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ORIGINAL CONTRIBUTION

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Multifold curdlan gel formation by dialysis into aqueous solutions of metal salts

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Abstract We have found that the dialysis of curdlan dissolved in alkaline solution into aqueous solutions of metal salts yielded multifold gel structures. Aqueous sodium chloride and potassium chloride as well as pure water induced isotropic gels. Aqueous calcium salts induced liquid crystalline gel with refractive index gradient/amorphous gel alternative structure. Aqueous salts of trivalent aluminum and ferric cations induced a rigid liquid crystalline gel, which shrank above a threshold concentration of each salt. On the other hand, Liesegang ring-like pattern was observed with aqueous solutions of mixed salts of calcium chloride and magnesium chloride. The patterns have been classified to discuss the mechanism of forming the variety of structures.

Keywords Curdlan · Liquid crystalline gel · Refractive index gradient · Liesegang ring · Dialysis

Introduction

Historically, in colloid science, the researchers have often brought up the issue of apparently complicated phenomena concerning the interfaces between various phases and have discovered simple principles. Among them, dialysis is one of the oldest subjects in colloid science. Most studies on dialysis have been performed in dilute solutions where the ideal gas approximation could be safely applied. In concentrated polymer solutions, however, we might find a treasure of issues of complex phenomena. In this report, we pick up a structure formation resulted from an interaction of a biopolymer and kinds of ionic solutions at the interface of a dialysis tube.

Curdlan, a polysaccharide consisting of β-1,3-glucan has been used as a thickener and a stabilizer in food engineering and construction engineering, and as an anti-cancer drug in pharmaceutics and clinics [1–4]. Curdlan is known to form gels induced by heat and calcium ions [5–10]. Recently, we found that the calcium-induced gel prepared with a dialysis consists of alternative layers of liquid crystalline gel with refractive index gradient and amorphous gel (LCG/AG) [11, 12]. This new material could be used as an optical component such as a polarizer and an indicator. In a preparative study, we found the gel structure pattern varies depending on the thermodynamic variables such as the species and concentrations of the extradialytic solution.

It is very interesting to investigate the condition of the multifold gel formation. Comprehensive results and discussion on the basis of the data obtained in a broad range of thermodynamic conditions could enable us to design gels with specific characteristics.

In this paper, we report newly found multifold gels prepared from curdlan solutions using different combinations of intradialytic solutions of curdlan dissolved in various alkaline solutions or dimethylsulfoxide and extradialytic solutions of water, salts of mono-, di-, and tri-valence cations or urea aqueous solution.

Experiment

Curdlan and metal salts were purchased from Wako Pure Chemicals. The molecular weight of curdlan was determined as 5.9×10^5 by a viscosity measurement in a previous paper [11]. Reagent grade sodium and potassium hydroxide, metal salts, dimethylsulfoxide and urea, and Milli-Q water were used for sample preparation. Table 1 shows the combinations of intradialytic solutions and extradialytic solutions used in this study.

In the systems I-0 to I-3, the intradialytic solutions are fixed to 5 wt% curdlan in 0.3 M aqueous sodium hydroxide, and in systems II-1 and II-2, the extradialytic solutions are fixed to 0.45 M aqueous calcium chloride. In the system II-2, aluminum chloride aqueous solution was also examined as the extradialytic solution. Gels were prepared by a dialysis with using a seamless cellulose tubing with the diameter of 28.6 mm purchased from Sanko Junyaku Co. Ltd. The volumes of the intradialytic and extradialytic solutions were 25 and 300 ml, respectively, otherwise indicated by brackets in Table 1. The dialysis was carried out at room temperature of 25°C for 48 h except that the extradialytic solution was exchanged twice and the total dialysis time was 6 days for the system I-1.

A round slice of the gel with the thickness of 3 mm was excised out perpendicularly to the long axis of the dialysis tubing. The gels were observed under both natural light and crossed nicols, the photographs of which were taken by a digital camera and they were analyzed by an image analyzing software (Image Pro, Planetron) to determine the shrinking ratio *R*, defined as the ratio of the difference between the cross-sectional area of the gel S_g and that of the original solution S_0 to S_0; $R \equiv (S_0 - S_g)/S_0$.

