

Humidity Dependence of Visible Absorption Spectrum of Gelatin Films Containing Cobalt Chloride

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SYNOPSIS

Gelatin films containing cobalt chloride were investigated to obtain information on the humidity dependence of optical absorbency in view of the coordinate state of cobalt ion and the polymer structure. The visible absorption spectra of the films exhibited that, with increasing relative humidity (RH), the absorption bands between about 550 and 750 nm decreased, accompanied by sharpening of the 693 nm peak. Further, the decrease of cobalt chloride in gelatin resulted in decreased absorption intensity at the 693 and 668 nm peaks, more than the overall lowering in intensity. The infrared spectral data indicated that most of the carboxyl groups of gelatin are linked to the complex even in the strongly hydrated state. In addition, the water content of the films containing cobalt chloride is a little less below 42% RH, but is much greater above 62% RH than that of the original gelatin film. These results indicated that there are cross-linked networks, composed of the hydrogen bonding and the coordination bond via the complex in the dry films; as water molecules are adsorbed by the film, they gradually destroy these bonds and swell the polymer. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

Recently, optical sensors for various chemical species and physical parameters have drawn considerable attention because of certain advantages, especially over electrochemical devices.^{1,2} Many of them made use of dye-doped polymers as a sensing membrane, which change the absorption or fluorescence spectrum, dependent on chemical and physical variables.³⁻⁵ Optical detection techniques are of interest also in the field of moisture measurement.⁶⁻⁹ Several approaches have been reported on the use of cobalt chloride (CoCl_2), which exhibits a strong hypsochromic shift with increasing the ambient humidity.^{10,11}

Recent workers reported the reproducibility, hysteresis effect, and temperature dependency of the curves of humidity vs. absorbance, using cellulose or acetylated cellulose films impregnated with CoCl_2 .¹² Few reports, however, have been published

that explain the relationship between the chemical structure and the humidity dependence of optical absorbency of polymer- CoCl_2 systems. This report describes the relative humidity (RH) dependence of the visible absorption spectrum and the water sorption of gelatin (GEL) films containing CoCl_2 , as well as the infrared (IR) spectrum of the films. The relations between the concentration of CoCl_2 and the absorbance of the films were measured quantitatively. The absorbance-RH curves of the film were discussed in view of the coordinate state of cobalt (Co) ion in the polymer.

EXPERIMENTAL

Preparation of Moisture-sensing Films

GEL (alkali-treated) and $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (guaranteed reagent) were purchased from Kishida Chemical and used as received. A definite volume of the aqueous solution, containing 3.8% m/Vol (g/100 mL) of the polymer and 0, 0.167, 0.333, and 0.667% m/Vol (g/100 mL) of CoCl_2 , respectively, was cast on a glass plate and dried at ca. 45°C to provide a moisture-

