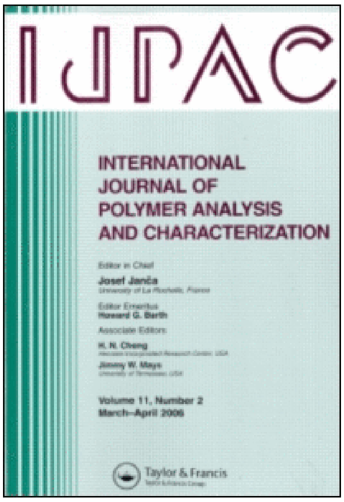

International Journal of Polymer Analysis and Characterization
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713646643

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To cite this Article Spychaj, T. , Bartkowiak, A. and Hunkeler, D.(1995) 'Metal-containing Hydrogels Based on Polysaccharides I. Dextran/epichlorohydrin/Mg 2', International Journal of Polymer Analysis and Characterization, 2: 1, 1 — 8

To link to this Article: DOI: 10.1080/10236669508233890

URL: http://dx.doi.org/10.1080/10236669508233890

Int. J. Polymer Analysis & Characterization
1995, Vol. 2, pp.1–8
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Printed in Malaysia

Metal-containing Hydrogels Based on Polysaccharides
I. Dextran/epichlorohydrin/Mg^{+2}

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(Received July 29, 1994; in final form November 30, 1994)

Magnesium-containing dextran hydrogels with spherical particle shape have been synthesized and characterized. Two types of cross-links create the gel structure: the first, strong covalent bridges formed by reaction of glycosidic hydroxyls with epichlorohydrin, and second, weak coordination links between Mg^{+2} cations and residual OH groups of dextran. Magnesium was introduced to the gel during their preparation. Dextran gels with Mg content up to 20 mg/g of dextran, evaluated by EDTA complexation method, have been characterized using inverse size exclusion chromatography (SEC) and scanning electron microscopy. Comparison of gel swellabilities in water revealed differences dependent on the metal content, with a minimum value for the hydrogel containing ca. 4 mg Mg/g. A strong influence of the Mg^{+2} content on shifts of SEC poly(ethylene glycol) calibration toward higher elution volumes was observed.

KEY WORDS Dextran/epichlorohydrin/Mg^{+2} hydrogels, SEC, SEM

INTRODUCTION

Polysaccharides bearing hydroxyl groups are often applied as base polymers for the preparation of gels cross-linked with covalent linkages by reaction with bifunctional reagents, for example epichlorohydrin or by complexation with polyvalent metal cations. The products of the reaction between dextran and epichlorohydrin are well known and widely applied as hydrogels in biochemistry, medicine, and used as porous packings for various kinds of chromatography (e.g., size exclusion or ion-exchange)[1]. These gels were introduced by Pharmacia Fine Chemicals under trade name Sephadex ca. thirty years ago [2,3].

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Presented at the 7th International Symposium on Polymer Analysis and Characterization, Les Diablerets, Switzerland, May 24–26, 1994.

Similarly, dextran/polyvalent metal complexes have been investigated and applied in various areas. The main field of applications of such complexes, especially those of iron, are found in medicine, for example, as magnetic carriers for diagnosis or cure agents, and for biological separations[4–9]. Polyvalent metal complexes with dextran may be used for visual markers in electron microscopy[10]. For the latter applications, submicron particles are typically used. The complexation ability of dextran plays an important role for the sorption of polyvalent metal compounds from aqueous solutions[11–14]. Nevertheless, the characteristics of hydrogels created by both covalent and complex networks have been unsatisfactory investigated. This paper presents results of investigation of hydrogels obtained from dextran and epichlorohydrin in the presence of magnesium sulfate.

EXPERIMENTAL

Materials and Gel Preparation

Dextran with a weight average molecular weight ca. 15,000 was obtained from "Polfa" Pharmaceutical Works (Kutno, Poland). Aqueous solutions $MgSO_4 \cdot 7H_2O$ (pure reagent, P.O.Ch. Gliwice, Poland) were prepared by dissolving the salt in sodium hydroxide solution.

