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Review

Functionality of cellulose by impregnation of inorganic substances

Y. Kurokawa[a]* & K. Hanaya[b]

[a]*Department of Engineering Science, Faculty of Engineering, Tohoku University, Aoba, Aramaki, Aoba-ku, Sendai 980, Japan;*
[b]*Research Institute for Science Education, Miyagi University of Education, Aramaki, Aoba-ku, Sendai 980, Japan*

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Functionalities of cellulose with inorganic substances were examined in the forms of membrane and fiber. Cellulose membranes impregnated with iron oxide, metal (Pd, Ru, Rh) particles, and silver particles acted as Donnan effective permselective membrane, hydrogenation–catalytic membrane, and surface-enhanced Raman scattering active substrate, respectively. Enzyme was entrap-immobilized onto cellulose–TiO_2 (ZrO_2) gel fiber via gel formation of cellulose with metal alkoxide. The entrap-immobilized fiber is stable in common solvents, phosphate and electrolyte solution over a wide range of pH values (4–10).

INTRODUCTION

Natural polymers such as cellulose have gained increasing interest in some aspects: high biological compatibility, renewability, biodecomposition in comparison to synthetic polymers. Many workers have so far reported improvements of cellulose by organic substitution of hydroxyl groups on the glucose residue (Kennedy *et al.*, 1989). The properties of cellulose derivatives depend on the degree of substitution and the distribution of substituents. On the other hand, there are many studies of mineral fillers (TiO_2, $BaSO_4$) in the paper making industry (Marchessault *et al.*, 1992), in which the importance of electrostatic interactions of cellulose with fillers is noted (Siffert & Metzger, 1991). However, there are few papers on the functionality of cellulose with inorganic substances. We have so far investigated the functionalities of cellulose by impregnating with inorganic substances. This paper is a mini-review of these results.

MATERIALS AND METHODS

Cellulose acetate (CA, acetyl content: 39.8% Wako Pure chemicals) was used exclusively for membrane and fiber. The former was prepared by varying the composition of cast solvents (acetone + formamide) (Kurokawa, 1975), and the latter by using acetone solution. The casting solution was prepared by using 39.8% acetyl content CA, formamide and acetone in the proportions 25:30:45 by weight. This solution was cast on a glass plate in a glove box. Cast membranes were gelled in an ice-cold bath for 3 h. Impregnation of inorganic substances into CA gel membrane was carried out via a counter-diffusion method (Kurokawa, 1982). The hydrous Ag_2O-impregnated membrane was obtained by inserting a CA membrane between 0.1 M $AgNO_3$ solution and 0.1 M NaOH solution. The impregnated membrane was next immersed in 0.1 M $NaBH_4$ solution. In this step, the color of impregnated membrane changed from white-grey to blue-grey. This indicated that the hydrous Ag_2O had been reduced to metal. Ag particle-impregnated membrane was dipped into an aqueous solution containing an analyte for 2 h. After removal from the solution, it was washed with 10–20 ml distilled water, then dried at room temperature for 2 h. Surface-enhanced Raman scattering (SERS) spectra were obtained by using a Spex 1403 spectrometer equipped with an Ar ion laser. Hydrous noble metal oxides were impregnated into the CA membrane by interfacing each side of the membrane with a 0.1 M metal salt solution and a 0.1 M NaOH solution. The

*Author to whom all correspondence should be addressed.

impregnated membrane was next immersed in 0.1 M $NaBH_4$ solution. The metal salts used were $RuCl_3$, $PdCl_2$ and $RhCl_3$. The metal particle-impregnated membrane was cut into small pieces. The pieces (0.05–0.2 g) were transferred into a flask containing an ethanol solution of the substrate. Hydrogenation rate measurements were performed by following the consumption of H_2 at constant pressure under room temperature. Reaction products were analyzed by gas chromatography (Hitachi 164 instrument) using a capillary column of PEG 400. The hydrous iron oxide-impregnated membrane was prepared by facing solution and a 0.1 M NaOH solution, respectively. Reverse osmosis (RO) properties of the impregnated membranes were examined by a continuous flow type (effective membrane area: 15 cm^2) under an operating pressure of 50 atm at 25°C. The CA acetone solution dispersed with enzyme (3 wt% of the former solution) was fed slowly through the nozzle into alkoxide acetone solution. After standing for 30 min, acetone was washed out from the fiber with pure water and phosphate solution. The enzymes which were immobilized included glucose oxidase, urease, invertase, L-amino acid oxidase, uricase (Kurokawa & Ohta, 1992; Kurokawa & Sano, 1992).