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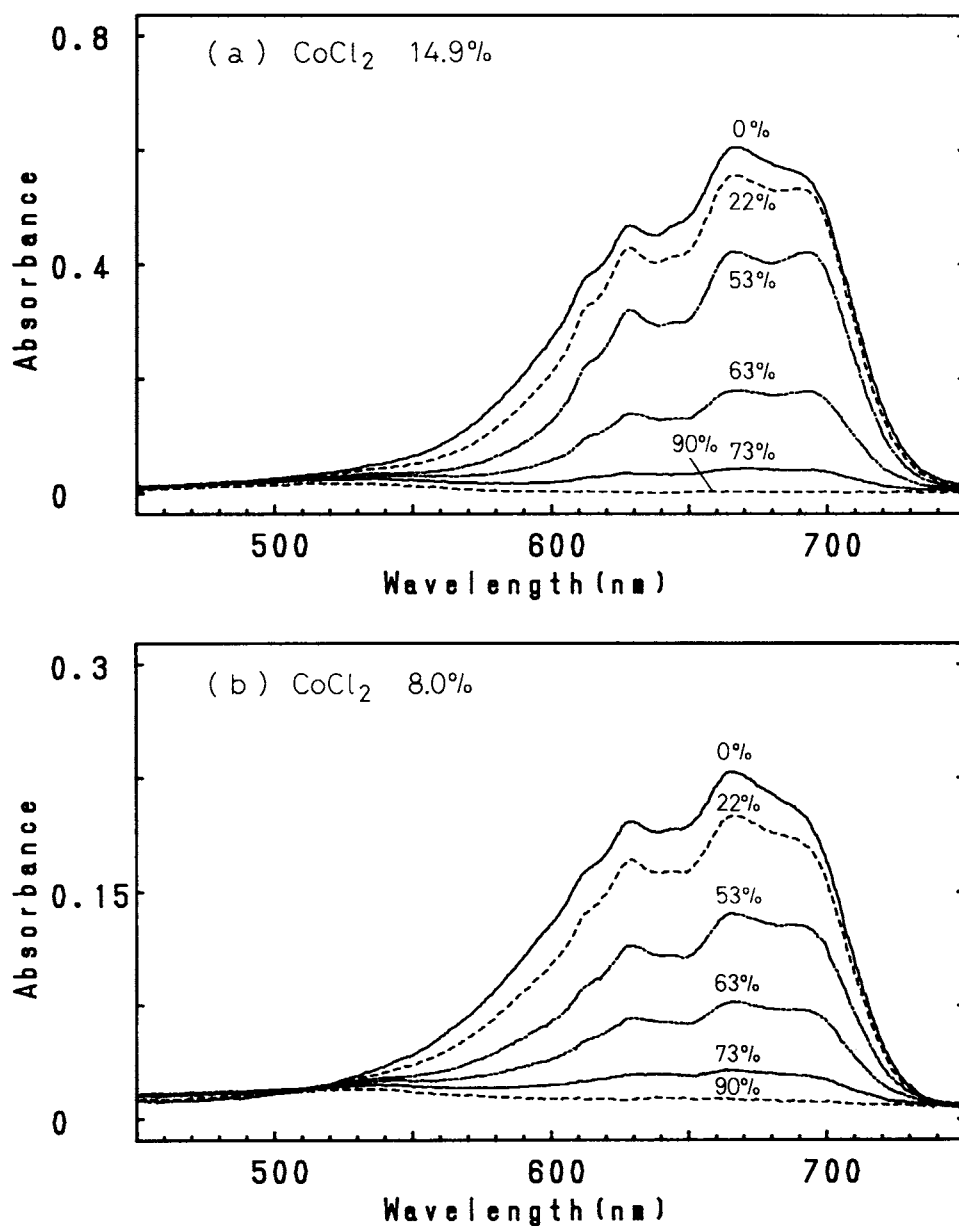


Figure 1 Humidity dependence of absorption spectra of a GEL film containing varying percentage concentrations of CoCl_2 : (a) 14.9%; (b) 8.0%; (c) 4.2%.

sensitive film containing 0, 4.2, 8, and 14.9% m/m (g/100 g film) of CoCl_2 . Assuming the density of all films to be equal to 1.0, the film thickness was calculated from the volume of the coated solution.

Measurement of Visible Absorption Spectrum

A 10 mm square cell capped with a stopper was used as a measuring cell, in which the film (thickness ca. 16 μm) coated on a 36 \times 13 mm glass plate was

placed diagonally. In a reference cell, a glass plate without film was placed. The two cells were mounted in a cell holder of a JASCO UVDEC-1 double-beam spectrometer to measure the change of the visible absorption spectrum in the transmission mode.

Measurement of Water Sorption

The content of water (g/g film) sorbed by the sample films was measured with a Cahn 2000 electrobalance.