Results and discussion

Figures 1 and 2 show the typical photographs observed under crossed nicols except for Fig. 1 [f], [f′], [f″] and Fig. 2 a, b and c, which were observed under natural light. From these figures the structure of the gels is roughly classified into the following seven patterns.

Pattern A (system I-3): No gelation and liquid crystal formation were observed in urea solution (not shown in Fig. 1).

Pattern B (System I-0 and I-1): Isotropic gels were observed in pure water and aqueous solutions of monovalence alkaline salts, as shown in Fig. 1 a and b.

Pattern C (System I-2a, I-2a′, II-1): LCG/AG alternative layers appeared in all the gels prepared in aqueous solutions of typical water-soluble calcium salts of chloride, nitrate and acetate, as shown in Fig. 1 (c1–c3). In the process of dialysis of curdlan dissolved in aqueous sodium hydroxide into aqueous calcium chloride, the outflux of hydroxide anions changes the conformation

Table 1 Intradialytic and extradialytic solutions of the system

System	Intradialytic fluid	Extradialytic fluid	Fig.No.
I – 0	5 wt% Curdlan0.3 M NaOH	H_2O	1a
I – 1	5 wt% Curdlan0.3 M NaOH	0.45 M NaCl [500 ml]0.45 M LiCl [500 ml]0.45 M KCl [500 ml]	1b
I –2a	5 wt% Curdlan0.3 M NaOH	0.18 M~1.08 M $CaCl_2$, $Ca(CH_3COO)_2$, $Ca(NO_3)_2$	1c
I – 2a	5 wt% Curdlan0.3 M NaOH	0.45 M $CaCl_2$, 1 M Urea0.45 M $CaCl_2$, 0.01 M Citric acid	
I – 2b	5 wt% Curdlan0.3 M NaOH	0.18 M~1.08 M $MgCl_2$, $SrCl_2$, $BaCl_2$, $CuCl_2$, $FeCl_2$, $CoCl_2$, $MnCl_2$, $CdCl_2$	1d
I – 2c	5 wt% Curdlan0.3 M NaOH	0.10 M~1.08 M $AlCl_3$, $FeCl_3$	1e
I – 2d	5 wt% Curdlan0.3 M NaOH	0.10 M~1.08 M $CaCl_2$ +0.10 M~1.08 M $MgCl_2$ 0.10 M~1.08 M $Ca(NO_3)_2$ +0.10 M~1.08 M $MgCl_2$ 0.10 M~1.08 M $Ca(CH_3COO)_2$ +0.10 M~1.08 M $MgCl_2$	1f
I – 2e	5 wt% Curdlan0.3 M NaOH	0.18 M $CaCl_2$ +0.18 M $AlCl_3$ 0.18 M $MgCl_2$ +0.18 M $AlCl_3$ 0.45 M $CuCl_2$ +0.18 M $AlCl_3$	2
I – 3	5 wt% Curdlan0.3 M NaOH	1 M Urea	
II – 1	5 wt% Curdlan0.3 M KOH	0.45 M $CaCl_2$	
II – 2	3wt% Curdlan 0.12 M LiCl in DMSO [50 ml]	0.45 M $CaCl_2$, 0.45 M $AlCl_3$	1g