RESULTS AND DISCUSSION

Transport properties of impregnated CA membrane

The selectivity normally achieved in reverse osmosis is generally related explicitly to ion size, valence, physical and chemical properties of the membrane. Membranes can be formed dynamically on a porous support by adding an appropriate colloidal additive to the feed solution. This membrane is referred to as a dynamic membrane (Freilich & Tanny, 1978). Its rejection is commonly presented as a Donnan effect (Lonsdale & Pusch, 1975). An important advantage of this membrane is the elimination of the fabrication step and the high product rate. However, it lacks reproducibility and stability. In order to improve these disadvantages, the composite membrane was prepared by the impregnation of the hydrous metal oxide into the porous cellulose acetate membrane (Ueno *et al.*, 1979; Kurokawa, 1982). This membrane showed the property of salt rejection by the Donnan electrochemical effect. It has a magnetic susceptibility although it is weak. It is interesting to attempt an examination of the magnetic effect on salt rejection (Kurokawa, 1982). Rare earth ions are trivalent and have a similar ionic radius but a different magnetic moment.

It has been reported that the rejection of rare earth ions by membranes is increased significantly by the formation of the corresponding rare earth metal chelate. This effect seems to be related to reduction in the solute diffusivity associated with the increased size of the rare earth ion complex. The impregnated CA membrane obtained resembles a brownish solid solution. It is brittle with bending but resists compression. It appears that impregnation occurs by the interaction between the OH of cellulose and the hydrous metal oxide. The hydrous iron oxide impregnated was identified as a mixture of α-FeOOH (Geothite) and Fe_2O_3 (Hematite) by X-ray diffraction. The impregnated CA membrane was powdered into fine particles and a magnetic susceptibility was determined by magnetic balance. Figure 1 shows the relation between the magnetic field and strength of magnetization. From this result, a magnetic susceptibility was calculated as 1.51×10^{-5} emu/g.

Figure 2 shows the RO performance of the membrane during 300 h. Salt rejection remained almost constant (Kurokawa, 1982, 1987). The CA membrane of asymmetric structure exhibited a decline in water flux with time due to the compaction of the porous layer. To improve this decline, the CA membrane was reinforced with a filler (e.g. silica gel), but this method did not lead to satisfactory improvement since affinity of the filler for CA was not good (Baum *et al.*, 1972). The impregnated CA membrane did not show a flux decline with time as shown in the figure.

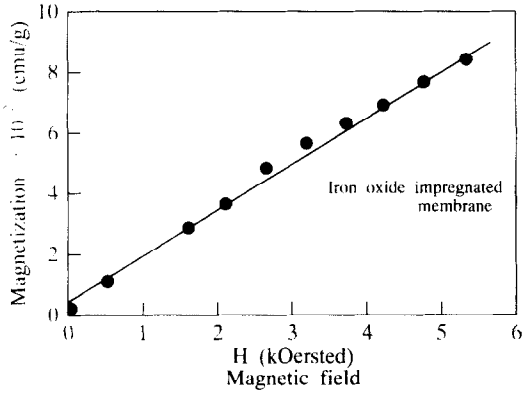

Fig. 1. The relation between magnetization and the magnetic field for ion oxide-impregnated CA membrane.

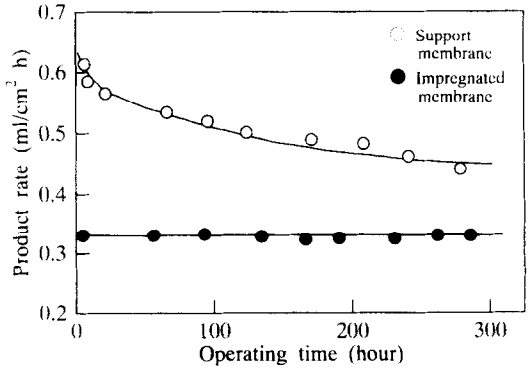

Fig. 2. Product rate with operating time at 25°C under 50 atm. Feed: 0.5 M NaCl. Membrane is the same as in Fig. 1.