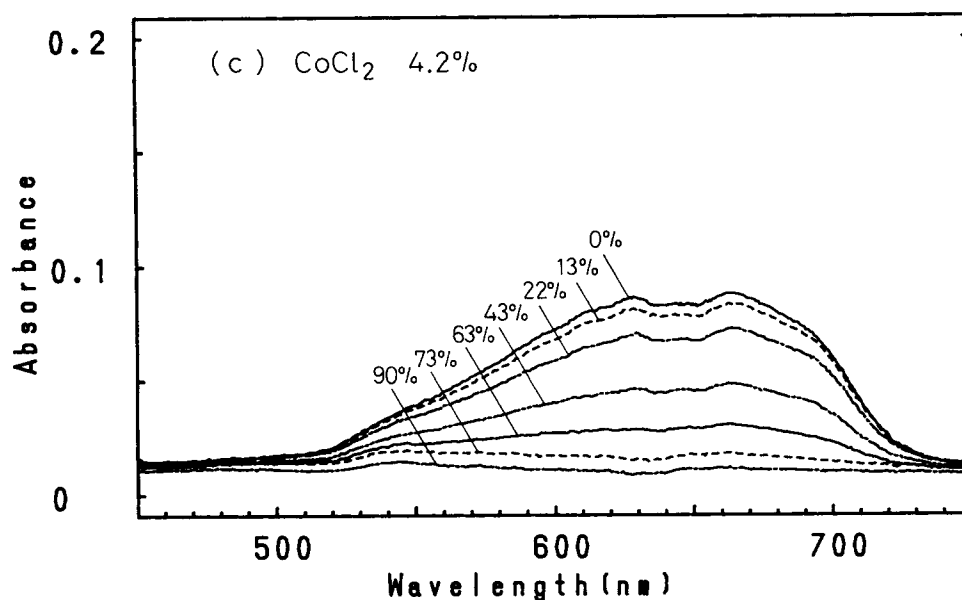


Figure 1 (continued from the previous page)

The film (thickness, ca. $24 \mu\text{m}$) coated on 18×18 mm glass plate was suspended in a measuring bottle with a nichrome wire and stirrup 40 cm long from the arm of the balance. The weight change as small as 0.01 mg could be measured. Since the weight of the glass plate (about 100 mg) was measured prior to casting the film, the weight of the dry film (about 7 mg) and the sorbed water, respectively, was known by weighing the sample after equilibration with air of 0% RH and another % RH.

Control of RH

The accurately humidified air was obtained by mixing dry air and water-saturated air using a Shin-ei SRH-1R humidity generator. For the optical absorbency measurement, the air stream of about 0.5 L min^{-1} was introduced through an inlet tube to the measuring cell and exhausted through an outlet tube. For the water-sorption measurement, the atmospheric RH was produced by flowing the humidified air at 5 L min^{-1} into the measuring bottle. All the tubes, as well as the cell holder and the measuring bottle, were temperature-controlled at $20.0 \pm 0.2^\circ\text{C}$ by circulated water. The humidity of the exhausted gas was measured frequently by a Tabai Espec EDP-1060 optical dew point sensor that afforded a calibration of RH determined by varying the ratio of mixed gas.

Measurement of IR Spectrum

IR spectra were measured by the use of a Nicolet 710 FTIR spectrometer with a resolution of 2 cm^{-1} . The sample for IR spectral measurement was prepared as follows: 0.1 mL of the GEL- CoCl_2 solutions prepared above was cast on a calcium fluoride plate ($35 \text{ mm } \phi$, 5 mm thick) and dried in room air. The absorption spectrum of the sample in the hydrated state was obtained by carrying out the measurement immediately after equilibration with water-saturated air. The spectrum in the dry state was obtained by carrying out the measurement after exposing the sample to the dry air in the measuring compartment for a long time. The scan times were 10 for the former and 100 for the latter.

RESULTS AND DISCUSSION

Humidity Dependence of Visible Absorption Spectrum

Absorption spectra of the GEL films containing varying concentration of CoCl_2 measured upon starting at 0% RH and ending at 90% RH are shown in the range of 450–750 nm in Figures 1(a)–(c). It can be seen in all the figures that the absorbance of the films decreases in the range from 550 to 750 nm

and the absorption maxima at ca. 520 nm appear distinctly when RH increased. These changes are apparently due to the alteration of Co ion from the 4-coordinate to the 6-coordinate form. It has been reported that the Co complex with GEL is formed in an aqueous solution; the carboxyl groups of acidic amino acid residues offer the principal site for the binding of the Co ion.¹³ The peptide groups of GEL also have been proposed to act as a ligand.¹⁴ Therefore, it is expected that these coordination bonds between the GEL and Co ion exist also herein in the films and participate in the change of the coordinate state of Co with increasing RH.

