Novel Approaches for Controlled Formose Reaction toward Selective Sugar Synthesis

A Doctoral Thesis

by

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Acknowledgement

This thesis presents a research that the author has worked at the Department of Macromolecular Science, Graduate School of Science, Osaka University, and supervised by Professor Takahiro Sato and Professor Akihito Hashidzume from 2012 to 2018.

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Contents

Chapter 1

General Introduction

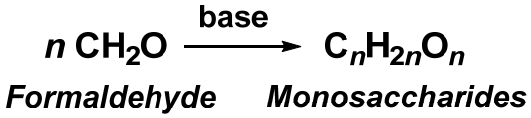
1-1. Background: Formose Reaction

Sugars play essential roles for our lives as carbohydrate foods, polysaccharide materials, genetic biomacromolecules, and so on. The sugars are generally produced via enzymatic reaction in living bodies such as photosynthesis. In contrast, industrial technology to produce sugars artificially and efficiently has not been developed well. The technique to produce sugars will enormously contribute to overcoming food crisis, carbohydrate production from waste materials even in space crafts, and industrial business to manufacture rare sugars for health food, drug discovery, and life science. In addition, understanding the mechanism to form carbohydrates from C1 or C2 compounds can be helpful to reveal the mystery how sugar was created on the earth in the prebiotic era, which would be close to origin of life. Although some protocols have been reported to synthesize sugar compounds,[1,2] they are still not applicable to industrial production because of low manufacturing efficiency and high cost. However, a limited chemical reaction to yield sugars readily is known as formose reaction.

Formose reaction yields monosaccharides under basic conditions via

oligomerization of formaldehyde (Scheme1-1), which was first reported by Butlerov in 1861.[3] Formose reaction has been studied for over a century in order to develop the technique to produce carbohydrates, or understand how sugar was synthesized on the earth in the prebiotic era.[4-7] To approach the challenge, selective sugar synthesis via formose reaction is one of the most important agendas. In 1970s and 80s, Shigemasa et al.[8,9] had intensively studied this topic and found several methods to obtain some specific sugars selectively.[10,11] Contemporaneously, Inoue et al.[12] reported selective formation of dihydroxyacetone, a three-carbon sugar, using a thiazolium-type catalyst. Recently, Benner et al.[13] reported that five-carbon sugars including ribose are stabilized by borate minerals, suggestive of a plausible mechanism of the prebiotic sugar formation. More recently, Lambert et al.[14] also reported that silicate minerals stabilize sugars, providing new aspects for selective formose reaction. In spite of these many efforts, the experimental methods and knowledge for selective formation of sugars via formose reaction have still been limited so far, because the reaction mechanism is too complicated to be controlled.

Scheme 1-1. Formose Reaction



According to the fundamental studies,[6,8,15] formose reaction proceeds through several reaction steps simultaneously, providing more than 30 species of sugars that include sugar alcohols. It is known that formose reaction, i.e., the oligomerization of formaldehyde, consists of the following steps: initiation, propagation, decomposition, disproportionation, and aldose–ketose isomerization, which correspond to acyloin-type condensation, (cross-)aldol reaction, retro-aldol reaction, (cross-)Cannizzaro reaction, and Lobry de Bruyn–van Ekenstein transformation, respectively (Scheme 1-2). The disproportionation step of Cannizzaro reaction yields sugar alcohols.

Scheme 1-2. Elementary Steps of Formose Reaction

Initiation Step
Acyloin-Type Condensation

$$2\ HCHO \longrightarrow (CH_2O)_2$$

Propagation Step
(Cross-)Aldol Reaction

$$(CH_2O)_m + (CH_2O)_n \longrightarrow (CH_2O)_{m+n}$$

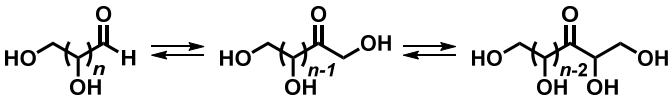
Decomposition Step
Retro-Aldol Reaction

$$(CH_2O)_{m+n} \longrightarrow (CH_2O)_m + (CH_2O)_n$$

Disproportionation Step
(Cross-)Cannizzaro Reaction

$$(CH_2O)_m + (CH_2O)_n \xrightarrow{H_2O} C_mH_{2m+2}O_m + C_nH_{2n}O_{n+1}$$

Aldose-Ketose Isomarization
Lobry de Bruyn-van Ekenstein Transformation



In addition, the time-course of formose reaction shows generally three stages: induction period, sugar-forming period, and sugar-decomposing period (Figure 1-1). In the induction period, formaldehyde is not consumed significantly, although Cannizzaro reaction slightly proceeds. In the sugar-forming period, formaldehyde is rapidly consumed, because glycolaldehyde, the dimer of formaldehyde formed in the initiation step, acts as a co-catalyst for formose reaction to promote the propagation step. During the sugar-forming period, propagation, disproportion, and isomerization steps rapidly proceed simultaneously, producing a variety of species of sugars and sugar alcohols. After full consumption of formaldehyde, the sugars formed are decomposed to not only smaller sugars via retro-aldol reaction but unknown products in the sugar-decomposing

period. Since the decomposing mechanism is very complicated, the structures and compositions of the decomposed products have not been still identified so far in spite of many studies of analytical chemistry. However, it is possible to know visually the onset of sugar-decomposing step from “yellowing point” at which the reaction mixture turns yellow. On the basis of the fundamental studies on formose reaction mechanism, if ones can control the second and third periods, in which consume formaldehyde rapidly and decompose the sugars formed, it is possible to obtain some specific sugars selectively by formose reaction.

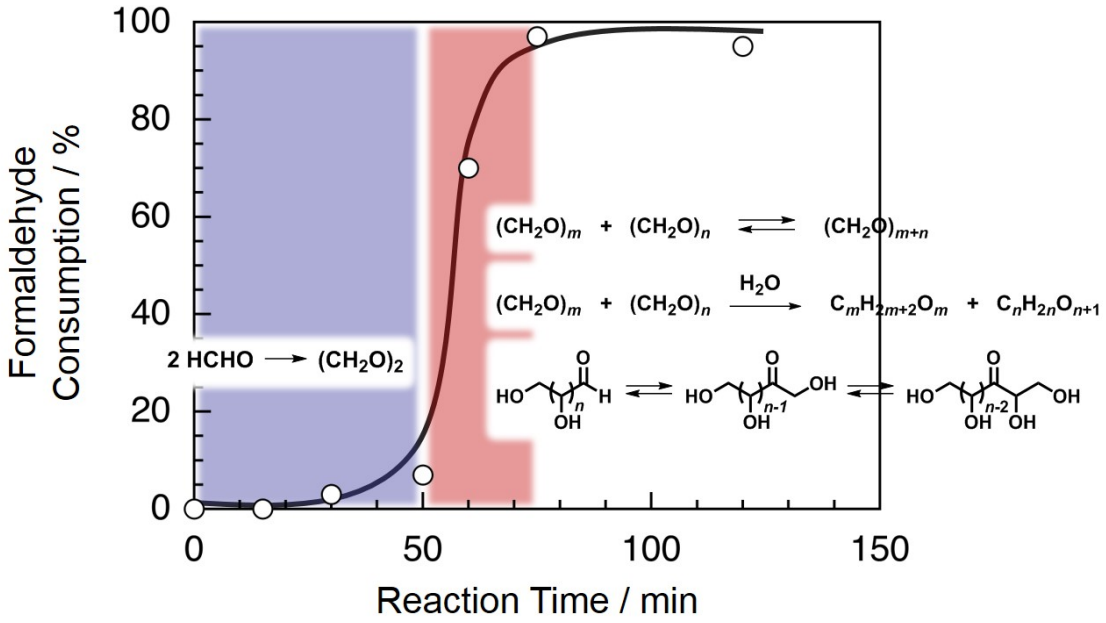


Figure 1-1. Typical time-conversion plots for formose reaction under a following condition: [formaldehyde] = 0.2 M; [calcium hydroxide] = 0.02 M; 60 °C. This data was obtained by the author. After an induction period, quick converting of formaldehyde, named sugar-forming period, is observed, triggered by the initiation step. During the sugar-forming period, propagation, disproportion, and isomerization steps rapidly proceed in parallel, resulting low selectivity in product.

1-2. Scope of This Thesis

In this thesis, therefore, the author reports novel approaches to control formose reaction so as to obtain sugars selectively with a focus on deceleration of the rapid sugar-forming period, which is mainly dominated by the propagation step. Although the

ultimate goal is to achieve selective and efficient synthesis of specific sugars, here the author has worked to obtain a formose reaction product with a narrower distribution than that obtained by the conventional formose reaction. In the following chapters, the author describes two novel approaches for "selective formose reaction", and discussed how effective the author's strategies are toward the ultimate goal.