Table 1. Physical properties of rare earth elements and rejection properties of membrane

Solute	Unpaired electron of cation	Ionic radius (Å) of cation	Magnetic moment of cation (debye unit)	RO rejection % (50 atm, 25°C)	
				Support CA membrane	Impregnated membrane[a]
$LaCl_3$	0	1.061	0	18.8	90.9
$CeCl_3$	1	1.036	2.56	16.3	93.5
$NdCl_3$	3	0.995	3.68	14.8	94.2
$GdCl_3$	7	0.938	7.94	12.0	93.9
$DyCl_3$	5	0.908	16.60	12.3	93.1
NaCl	0	0.980	–	6.2	37.2
$CaCl_2$	–	1.060	–	11.9	68.8

[a]Impregnated membrane: hydrous iron oxide–CA.

Separation of rare earth ions from each other is difficult since they have a similar ionic radius and chemical properties as shown in Table 1. However, the magnetic moment differs greatly. Therefore, RO rejection of rare earth ions was attempted in anticipation of a magnetic effect on rejection. Results are given in column 5 of Table 1 for both CA support membrane and the impregnated membrane. The impregnated membrane shows higher rejection compared to a support CA membrane. However, it has broad pore distribution owing to hydrolysis by alkali (Kurokawa & Ueno, 1982). Despite broadening of pores by impregnation, the impregnated membrane gives a high salt rejection. This may be due to the Donnan electrochemical effect based on charging of the membrane by impregnation. The larger the valence, the greater the effect. The impregnated membrane has been estimated to be a cation exchanger of low fixed charge densities from a comparison between experimental and theoretical membrane potentials according to the fixed charge theory. As for rare earth ions, there is no change of rejection in the range of experimental error (±1%). A magnetic effect on rejection might be expected if the membrane which has a higher magnetic moment is used.

The authors evaluated the permeability ratio from the membrane potential using the equation of Hodgkin & Katz, assuming the Cl^- permeability to be unity (Kurokawa & Ueno, 1982). The order of salt rejection was consistent with the order of the permeability ratio determined from the membrane potential. Figure 3 shows the relationship between the membrane potential in the systems of NaCl, $CaCl_2$ and Na_2SO_4 solutions and the concentration ratio referring to the standard concentration of 5×10^{-2} N. The membrane potentials vary non-linearly with concentration ratios and are similar. The salt, such as the divalent ion, shows the higher membrane potential. Potential curves for NaCl and $CaCl_2$ solutions present a negative slope, but the Na_2SO_4 solution presents a positive slope. A negative slope indicates that the membrane is anion-selective. A positive slope indicates that the membrane is cation-selective. This may be attributed to the strong adsorption on CA membrane which leads to a stage in which a

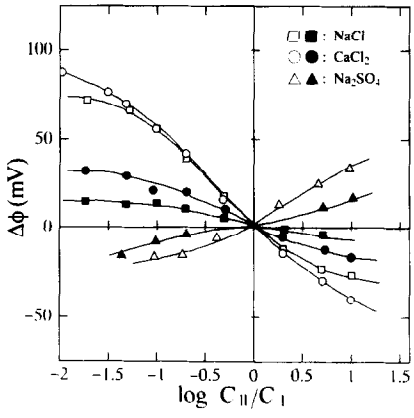

Fig. 3. Membrane potential arising across membrane with concentration ratio: □, NaCl; △, Na_2SO_4. Open symbols denote support CA membrane and closed symbols denote the impregnated CA membrane. Membrane is the same as in Fig. 1.

negative charge is fixed on the membrane. Such behavior is not peculiar to this system. Siddigi *et al.* found that the surface charge on ferrocyanide membranes was reversed from negative to positive by adsorption of di- and trivalent cations (Siddigi *et al.*, 1971). The impregnated membrane may consist of a tridimensional polymeric network of hydrated metal oxide containing an ionic group. The impregnated membrane may expel the co-ion by electrostatic repulsion according to the Donnan ion exclusion effect. The apparent transport numbers of cations are calculated from the slope at the point of $\log C_{II}/C_I = 0$. The results are $t^+(NaCl) = 0.20$, $t^+(Na_2SO_4) = 0.6$ for hydrous iron oxide impregnated membrane; and $t^+(NaCl) = 0.5$, for support CA membrane. The support CA membrane is nearly non-selective. The fixed charge on the membrane can be estimated from the fixed charge theory of membrane potentials. The fixed charge of the impregnated membrane is approximately 10 times that of the support CA membrane. Gas permeabilities through iron oxide-impregnated membrane have been investigated for H_2, He, CH_4, N_2, O_2 and CO_2 gases. Selectivity did not appreciably change but permeability did appreci-

ably compared to CA membrane without impregnation (Suzuki *et al.*, 1984).