The gel preparation procedure was as follows: dextran was dissolved in an aqueous alkali salt solution, and was dispersed in poly(vinyl acetate) (M. W. about 45,000, BDH Chemicals Ltd, Poole, England) containing toluene and cross-linked with epichlorohydrin (pure reagent, VEB Jenapharm-Labochemie, Apolda, Germany) at 50°C[15]. Concentration of poly(vinyl acetate) in toluene solution was kept constant 4 wt %. The ratio of epichlorohydrin to dextran was varied from 1.5–2.25/1 mol/mol. Concetration of $MgSO_4 \cdot 7H_2O$ was changed from 5 to 40 wt % in relation to dextran (i.e., 33–263 mequiv. Mg/mol of dextran). The products were filtered, washed with toluene, then with mixtures of toluene and methanol, and finally with methanol.

Magnesium Content Determination

The gel was ashed by combustion of 0.5 g at 600°C for 4 h. The ash was suspended in 100 cm^3 of 0.01 M H_2SO_4, buffered to pH 10 and then titrated with 0.01 M EDTA solution using lead chromate steam black indicator.

Size Exclusion Chromatography

Inverse size exclusion chromatography was used for pore size characterization of the gels using water as the eluent. Knauer HPLC instrument was equipped with a glass high-pressure column (300 mm in length, 5-mm i.d.). Spherical shape particles (dry size fraction of 150–250 μm) were swelled in water and gravimetrically packed into the column. Flow rate of an eluent was kept 0.5 cm^3/min. A differential refractometer was used for the detection poly(ethylene glycol) standards. Poly(ethylene glycol) samples with $M_w/M_n < 1.20$ of various origin were applied as standard solutions.

Scanning Electron Microscopy

SEM was applied for visualization and comparison of the particular gel particles (ComScan Series-4, Cambridge Scanning Co. Ltd.). Dry particles of a given gel coated with gold under vacuum were used for SEM analysis.

RESULTS AND DISCUSSION

Creation of the porous structure in the gel system, dextran/epichlorohydrin/aqueous NaOH/$MgSO_4 \cdot 7H_2O$, is based on the interaction between dextran hydroxylic groups and epichlorohydrin, as well as magnesium cation with hydroxyls. Strong covalent ether-type bridges are formed in effect by OH groups reaction with epichlorohydrin. An interpenetrating complex type of network, resulting from the coordination between magnesium cations and oxygen atoms of the surrounding hydroxyls of the polysaccharide, is rather weak in comparison with covalent cross-links[16].

Three different concentrations of the covalent cross-linker were used for gel syntheses (Table I). Standard ratio of epichlorohydrin to dextran 1.5/1 mol/mol corresponding theoretically to the reaction of 50% OH groups in covalent cross-linking, was selected to investigate the influence of magnesium salt concetration on hydrogel properties. Table I presents data on the Mg content in dry gels in comparison with the theoretical amount of Mg introduced to the system during preparation. Effectiveness of Mg complexation and swellability for the gels are included in Table I as well.

Two-component gels without magnesium swell in water in proportion to the molar ratio of the components (4.3-3.6 cm^3/g, respectively for 1.5/1–2.25/1 mol/mol epichlorohydrin/dextran). These data are in good agreement with corresponding Sephadex G gels G25-G15[1].

Introduction of magnesium into dextran gel changed its structure and properties. The concentration range of Mg^{+2} salt added to hydrogels was varied from ca. 33–263 m equiv.

TABLE I

Characteristics of dextran/epichlorohydrin hydrogels synthesized in the presence of $MgSO_4 \cdot 7H_2O$

Sample	Cocentration of Mg^{+2} salt, wt %/dextran	Mg added mequiv./mol	Mg added mg/g	Mg determined mequiv./mol	Mg determined mg/g	Effectiveness of Mg complexation %	Swellability in water bed volume cm^3/g
		epichlorohydrin/dextran 1.5/1 (mol/mol)					
1	0	0	0	0	0	—	4.3
2	5	32.9	4.9	27.5	4.1	84	3.5
3	10	65.8	9.9	44.7	6.7	68	4.5
4	20	131.5	19.7	67.1	10.1	51	5.5
5	30	197.2	29.6	96.9	14.5	49	5.0
6	40	262.9	39.4	131.8	19.8	50	6.0
		epichlorohydrin/dextran 1.875/1 (mol/mol)					
7	0	0	0	0	0	—	3.8
8	5	32.9	4.9	23.0	3.5	70	3.4
		epichlorohydrin/dextran 2.25/1 (mol/mol)					
9	0	0	0	0	0	—	3.6
10	5	32.9	4.9	21.5	3.2	66	3.3