In Chapter 2, the author focuses on the size of reaction medium to control the propagation step. The concept of this approach is "nanometer-scale flask", which confines precisely controlled the number of monomer molecules, i.e. formaldehyde (Figure 1-2). By controlling the number of reactants in separated reaction media, oligomerization degree of formaldehyde may be controlled, resulting in a narrower product distribution. Indeed, many publications on chemical reactions in nanometer-scale reaction media have recently been reported to achieve regioselective,[16] stereoselective,[17,18] molecular weight-controlled reactions,[17,18] or catalytic effects.[19,20] As the nanometer-scale flask, in this chapter, reverse micelles are used, which provide water droplets of a narrow size distribution in nanometer scale.[21] The author reports the kinetic study of formose reaction in reverse micelles and discuss the effect of reverse micelles as well as the product analysis in the reaction.

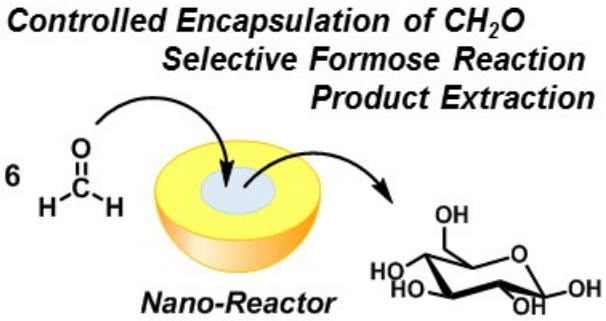


Figure 1-2. Conceptual illustration of selective formose reaction in "nano-size flask".

In Chapters 3 and 4, the author applys boronic acid chemistry into formose reaction in order to suppress the rapid propagation and decomposition steps (Figure 1-3). Since boronic acids form dynamic covalent bonding of boronate ester with dialcoholic compounds such as sugars, many scientists have utilized this dynamic covalent bonding to make sensor molecules for sugar compounds[22-24] or sugar-responsive materials.[25,26] Boronic acid compounds can stabilize sugars formed by formose reaction through boronate ester formation, because the propagation and decomposition step of formose reaction are aldol and retro-aldol reaction, respectively, in which ketone or aldehyde groups act as active species. In these chapters, hence, the author study formose reaction in the presence of low molar mass and polymeric boronic acid compounds. As polymeric compounds, anionic and nonionic polymers possessing boronic acid moieties are used in Chapters 3 and 4, respectively.

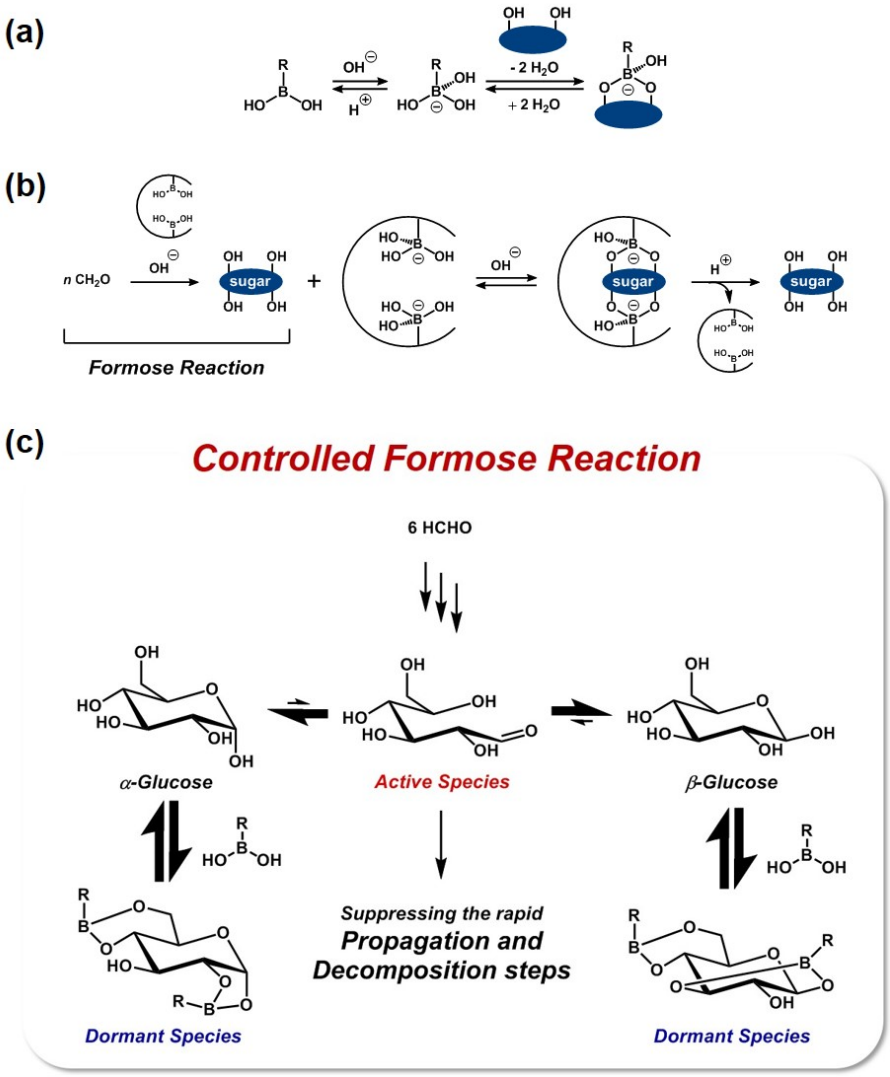


Figure 1-3. Reversible boronate ester formation of boronic acid with dialcoholic compound such as sugar under basic aqueous solution (a), a conceptual illustration of selective formose reaction controlled by boronic acid compound to stabilize sugar formed (b), and an expected mechanism of controlled formose reaction by boronic acid compound as an example when to form glucose (c).

In Chapter 5, finally, the author summarizes Chapters 2 – 4. Herethe authorI describes the effective factors for selective formose reaction, which have been understood in my doctoral study.

1-3. References

(1) Hudlicky, T.; Entwistle, D. A.; Pitzer, K. K.; Thorpe, A. J. *Chem. Rev.* **1996,** *96*, 1195-1220.

(2) Northrup, A. B.; MacMillan, D. W. C. *Science* **2004**, *305*, 1752-1755.

(3) Butlerow, A. *Ann. Chem.* **1861**, *120*, 295-298.

(4) Mizuno, T.; Weiss, A. H. *Adv. Carbohydr. Chem. Biochem.* **1974**, *29*, 173-227.

(5) Weiss, A. H.; Socha, R. F.; Likholobov, V. A.; Sakharov, M. M. *Appl. Cat.* **1981**, *1*, 237-246.

(6) Delidovich, I. V.; Simonov, A. N.; Taran, O. P.; Parmon, V. N. *ChemSusChem* **2014**, *7*, 1833-1846.

(7) Kim, H.-J.; Ricardo, A.; Illangkoon, H. I.; Kim, M. J.; Carrigan, M. A.; Frye, F.; Benner, S. A. *J. Am. Chem. Soc.* **2011**, *133*, 9457-9468.

(8) Shigemasa, Y.; Shimao, M.; Sakazawa, C.; Matsuura, T. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 2099-2102.

(9) Shigemasa, Y.; Taji, T.; Eaki, E.; Nakashima, R. *Bull. Chem. Soc. Jpn.* **1981**, 5*4*, 1403-1409.

(10) Shigemasa, Y.; Nagae, O.; Sakazawa, C.; Nakashima, R.; Matsuura, T. *J. Am. Chem. Soc.* **1978**, *100*, 1309-1310.

(11) Shigemasa, Y.; Kawahara, M.; Sakazawa, C.; Nakashima, R.; Matsuura, T. *J. Cat.* **1980**, *62*, 107-116.

(12) Matsumoto, T.; Yamamoto, H.; Inoue, S. *J. Am. Chem. Soc.* **1984**, *106*, 4829-4832.

(13) Ricardo, A.; Carrigan, M. A.; Olcott, A. N.; Benner, S. A. *Science* **2004**, *303*, 196.

(14) Lambert, J. B.; Gurusamy-Thangavelu, S. A.; Ma, K. *Science* **2010**, *327*, 984-986.

(15) Partridge, R. D.; Weiss, A. H.; Todd, D. *Carbohyd. Res.* **1972**, *24*, 29-44.

(16) Yoshizawa. M; Tamura, M; Fujita, M. *Science*. **2006**, *312*, 251-254.

(17) Serizawa, T; Hamada, K; Akashi, M. *Nature*, **2004**, *429*, 52-55.

(18) Uemura, T; Ono, Y; Kitagawa, K; Kitagawa, S. *Macromolecules.* **2008**, *41*, 87-94.

(19) Hashidzume, A.; Fujimoto, T.; Masaoka, M.; Sanada, Y.; Sato, T. *Kobunshi Ronbunshu* **2010**, *67*, 312-317.

(20) Fang, X.; Liu, Z.; Hsieh, M. F.; Chen, M.; Liu, P.; Chen, C.; Zheng, N. *ACS Nano* **2012** *6*, 4434-4444.

(21) Uskokovic, V.; Drofenik, M. *Surf. Rev. Lett.* **2005**, *12*, 239-277.

(22) James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1910-1922.

(23) James, T. D.; Phillips, M. D.; Shinkai, S. *Boronic Acids in Saccharide Recognition*; The Royal Society of Chemistry: Cambridge, UK, 2006.

