rheological properties of hydrogel slabs

Three parameters can be extracted from the time-based gelation profile (typical examples are shown in Fig. 2), namely the final shear storage modulus (G'), the gelation time and lag time. G', taken from the plateau value of the kinetic curve, gives information on the stiffness of the hydrogel. The gelation time is arbitrarily defined as the time required for G' to increase from 10 to 90% of its plateau value, and the lag time is defined as the time required for G' to reach 10% of its plateau value. Conventionally, the gelation time is taken as time when G' equals the loss modulus G'' (Nijenhuis and Mijs, 1998) that reflects the viscous properties of the gels. However, this was not applicable for the dex-HEMA gels as it was observed that G' is much greater than G'' (data not shown), and consequently there is no obvious crossover point. As the purpose of the rheological study was to verify the elastic property of macro-gel and microspheres, only G' results are thus presented. Both the gelation and the lag time reflect the rate of the polymerization reaction. The shorter the gelation and lag time, the faster the polymerization reaction is.

For most of the formulations listed in Table 1, the outcomes from the gelation profile, G', gelation time and lag time were found to be similar to those of Formulation 1, prepared under standard conditions. However, there are several formulations that showed distinctive variations, which are summarized in Table 2.

The gelation profiles of Formulations 1, 4, and 11, which were prepared at different KPS concentrations, are presented in Fig. 2a. The results show that, as expected, with decreasing KPS concentration, the gelation proceeds more slowly. Fig. 2a also shows that the final G' for the gels prepared with 0.2 and 0.5 KPS mmol/l is significantly smaller than that of the gels prepared at KPS 4.9 mmol/l. The low G' of the gel prepared at 0.2 KPS mmol/l compared with the gel prepared at 4.9 mmol/l was likely caused by the low methacrylate conversion which in turn resulted in a low crosslink density. Interestingly, the gel prepared at 0.5 KPS mmol/l had the same final G' as the gel prepared at 0.2 mmol/l, despite its higher methacrylate conversion. Likely, the molecular weight of the polymerized methacrylate groups for the gel prepared at higher KPS (0.5 mmol/l) is lower than that of the polymerized methacrylate in the gel prepared at 0.2 mmol/l. A lower molecular weight decreases the chain functionality of the crosslinks. It has been reported that a lower chain functionality results in a lower Young's modulus of the gels (Barrhowell and Peppas, 1985). In the gel prepared at 0.2 mmol KPS, the lower methacrylate conversion might be compensated by a higher methacrylate molecular weight, resulting in a gel with nearly the same final G' as the 0.5 mmol KPS gel. However, the higher final G' of the gels prepared at 4.9 mmol/l KPS than 0.5 mmol is unexpected since both gels have the same methacrylate conversion (Table 1) and the higher KPS concentration should result in a lower molecular weight of the polymerized methacrylates corresponding to lower final G' (Barrhowell and Peppas, 1985). This discrepancy requires further investigation.

The effect of polymerization temperature on the gelation kinetics is shown in Fig. 2b. It can be seen that when the polymerization temperature was elevated from 21 to 50 °C, the time for G' to reach the plateau became shorter. This is logical since at higher temperature more radicals were formed which in turn caused a faster methacrylate conversion. It is very interesting to note that the hydrogel formed at 50 °C had a lower G' than observed for the gels formed at lower temperatures. An increase in reaction temperature increases the polymerization rate and decreases the polymer molecular weight (Odian, 1991). A lower molecular weight of the polymerized methacrylate groups reduces the junction functionality of the hydrogel network

Table 2
Gelation and rheological data of different dex-HEMA hydrogels

Formulation	T (°C)	$[KPS]_{dex}$ (mmol/l)	G' (MPa)	Gelation time (s)	Lag time (s)
1	37	4.9	0.12 ± 0.02	208 ± 16	11.7 ± 0.2
11	37	0.5	0.09 ± 0.01	408 ± 24	275 ± 15
4	37	0.2	0.09 ± 0.01	700 ± 155	468 ± 118
7	21	4.9	0.13 ± 0.02	545 ± 90	100 ± 0.4
8	50	4.9	0.07 ± 0.01	126 ± 12	4.2 ± 0.1