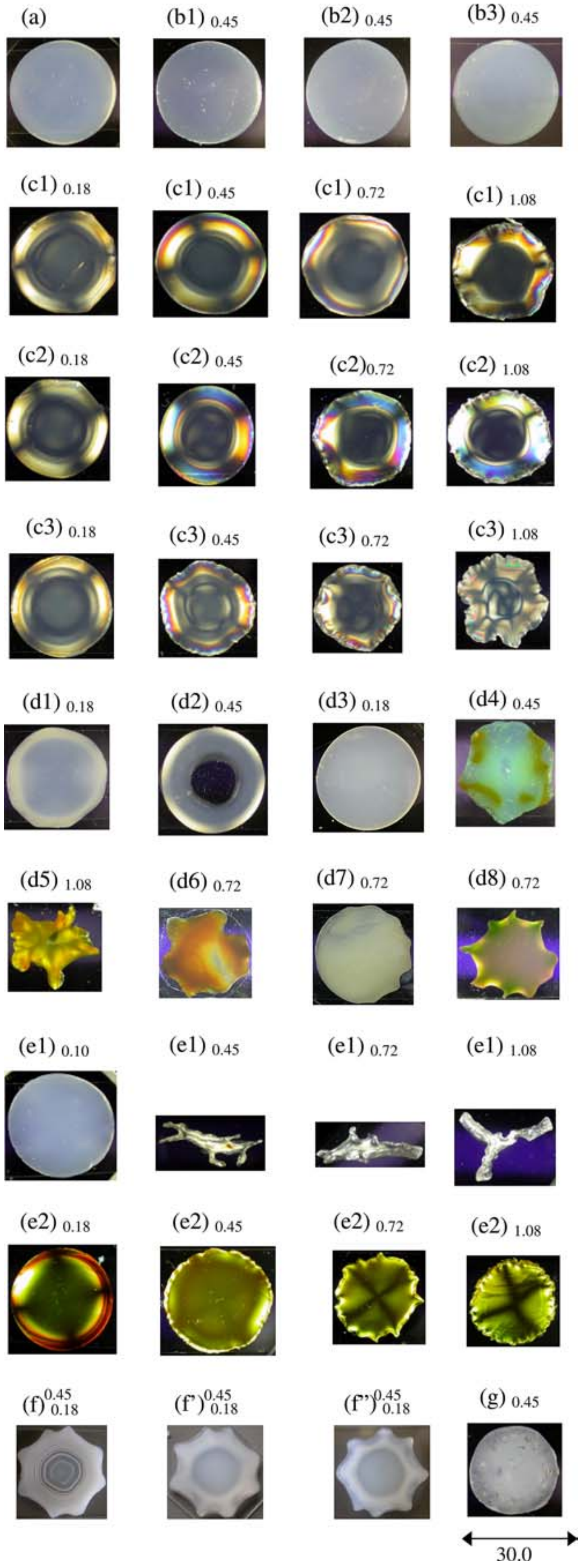

Fig. 1 Photographs of curdlan gel observed under different preparation conditions. The extradialytic solution of **a, b1, b2, b3, c1, c2, c3, d1, d2, d3, d4, d5, d6, d7, d8, e1, e2** and **f** are water, aqueous LiCl, NaCl, KCl, $CaCl_2$, $Ca(NO_3)_2$, $Ca(CH_3COO)_2$, $MgCl_2$, $SrCl_2$, $BaCl_2$, $CuCl_2$, $FeCl_2$, $MnCl_2$, $CdCl_2$, $CoCl_2$, $AlCl_3$, $FeCl_3$, mixed salts of 0.45 M$CaCl_2$ and 0.18 M$MgCl_2$, respectively. **f′** and **f″** are the photographs of the curdlan gel prepared by immersion of the gel shown in **f** into 5.5 M NH_4Cl and 26 mM EGTA, respectively. The photographs of **f**, **f′** and **f″** were observed under natural light and others were observed under crossed nicols. The *decimals behind the bracket* denote the molarity of the salt. **g** is the photograph of the gel made from the curdlan dissolved in dimethylsulfoxide (DMSO). The *scale* is shown in mm