(24) Zhai, W.; Sun, X.; James, T. D.; Fossey, J. S. *Chem. Asian J.* **2015**, *10*, 1836-1848.

(25) Ma, R.; Shi, L. *Polym. Chem.* **2014**, *5*, 1503-1518.

(26) Brooks, W. L. A.; Sumerlin, B. S. *Chem. Rev.* **2016**, *116*, 1375-1397.

Chapter 2

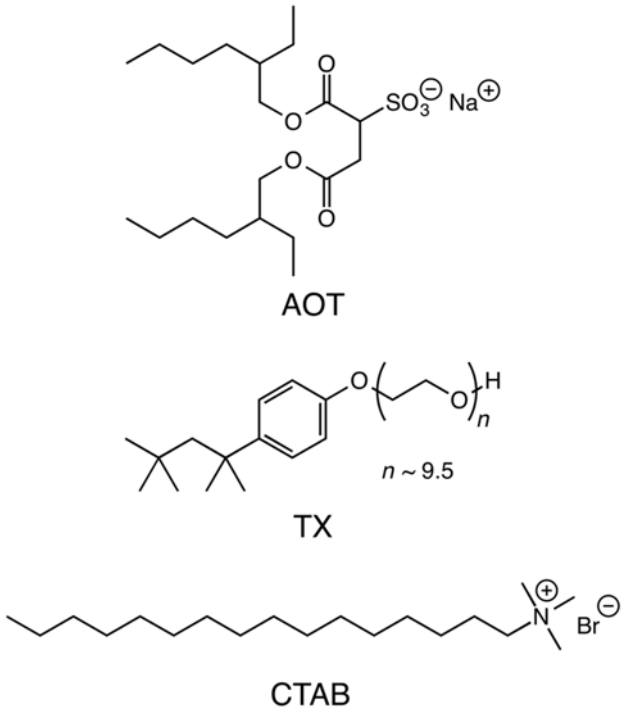
Formose Reaction Accelerated in Aerosol-OT Reverse Micelles

When an aqueous solution of formaldehyde is heated under basic conditions, a mixture of monosaccharides and sugar alcohols, i.e., "formose", is obtained.[1] This reaction is called "formose reaction". Since formose is a complicated mixture including non-natural monosaccharides, e.g., L- and branched isomers,[2,3] formose reaction is of no practical use. From late 1970's to 1980's, a few research groups in Japan devoted their efforts to formose reaction to obtain selectively useful monosaccharides. Shigemasa et al.[4-6] studied optimization of the conditions for selective formose reaction. They first isolated a seven-carbon branched sugar alcohol from a mixture obtained by formose reaction catalyzed with barium hydroxide in methanol.[7-13] Matsumoto and Inoue[14-16] successfully obtained dihydroxyacetone, i.e., a three-carbon monosaccharide, using a thiazolium catalyst in ethanol in the presence of triethylamine. After their pioneering works, only a few researches have been conducted on selective formose reaction.[17,18]

The author's group has first focused on the importance of reaction medium for formose reaction.[19] It is likely that five or six formaldehyde molecules occupy space of nanometer scale. Well-defined space of nanometer scale is provided as water pools in

reverse micelles of surfactants. Since the radius of water pools in reverse micelles of an ionic surfactant is practically proportional to the molar ratio of water and surfactant, the author focuses on reverse micelles as nanometer scale reaction media. The water pools of nanometer scale may also act as reaction media different from bulk water. In this chapter, formose reaction is investigated in water pools of reverse micelles formed from surfactants, i.e., anionic aerosol OT (AOT), nonionic triton X-100 (TX), and cationic hexadecyltrimethylammonium bromide (CTAB) (Scheme 2-1).[20]

Scheme 2-1. Structures of AOT, TX, and CTAB



2-2. Experimental Section

Materials. Aerosol OT (AOT) was purchased from Tokyo Chemical Industry and used without further purification. Triton X-100 (TX) and hexadecyltrimethylammonium bromide (CTAB) were purchased from Wako Pure Chemical Industries and used without further purification. An aqueous solution of formaldehyde (36 wt%) and calcium hydroxide were purchased from Sigma Aldrich Japan. Isooctane and 1-hexanol were purchased from Wako Pure Chemical Industries and used without further purification. 3,7-Diamino-2,8-dimethyl-5-phenyl phenazinium chloride (Safranine T), used as a fluorescent probe, was purchased from

Tokyo Chemical Industry. Potassium bromide, used as a quencher, was purchased from Sigma Aldrich Japan. Water was purified by a Millipore Milli-Q system. Other reagents were used without further purification.

Measurements. UV-Vis spectra were recorded on a JASCO V-550 spectrometer.

Dynamic light scattering (DLS) measurements were performed using an ALV/SLS/DLS-5000 light scattering instrument equipped with an ALV-5000 multiple τ digital correlator and a Nd:YAG laser operating at 532 nm at 30, 45, and 60 °C. The intensity autocorrelation functions $\{g^{(2)}(t)\}$ obtained were analyzed by the CONTIN algorithm to estimate the spectrum $\{A(\tau)\}$ of the relaxation time (τ) at each scattering angle θ or the magnitude of the scattering vector (k) expressed as

$$k = \frac{4\pi n_0}{\lambda} \sin\left(\frac{\theta}{2}\right) \tag{1}$$

where n_0 is the refractive index of the solvent, and λ is the wavelength of the incident light. The n_0 value of isooctane at 20 °C was used for all analyses. The hydrodynamic radius R_H can be calculated by

$$R_H = \frac{k_B T}{6\pi\eta_0} \left(\lim_{k\to 0} \frac{\Gamma}{k^2}\right) \tag{2}$$

where $k_B T$ is the Boltzmann constant multiplied by the absolute temperature, and η_0 is the solvent viscosity, which was determined for isooctane by viscometry using an Ubbelohde-type viscometer.

Fluorescence spectra for Safranine T were recorded with excitation at 520 nm on a Hitachi F-4500 fluorescence spectrometer at 30, 45, and 60 °C. The slit widths for excitation and emission sides were kept at 2.5 nm during measurements.

Formose Reaction. A typical procedure of formose reaction in water pools of reverse micelles is described below. An aqueous solution (1.8 mL) containing 200 mM formaldehyde and 20 mM calcium hydroxide was added to a solution of AOT in isooctane (100 mM, 100 mL) to adjust *w*. The mixture was warmed with a water bath thermostated at 60 °C. After predetermined times, aliquots of the reaction mixture (10 mL) were taken out by a syringe, and the reaction was terminated by rapid cooling with an ice water bath followed by neutralization with aqueous HCl (2.0 M, 0.2 mL). Water (10 mL) was added to the mixture to extract formaldehyde remaining, and then the mixture was centrifuged at 4000 rpm for 15 min. The aqueous layer obtained was washed with dichloromethane (10 mL) once. Using the clear aqueous layer obtained, the concentration of formaldehyde was determined by the acetylacetone method.[21,22]

As a reference experiment, formose reaction was also carried out in aqueous media as follows. An aqueous solution of formaldehyde (200 mM, 18 mL) and calcium hydroxide (0.027 g, 3.6×10^{-4} mol) were placed in a 50 mL recovery flask. The flask was warmed with a water bath thermostated at 60 °C. After predetermined times,

aliquots of the reaction mixture (1.0 mL) were taken out by a syringe, and the reaction was terminated by rapid cooling with an ice water bath followed by neutralization with aqueous HCl (2.0 M, 0.2 mL). Water (ca. 40 mL) was added to the mixture, and then the concentration of formaldehyde was determined by the acetylacetone method.[21,22]

2-3. Results and Discussion

Characterziation of Reverse Micelles. Before the study on formose reaction, the sizes of water pools of reverse micelles formed from the surfactants were evaluated under several conditions. In the cases of AOT and CTAB, since the hydrophilic groups are small enough compared to reverse micelles, R_w was calculated from the hydrodynamic radii of water-free reverse micelles and those containing water pools ($R_{H,0}$ and R_H, respectively) as

$$R_w = R_H - R_{H,0} \quad (3)$$

Here $R_{H,0}$ and R_H were measured by DLS at different temperatures and w (= [water]/[surfactant]).[23,24] Figure 2-1 shows R_w as a function of w at 30, 45, and 60 °C. In both cases of AOT and CTAB, R_w is almost proportional to w independent of temperature. These observations indicate that the area per surfactant molecule (i.e., AOT and CTAB) is almost constant independent of w and temperature in the whole range

examined. At w = 10, AOT and CTAB reverse micelles contain water pools of $R_w \approx 3$ and 2 nm, respectively. On the other hand, since TX possesses oligo(ethylene glycol) chain as the hydrophilic group, which is larger compared to the hydrophobic group, nonylphenyl group, it is not possible to determine R_w from DLS data unlike the cases of AOT and CTAB. Thus, apparent values of R_w were estimated by the steady state fluorescence quenching technique using a pair of water soluble fluorophore and quencher, i.e., safranin T and potassium bromide.[25] When both the fluorophore and quencher are dissolved in water pools of reverse micelles, the fluorescence from the fluorophore will be quenched by the quencher. At a constant fluorophore concentration, the fluorescence intensity (I) decreases with increasing the quencher concentration ([Q]). Given a Poisson distribution of the quencher molecules between water pools and static quenching, there is a relationship between I and [Q]:

$$\ln (I_0/I) = [\mathrm{Q}]/^{26} \tag{4}$$

where I_0 denotes the fluorescence intensity in the absence of the quencher and ^{26} denotes the molar concentration of water pools. Assuming that all the water molecules added form spherical water pools of the density of unity, apparent R_w values are calculated by

$$R_w = \left(\frac{3V_w}{4\pi N_A [\mathrm{WP}] V_{soln}} \right)^{-1/3} \tag{5}$$

to be compared with those for AOT and CTAB reverse micelles. Here V_w and V_{soln} denote the volumes of the water added and solution, respectively. As can be seen in Figure 2-1, R_w for water pools of TX reverse micelles is nearly constant at ca. 1 nm independent of w and temperature.