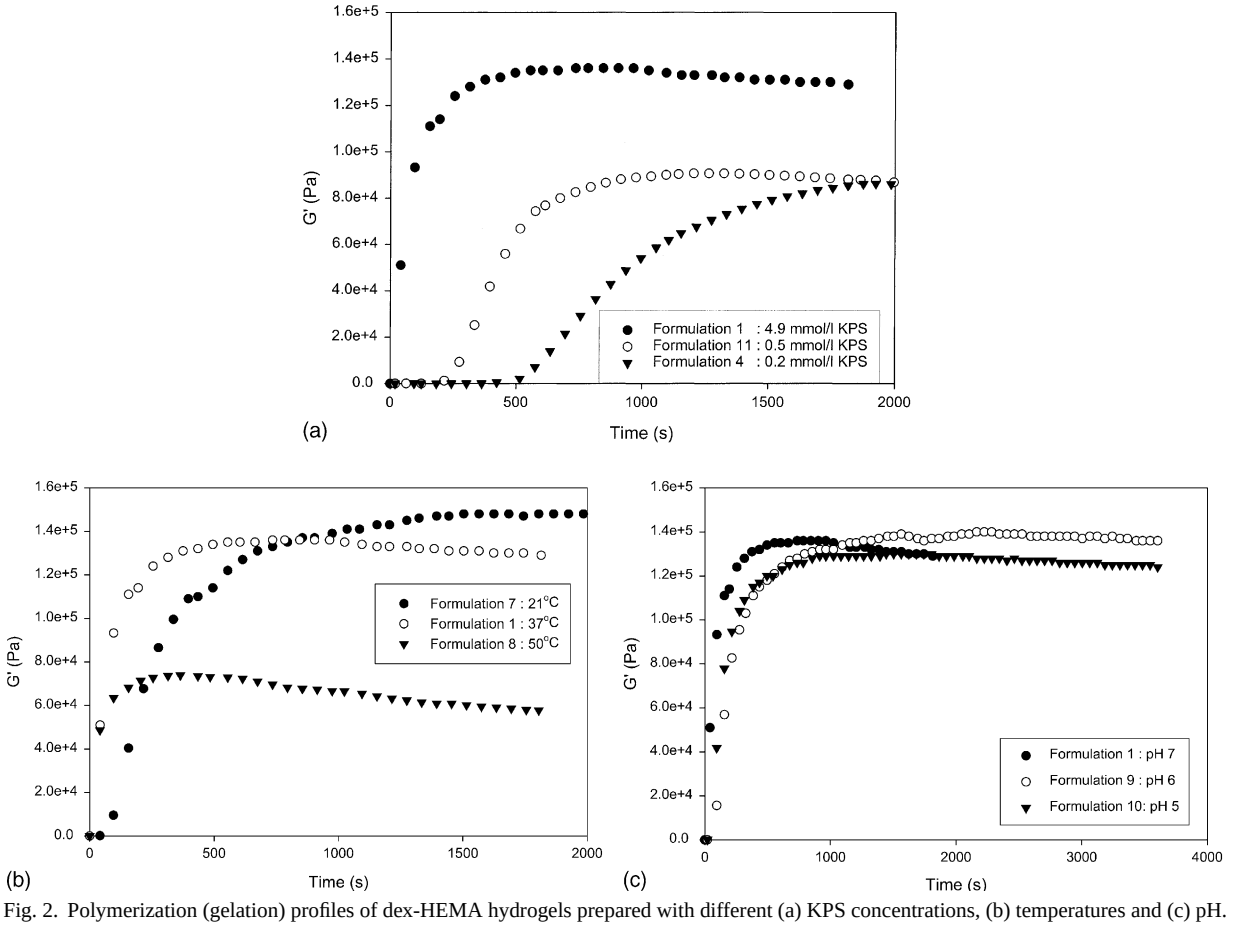


Fig. 2. Polymerization (gelation) profiles of dex-HEMA hydrogels prepared with different (a) KPS concentrations, (b) temperatures and (c) pH.

(Barrhowell and Peppas, 1985), which in turn results in a lower mechanical rigidity of the network.

