

Emulsion Pathway for Gelatin Cross-Linking

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SYNOPSIS

A new method for gelatin cross-linking is suggested that starts with the generation of an emulsion between an aqueous solution containing gelatin and a surfactant, and a monomer (MMA) that contains an initiator (AIBN). The emulsion is prepared at 50°C under intense stirring, but is brought, subsequently, to room temperature. There are conditions under which the gellike emulsions thus obtained are transparent and extremely stable at room temperature. A slow cross-linking reaction occurs in the latter gels that leads to a material that is insoluble in water. The swelling of this material in water, which is very large after 5 days, reduces appreciably after 25 days of reaction. The cross-linking occurs only when MMA contains the initiator. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

In a previous paper,¹ a method for the preparation of gelatin-based composite polymers, via a gel-emulsion pathway, has been reported. The gels have been prepared by mixing mechanically at 50°C an aqueous solution of gelatin and a surfactant (sodium dodecylsulfate, SDS) with a monomer (methyl methacrylate, MMA) containing an initiator (AIBN). These gels can be classified into two classes: liquidlike gels with low stability, which separate into two phases after 0.5–2 h at room temperature, and solidlike gels, with high stability at room temperature. The gels have been polymerized to generate composites and the details were presented in Ref. 1. Some of the solidlike gels are transparent and extremely stable. Recently, we have found that the transparent gels can be used not only for the radical polymerization of MMA at 50°C to obtain gelatin-based composites, but also for the cross-linking reaction of gelatin at room temperature without using the conventional cross-linking reagents.

Cross-linking of gelatin, which is a protein, is most commonly carried out in aqueous solutions by using various cross-linking reagents with two or more functional groups, such as 2,4-dichloro-6-hy-

droxy-*s*-triazine,² poly[*p*(chloroacetyl-amino)methacrylanilide],³ bis(isomaleimide),⁴ epoxides,⁵ aldehydes,⁶ and ketones.⁷ Much of the literature on this subject is in patent form.

The present study shows that a gellike emulsion can be used to cross-link the gelatin by a radical process. This cross-linking reaction, being slow, can occur only in gels that remain stable for a long time. The gels that are transparent at room temperature are most suitable for cross-linking. The cross-linked gelatins are insoluble in water, cyclohexane, or toluene. Their swelling in water depends on the cross-linking time and becomes smaller for longer times. Infrared spectroscopy was used to examine the cross-linked materials. From weight measurements, it has been found that only about 3–4% of MMA is fixed in the cross-linked gelatin. However, in the absence of AIBN, no cross-linking of gelatin has been observed in the transparent gels even after several months. Similarly, gels containing a nonpolymerizable molecule, such as ethyl isovalerate, instead of MMA, could not generate cross-linked gelatin. Scanning electron microscopy (SEM) was used to examine the morphologies of the cross-linked gelatins.

EXPERIMENTAL

Materials

Methyl methacrylate (Aldrich Chemical Co.) was purified by distillation under reduced pressure, and

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the initiator, azobisisobutyronitrile (AIBN, Alfa Chemical Co.), was purified by recrystallization in methanol. Sodium dodecylsulfate (SDS, Aldrich) and gelatin (300 bloom, Alfa Chemical Co.) were used as received. Water was deionized and double-distilled.

The Swelling Test

Samples of dried composites were weighed and placed in glass tubes containing the respective liquid. The weights of the swollen samples were determined after 72 h. The swelling ratio of the samples was calculated using the expression $S = (W_S - W_0)/W_0$, where W_0 and W_S are the weights of the dried (after swelling) and swollen samples, respectively.

Instruments Used

Infrared absorption spectra were obtained using a Mattson Alpha Centauri FTIR instrument, and the morphologies of the cross-linked gelatins were investigated with SEM (Amray 100A).

Gel Preparation

Two stock solutions have been prepared by dissolving the surfactant (SDS) in water and the initiator (AIBN) in the monomer (MMA), respectively.