The specific absorption maxima appear at 630, 668, and 693 nm in all the spectra of the films containing a different concentration of CoCl_2 . These maxima hardly show any wavelength shift, but change the relative intensities when the CoCl_2 concentration in the film is changed. In both spectra of GEL- CoCl_2 -14.9% and -8.0%, the absorbance of the maximum at 693 nm increases when RH increased. The absorbance of this maximum for GEL- CoCl_2 -14.9% is larger at the same RHs than that for GEL- CoCl_2 -8.0%. The maximum is probably attributed to the CoXCl_3 entity. It has been demonstrated that addition of water to an acetone solution of CoCl_2 makes the absorption spectrum resemble to that of the CoCl_3^- entity, which has the maximum at 685 nm.¹⁵ This wavelength is very close to 693 nm found in the present spectra. It seems that the water molecule, sorbed in the film, knocks a chloride ion away from a Co complex; the chloride ion attacks another complex and eliminates a relatively weak ligand like the peptide group of GEL, generating the CoXCl_3 entity. In addition, the absorbance of the maxima at 668 nm, as well as that at 693 nm, becomes larger the higher the CoCl_2 concentration. Assuming that the maximum at 668 nm is ascribed to the CoX_2Cl_2 entity, these spectral changes indicate that the Co complex possessing one chloride are present in the film and increased with decreasing CoCl_2 concentration. The above conclusion is contrary to the report that the 4-coordinated form of CoCl_2 is the $\text{CoX}_4-n\text{Cl}_n$ ($n \geq 2$) entities in organic solutions.¹⁵ It is supposed that, in the GEL film, the carboxyl groups of the polymer, which have a strong ligating ability with their chelating effect, easily enter the CoX_2Cl_2 group by eliminating a chloride, producing the CoX_3Cl group.

The RH dependencies of the absorbance at 668 nm of the GEL- CoCl_2 composite films are shown in Figure 2. The total change of absorbance was 0.076, 0.215, and 0.599, respectively, at a CoCl_2 concentra-

tion of 4.2, 8.0, and 14.9%. The increasing degree of the total change is much larger than that expected from a simple proportion between the absorbance and the CoCl_2 concentration. The curves of absorbance vs. % RH differ from each other also in shape. Both curves for GEL- CoCl_2 -8.0% and -14.9% are steep in the range from 53 to 73% RH, while the curve for GEL- CoCl_2 -4.2% is smooth in the whole range of % RH. These features indicate that the coordinate states of the Co ion in the films containing different concentrations of CoCl_2 are different. It is also noteworthy that, on the curve for GEL- CoCl_2 -14.9%, the absorbance during the desiccation process is lower below 53% RH, but is slightly higher above 53% RH than that during the humidification process. A similar behavior is also exhibited by the curve for GEL- CoCl_2 -8.0%. This anomalous hysteresis effect will be discussed below.

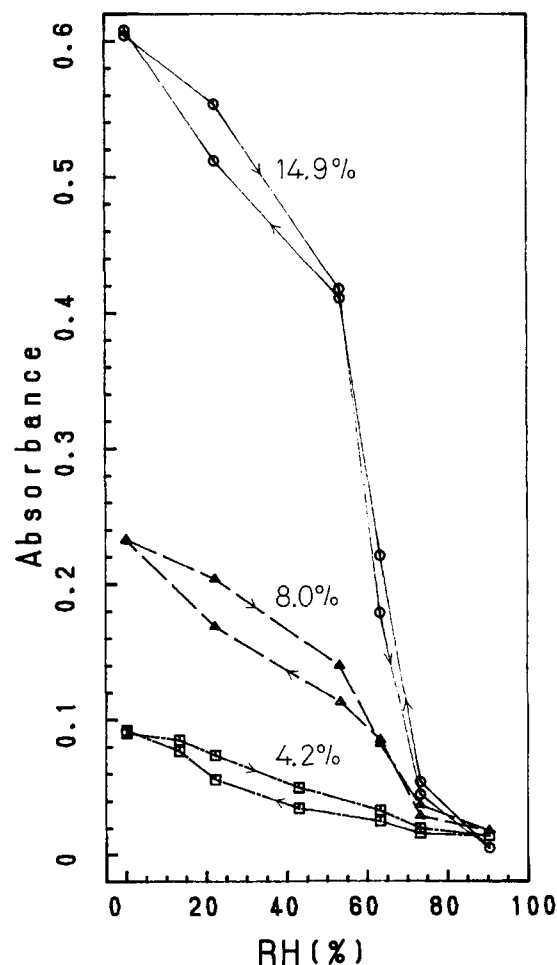


Figure 2 Absorbance at 671 nm vs. relative humidity for GEL films containing various percentage concentrations of CoCl_2 .