Ag particle-impregnated CA membrane as a SERS active substrate

The majority of SERS in aqueous solution have been carried out for Ag electrode surfaces. Some investigators have developed practical SERS active substrate that can be easily prepared and that can give data with reproducibility and accuracy for analytical purposes (Otto *et al.*, 1992). The observation of SERS by molecules adsorbed on Ag colloid has been the subject of considerable interest in recent years. Otto *et al.* have successfully indicated that the SERS spectra of DNA components and some of their derivatives on Ag colloid can be obtained at concentration levels as low as 10^{-5}–10^{-7} M (Otto *et al.*, 1986). However, the procedure necessitates the use of surface active agent to stabilize Ag colloid which often interferes with the spectra. The signal changes depending on the extent of the aggregation induced by the analyte addition on Ag colloid. Here, the authors prepared SERS active cellulose gel membrane impregnated with Ag particles and tried to detect biorelated materials (Kurokawa & Imai, 1991; Ishikawa *et al.*, 1994).

The impregnated membrane resembles a solid solution which has a thickness of 100–120 μm. It contains fine Ag particles diameters in the range of 7–15 nm. The particle content is in the range of 5–20 wt%. Extent of dispersion is characterized by a color which can vary from yellow (monodispersed) through blue to gray (aggregated). This may be caused by clustering of particles into the water domain of polymer through the diffusion period. The impregnated membrane is stable over a few years because the particles are stabilized by the gel network of polymer. The intimate structure of polymer and Ag particles may facilitate SERS observation. Figure 4 shows an electron micrograph of a sample dissolved in acetone (*c.* 10^{-6} M), indicating the presence of aggregated particles.

Figure 5 shows the SERS spectrum of uridine which has been already reported by some investigators using colloids (Oh & Kim, 1988; Sanchez-Cortes & Carcia-Ramos, 1992; Otto *et al.*, 1986). It is known that uridine has two forms at different acidities in solution and that the molecule is deprotonated at N–H of the uracil ring at pH > 10 and has a neutral form at pH < 9. Three marker bands were observed at 590, 1035 and 1293 cm^{-1} for the deprotonated form (Oh & Kim, 1988). Oh & Kim have concluded that uridine has the deprotonated form on Ag colloids, because the three marker bands were observed at 598, 1035 and 1288 cm^{-1} in the SERS spectrum (Oh & Kim, 1988). The two marker bands observed at 598 and 1288 cm^{-1} in the Ag colloid correspond to the 591 and 1270 cm^{-1} bands, respectively, in the present work. Although the

Fig. 4. TEM photograph of Ag fine particles in Ag particle-impregnated CA membrane.

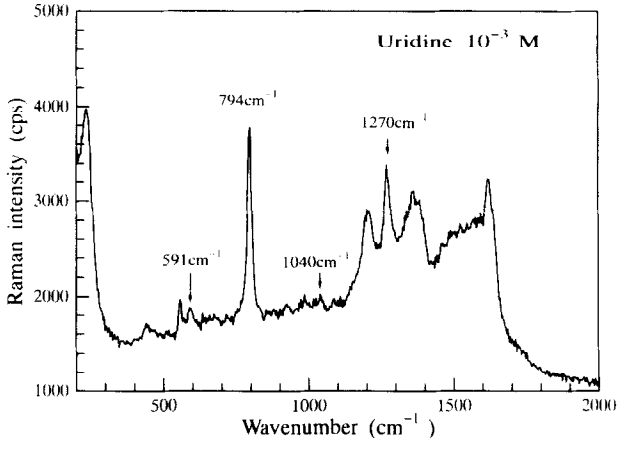

Fig. 5. SERS spectrum of uridine adsorbed on Ag particle-impregnated membrane. Concentration of test solution: 10^{-3} M.