Further, the aqueous solutions of SDS were introduced in glass tubes containing gelatin. The glass tubes were placed in a water bath at 50°C for 30 min to 1 h and stirred continuously with a mechanical mixer (Mistral Mixer) until the solution became homogeneous. The monomer containing AIBN was then introduced in the glass tubes. Gellike emulsions were finally obtained by the intensive stirring of the aqueous solution of gelatin and SDS with the monomer containing the initiator under heating at 50°C for about 15 min.

RESULTS AND DISCUSSION

Figure 1 is a phase diagram, taken from our previous paper,¹ for gellike emulsion formation in which the concentration of gelatin in the entire system, C_g (g/mL), is plotted vs. the volume ratio of aqueous solution of SDS and monomer MMA, C_{WM} (mL/mL), at the constant SDS concentration (C_{SDSW}) of 1.0 mol/L in the water solution. Gels form in regions I and III, but do not in region II. In the gel-forming domain and at low gelatin concentrations, the gels are liquidlike and separate into two phases after 0.5–2 h at room temperature. When the contents of gelatin and water are higher, the gels have higher stability and a solidlike behavior at room temperature.

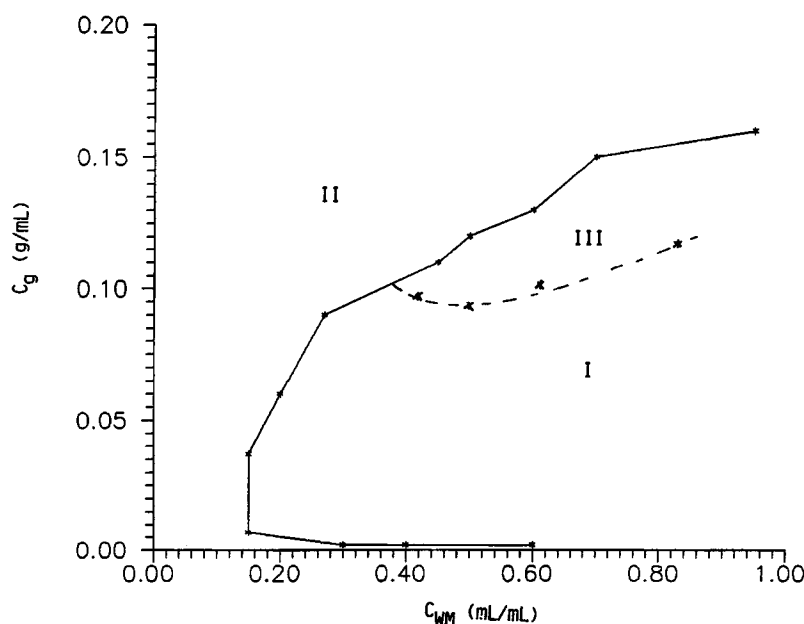


Figure 1 Phase diagram of the system composed of the monomer MMA and an aqueous solution of SDS and gelatin in the representation gelatin concentration (C_g) in the gel against volume ratio (C_{WM}) of aqueous solution of dispersant (SDS) to MMA. Region I is the gel region, region II is the two-phase region, and region III is the transparent gel domain. In Figure 1, the concentration of the aqueous solution of SDS is 1.0 mol/L.

Transparent gels that are highly stable at room temperature have been found in region (III). Their storage for many months at room temperature did not lead to two phases. They melt above 36°C and separate into two phases, but form again transparent gels under careful mixing at 50°C followed by cooling to room temperature. This behavior of the transparent gels with respect to heating and mixing suggests that a physical three-dimensional infinite network of gelatin is formed. We have not expected that this physically cross-linked gelatin network will transform into a chemically cross-linked network, because no conventional cross-linking reagent was present in the system. Surprisingly, we found that after several days the transparent gels did not dissolve in water or melt at 50°C. This fact suggested that a chemical cross-linking occurred and stimulated us to investigate this phenomenon. It should be again emphasized that the high stability of the transparent gels allowed the slow chemical cross-linking of gelatin to take place.