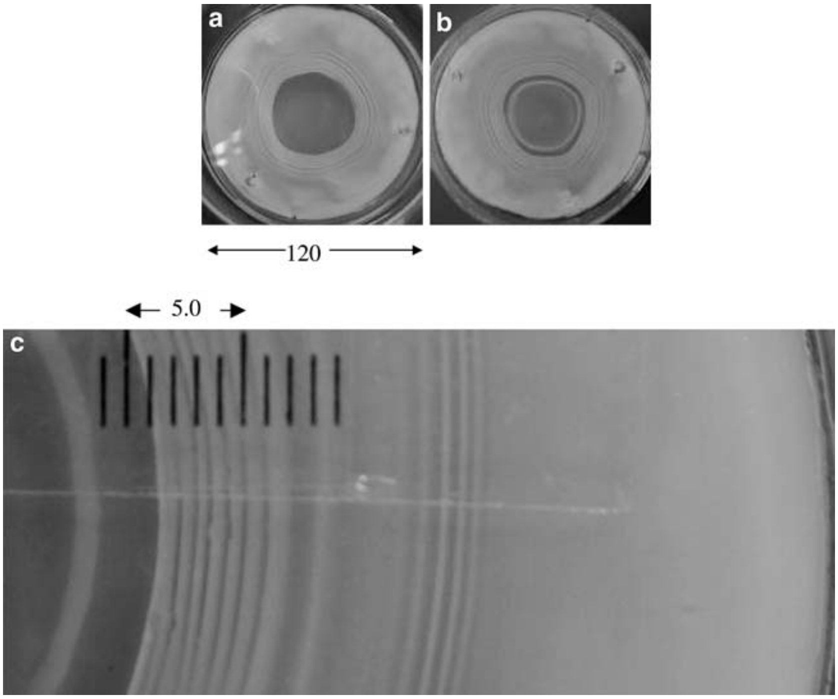

Fig. 2 Curdlan gels for the extradialytic aqueous solution of mixed salts of 0.45 M $CaCl_2$ and 0.18 M $MgCl_2$ observed under natural light for the dialysis time of 8 days (**a**) and 14 days (**b**). **c** shows an enlarged photograph of **b**. The scale is shown in mm

of the curdlan molecules from random coil to triple helix due to the pH change and the influx of calcium cations cross-links the helical curdlan molecules inter-molecularly. This flow process combined by a steric hindrance of the mesogens consisted of triple helix-dominant domains results in LCG/AG, that is, the increase in rigidity of each curdlan molecule induced by lowering pH prevent from ordering [10]. The shrinking ratio *R* was larger in the order of acetate, nitrate and chloride. The ratio of the LCG layer thickness and the gel diameter was larger in the order of nitrate, acetate and chloride. The salt concentration dependence of *R* was not large in the experimental range between 0.18 and 1.08 M, as typically shown for calcium chloride in Fig. 3a (not shown for calcium nitrate and calcium acetate). The birefringence of the outermost LCG layer was larger in the order of acetate, nitrate and chloride. At this stage, we cannot confirm any specific relationship between these orders and that of Hofmeister's series. The gel made from curdlan dissolved in KOH was LCG/AG and no difference from the gel made from curdlan dissolved in NaOH of Fig. 1 (c1) was found (not shown). Therefore, LCG/AG formation does not depend on the alkaline species. Because only aqueous calcium salt induced LCG/AG alternative layer as shown in Fig. 1, this unique pattern is considered to be specific to calcium salts. On the other hand, the difference of shrinking ratio, the ratio of the LCG layer thickness and the gel diameter and the birefringence among calcium salts with different anionic groups of the salts suggest a certain effect of anionic group in the calcium salt. According to