second marker band at 1035 cm^{-1} for the Ag colloid is weak in the impregnated membrane spectrum, the band seems to be observed at approximately 1040 cm^{-1}. Therefore, it is concluded that the molecule in the impregnated membrane probably has the same form as that in the Ag sols. Oh and Kim observed weak peaks at 872 and 1109 cm^{-1} which were assigned to the vibrational modes of the ribose ring, and then suggested the appearance of the two bands as evidence for the flat orientation of the molecule on the surface of Ag colloids (Oh & Kim, 1988). In the spectrum for the impregnated membrane, no ribose bands were observed in the frequency region corresponding to their observation. Therefore, it is considered that only the uracil ring is adsorbed on the silver surface and the ribose ring is separate from the surface in this impregnated membrane. The band at 794 cm^{-1} is assigned to the breathing mode of the uracil ring in uridine and is the most intense band in its SERS spectrum. This band was observed for the

membrane dipped into 10^{-6} M solution, thus it can be used as a key band to detect uridine.

Quinacrine, ellipticine and nicotine are alkaloids. The first two contain a polycyclic aromatic ring system which interacts with DNA by intercalation between base pairs. Quinacrine and ellipticine are drugs used in the treatment of malaria and tumor in clinical medicine, respectively. Nicotine is a one of the substances which causes lung cancer. Normal Raman spectra cannot be obtained because of their fluorescence. The SERS spectra are accompanied by their strong quenching of fluorescence. Figure 6 shows SERS spectra of quinacrine hydrochloride which was permeated into the membrane in a 1×10^{-4} M aqueous solution. There are intense Raman bands in the range 1250–1500 cm^{-1}. There are no reports on the vibrational assignments of quinacrine hydrochloride. Therefore, these bands were not clearly assigned but they are probably due to in-plane ring modes, taking into account the observed frequency range. Since no strong Raman bands are observed below 1100 cm^{-1} where out-of-plane modes are expected, the molecule does not lie flat on the Ag surface at least, according to the surface selection rule proposed by Moskovits (1982). The strongest band at 1370 cm^{-1}, which may be assigned to an in-phase breathing mode of three rings in the molecule, can be used as a key band for the detection of quinacrine. Figure 7 shows a logarithmic plot of the intensity of this band vs the concentration of quinacrine in the solution. Although the figure shows some scatter, the curve is approximately linear over nearly four orders of magnitude. The curve suggests the existence of some kind of adsorbed species on the Ag surface, because the relationship denotes an obedience to the Freundlich-type adsorption equation (Atkins, 1990). The impregnated membrane can probably be utilized as a preferred test substrate in detecting trace amounts of SERS active substances in aqueous solution.

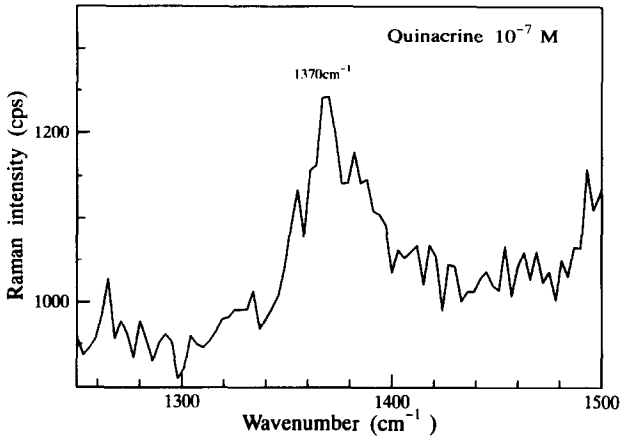

Fig. 6. SERS spectrum of quinacrine hydrochloride adsorbed on Ag particle-impregnated membrane. The concentration of test solution: 10^{-7} M.

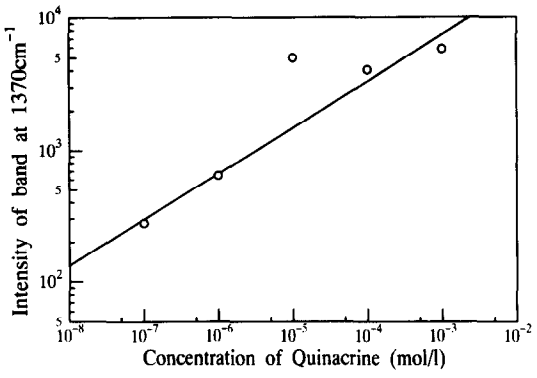

Fig. 7. Plot of concentration of quinacrine hydrochloride vs intensity of the band at 1370 cm^{-1}

Catalytic impregnated CA membrane

It is reported that Pd/CA membrane prepared from soluble metal salt and CA shows catalytic activities under mild conditions in a reaction such as hydrogenation of C_2H_4 and oxidation of CO (Hwang & Shim, 1992). The disadvantage of the homogeneous hydrogenation, the difficult separation of catalyst and hydrogenated product, can be eliminated by fixation of the catalyst to a support such as polymer. For hydrogenation in food technology, synthetic polymers are not permitted. Therefore, natural polymers such as cellulose play a key role in an approach to hydrogenation of unsaturated fatty acids (Bayer *et al.*, 1986).