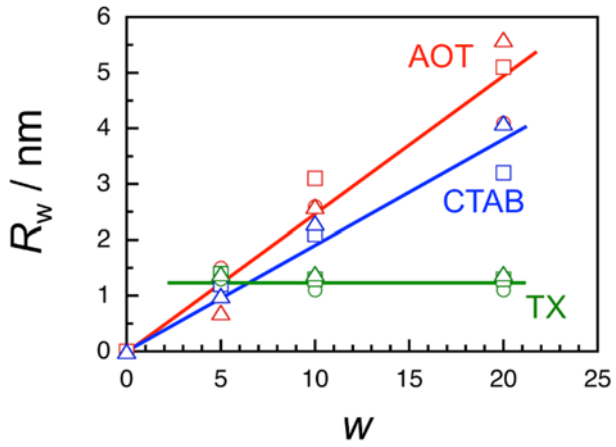


Figure 2-1. R_w as a function of w for AOT (red), TX (green), and CTAB reverse micelles (blue) at 30 (circle), 45 (square), and 60 °C (triangle).

Formose Reaction in Reverse Micelles. Formose reactions were carried out at 60 °C using mixtures prepared by mixing a 100 mM solution of a surfactant in isooctane or in a mixed solvent of isooctane and 1-hexanol (5/1, v/v) and an aqueous solution containing 200 mM formaldehyde and 20 mM calcium hydroxide to adjust w to 10. After predetermined times, aliquots of the reaction mixture were taken and neutralized

with 100 mM hydrochloric acid to terminate the reaction. The remaining unreacted formaldehyde was then extracted with water several times. Using the combined water phase, the concentration of unreacted formaldehyde was determined by the acetyl acetone method[21,22] to evaluate the conversion of formose reaction. Figure 2-2 demonstrates the time–conversion plots for the formose reactions in water pools of AOT, TX, and CTAB reverse micelles and that in an aqueous solution. In the formose reaction in an aqueous solution, i.e., a reference experiment, after an induction period of 0 – 50 min, the conversion commenced to increase rapidly and reached a quantitative conversion at 75 min. In the formose reactions in AOT, TX, and CTAB reverse micelles, on the other hand, no induction period was observed, indicative of acceleration of the formation of glycolaldehyde. The acceleration was most remarkable in the case of AOT reverse micelles. It is noteworthy that the conversion leveled off and did not reach a quantitative one. The saturated conversions were ca. 65, ca. 35, and ca. 25 % for AOT, TX, and CTAB reverse micelles, respectively.[27] On the basis of these observations, AOT provides the most efficient medium for formose reaction among the surfactants examined in this study.

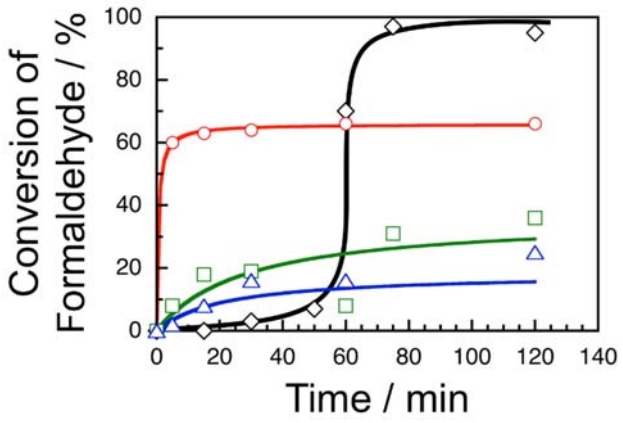


Figure 2-2. Time–conversion plots for formose reactions in an aqueous solution (black) and in water pools of AOT (red), TX (green), and CTAB reverse micelles (blue) at 60 °C. The curves are drawn for eye-guide.

Formose reactions in water pools of AOT reverse micelles were investigated at varying *w* and temperatures. Figure 2-3 shows time–conversion plots for AOT reverse micelles of different *w* at 30, 45, and 60 °C. This figure indicates that there is no induction period at all the *w* and temperatures examined. It should be noted here that the saturated conversion is lower at a larger *w*. Given a second order reaction for formaldehyde, the time–conversion plots were analyzed by

$$\mathrm{Conv} = \frac{\mathrm{Conv}_{\max} k' c_0 t}{k' c_0 t + 1} \tag{6}$$

where Conv and $\mathrm{Conv}_{\max}$ denote the conversion at time *t* and the saturated conversion, respectively, and k_2 and c_0 are the *pseudo*-second-order rate constant and the initial concentration of formaldehyde (= 200 mM), respectively.[28] As can be seen in Figure 2-

3, the best fitted curves agree well with the experimental data. Values of $Conv_{max}$ and k_2 obtained from the fitting were plotted in Figure 2-4 against w at different temperatures. This figure indicates that both $Conv_{max}$ and k_2 decrease with increasing w at all the temperatures examined, indicating that formose reaction proceeds more efficiently in AOT reverse micelles of a smaller w. Since formose reaction did not proceed in aqueous solutions at 30 °C and the same concentrations of formaldehyde and calcium hydroxide, it is concluded that water pools of AOT reverse micelles act as an effective medium for formose reaction.

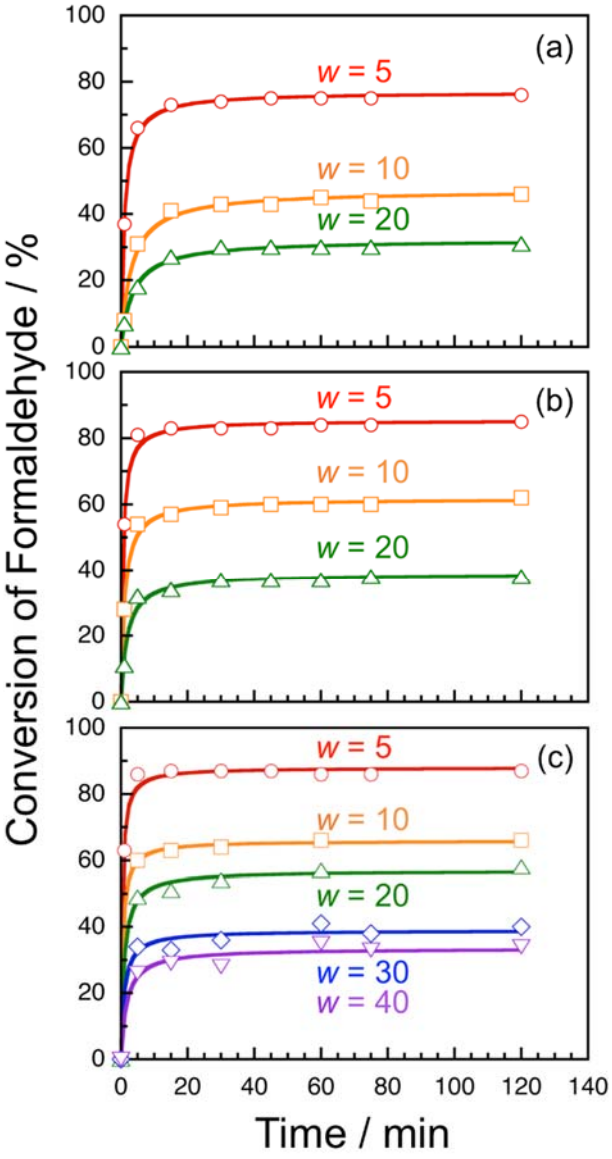


Figure 2-3. Time–conversion plots for formose reactions in AOT reverse micelles of different *w* at 30 (a), 45 (b), and 60 °C (c). Curves indicate the best fits using eq 1.