Mg/mol of dextran (Table I). The level of the complexed metal in some gels was found to be in the range of 28–132 m equiv. Mg/mol of dextran, respectively. Effectiveness of Mg^{+2} complexation was found to be ca. 84% for the lowest magnesium sulfate concentration (sample 2, Table I) to ca. 50% for higher levels of the salt introduced to the system (samples 4,5 and 6). If the concentration of residual hydroxyl groups after covalent cross-linking decreased, the amount of Mg in the gel structure was also decreased (respectively, samples 2,8 and 10 in Table I).

Very specific and rather suprising swellability behavior of the hydrogels was observed. Gel 2, prepared using 5 wt% of $MgSO_4 \cdot 7H_2O$, was found to have the minimum bed volume (3.5 cm^3/g, i.e., 81% of the bed volume as compared to gel 1). A further increase of Mg salt concentration applied to the gels cross-linked with the same concentration of epichlorohydrin caused a gradual increase of particle swellabilities (samples 2–6 in Table I).

To characterize the porous structure of the hydrogels, inverse SEC was used. Figure 1 presents the SEC calibration dependency $\log M = f(V_e)$ (where M is the molecular weight, and V_e is the elution volume) for two-component hydrogels: dextran/epichlorohydrin (see Table I, gels 1,7 and 9), together with gels containing lower amounts of magnesium (gels 2,8 and 10). In general, V_o values and overall calibration shift to higher elution volumes with increased degree of cross-linking via covalent, as well as coordination links (Figs. 1 and 2).

Ideal size exclusion chromatography is expressed by

$$V_{eo} = V_o + K_D \cdot V_i \tag{1}$$

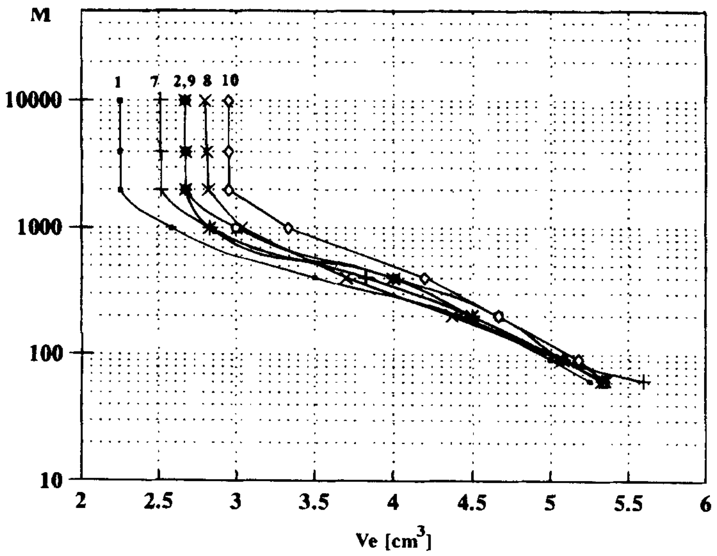

FIGURE 1 Inverse SEC poly(ethylene glycol) calibrations for dextran/epichlorohydrin hydrogels with various covalent cross-link densities (samples 1,7 and 9); and dextran/epichlorohydrin/Mg^{+2} gels with a constant $MgSO_4 \cdot 7H_2O$ concentration (5 wt %) and respectively different epichlorohydrin concentrations (gels 2,8 and 10), (see Table I).