The samples for cross-linking were prepared as follows: Transparent gellike emulsions were first prepared as described in the Experimental part. The transparent gels heated at 50°C were poured into aluminum dishes and allowed to cool to room temperature. The samples employed for the cross-linking reaction were prepared in sheetlike form of 2–5 mm thickness and then placed in closed glass containers at room temperature. To follow the cross-

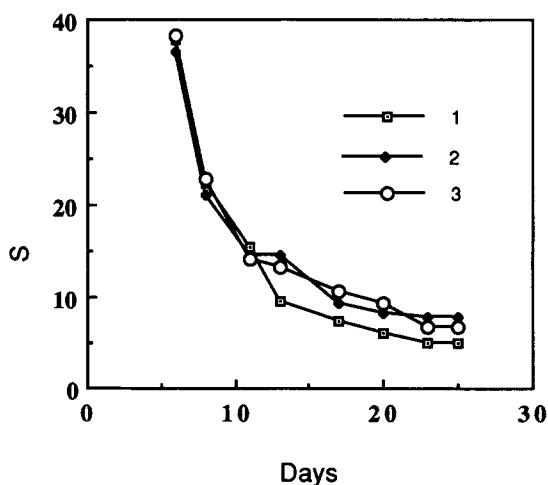


Figure 2 The swelling of cross-linked gelatins at room temperature as a function of cross-linking time. The compositions of samples 1 and 2 are $C_g = 0.09$ and 0.11 g/mL, $C_{WM} = 0.50$ mL/mL, $C_{SDSW} = 1.00$ mol/L, and $C_{AIBN} = 0.25$ mol/L. The composition of sample 3 is $C_g = 0.13$ g/mL, $C_{WM} = 0.67$ mL/mL, $C_{SDSW} = 1.00$ mol/L, $C_{AIBN} = 0.25$ mol/L.

Table I The Swelling of Cross-Linked Gelatin after 12 Days of Cross-Linking Reaction at Room Temperature

TH	Solvent	
	Cyclohexane	Toluene
Sample ^a		
1	0.027	0.036
2	0.028	0.034
3	0.025	0.035

^a The compositions of samples correspond to those in Figure 2.

linking reaction, swelling tests have been carried out after various cross-linking times. The samples used in the swelling tests were immersed in water for 72 h and dried in vacuum for 12 h. Figure 2 presents the swelling behavior of the gelatin after various times. The swelling during the first 10 days of reaction was relatively large and reached the equilibrium value after about 23 days of reaction.

The chemically cross-linked gelatins are not soluble in water, cyclohexane, or toluene. In addition, from Table I, one can see that they absorb very small amounts of the hydrophobic molecules cyclohexane and toluene. This fact indicates that MMA is not much involved in the cross-linking system. In our previous study,¹ it was shown that the composite polymers of gelatin and PMMA absorbed relatively large amounts of cyclohexane and toluene when most of the MMA was involved in polymerization. To determine how much MMA was included in the cross-linked gelatin, weighing tests were conducted as follows: Samples of cross-linked gelatin were first dried for 24 h at the ambient temperature, followed by drying for 12 h under vacuum at room temperature and, finally, by weighing. Only about 3–4% of MMA remained in the cross-linked gelatins.

The infrared spectra of the pure gelatin, PMMA, and dried cross-linked gelatins in Figure 3 show that some of the MMA molecules are present in the cross-linked materials. The peaks at 2925 and 2850 cm^{-1} (in spectrum a) can be assigned to the antisymmetric and symmetric vibrations of $-\text{CH}_2-$ in PMMA.⁸ The peak at 1735 cm^{-1} can be assigned to $\text{C}=\text{O}$ in PMMA.⁸ These peaks are not present in the IR spectrum of pure gelatin, but can, however, be observed with reduced intensities in the spectra c, d, and e of dried cross-linked gelatins. This indicates that a small amount of MMA is involved in the cross-linked gelatins. The IR spectrum f of a composite polymer based on gelatin and MMA is nearly identical to that of PMMA when most of the