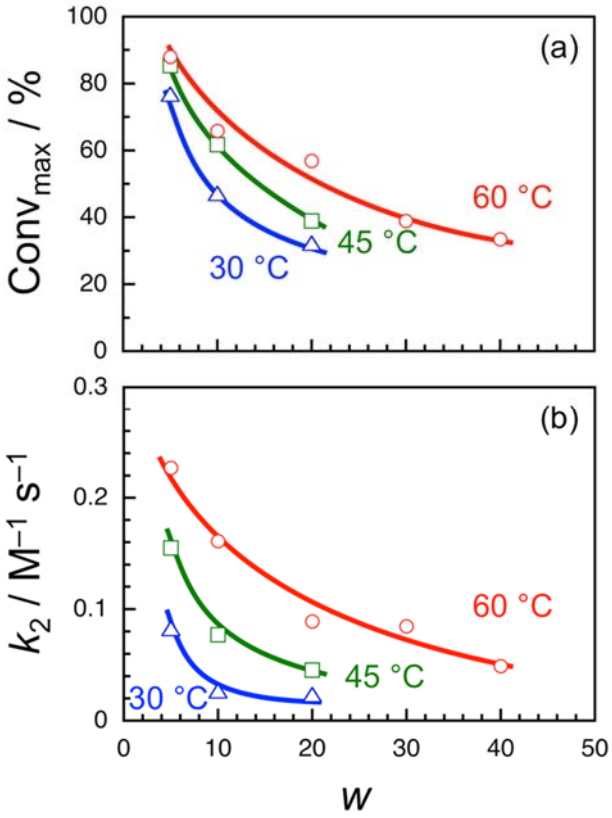


Figure 2-4. $Conv_{max}$ and k_2 as function of w for formose reactions in water pools of AOT reverse micelles at 30 (triangle), 45 (square), and 60 °C (circle). The curves are drawn for eye-guide.

Here it is worthy of discussion about R_w of AOT reverse micelles of $w = 5$. As can be seen in Figure 2-1, R_w is ca. 1 nm for water pools of AOT reverse micelles of $w = 5$. Even though a water pool of $R_w \approx 1$ nm contains one formaldehyde molecule on an average under the conditions used (i.e., at 200 mM formaldehyde), the values of $Conv_{max}$ and k_2 were the largest at $w = 5$. These observations indicate that the contents are

frequently exchanged between water pools of AOT reverse micelles during the reaction.

On the interface between the surfactant molecules and the water phase in reverse micelles, there is a layer of water molecules, of which the mobility is restricted, because of hydration of the hydrophilic group of surfactant (i.e., the surfactant head).[29,30] It is likely that the thickness of the layer of restricted water is approximately several nm.[31,32] The water molecules in interfacial layer are more polar than those in the bulk water phase because of polarization caused by hydration of the surfactant head. Since R_w of AOT reverse micelles is practically proportional to w (Figure 2-1), the fraction of water molecules in the interfacial layer increases with decreasing w. As can be seen in Figure 2-4, $Conv_{max}$ and k_2 values are larger at a smaller w. It can be thus concluded that the interfacial layer of restricted water provides an efficient medium for formose reaction.

It is important to characterize the product of formose reaction in AOT reverse micelles. However, it was not possible to purify the product because the reaction mixture contained a large amount of AOT. After a large fraction of AOT was removed, the mixture obtained was measured by high-performance liquid chromatography, NMR, and mass spectroscopy, but no signals ascribable to the products, i.e., sugars or sugar alcohols, were observed because of the residual AOT. Formose reaction was thus carried out in AOT reverse micelles of $w = 5$ using formaldehyde-^{13}C as starting material

at 30, 45, and 60 °C for 60 min, and the product was characterized by ^{13}C NMR after removal of a large fraction of AOT. Figure 2-5 compares ^{13}C NMR spectra for the products of formose reaction carried out using formaldehyde-^{13}C at 30, 45, and 60 °C. In these spectra, signals at 1.5 and 119.5 ppm are due to the methyl and nitrile carbons in acetonitrile, the internal standard, respectively. All the spectra contain two intense signals at ca. 63 and 75 ppm, although the ratios of signal intensities are different at different temperatures. The signal at ca. 63 can be assigned to ethylene glycol, which may be derived from glycolaldehyde through Cannizzaro reaction. (The author could not assign the signal at ca. 75 ppm comparing to signals for various sugar and sugar alcohols.) On the basis of these spectra, the author can conclude that ethylene glycol is formed as a major product.

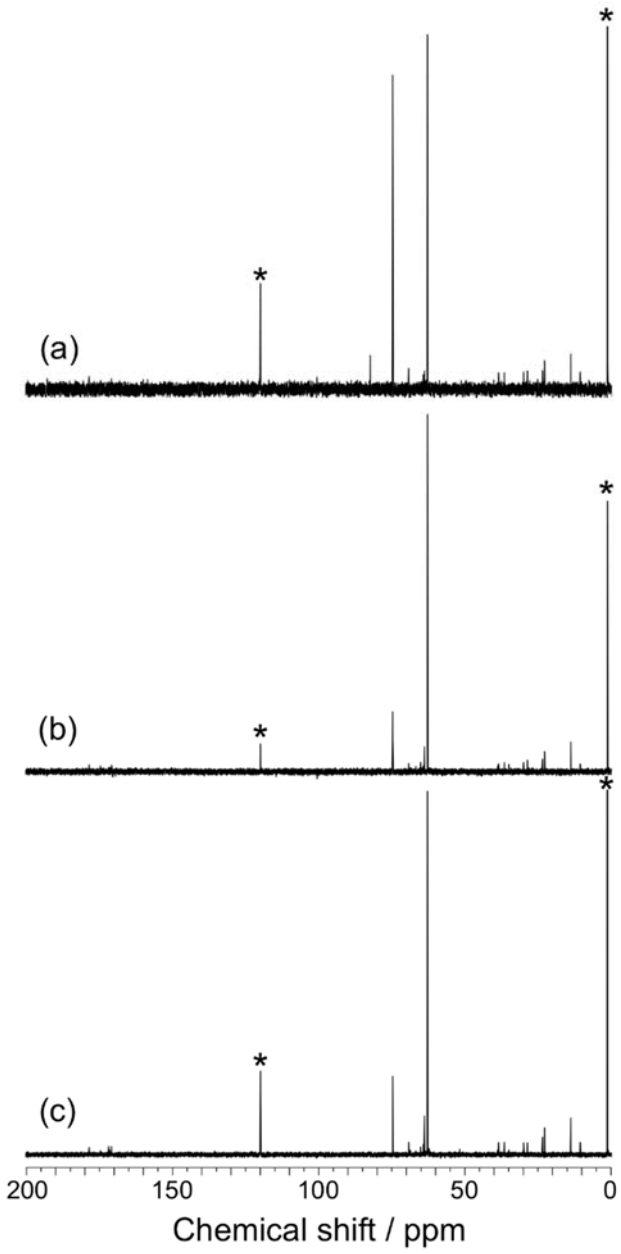


Figure 2-5. ^{13}C NMR spectra for the products of formose reaction carried out in AOT reverse micelles of $w = 5$ using formaldehyde-^{13}C at 35 (a), 45 (b), and 60 °C for 60 min (c). Asterisks represent the signals of acetonitrile, i.e., the internal standard.

2-4. Conclusion

Formose reaction in reverse micelles of AOT, TX, and CTAB was investigated. The formose reaction did not indicate the induction period, which is shown in conventional formose reaction, indicating that the formation of glycolaldehyde was accelerated in reverse micelles. AOT was the most effective among the surfactants examined. The values of $Conv_{max}$ and k_2 were larger at smaller w, indicating that the interfacial water layer is a medium that accelerates formation of glycolaldehyde in formose reaction. The ^{13}C NMR spectra for the products of formose reaction using formaldehyde-^{13}C were indicative of the formation of ethylene glycol as a major product.

2-5. References and Notes

(1) Butlerow, A. *Ann. Chem.* **1861**, *120*, 295-298.

(2) Mizuno, T.; Weiss, A. H. *Adv. Carbohydr. Chem. Biochem.* **1974**, *29*, 173-227.

(3) Weiss, A. H.; Socha, R. F.; Likholobov, V. A.; Sakharov, M. M. *Appl. Cat.* **1981**, *1*, 237-246.

(4) Matsuura, T.; Shigemasa, Y.; Sakazawa, C. *Chem. Lett.* **1974**, 713-714.

(5) Shigemasa, Y.; Shimao, M.; Sakazawa, C.; Matsuura, T. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 2099-2102.

(6) Shigemasa, Y.; Saimoto, H. *Trends Glycosci. Glycotechnol.* **1990**, *2*, 119-123.

(7) Shigemasa, Y.; Nagae, O.; Sakazawa, C.; Nakashima, R.; Matsuura, T. *J. Am. Chem. Soc.* **1978**, *100*, 1309-1310.

(8) Shigemasa, Y.; Kawahara, M.; Sakazawa, C.; Nakashima, R.; Matsuura, T. *J. Cat.* **1980**, *62*, 107-116.

(9) Shigemasa, Y.; Akagi, S.; Nakashima, R.; Saito, S. *Carbohydr. Res.* **1980**, *80*, C1-C3.

(10) Shigemasa, Y.; Hamada, T.; Hirabayashi, M.; Waki, E.; Nakashima, R.; Harada, K.; Takeda, N.; Suzuki, M. *Chem. Lett.* **1981**, 899-902.

(11) Shigemasa, Y.; Oogaki, K.; Ueda, N.; Nakashima, R.; Harada, K.; Takeda, N.; Suzuki, M.; Saito, S. *J. Carbohydr. Chem.* **1983**, *1*, 325-329.

(12) Shigemasa, Y.; Sasaki, Y.; Ueda, N.; Nakashima, R. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 2761-2767.