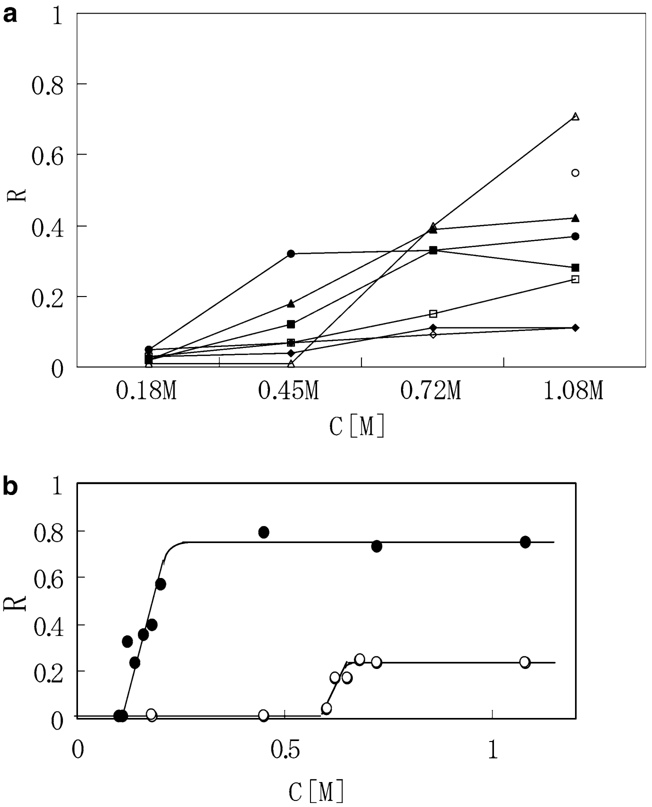

Fig. 3 Shrinking ratio as a function of molarity of salts: **a** $CaCl_2$ (*open diamond*), $MgCl_2$ (*open square*), $SrCl_2$ (*open circle*), $CuCl_2$ (*filled square*), $FeCl_2$ (*filled triangle*), $CoCl_2$ (*open triangle*), $MnCl_2$ (*filled circle*), and $CdCl_2$ (*filled diamond*), **b** $AlCl_3$ (*filled circle*) and $FeCl_3$ (*open circle*). The solution did not gel below 1 M in aqueous $SrCl_2$

the measurement of the calcium concentration of the gel [10], an assumption of the simple ionic bondings between a calcium cation sandwiched by two oxide anions at 6C carbons of glucose units of different curdlan molecules cannot explain the abundance ratio of glucose units and calcium cations in the gel. Therefore, a certain chelating complex including calcium cations, glucose units, and anionic groups of salts might be suggested for gel network formation, resulting in the different characteristics.

In case that 1 M urea was added to the extradialytic calcium solution, the birefringence did not change (not shown). Therefore, urea did not affect the calcium-induced LCG formation. On the other hand, in case that 10 mM citric acid was added to the extradialytic solution, the birefringence of the gel decreased considerably,