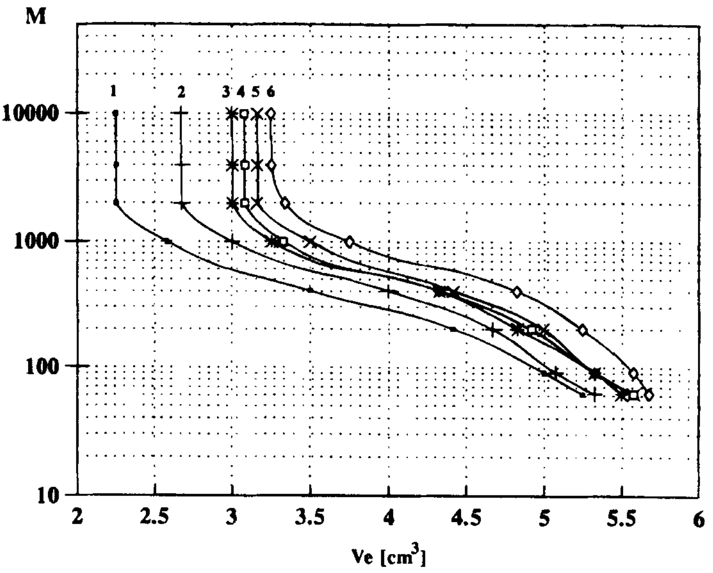

FIGURE 2 Inverse SEC poly(ethylene glycol) calibration for dextran/epichlorohydrin (1) and dextran/epichlorohydrin/Mg^{+2} hydrogels with different magnesium content (2,3,4,5 and 6) and a constant ratio of epichlorohydrin/dextran 1.5/1 mol/mol. (see Table I).

where V_o is the interstitial volume, K_D is the solute distribution coefficient ($0 \leq K_D \geq 1$), V_i is the volume of an eluent inside the gel pores, and V_{eo} is the elution volume for a given solute under ideal size exclusion conditions. In cases where nonexclusion phenomena play a role, a modified equation is applied[17]:

$$V_e = V_o + K_D \cdot K_p \cdot V_i \tag{2}$$

where K_p is the distribution coefficient reflecting interactions between solute and gel. In the first approximation, one can assume that for the same ratio of dextran/epichlorohydrin-gel particles prepared without or in presency of different Mg salt concentration should be similar in pore sizes (i.e., V_0 and V_i values are respectively the same). Using this assumption, one can evaluate the influence of complexed Mg cation on nonexclusion effects in size exclusion separation of poly(ethylene glycol) solutes.

Significant shifts of the calibration curves for magnesium-containing gels, as compared with that for standard dextran/epichlorohydrin hydrogel (1/1.5 mol/mol) (Fig. 2), may have been caused by solute-gel interactions. Table II presents SEC calibration data measured for poly(ethylene glycol) standards together with calculated K_p coefficients, assuming the separation on the standard dextran/epichlorohydrin gel was governed by a pure size exclusion mechanism. Figure 3 gives the relation between K_p and Mg content in the hydrogels.

Results of the SEC analysis and calculated K_p values demonstrate that higher content of magnesium in the gels causes an increased degree of nonexclusion, that is sorption/complexation effects during PEG separation (e.g. $K_p = 2 \rightarrow 4$ for $M_{PEG} = 1000$).

The gel particles are spherical as seen from Figure 4. SEM revealed increasing shape and surface defectiveness of the particles with increased level of magnesium. For exam-

TABLE II

SEC poly(ethylene glycol) calibration data for dextran/epichlorohydrin/Mg^{+2} hydrogels. K_p values were calculated from Equation (2) using $V_0 = 2.25$ cm³ and $V_i = 5.25$ cm³

Sample	M_{PEG}, *g/mol*	V_e, cm3	K_D	K_p
1	1000	2.6	0.07	1.0
	400	3.5	0.24	1.0
	200	4.4	0.41	1.0
	106	5.0	0.52	1.0
2	1000	3.0	0.07	2.04
	400	4.0	0.24	1.39
	200	4.65	0.41	1.11
	106	5.1	0.52	1.04
3	1000	3.25	0.07	2.72
	400	4.3	0.24	1.63
	200	4.8	0.41	1.18
	106	5.3	0.52	1.12
4	1000	3.3	0.07	2.86
	400	4.3	0.24	1.63
	200	4.9	0.41	1.23
	106	5.3	0.52	1.12
5	1000	3.5	0.07	3.40
	400	4.4	0.24	1.71
	200	5.0	0.41	1.28
	106	5.3	0.52	1.12
6	1000	3.75	0.07	4.08
	400	4.8	0.24	2.02
	200	5.25	0.41	1.39
	106	5.6	0.52	1.23