(13) Shigemasa, Y.; Ueda, T.; Saimoto, H. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 389-394.

(14) Matsumoto, T.; Komiyama, M.; Inoue, S. *Chem. Lett.* **1980**, 839-842.

(15) Matsumoto, T.; Inoue, S. *J. Chem. Soc., Chem. Commun.* **1983**, 171-172.

(16) Matsumoto, T.; Yamamoto, H.; Inoue, S. *J. Am. Chem. Soc.* **1984**, *106*, 4829-4832.

(17) Ricardo, A.; Carrigan, M. A.; Olcott, A. N.; Benner, S. A. *Science* **2004**, *303*, 196.

(18) Lambert, J. B.; Gurusamy-Thangavelu, S. A.; Ma, K. *Science* **2010**, *327*, 984-986.

(19) Hashidzume, A.; Fujimoto, T.; Masaoka, M.; Sanada, Y.; Sato, T. *Kobunshi Ronbunshu* **2010**, *67*, 312-317.

(20) Masaoka, M.; Michitaka, T.; Hashidzume, A. *Beilstein J. Org. Chem.* **2016**, *12*, 2663-2667.

(21) http://www.maff.go.jp/nval/kijyun/pdf/ST06130.PDF.

(22) Maruo, Y. Y.; Nakamura, J.; Uchiyama, M. *Talanta* **2008**, *74*, 1141-1147.

(23) Dokter, A. M.; Woutersen, S.; Bakker, H. J. *J. Chem. Phys.* **2007**, *126*, 124507.

(24) Mitra, R. K.; Sinha, S. S.; Pal, S. K. *Langmuir* **2008**, *24*, 49-56.

(25) Nandi, S.; Ghosh, S. K.; Bhattacharya, S. C. *Colloids Surf., A* **2005**, *268*, 118-123.

(26) Mountjoy, G.; Holland, M. A.; Wallidge, G. W.; Gunawidjaja, P.; Smith, M. E.; Pickup, D. M.; Newport, R. J. *J. Phys. Chem. B* **2003**, *107*, 7557-7566.

(27) At present, the author is not sure why formose reaction in reverse micelles exhibits a saturated conversion. This may be because the catalyst is deactivated in the first 20 – 30 min.

(28) Formose reaction starts with the formation of glycolaldehyde from two formaldehyde molecules, which is the rate-determining step in conventional formose reaction. It is thus reasonable to analyze the time-conversion data based

on second-order reaction as a first approximation. Since the conversion does not reach 100 % for all the cases examined in formose reaction in the presence of AOT reverse micelle, the author modified the conventional equation of second-order reaction to obtain eq 1.

(29) Hasegawa, M.; Sugimura, T.; Suzaki, Y.; Shindo, Y.; Kitahara, A. *J. Phys. Chem.* **1994**, *98*, 2120-2124.

(30) Hasegawa, M.; Sugimura, T.; Shindo, Y.; Kitahara, A. *Colloids Surf., A* **1996**, *109*, 305-318.

(31) Pieniazek, P. A.; Lin, Y.-S.; Chowdhary, J.; Ladanyi, B. M.; Skinner, J. L. *J. Phys. Chem. B* **2009**, *113*, 15017-15028.

(32) Chowdhary, J.; Ladanyi, B. M. *J. Phys. Chem. B* **2009**, *113*, 15029-15039.

Chapter 3

Formose Reaction Controlled by Boronic Acid Compounds

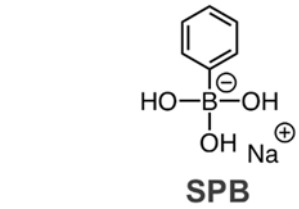
3-1. Introduction

Formose obtained by heating an aqueous solution of formaldehyde under basic conditions is a complicated mixture containing non-natural monosaccharides and sugar alcohols.[1-3] It is an important challenge to enhance the selectivity of formose reaction.[4-13] In Chapter 2, the author employed reverse micelles of surfactants as reaction media for formose reaction because the size of water pools in reverse micelles can be well controlled by varying the molar ratio of water and surfactant. Consequently, the author observed acceleration of the formation of glycolaldehyde and the formation of ethylene glycol as a major product.[14]

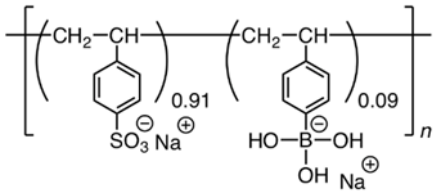
Monosaccharides and sugar alcohols are compounds possessing two or more hydroxy groups. It is known that they form esters with boronic acid compounds. The ester formation can be applied to construction of artificial molecular sugar sensors[15-19] and sugar-responsive polymer materials.[20] As described in Chapter 1, formose reaction proceeds through the three periods, i.e., the induction period, sugar-forming period, and sugar-decomposing period. In the sugar-forming and sugar-decomposing periods, a

variety of monosaccharides and sugar alcohols are formed.[21-23] Thus, the author employs boronic acid compounds to control the sugar-forming and sugar-decomposing periods. It is likely that the formation of boronic acid esters prevents further reaction of the products obtained. Another advantage of the use of boronic acid compounds is that the ester formation may provide the selectivity because the stability of boronic acid esters is dependent on the relative arrangement of two hydroxy groups, i.e., the species of sugars or sugar alcohols.[16,17] In this chapter, formose reaction is investigated in the presence of boronic acid compounds. The author utilizes low molecular weight and macromolecular boronic acid compounds, i.e., sodium phenylboronate (SPB) and a copolymer of sodium 4-vinylphenylboronate and sodium 4-styrenesulfonate (pNaSS/VPB) (Scheme3-1). The author describes that these boronic acid compounds provide different selectivities.[24]

Scheme 3-1. Structures of the Boronic Acid Compounds Used in This Study (i.e., SPB and pNaSS/VPB)



SPB



$M_w = 1.4 \times 10^4$, $M_w/M_n = 3.31$

pNaSS/VPB

3-2. Experimental section

Materials. 2-Acrylamide-2-methylpropanesulfonic acid (AMPS), phenylboronic acid, and 4-vinylphenylboronic acid (VPB) were purchased from Tokyo Chemical Industry. An aqueous solution of formaldehyde (36 wt%), calcium hydroxide, and sodium 4-styrenesulfonate (NaSS) were purchased from Sigma Aldrich Japan. 4,4′-Azobis(4-cyanovaleric Acid) (ACVA) was purchased from Wako Pure Chemical Industries. Water was purified by a Millipore Milli-Q system. Other reagents were used without further purification.

Sodium phenylborate (SPB) was prepared by neutralization of phenylboronic acid with an equimolar amount of sodium hydroxide.

VPB (0.45 g, 3.0 mmol), NaSS (6.2 g, 30 mmol) and ACVA (0.12 g, 0.44 mmol) were dissolved in a mixed solvent of water (30 mL) and DMF (12 mL) under an argon atmosphere. The reaction mixture was warmed with an oil bath thermostated at 60 °C for 24 h. The polymerization was quenched by rapid cooling with an ice-water bath. An aqueous solution of sodium hydroxide (1 M, 20 mL) was added to the reaction mixture for neutralization. The neutralized polymer sample was purified by dialysis for a week.

Formose Reaction for Time–Conversion Experiments. Calcium hydroxide (23.7 mg, 0.32 mmol), a cocatalyst (glyceraldehyde or fructose, 0.11 mmol), and a boronic acid compound (SPB or pNaSS/VPB) were added to an aqueous solution of formaldehyde (200 mM, 16 mL). The reaction mixture was warmed with a water bath thermostated at 60 °C. After a predetermined time, aliquots (0.1 mL) of the reaction mixture were taken. To the aliquots was added water (5 mL), and then the concentration of formaldehyde was determined by the acetylacetone method.[25,26]

Preparation of the Product for Characterization. Calcium hydroxide (23.7 mg, 0.32 mmol), a cocatalyst (glyceraldehyde or fructose, 0.11 mmol), and a boronic acid compound (SPB or pNaSS/VPB) were added to an aqueous solution of formaldehyde

(200 mM, 16 mL). The reaction mixture was warmed with a water bath thermostated at 60 °C. The reaction was then quenched with hydrochloric acid (1 M, 3 mL) at 0 °C using an ice bath. For the reactions using the polymer samples, formose samples were purified by dialysis (1 L) overnight. Ion-exchange resins (Amberlite IR-120H and IRA-410) were added to the reaction mixture, and the mixture was stirred for 2 – 3 h. After filtration and evaporation, the product for characterization was recovered.

Measurements. Size exclusion chromatography equipped with an online multi-angle light scattering detector (SEC-MALS) measurements were performed on a JASCO GPC-101 system equipped with a Wyatt DAWN HELEOS-II multiangle light scattering photometer and a Shodex SB-806M HQX2 column using 0.1 M NaCl as eluent at 30 ºC with a flow rate of 1.0 mL min^{-1}.

UV-Vis spectra for the acetylacetone method were recorded on a Jasco V-500 spectrophotometer.