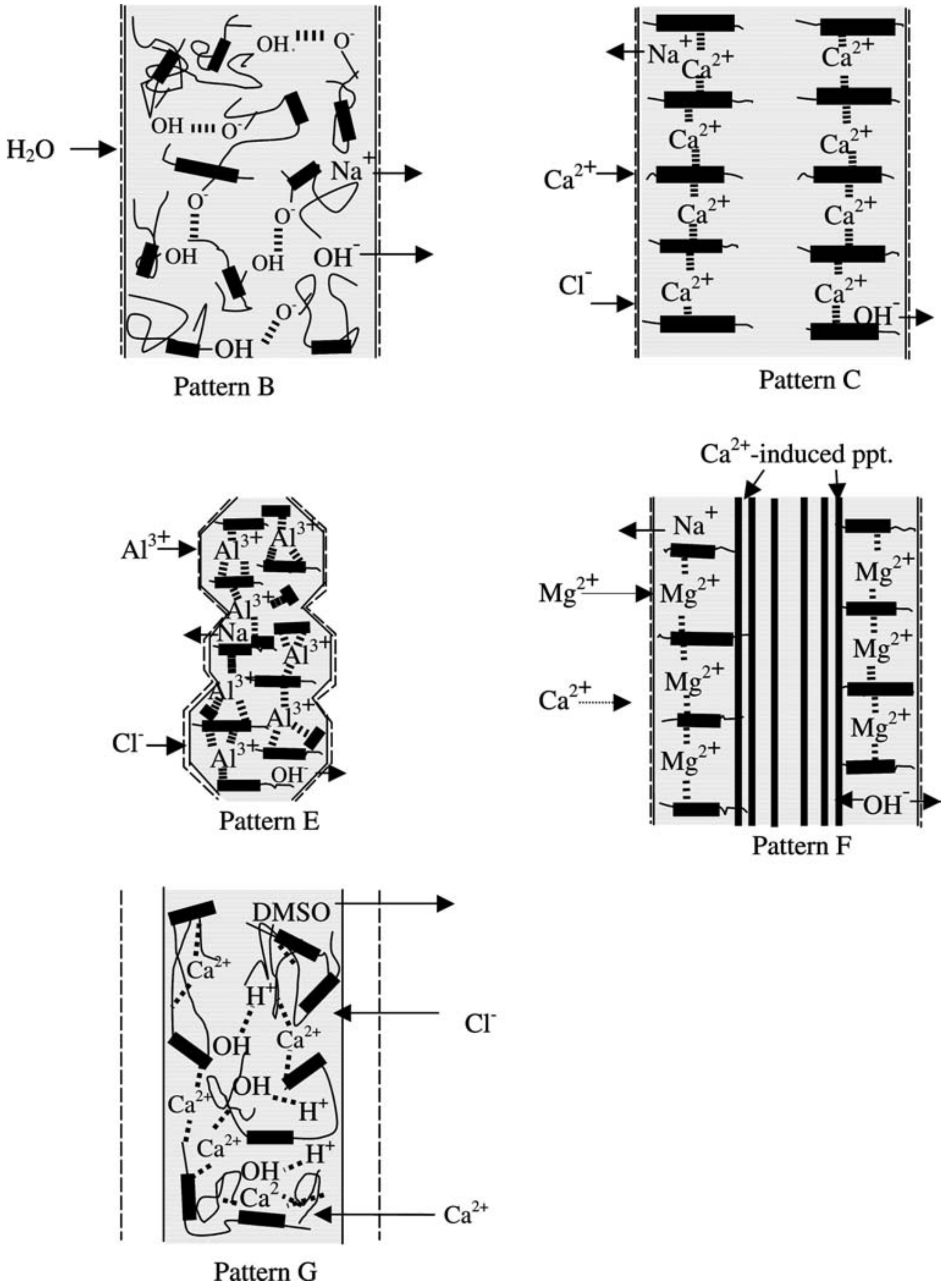

Fig. 4 Illustration of different gelation processes. The *outermost dashed lines* denote the dialysis tubing. The *shaded portion* is the gel

the transparency of the gel increased, the thickness of the AG layer increased slightly but the shrinking ratio did not change. These results suggest that a more homogeneous structure consisting of not very oriented mesogens is yielded with the addition of citric acid whose pH buffer function decreases pH of the solution.

Pattern D (system I-2b): In the aqueous solutions of most di-valence salts of Mg, Sr, Ba, Cu, Fe, Co, Mn and Cd, the curdlan solution gelled and the gels showed a weak birefringence in each restricted condition but did not show as much birefringence as calcium salts, and no alternative layers, as typically shown in Fig. 1 (d1–d8). The gels were slightly turbid for salts of typical metals and colored for the salts of transition metals. The time for gelation was smaller with increasing salt concentration except for aqueous barium chloride in which the solution gelled only at 0.18 M salt in a month and the viscosity was smaller at higher salt concentration above 0.18 M, probably resulting from degradation of curdlan. R gradually increased with the increase of salt concentration except for Co-induced gel, as shown in Fig. 3a. For Co-induced gel, R abruptly increased above a threshold concentration of 0.45 M. Individual properties are as follows: a gel with weak birefringence surrounded by a thin turbid layer was observed in aqueous magnesium chloride. Shrinking ratio was the largest at 0.72 M salt. The rigidity of Sr-induced gel was larger than that of Mg-induced one as observed with a hand touch. It is hoped that rheological properties are quantified to understand the relationship between the mechanical characteristics and the molecular conformation. Only an outer ring with weak birefringence gelled but the core remained a solution in Sr-induced gel at salt concentration lower than 1 M. Ba-induced gel only observed at 0.18 M was transparent isotropic gel. In concentrated solutions of aqueous $CuCl_2$, $FeCl_2$ and $CoCl_2$, weak liquid crystal structure was observed. R was the largest in aqueous $CoCl_2$ among these. Mn- and Cd-induced gels were brown and white gels, respectively, and no liquid crystallinity could be observed because of the strong color.