It has been proposed that unprotonated TEMED catalyzes the decomposition of the peroxydisulfate anion into radicals (Feng et al., 1988). Hence the degree of protonation of TEMED (pK_as are 8.8 and 10.3 at 37 °C (Sigma, UK)), and thus the pH, may affect the polymerization rate. The polymerization was done at pH 5, 6 and 7. Since the concentration of the unprotonated TEMED (which is suggested to catalyze the decomposition of the peroxydisulfate anion) decreases by a factor 100 from pH 7 to 5, a substantial effect on the polymerization kinetics was expected. However, Fig. 2c shows that there was no significant effect of the pH on the gelation profile of the formulations, which strongly suggests that not the unprotonated TEMED, as proposed in the literature (Feng et al., 1988), but the $TEMEDH_2^{++}$ cation catalyzes the decomposition of the peroxydisulfate anion.

3.4. Mechanical characterization of microspheres by the micromanipulation technique

Fig. 3 shows a typical force versus displacement curve, obtained by the micromanipulation technique, for compressing a single hydrated dex-HEMA microsphere. The force transducer probe started to move downward at point A and touched the microsphere at B. The compression commenced immediately and the force started to increase until the microsphere underwent large deformation (point C).

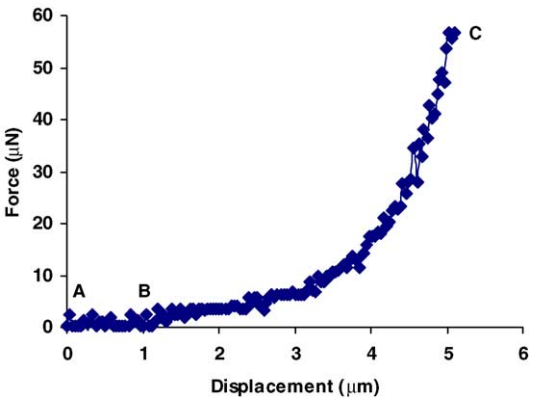


Fig. 3. A typical force versus displacement graph obtained from compression of a hydrated microsphere by micromanipulation. The diameter of the microsphere was 5.8 μm.

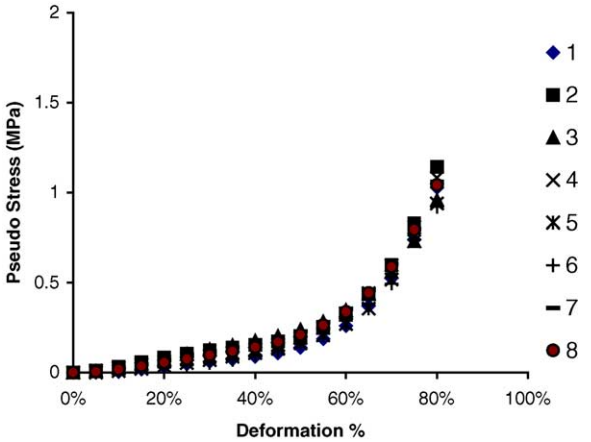
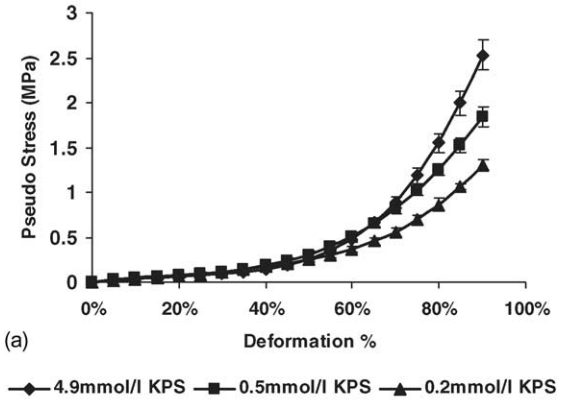


Fig. 4. Pseudo-stress versus deformation for repeatedly compressing a typical dex-HEMA microsphere (Formulation 1) 8 times.

It appears from the force–displacement curve in the region BC that the hydrated microsphere was only deformed under compression and did not show any rupture behaviour as the force profile does not show any sudden decrease with the displacement (Sun and Zhang, 2001; Stenekes et al., 2000b). In order to identify whether the microspheres are mainly elastic or elastic–plastic, single microspheres were compressed repeatedly and the results in pseudo-stress (the force divided by the cross-section area of each microsphere before it was deformed) versus deformation (the ratio between the displacement of the microsphere and its diameter) are presented in Fig. 4. As can be seen, all the curves overlap, which indicates that for the deformations investigated the dex-HEMA hydrogel microspheres are mainly elastic.