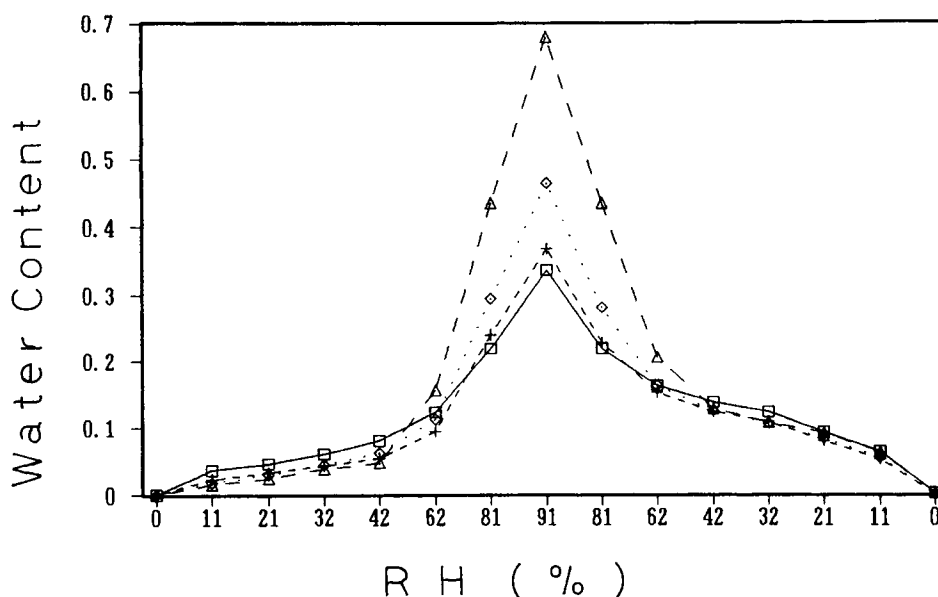


Figure 3 Water contents (g/g film) in GEL films with and without CoCl_2 at varying relative humidity that was changed from 0% to 91% and back to 0%. The percentage concentrations of CoCl_2 are (—) 0%, (---) 4.2%, (\cdots) 8.0%, and (-·-) 14.9%, respectively.

Water-vapor Sorption

It seems very important to investigate the water-sorption isotherm of the films containing CoCl_2 by varying its concentration. The water content (g/g film) at various RHs are shown in Figure 3, when RH changes 0% to 91% and back to 0%. The water content of the GEL films containing CoCl_2 below 42% RH is a little less than that of the original GEL film. It seems that the coordination bond between GEL and the Co ions makes the film hard and, hence, slightly depresses the water sorption. On the other hand, at higher humidities than 62% RH, the water content in the composite films is greatly enhanced with the increase of the concentration of CoCl_2 ; i.e., the ability of CoCl_2 of making the film hygroscopic develops in the humidity range above 62% RH. These results indicate that water molecules, sorbed by the polymer above 62% RH, destroy the hydrogen bonding and the coordination bond via the Co complex between the polymer chains and drastically swell the polymer.

In addition, the curves of water-vapor sorption exhibit the hysteresis, as can also be seen in Figure 3. In both curves of GEL- CoCl_2 -0% and -14.9%, the water content during desorption is almost the same at 81% RH, but is somewhat greater below 62% RH than that during adsorption. In both curves of GEL- CoCl_2 -4.2% and -8.0%, the water content

during desorption is a little less at 81% RH, but is somewhat greater below 62% RH than that during adsorption. The measurement of water sorption on GEL by Rao and Das¹⁶ showed that the hysteresis loop in the low-RH region is larger than that in the middle- and high-RH regions, particularly after several cycles of adsorptions and desorptions. Their results seem consistent with ours. They explained the hysteresis on the basis of the cavity theory. Taking into account their explanations, it is supposed that the cavity necks shrink more than does the body in the low-RH region, which results in an increased entrapping effect and, hence, a large hysteresis. It is well known that there are cross-linked networks, composed of multiple hydrogen bonds, in GEL film.¹⁷ It seems that the network contributes to the formation of the cavity, which entraps water molecules.