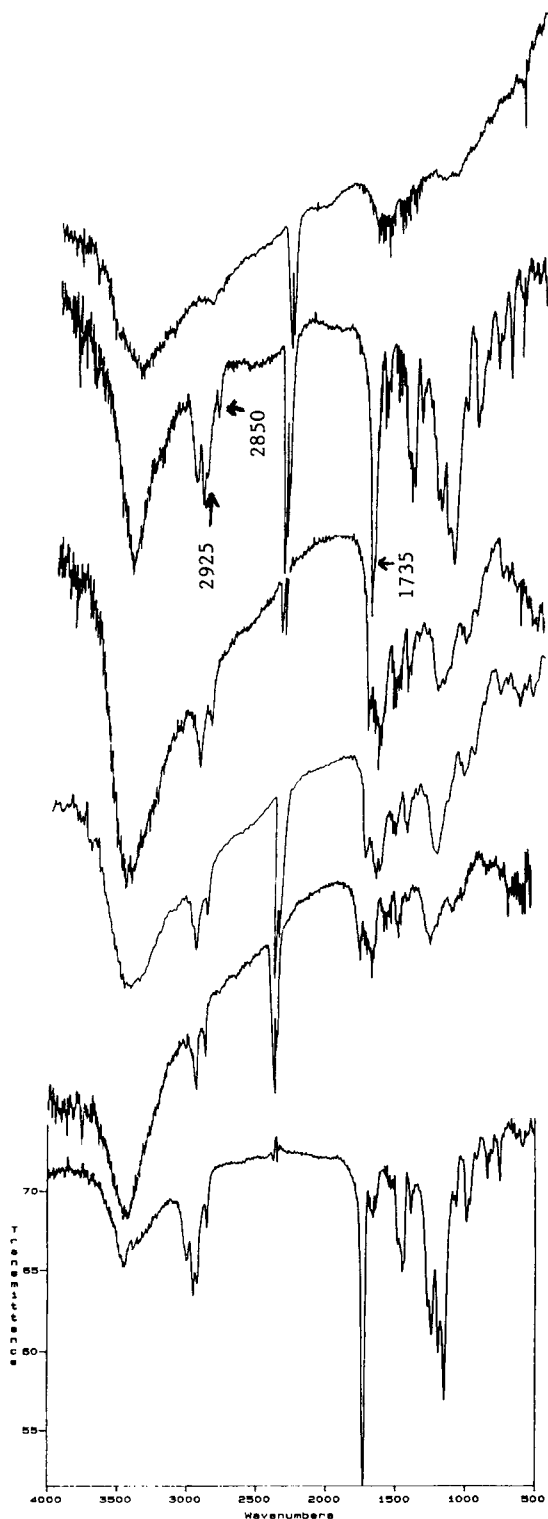


Figure 3 The FTIR spectra of (a) gelatin, (b) PMMA, and cross-linked gelatins c, d, and e for the samples 1, 2, and 3 of Figure 2. The FTIR spectrum f is that of a composite polymerized for 24 h at 50°C with the composition $C_g = 0.15$ g/mL, $C_{WM} = 0.5$ mL/mL, $C_{SDSW} = 1.00$ mol/L, and $C_{AIBN} = 0.25$ mol/L.

MMA is involved in polymerization. Figure 4 presents the IR spectra b, e, and f of Figure 3, at a larger scale around the wavenumber of 3000 cm^{-1} . One can observe that the locations of the peaks and their shapes are similar for the composite and for the pure PMMA, but somewhat different for the cross-linked gelatin. This suggests that MMA reacts with some active groups on the gelatin molecules. The structure of the cross-linked gelatin is not yet clear. The gel-