Pattern E (system I-2c): Liquid crystalline gels were observed for salts with tri-valence cations of $AlCl_3$ and $FeCl_3$, although the birefringence of the gel was much weaker for $AlCl_3$ and slightly weaker for $FeCl_3$ than for $CaCl_2$ in the low salt concentrations, as shown in Fig. 1 (e1–e2). The shrinking ratio R decreased sharply above each threshold salt concentration in the ranges of 0.1 – 0.2 M and 0.6 – 0.68 M, respectively, for $AlCl_3$ and $FeCl_3$, as shown in Fig. 3 b, incontrast to Fig. 3a for di-valence salts. The gels were very rigid in the high salt concentrations.

Pattern F (I-2d, I-2e): For extradialytic solutions containing mixed salts of Ca salt and $MgCl_2$, a series of concentric rings appeared, though the gel was shrunken inhomogeneously, as shown in Fig. 1f observed under natural light. The gel has a weak birefringence similar to the Mg-induced gel of Fig. 1 (d1). To observe the pattern more in detail and to avoid the inhomogeneous shrinking, 0.11 ml of the curdlan solution prepared for Fig. 1f was sandwiched between two circular glass plates having the diameter of 120 mm so as to make the gel height to be 1 mm, and immersed into 600 ml of the same extradialytic solution as for Fig. 1f. The circumferential part of the curdlan solution was then instantaneously gelled after the immersion, and played the role of dialysis tubing after that. This method is similar to the microencapsulation by means of insolubilization reaction [11]. Figure 2 shows the gel observed in 8 days (a) and 14 days (b) after the immersion. The pattern is similar to the Liesegang rings. Figure 2c shows an enlarged photograph of b. The right hand side is the surface of the gel and the left hand side is the center of the gel. A thick turbid layer is observed at the right hand and then a series of turbid rings with each 0.5 mm separation appears. Next a turbid layer with around 4-mm thick, a series of turbid rings with roughly constant separation, a transparent layer with 2 mm and finally a turbid ring appears. This pattern cannot coincide with the space law characteristic to the Liesegang phenomena [13–15], but is more complicated. To clarify the mechanism of forming this pattern, parts of the gel were immersed in 5.5 M aqueous ammonium chloride, which dissolved hydroxides or 26 mM aqueous ethylene glycol bis(2-aminoethyl ether)-N,N,N′N′-tetraacetic acid (EGTA) which chelated only calcium cations but not magnesium cations for 24 h. Then the characteristic rings completely disappeared for both treatments as shown in Fig. 1 (f′) and (f″), but the birefringence of the gel did not change. For extradialytic solutions containing two kinds of salts $AlCl_3/MgCl_2$ and $MgCl_2/CuCl_2$, the gel appeared to be the same as that for the extradialytic solutions containing only $AlCl_3$ as shown in Fig. 1 e1 and $CuCl_2$as shown in Fig. 1 d3, respectively. The diffusion coefficient of calcium cations in water is much larger than that of magnesium cations. This is closely related to the ionic radius. We note that the descending order of the hydrated ionic radius is $Al^{3+} > Ca^{2+} > Cu^{2+} > Mg^{2+}$. Therefore, the diffusion coefficient of the cations in curdlan solutions could be assumed to be smaller in this order. The observed gels for the mixed salts of $CaCl_2/MgCl_2$, $AlCl_3/MgCl_2$ and $MgCl_2/CuCl_2$ are attributed to the gelation induced by Mg^{2+} cation, since this cation has the largest diffusion coefficient. It is well known that the Liesegang phenomena result from periodic precipitations of a diffusive component on rigid gels [14, 15]. Although the space law was not exactly confirmed for $CaCl_2/MgCl_2$, the characteristic pattern might be yielded by precipitates of $Ca(OH)_2$ on Mg-induced gel because of the large difference of diffusion coefficients of Ca^{2+} and Mg^{2+} and low solubility of $Ca(OH)_2$. For $AlCl_3/MgCl_2$ and $MgCl_2/CuCl_2$, chelating complexes consisted of Al^{3+} or Cu^{2+} might prevent from precipitating.