The hysteresis behavior in water sorption is somewhat different to that in the humidity dependencies of the absorbance, described above. The fact that the absorbance during desiccation is slightly greater above 63% RH than that during humidification suggests the following: Almost all the Co complexes come to interact with the medium in the high-RH region and the water molecules, bonded to the complex, can move more easily during desiccation than during humidification. It seems that the complexes, shielded by the polymer chains, are lib-

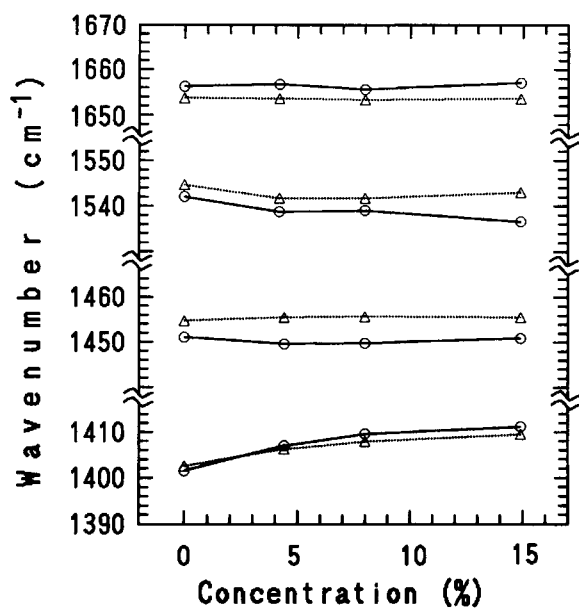


Figure 4 The frequency of the main absorption bands for the IR spectra of the GEL films in the (—) dry and (···) hydrated states as a function of the concentration of CoCl_2 .

erated in the strongly hydrated state in the high-RH region.

IR Spectrum

The IR spectrum provides further information about the interaction between the polymer and the Co ion and the hydration process of the composite films. Figure 4 is a plot of the frequency of the key absorption bands for the IR spectra of the GEL films in the dry and hydrated states as a function of the CoCl_2 concentration. The most pronounced and unambiguous change is observed in the absorption band around 1405 cm^{-1} . For both the dry and hydrated films, the frequency increases monotonously with increasing the CoCl_2 concentration and reaches to a maximum at the concentration of 14.9%. Considering the principal site for the binding of the Co ion, this band seems to be ascribed to the carboxyl groups of aspartyl and glutamyl residues of GEL. It is supposed that the absorption band at 1402 cm^{-1} for the original GEL film in the dry state is asso-