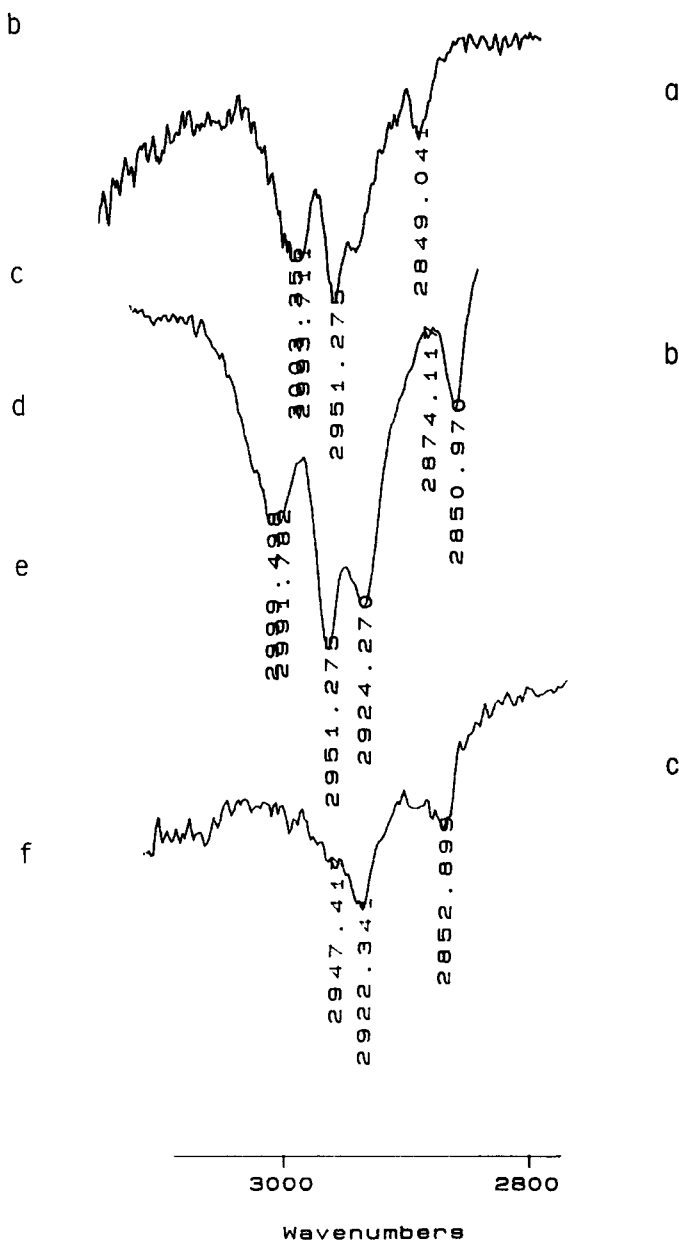
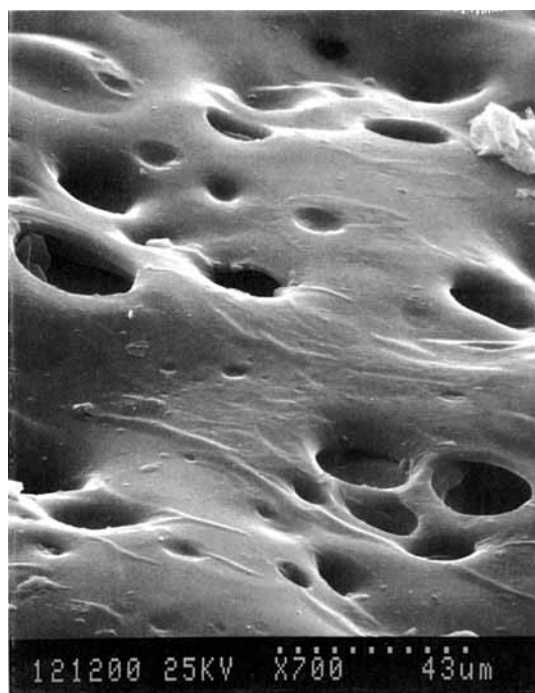
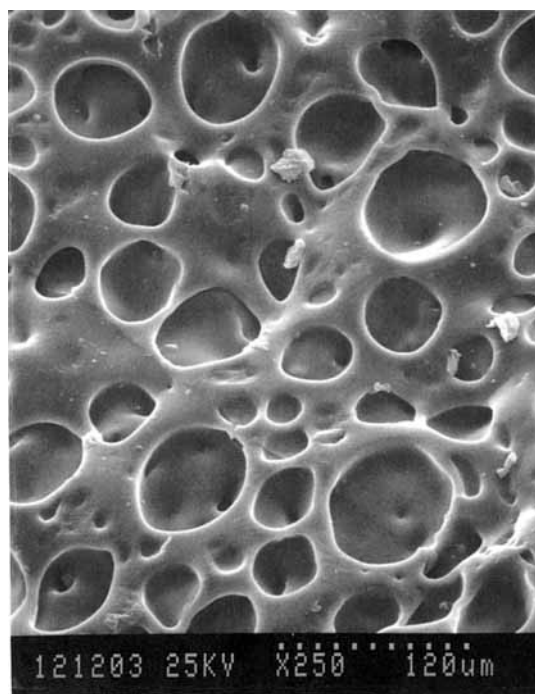