HPLC measurements for the formose samples were carried out on a Jasco LC-2000 Plus system equipped with a Jasco PU-2080 pump and a Shodex 5NH2P-50 4D column. A mixed solvent of water and acetonitrile (1/3, v/v) was used as eluent at a flow rate of 0.6 mL min^{-1}. The sample signals were detected using a Jasco RI-2031

detector.

^{1}H and ^{13}C NMR spectra were measured on a JEOL JNM ECS400 or ECA500 spectrometer using $CDCl_3$ or D_2O as a solvent at 25 °C. Acetonitrile was used as an internal standard. Chemical shifts in ^{13}C NMR were referenced to the signal due to the methyl carbon of acetonitrile (δ = 1.47 ppm).

Negative-ion ESI-MS data were recorded on a Thermo Fisher Scientific LTQ Orbitrap XL mass spectrometer, using methanol as a solvent. Mass numbers were calibrated with four peptides, i.e., Angiotensin II ($[M+H]^+$ 1046.5418), Angiotensin I ($[M+H]^+$ 1296.6848), Substance P ($[M+H]^+$ 1347.7354), and Bombesin ($[M+H]^+$ 1619.8223).

3-3. Results and Discussion

SPB and pNaSS/VPB were used as a low molecular weight boronic acid compound and a boronic acid polymer, respectively. The copolymer was prepared by radical copolymerization at a molar ratio of 1:10 in monomer feed. Formose reaction was carried out using a solution containing 200 mM formaldehyde and 20 mM calcium hydroxide at 60 °C. Fructose or glyceraldehyde was employed as a cocatalyst because formose reaction did not proceed in the presence of a boronic acid compound without

cocatalyst. Conversions of formose reaction were determined by the acetylacetone method.[25,26] Figure 3-1a shows a typical example of the time–conversion plots for formose reactions in the presence of SPB. In the presence of 5 mM SPB, the time–conversion plots are almost the same as those for formose reaction in the absence of boronic acid compounds, indicative of no effect of SPB at a lower concentration. At 8, 10, and 12 mM, the conversion shows a small onset and then increases gradually. After ca. 30 min, the conversion starts to increase significantly and then reaches a quantitative conversion. At concentrations ≥ 15 mM, the conversion is ca. 30 % even after 90 min. These data indicate that SPB retards formose reaction. Figure 3-1b shows the time–conversion plots for formose reactions carried out in the presence of pNaSS/VPB. Formose reaction is retarded more significantly at a higher concentration. At 30 g L^{-1}, the conversion was ca. 40 % even after 180 min, indicating that pNaSS/VPB also retards formose reaction. Figure 3-2 compares the time–conversion data for SPB and pNaSS/VPB. This figure indicates that pNaSS/VPB retards formose reaction more significantly than does SPB at similar concentrations of boronic acid residues. In a separate experiment, formose reaction did not proceed significantly in the presence of 100 g L^{-1} poly(sodium 4-styrenesulfonate) presumably because sulfonate residues capture calcium ions. These observations indicate that the stronger retardation effect of

pNaSS/VPB is ascribable to both boronic acid and sulfonate residues.

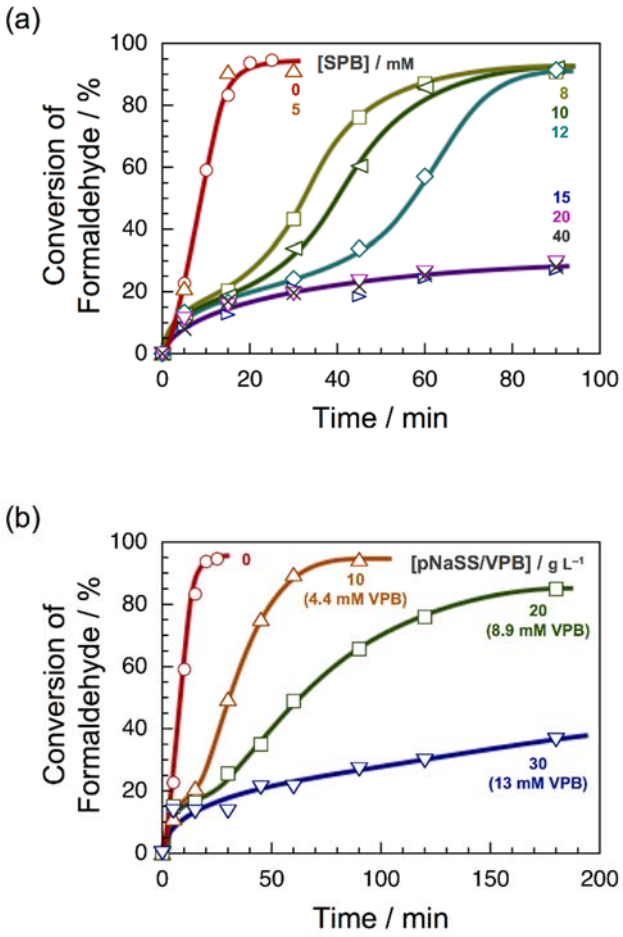


Figure 3-1. Time–conversion plots for formose reaction using 200 mM formaldehyde, 20 mM calcium hydroxide, and 6.9 mM cocatalyst in the presence of SPB (a) and pNaSS/VPB (b); fructose and glyceraldehyde was used as a cocatalyst for SPB and pNaSS/VPB, respectively. The curves are drawn for eye-guide.

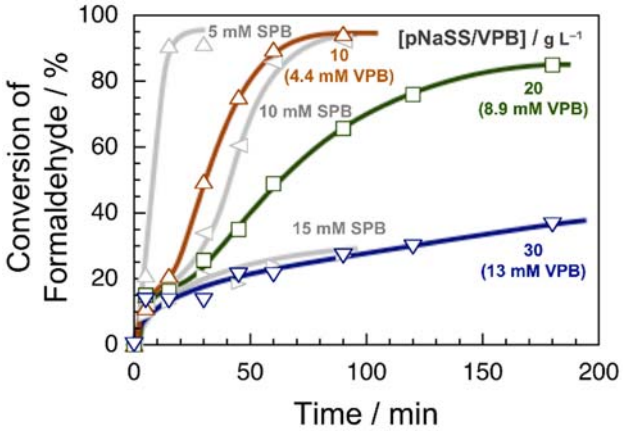


Figure 3-2. Time–conversion plots for formose reaction using 200 mM formaldehyde, 20 mM $Ca(OH)_2$, and 6.9 mM cocatalyst in the presence of SPB and pNaSS/VPB; fructose and glyceraldehyde was used as a cocatalyst for SPB and pNaSS/VPB, respectively. The curves are drawn for eye-guide.

The product was purified by dialysis against water and treatment with ion-exchange resins, and then recovered the product by freeze-drying, and characterized by HPLC using an amino column and a mixed solvent of water and acetonitrile, as can be seen in Figure 3-3. As reference, this figure also contains an HPLC chart for standard samples, i.e., two-, three-, four-, five-, and six-carbon sugar alcohols (ethylene glycol, glycerol, erythritol, D-arabinitol, and D-mannitol, respectively) (Figure 3-3a). In Figures 3-3b – 3-3d, the signals at ca. 5 min may be due to impurities, e.g., inorganic salts. As can be seen in Figure 3-3b, the HPLC chart for formose reaction in the absence of boronic acid compounds contains a number of signals in a wide range of elution time,

indicative of formation of a complicated mixture. On the other hand, the HPLC chart for SPB indicates a broad signal in the region of smaller carbon numbers (Figure 3-3c), and the chart for pNaSS/VPB exhibits signals in the region of larger carbon numbers (Figure 3-3d). These data are suggestive of modest selectivity of formose reaction in the presence of boronic acid compounds.

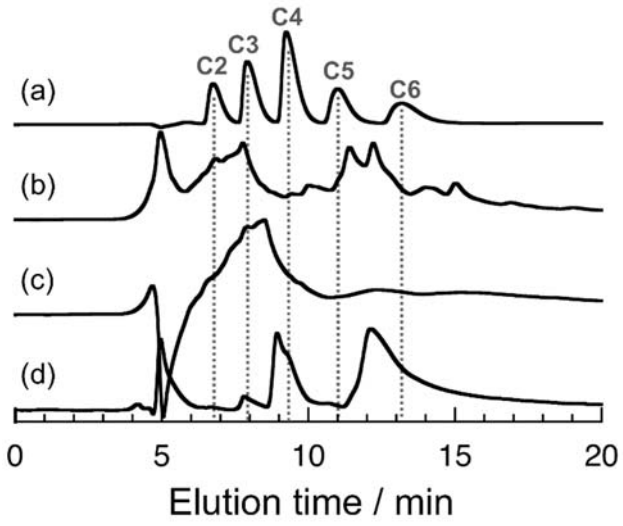


Figure 3-3. HPLC charts for standard samples (a) and products of formose reaction in the absence (b) and presence of 10 mM SPB (c) and 20 g L^{-1} pNaSS/VPB (d); the reaction times were 20, 90, and 180 min for (b), (c), and (d), respectively.