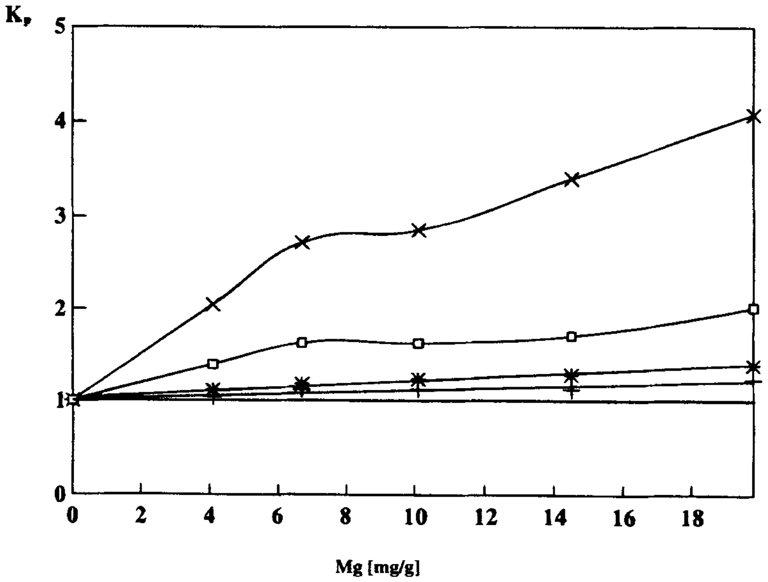

FIGURE 3 Dependence between K_p distribution coefficient and magnesium content in the dextran/epichlorohydrin Mg^{+2} hydrogels: x: $M_{PEG} = 1000$; □: $M_{PEG} = 600$; *: $M_{PEG} = 200$; +: $M_{DEG} = 106$.