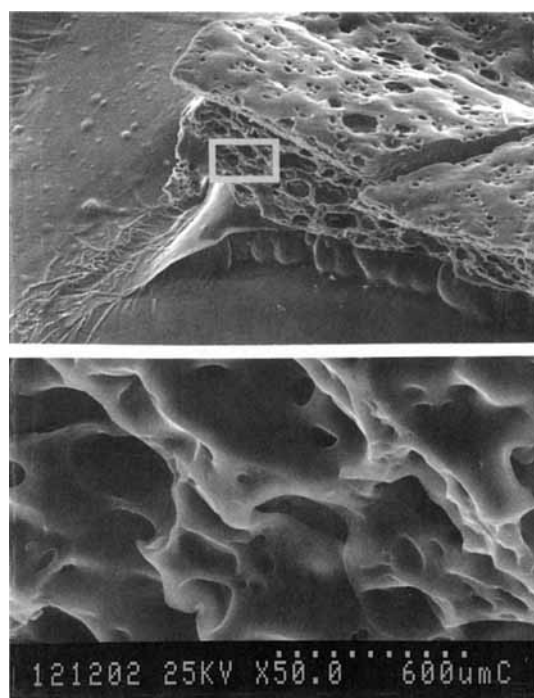
Figure 4 FTIR spectra around the wavenumber of 3000 cm^{-1} of (a) PMMA, (b) the composite polymer, whose composition is that of specimen f in Figure 3, and (c) cross-linked gelatin corresponding to sample 3 in Figure 2.



(a)



(b)



(c)

Figure 5 Electron micrographs of samples 2 and 3 (Fig. 2) of cross-linked gelatins.

atin cross-linking reaction may occur via bridges of MMA oligomers among gelatin molecules: gelatin-(MMA)_n-gelatin.

Another possibility is a cross-linking among gelatin molecules through a radical reaction initiated by AIBN. It should be noted that the gels could not

be cross-linked in the absence of AIBN and that the gelatin in gels containing a nonpolymerizable molecule, such as ethyl isovalerate (instead of MMA), could not be cross-linked. These facts indicate that gelatin cross-linking in gellike emulsions occurs only when the oil phase is a reactive monomer that contains an initiator.

The SEM pictures of cross-linked and dried gelatins presented in Figure 5 reveal their porous structure. A porous structure can be also recognized in picture c, which represents the cross section of the specimen in picture a.

CONCLUSION

A new method of cross-linking gelatin is developed via a gellike emulsion pathway. The gels have been prepared by mechanical mixing at 50°C an aqueous solution of surfactant and gelatin with a monomer (MMA) containing an initiator (AIBN). Some of these emulsions that contain a relatively large amount of gelatin are transparent and highly stable at room temperature. A slow cross-linking reaction occurs in these transparent emulsion gels at room temperature. The cross-linked gelatin is insoluble in water, cyclohexane, and toluene. Their swelling in water depends on the cross-linking time and attains equilibrium after 25 days. Infrared spectroscopy suggests that some MMA molecules are involved in the cross-linking probably as MMA oligo-

mer bridges among gelatin molecules. The SEM pictures show that the cross-linked gelatin has a porous structure.

NOTATIONS

C_g	the concentration of gelatin in the entire gel (g/mL)
C_{SDSW}	the concentration of SDS in water (mol/L)
C_{AIBN}	the concentration of AIBN in monomer MMA (mol/L)
C_{WM}	the volume ratio of aqueous solution of SDS to monomer MMA (mL/mL)

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