We have prepared reactive impregnated membranes with uniformly dispersed fine metal particles by a counter-diffusion method. Their catalytic activities and selectivities have been examined in the hydrogenation of unsaturated compounds (Kurokawa & Ishikawa, 1993). Here, 2-methyl-4-chromon(2-methyl-4H-1-benzopyran-4-one) and 3-methyl-4-chromon (3-methyl-4H-1-benzopyran-4-one) were used as substrates. The data are given in Table 2. The reaction scheme for hydrogenation is shown in Fig. 8. there are three reaction paths, a saturated ketone production path by 1,2-hydrogen

Table 2. Hydrogenation of 2-methyl-4-chromon and 3-methyl-4-chromon

Catalyst	Product (%)							
	2-methyl-4-chromon				3-methyl-4-chromon			
	II	III	IV	V	II	III	IV	V
Raney Ni	94	6	0	0	90	10	0	0
Ni/CA	72	28	0	0	86	14	0	0
Rh-black	20	61	19	0	18	63	19	0
Rh/CA	10	24	48	18	10	32	54	4
Pd black	12	73	15	0	11	49	40	0
Pd/CA	3	28	69	0	19	40	41	0
Pt/black	41	17	0	42	66	26	0	8
Pt/CA	43	25	14	12	66	23	8	3

See scheme (Fig. 8). Temperature = 25 ± 0.1°C.

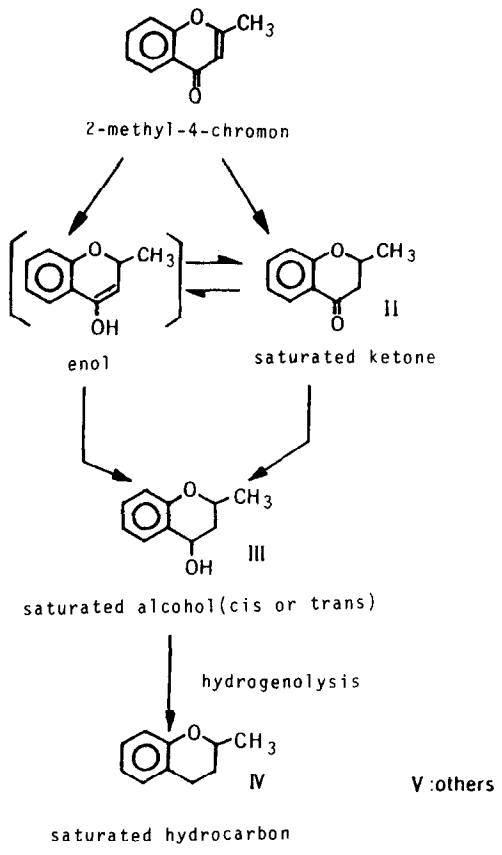

Fig. 8. A scheme for the hydrogenation reaction of chromon.

addition, and a saturated hydrocarbon production path by hydrogenolysis of saturated alcohol. Raney/Ni, Ni/CA produce chiefly saturated ketone and Ni/CA produces saturated alcohol. Rh and Pd black catalysts yield mainly saturated alcohol, Rh/CA and Pd/CA yield unknown compounds but also produce saturated ketone considering the published reaction mechanism of hydrogenation (Phillip & Mentha, 1956). From the table, it can be seen that Ni and Pt catalysts yield saturated ketone through the preferential reaction of 1,2-hydrogen addition, Rh and Pd catalysts yield saturated alcohol through the preferential reaction of 1,4-hydrogen addition. Despite the difference in state (black, distributed), the same metal denotes similar behavior.