Micromanipulation was carried out on five microsphere samples which were prepared under different conditions, the corresponding hydrogel slabs of which showed significant variation in their G' values. The relationships between the pseudo-stress and deformation of microspheres are plotted in Fig. 5a and b. It can be clearly seen that the pseudo-stress for a given deformation of the microspheres increased with the KPS concentration, particularly at higher deformation (Fig. 5a). Fig. 5b shows that there is no significant difference in the relationship of the pseudo-stress versus deformation between the microspheres polymerized at 21 and 37 °C. However, when the temperature was increased to 50 °C, the microspheres formed were weaker.



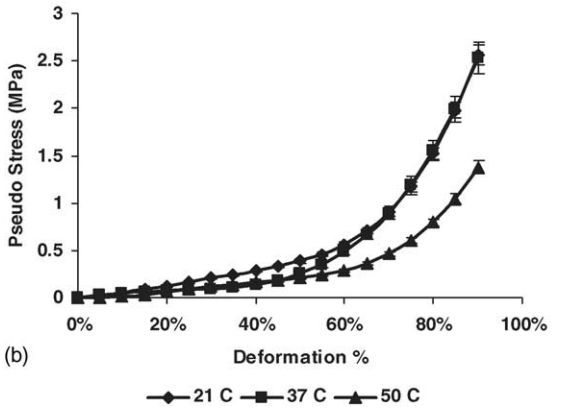


Fig. 5. Pseudo-stress versus deformation for compressing single microspheres made with different (a) KPS concentrations and (b) polymerization temperatures. The number of microspheres measured for each formulation is 30. The error bars represent two times of the standard error of the mean.

3.5. Young's modulus (E) of dextran hydrogel slabs and microspheres

From the rheological study, the Young's modulus (E) of macroscopic hydrogel slabs was calculated by $E = 3G'$ (Flory, 1953). From the pseudo-stress versus deformation profile obtained from micromanipulation measurements of single microspheres, the Young's modulus (E) of the microspheres was estimated using the initial slope of the curve (Stenekes et al., 2000b), as demonstrated in Fig. 6. The Young's moduli from both approaches are presented in Table 3. The table shows that the Young's moduli (E) of dex-HEMA hydrogels of the same composition in the form of slabs and microspheres are in good agreement, which indicates that the rheological measurements on macroscopic hydrogels have validated the micromanipulation method (in agreement with Stenekes et al., 2000b).

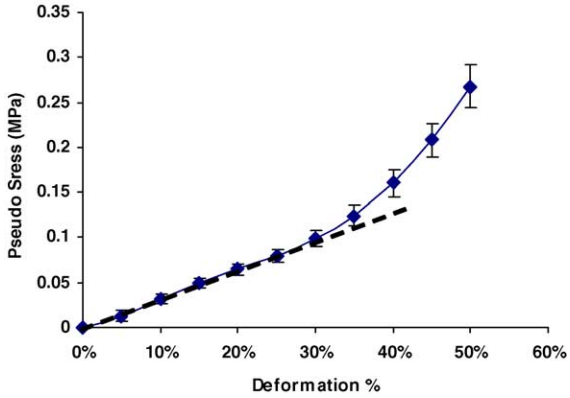


Fig. 6. Initial section of mean pseudo-stress versus deformation for compressing 30 microspheres of Formulation 1. Error bars indicate two times of the standard error of the mean. The dotted line indicates the initial slope of the profile.

3.6. Average molecular weight between crosslinks, crosslink density and pore size

The Young's modulus of the dex-HEMA hydrogels was used to estimate the average molecular weight between the crosslinks (M_c) and the effective crosslink density (ν_e). By assuming that the end-to-end distances of the chains are Gaussian, that the network deformation is isothermal and affine, and that dangling ends are absent, M_c was determined using Eq. (1) derived from the rubber elasticity theory (Flory, 1953; Ward and Hadley, 1993; Peppas et al., 2000):

$$M_c = \frac{3\rho RT}{E} \quad (1)$$

where ρ is the specific density (kg/m^3), R is gas constant, T is absolute temperature, and E is Young's modulus. The specific density of the dextran microspheres was calculated from the partial specific volume of dextran and their equilibrium water content (Stenekes et al., 2000b).