Pattern G (II-2): The curdlan dissolved in dimethylsulfoxide (DMSO) formed a rigid gel without liquid crystals with extradialytic solutions of aqueous $CaCl_2$ and $AlCl_2$, as shown in Fig. 1 g. The gel was observed floating in a fluid in the dialysis tubing and the gel surface was away from the dialysis tubing. This is in striking contrast to the other gels whose surfaces exhibit weak adhesion to the dialysis tubing. Higher affinity of curdlan with calcium cations and DMSO might disturb the inflow of water into the gel. Since curdlan is considered to be at random coil state in DMSO just like shizophillan [16], no triple helix formation could occur. This suggests the unique LCG/AG cannot be formed only by calcium cations but need OH^- flow.

According to these results, followings are suggested.

1. Comparing Pattern A–E, hydrogen bonding or ionic bonding or chelating is required for gelation of curdlan by dialysis at room temperature.
2. From Pattern C–E, multi-valence cations induce LCG at each restricted condition. The condition for yielding LCG and the degree of ordering depends on the ionic species. The shrinking occurs moderately by increasing salt concentration for di-valence cations except for cobalt cations, and abruptly above a threshold salt concentration for tri-valence cations. The abrupt change of shrinking ratio might be related to larger Coulombic force of tri-valence cations.
3. Alternative concentric layers of liquid crystalline gel with refractive index gradient and amorphous gel (LCG/AG) appear especially remarkably for aqueous calcium salts. This suggests the necessity of atomic properties inherent to calcium for the unique regular pattern formation. The difference of birefringence, the shrinking ratio and the thickness of the liquid crystalline layer depending on the anionic groups of calcium salts suggest a chelating complex containing anionic group in cross-linking inter-molecularly.
4. From Pattern F, a series of rings appears when mixed salts are used in the extradialytic solution. The rings should be a precipitation of calcium hydroxides, just like the Liesegang phenomena [13–15]. It is speculated that because of the larger diffusion rate of magnesium cations compared with the calcium cations, magnesium gel is formed first and then the slower diffusive component of calcium cations reacted with the curdlan solution to make precipitates of calcium hydroxide.
5. From Pattern A and G, to dissolve curdlan with strong alkaline solution is necessary for forming LCG. A considerable pH change of the curdlan solution associated with the dialysis, which change the conformation of curdlan from random coil to triple helix might be important for the molecular ordering.

Coupling of molecular ordering and cross-linking induce multifold structural patterns. The mechanisms for forming variety of typical structural patterns are speculated as in Fig. 4. When the original curdlan is a random coil and pH change occurs during the dialysis, hydrogen bondings induce an isotropic gel as Pattern B, unless they are not disturbed by chemical species such as urea. When the curdlan conformation changes from random coil to triple helix during dialysis and cross-linker cations with appropriate size fit in the intermolecular interstitial area of curdlan, LCG is induced, as Pattern C. Especially, inflow of calcium salt and outflow of alkaline hydroxide induce a unique LCG/AG. When the Coulombic force at the cross-linking points is very strong as in the case of tri-valence cations, a gel shrinking occurs sharply above a threshold salt concentration, just like the counter ion effect to induce a considerable shrinking, as Pattern E. When mixed metal salts are used as the extradialytic solution, the smaller and more diffusive salt permeates into the solution initially to make a gel and then another larger and less diffusive species react the solution involved in the gel to yield precipitates, resulting in Liesegang ring-like concentric pattern, as Pattern F. Curdlan dissolved in DMSO forms a Ca-induced but homogeneous rigid gel as Pattern G, which is much different from Pattern B.

Multifold structures similar to those found for curdlan solutions are possible with using combinations of concentrated semi-flexible or rod-like polymer intradialytic solutions and concentrated extradialytic ionic solutions, which are published soon.

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