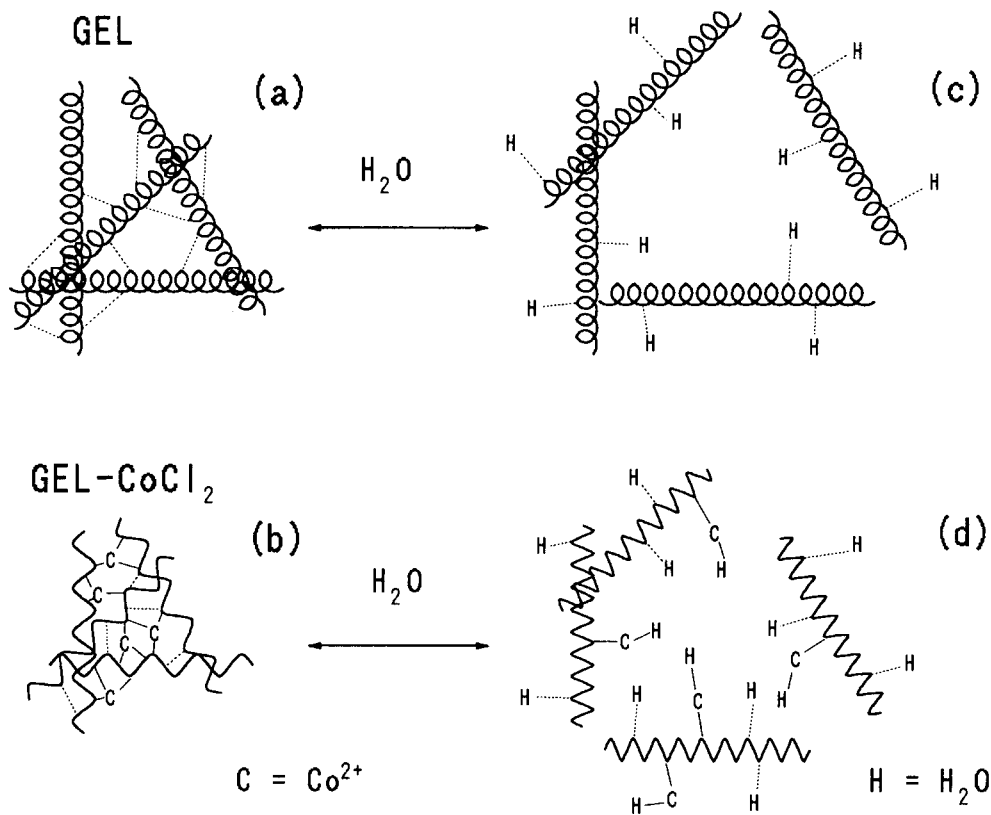


Figure 5 Hydration process of GEL films with and without CoCl_2 .

ciated with C—O—H bending modes; on the other hand, the band at 1411 cm^{-1} for GEL-CoCl₂-14.9% in the dry state represents COO⁻ symmetric stretching modes. According to the reported amino acid composition of cod skin collagen,¹⁸ the percentage ratio and the molar concentration of the aspartyl and glutamyl residues is 12.9% of the total residues and 1.43 mmol/g polymer, respectively. On the other hand, the molar concentration of CoCl₂ is 0.33, 0.67, and 1.35 mmol/g polymer, respectively, corresponding to the percentage concentration of 4.2, 8.0, and 14.9%. The molar concentration of the carboxyl groups of GEL is somewhat larger than that of CoCl₂, which explains the frequency change of the 1405 cm^{-1} band in Figure 4. Further, this reveals that most of the carboxyl groups are coordinated to the Co ions.

The amide II bands in the vicinity of 1540 cm^{-1} , for the dry films, are fairly affected by the CoCl₂ concentration, while the amide I bands in the vicinity of 1655 cm^{-1} are not affected as much. Since no absorption band has been observed near 1540 cm^{-1} in the spectra of poly-L-proline,¹⁹ the former is probably related to the amide NH of amino acid residues other than prolyl and hydroxyprolyl ones. In contrast, the latter is composed of many components related to various kinds of carbonyl groups. The amide II bands show a low-frequency shift of ca. 8 cm^{-1} , suggesting that the binding of the Co ion weakens the strength of hydrogen bonds between the peptide groups of GEL. In addition, this band increases the frequency when the film is hydrated, indicating increased strength of hydrogen bonding of the peptide groups. Similarly, the amide I bands and the 1335 cm^{-1} bands (CH bending) shift to lower and higher frequencies, respectively, when the films hydrated. Researchers in the past have reported the change of IR spectra of collagen as a function of RH and have obtained similar results.²⁰ It seems that water molecules, sorbed in the film, break the coordination bond between the Co ion and (probably the peptide groups of) GEL and are attached to the peptide backbone. On the other hand, the 1405 cm^{-1} band is not much influenced by hydration, which indicates that the carboxyl groups, linked to the Co complex, are not replaced by water molecules sorbed in the film.