The effective crosslink density (ν_e) was calculated using the following equation (Flory, 1953; Uzun et al.,

Table 3
Young's moduli (E) obtained from micromanipulation measurements of hydrogel microspheres and rheological data of hydrogel slabs

Formulation	T (°C)	$[KPS]_{dex}$ (mmol/l)	Micromanipulation (microspheres) (E) (MPa)	Rheological data (hydrogel slabs) (E) (MPa)
1	37	4.9	0.45 ± 0.05	0.37 ± 0.06
11	37	0.5	0.28 ± 0.05	0.27 ± 0.04
4	37	0.2	0.24 ± 0.05	0.26 ± 0.04
7	21	4.9	0.53 ± 0.07	0.43 ± 0.06
8	50	4.9	0.24 ± 0.03	0.22 ± 0.03

Table 4
Calculated value of the density of dex-HEMA gels (ρ), average molecular weight between crosslinks (M_c), polymer volume fraction (v), effective crosslink density (ν_e) and pore size (ξ), at varying KPS initiator concentrations and polymerization temperatures

Formulation	T (°C)	$[KPS]_{dex}$ (mmol/l)	Density (ρ) (g/cm^3)	M_c (kg/mol)	$\nu_e \times 10^{-5}$ (mol/cm^3)	v	ξ (nm)
1	37	4.9	1.16	18.9 ± 2.1	6.1 ± 0.7	0.18	17.3 ± 1.9
11	37	0.5	1.15	30.1 ± 5.4	3.8 ± 0.3	0.17	22.2 ± 4.0
4	37	0.2	1.12	33.5 ± 6.9	3.3 ± 0.2	0.13	25.7 ± 5.3
7	21	4.9	1.16	15.9 ± 2.1	7.3 ± 0.8	0.18	15.9 ± 2.1
8	50	4.9	1.13	34.3 ± 4.3	3.3 ± 0.4	0.15	24.7 ± 3.0

2003):

$$\nu_e = \frac{\rho}{M_c} \quad (2)$$

The mean pore size of the hydrogel network of the microspheres (ξ) was estimated using the following formula (Peppas et al., 1985):

$$\xi = 0.071(v)^{-1/3} {M_c}^{1/2} \quad (3)$$

where v is the polymer volume fraction that can be derived from their water content (Peppas and Merrill, 1976).

The results of M_c and ν_e estimated for the microspheres made of the formulations described in Table 3 are shown in Table 4. From this table it appears that the lower the KPS concentration, the greater the M_c and thus the lower the crosslink density is. Further, with increasing polymerization temperature, M_c increases and the crosslink density decreases. The mean pore sizes of the different microspheres are also summarized in Table 4. In agreement with the M_c data, the calculated mean pore size decreased with increasing KPS concentration but increased with the polymerization temperature. Overall, the average pore size of the dex-HEMA hydrogel is slightly greater than that of the dex-MA hydrogels (Stenekes et al., 2000b). However, it should be pointed out that the calculations were done for an ideal network. The dextran networks are certainly not ideal. Therefore, the M_c/pore size data presented can only be used in a relative sense.

4. Conclusions

This study shows that single dex-HEMA microspheres are mainly elastic up to a deformation of 80%. The Young's moduli of the different microspheres derived from the micromanipulation data, were consistent with those from the rheological data. Increased KPS initiator concentrations caused a faster polymerization rate, and the formed hydrogel was stronger (greater G' or E value), had a smaller molecular weight between crosslinkings (M_c) and a smaller pore size. However, although an increase in polymerization temperature also led to a faster reaction rate, the formed hydrogel was weaker, had a larger molecular weight between crosslinkings (M_c) and a bigger pore size. The pH of the polymerizing solution did not have a significant effect on both the polymerization rate and the hydrogel network properties.

This study shows that the network characteristics are dependent on the polymerization conditions. These altered network properties likely results in different re-

lease profiles of entrapped proteins, which is the subject of present investigations.

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