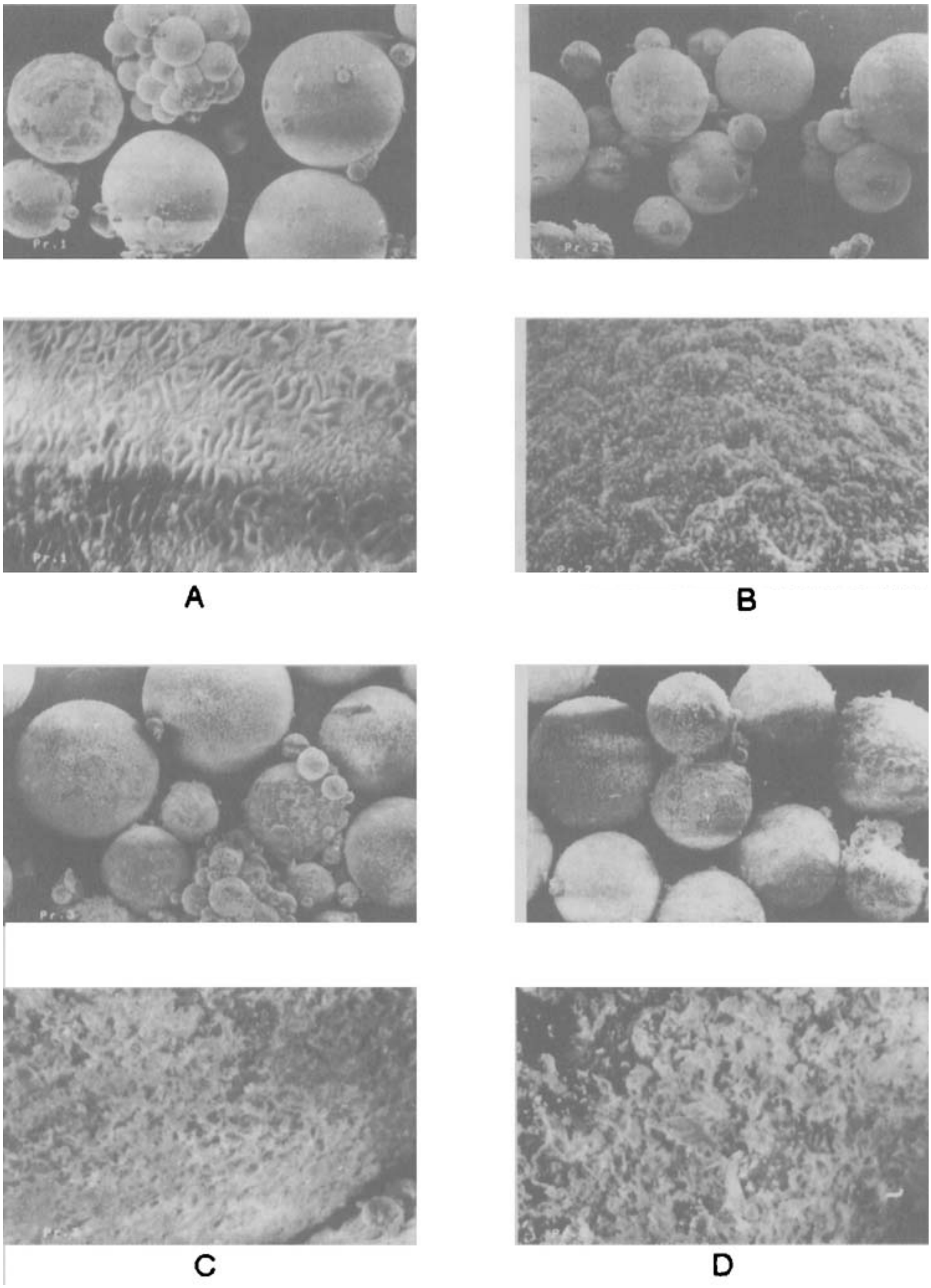

FIGURE 4 Two magnifications of SEC photomicrographs for selected dextran/epichlorohydrin (1/1.5 mol/mol)/Mg^{+2} hydrogel particles: without Mg^{+2} (A) (sample 1 from Table I) and prepared in the presence of 5 (B), 10 (C) and 30 (D) wt % of $MgSO_4 \cdot 7H_2O$, respectively, samples 2,3 and 5 from Table I.

ple gel sample 10 prepared using 40 wt % of magnesium salt (Table I) was nonspherical. Visible differences in surface coarseness which increased with higher magnesium content in the hydrogels are evident at higher SEM magnification.

CONCLUSIONS

Spherically shaped composite hydrogels were synthesized with magnesium content ranging from ca. 28–132 mequiv. Mg/mol of dextran (4–20 mg Mg/g). Mg coordinatively linked with OH groups of the covalently cross-linked dextran caused substantial changes of the hydrogel properties. Minimum swellability in water for the three component gel (dextran/epichlorohydrin/Mg^{+2}) was found for the material containing ca. 4 mg Mg^{+2}/g. Poly(ethylene glycol) solutes separated on the composite gels are retarded longer in an SEC column in comparison with those separated on the two-component dextran/epichlorohydrin standard hydrogel. A correlation was found between the distribution coefficient K_p, which reflects nonexclusion phenomena during SEC separation, and Mg content in the gel.

SEM confirmed the spherical shape of the particles and revealed differences in particle surface coarseness. With increasing content of magnesium, dry gel particles contained more defects and their surface resembled an inorganic "ceramic-type" appearance. For the highest investigated Mg level (20 mg Mg/g), hydrogel particles lost spherical shape.

Potential area of application of the investigated materials may be for controlled Mg delivery from the hydrogel, e.g:, for medical purposes.

Acknowledgments

The authors appreciate collaboration of Professor A. Bledzki and Mr. K. Goracy from the Institut für Werkstofftechnik, Universität—Gesamthochschule Kassel, Germany, for the SEM analysis of the gel particles, and the Polish State Committee for Scientific Research for partial support of the work (grant 7520402806).

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