Structure of Gelatin-CoCl₂ Films

The water sorption was depressed in the low-RH region by addition of CoCl₂, as described above. In this connection, from the low-frequency shift of the

amide II band, it was suggested that the binding of the Co ion weakens the strength of hydrogen bonds between the peptide groups of GEL. These situations are illustrated in Figure 5(a) and (b). It has been reported that the GEL film prepared from the solution by evaporation at room temperature and 40°C, respectively, has a collagen- and α -fold structure.¹⁷ It seems that these helical conformations exist in the present GEL films, whereas addition of CoCl₂ results in the formation of interchain cross-links via the Co complex, which restricts the natural tendency to the assumption of the helical conformation. This effect of CoCl₂ is similar to that of chemical cross-linking agents.²¹ Therefore, it seems that there are cross-linked networks, composed of the hydrogen bonding and the coordination bond via the Co complex, in the dry films of GEL-CoCl₂; as water molecules are adsorbed by the film, they gradually destroy these bonds and swell the polymer, as shown in Figure 5(c) and (d). The IR spectral data also supported the above conclusion, because of the frequency shift of the absorption bands corresponding to increased strength of the hydrogen bonds.

The absorption spectra of the GEL-CoCl₂ films as a function of RH demonstrated that, with increasing RH, the 4-coordinate form of the Co ion changes to the 6-coordinate one, accompanied by the replacement of relatively weak ligands by water molecules. The IR spectral data indicated that most of the carboxyl groups of GEL are linked to the Co complex even in the strongly hydrated state, and, hence, water molecules, sorbed in the film, eliminate the peptide groups of GEL. It seems that these exchanges of the ligands result in the formation of the CoXCl₃ and CoX₃Cl entities, which was predicted from the spectral difference for the films containing varying concentrations of CoCl₂.

REFERENCES

1. W. R. Seitz, *Anal. Chem.*, **56**, 16A (1984).
2. W. E. Morf, K. Seiler, B. Lehmann, Ch. Behringer, K. Hartman, and W. Simon, *Pure Appl. Chem.*, **61**, 1613 (1989).
3. O. S. Wolfbeis, H. E. Posch, and H. W. Kroneis, *Anal. Chem.*, **57**, 2556 (1985).
4. D. M. Jordan and D. R. Walt, *Anal. Chem.*, **59**, 437 (1987).
5. C. Zhu, F. V. Bright, and G. M. Hieftje, *Appl. Spectrosc.*, **44**, 59 (1990).
6. K. Wang, K. Seiler, J. P. Haug, B. Lehmann, S. West,

- K. Hartman, and W. Simon, *Anal. Chem.*, **63**, 970 (1991).
7. Y. Sadaoka, M. Matsuguchi, and Y. Sakai, *Sens. Actuators A*, **26**, 489 (1991).
8. S. Muto, A. Fukasawa, M. Kamimura, F. Shinmura, and H. Ito, *Jpn. J. Appl. Phys.*, **28**, L1065 (1989).
9. C. Zhu, F. V. Bright, W. A. Wyatt, and G. M. Hieftje, *J. Electrochem. Soc.*, **136**, 567 (1989).
10. A. P. Russell and K. S. Fletcher, *Anal. Chim. Acta*, **170**, 209 (1985).
11. D. S. Ballantine and H. Wohtjen, *Anal. Chem.*, **58**, 2883 (1986).
12. F. Boltinghouse and K. Abel, *Anal. Chem.*, **61**, 1863 (1989).
13. W. U. Malik and M. Muzaffaruddin, *Experientia*, **21**, 232 (1965).
14. J. Bello and H. R. Bello, *Nature*, **192**, 1184 (1961).
15. L. I. Katzin and E. Gebert, *J. Am. Chem. Soc.*, **72**, 5464 (1950).
16. K. S. Rao and B. Das, *J. Phys. Chem.*, **72**, 1223 (1968).
17. D. C. Robinson and M. J. Bott, *Nature*, **168**, 325 (1951).
18. F. Laszlo and B. R. Olsen, *Eur. J. Biochem.*, **11**, 140 (1969).
19. T. Isemura, H. Okabayashi, and S. Sakakibara, *Biopolymers*, **6**, 307 (1968).
20. H. Susi, J. S. Ard, and R. J. Carroll, *Biopolymers*, **10**, 1597 (1971).
21. I. H. Coopes, *J. Polym. Sci. A-1*, **6**, 1991 (1968).

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