This difference in selectivity may be due to characteristic properties of the metal itself. The reaction with catalytic membrane does not stop in one step but proceeds in a further hydrogenation step which produces saturated alcohol and hydrocarbon. In the Rh catalyst, non-identified products were found which may be due to hydrogenation of the benzene ring. These results reflect an enhancement of hydrogenation by decreasing particle size. Data on 3-methyl-4-chromon are given in Table 2. Its catalytic behavior is very similar to that of 2-methyl-4-chromon. The main products are saturated ketone in Ni and Pt catalysts, and saturated alcohol in Rh and Pd catalysts. Rh/CA shows the size effect of the catalyst, however, Pd/CA does not show this so much. Such phenomena should be correlated with changes in particle sizes of metal catalysts and support CA membrane. This simple method of preparation should produce well-dispersed metal particles supported on a polymer and avoids the need for immobilization procedures used in other catalyst preparations.

Gel fiber formation of CA with metal alkoxide and its application to enzyme-immobilization

We have been investigating the formation of a polymer–inorganic composite and its properties (Kurokawa & Ono, 1983). It was noted that a homogeneous gel is formed when an organic solution of CA is in contact with an alkoxide solution. It has been reported that an oil-like substance is formed from contact of the polysaccharide solution and titanium triethanolamine, but the reaction mechanism is not well established (Kiefer & Touey, 1965). Hydrated TiO_2 and ZrO_2 have been shown to be suitable as matrices on which enzymes are immobilized with retention of enzyme activity (White & Kennedy, 1980). The immobilized enzyme on TiO_2 is prepared by hydrolysis of a $TiCl_4$ solution co-existing with the enzyme. Hydrated TiO_2 is very fine, and a finite amount of enzyme then leaks from the matrix by washing or during reactions. In addition, it is difficult to form it into different shapes. Alginate and carrageenan gels are widely employed to immobilize enzymes (Chibata, 1990). The lack of stability of alginate gels to phosphate buffer is a serious problem. Although interest in the use of cellulose as a matrix for enzyme-immobilization has existed for a long time, cellulose has not been applied to a significant degree (Gemeiner *et al.*, 1993). We examined the gel formation of CA with an organometallic compound and its application to enzyme immobilization.

The gel formation between alkoxide and CA may be due to co-ordination bonding between the hydroxyl group on pyranose rings and the polyvalent metal (Kurokawa *et al.*, 1994). The mixture of cellulose triacetate with titanium and of cellulose diacetate with sodium alkoxide did not give good gel formations. In addition to the above reaction, one part of the alkoxide entrapped may undergo multiple hydrolysis reactions followed by contact with water. The fiber is stable in common solvents, phosphate and electrolyte solutions over a wide range of pH values. Rigidity or hardness of the fiber is controlled by the alkoxide content of the coagulation solution. The lower the alkoxide content, the softer the fiber. A higher alkoxide content produces a more rigid fiber.

The fiber contains 5–20 wt% metal oxide although this depends on the preparation conditions. It shows no X-ray diffraction pattern, indicating very fine amorphous particles. Then, it has an amphoretic property acting both on anion and cation exchangers depending

on the pH of the solution. The uptake of phosphate increases with a decrease of pH and that of potassium increases with an increase of pH. Some immobilized enzymes denote the shifts of optimum pH value towards alkalinity. This may be due to an amphoretic property of the metal oxide described above. Namely, the fiber provides a microenvironment for the immobilized enzyme that has a higher H^+ concentration (lower pH) than the concentration in the surrounding solution where pH is actually measured. For example, the pH profile of invertase activity is shown in Fig. 9. It is considerably widened due to diffusional limitations. The thermal stability of immobilized enzymes is one of the most important criteria for their application. The immobilized enzyme on fiber is more heat-resistant than a native and immobilized one on alginate gel as shown in Fig. 10.

As for the stabilities of the immobilized enzymes, Fig. 11 illustrates the effects of repeated runs on residual activities. The high stability of fiber-entrapped enzyme is in marked contrast with the rather poor durability of alginate-entrapped enzyme. Figure 12 shows the activity of the immobilized enzyme in the continuous reaction system. It retains a large proportion of its activity after 40 days. This new immobilization method has advantages over other methods in that it can be easily performed under mild conditions. It can also be easily tailored in the forms of powder, beads and fibers. This composite fiber is insoluble in all common solvents and in high aqueous ionic solutions.

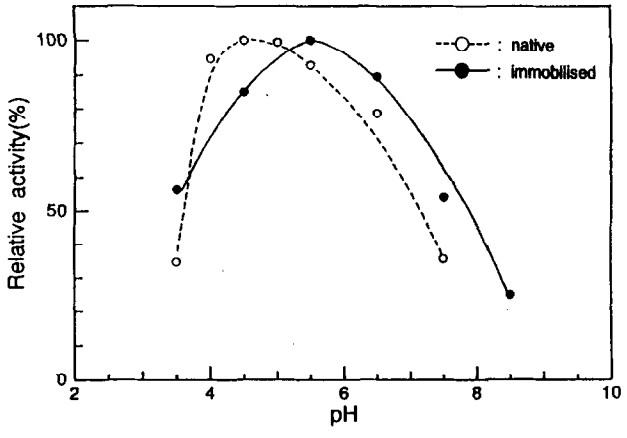

Fig. 9. The pH profile of invertase, native and immobilized enzymes.

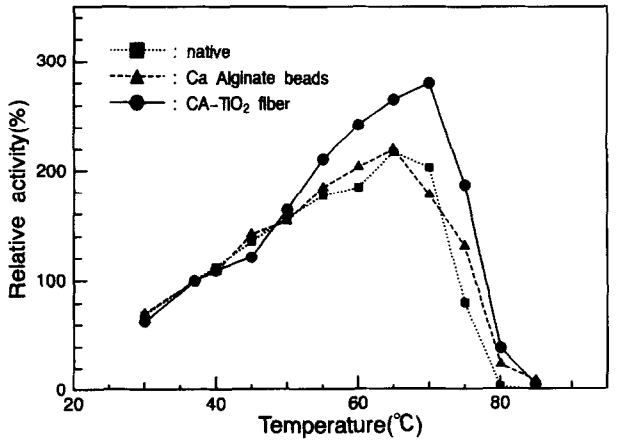

Fig. 10. Comparison of thermal stability of invertase.

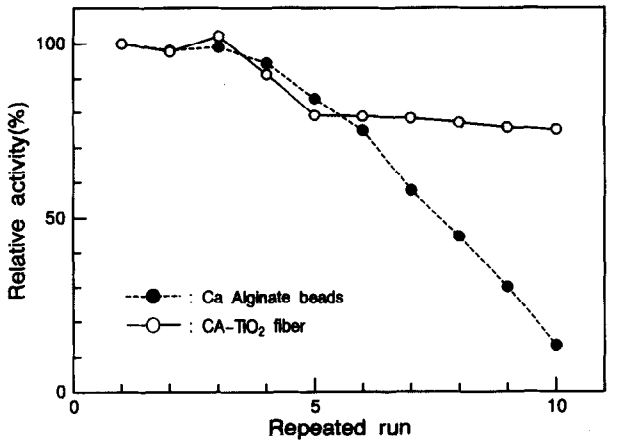

Fig. 11. The effects of repeated runs on the residual activity.

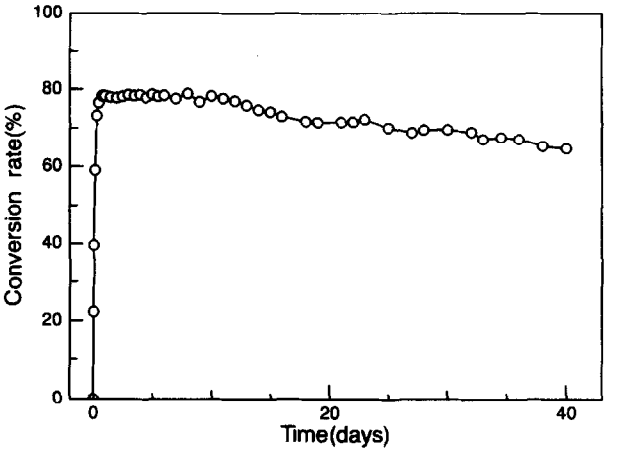

Fig. 12. Activities of invertase on CA-TiO_2 in the continuous reaction system.

CONCLUSION

We have investigated the interaction of cellulose with inorganic substances and examined the properties of the resultant impregnated membrane and fiber, permselectivities, catalytics, SERS activities, and enzyme immobilization. It seems that the last two properties are of great practical values. Ag particle-impregnated membranes are applicable to the detection of biomaterial substance in the 10^{-5}–10^{-8} M region. Enzyme-immobilized fiber is stable in phosphate solution and electrolyte solution over a wide range of pH values (4–10).

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