Figure 3-4 compares ^{1}H and ^{13}C NMR spectra for the products obtained in the presence of SPB and pNaSS/VPB. The ^{1}H and ^{13}C NMR spectra for SPB exhibit broad signals, similar to those for formose reaction without boronic acid compounds. Since it

was difficult to remove SPB from the reaction mixture, the NMR sample contained SPB, which might cause the broadening of NMR signals presumably because of equillibria of the formation of boronic acid esters. On the other hand, the ^{1}H and ^{13}C NMR spectra for pNaSS/VPB show well-resolved signals, indicating that formose reaction provides products with a significant selectivity in the presence of pNaSS/VPB. Figure 3-5 displays typical examples of electrospray ionization mass spectroscopy (ESI-MS) data for the products of formose reaction in the presence of SPB and pNaSS/VPB. The spectrum for SPB shows a series of signals at m/z = 215, 245, and 275, indicating an interval of 30 mass units, which corresponds to the formaldehyde unit. These signals are ascribable to potassium adducts of boronic acid esters formed from SPB and three-, four-, and five-carbon sugars. On the other hand, the spectrum for pNaSS/VPB contains a series of signals at m/z = 145, 175, 205, 235, and 265. These signals are assignable to sodium adducts of four- to eight-carbon sugar alcohols. On the basis of these characterization data, the author can conclude that formose reaction in the presence of SPB produces favorably sugars of a small carbon number whereas formose reaction in the presence of pNaSS/VPB produces preferably sugar alcohols of a larger carbon number.

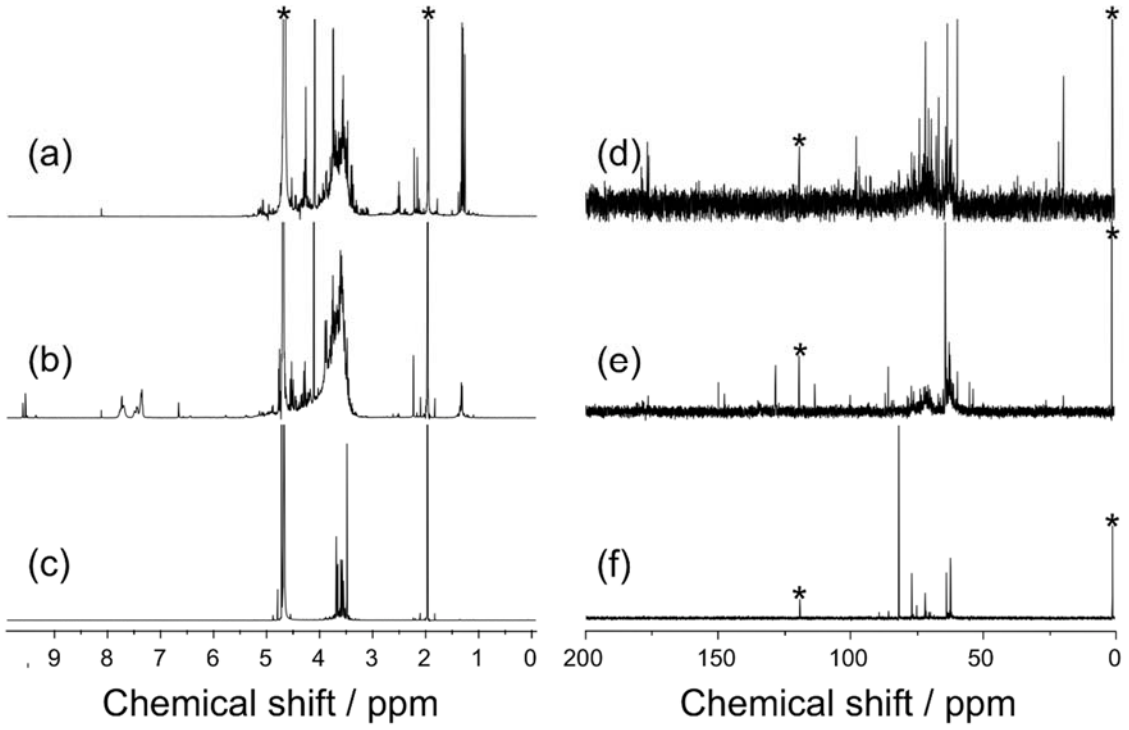


Figure 3-4. ^{1}H (a, b, and c) and ^{13}C NMR spectra (d, e, and f) for the products of formose reaction in the absence (a and d) and presence of 10 mM SPB (b and e) and 20 g L^{-1} pNaSS/VPB (c and f); the reaction times were 20, 90, and 180 min for (a and d), (b and e), and (c and f), respectively. Asterisks denote the signals of internal standard, i.e., acetonitrile.

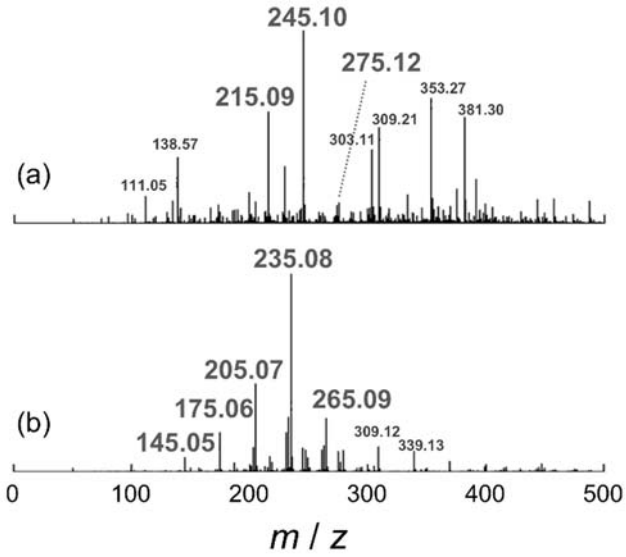


Figure 3-5. ESI-MS data for the products of formose reaction in the presence of 10 mM SPB (a) and 20 g L^{-1} pNaSS/VPB (b); the reaction times were 90 and 180 min for (a) and (b), respectively.

Here the author discusses the effect of SPB and pNaSS/VPB on formose reaction. As shown in Figure 3-1, both SPB and pNaSS/VPB retarded formose reaction, indicating that boronic acid compounds form esters with the sugars and sugar alcohols formed and the esters somehow protect the sugars or sugar alcohols from further reaction. The ESI-MS data of products elucidated that the formose reactions in the presence of SPB and pNaSS/VPB exhibited different selectivities; sugars of a small carbon number were formed preferably in the presence of SPB, whereas sugar alcohols of a larger carbon number were obtained favorably in the presence of pNaSS/VPB (Figure 3-5). The

selectivity of product should be dependent on the stability of esters. It is thus likely that SPB forms rather stable esters with sugars of a small carbon number and pNaSS/VPB forms relatively stable esters with sugar alcohols of a larger carbon number. A detail mechanism of selective formose reaction assisted with boronic acid compounds should be investigated in the near future.

3-4. Conclusion

In order to control formose reaction, the author carried out formose reactions in the presence of SPB and pNaSS/VPB. The time–conversion data indicate that SPB and pNaSS/VPB retarded formose reaction. The characterization data by HPLC, NMR, and ESI-MS for the products indicated that sugars of a small carbon number were formed favorably in the presence of SPB, whereas sugar alcohols of a larger carbon number were formed preferably in the presence of pNaSS/VPB.

3-5. References

(1) Butlerow, A. *Ann. Chem.* **1861**, *120*, 295-298.

(2) Mizuno, T.; Weiss, A. H. *Adv. Carbohydr. Chem. Biochem.* **1974**, *29*, 173-227.

(3) Weiss, A. H.; Socha, R. F.; Likholobov, V. A.; Sakharov, M. M. *Appl. Cat.* **1981**, *1*, 237-246.

(4) Shigemasa, Y.; Nagae, O.; Sakazawa, C.; Nakashima, R.; Matsuura, T. *J. Am. Chem. Soc.* **1978**, *100*, 1309-1310.

(5) Shigemasa, Y.; Kawahara, M.; Sakazawa, C.; Nakashima, R.; Matsuura, T. *J. Cat.* **1980**, *62*, 107-116.

(6) Shigemasa, Y.; Akagi, S.; Nakashima, R.; Saito, S. *Carbohydr. Res.* **1980**, *80*, C1-C3.

(7) Shigemasa, Y.; Hamada, T.; Hirabayashi, M.; Waki, E.; Nakashima, R.; Harada, K.; Takeda, N.; Suzuki, M. *Chem. Lett.* **1981**, 899-902.

(8) Shigemasa, Y.; Oogaki, K.; Ueda, N.; Nakashima, R.; Harada, K.; Takeda, N.; Suzuki, M.; Saito, S. *J. Carbohydr. Chem.* **1983**, *1*, 325-329.

(9) Shigemasa, Y.; Sasaki, Y.; Ueda, N.; Nakashima, R. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 2761-2767.

(10) Shigemasa, Y.; Ueda, T.; Saimoto, H. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 389-394.

(11) Matsumoto, T.; Komiyama, M.; Inoue, S. *Chem. Lett.* **1980**, 839-842.

(12) Matsumoto, T.; Inoue, S. *J. Chem. Soc., Chem. Commun.* **1983**, 171-172.

(13) Matsumoto, T.; Yamamoto, H.; Inoue, S. *J. Am. Chem. Soc.* **1984**, *106*, 4829-4832.

(14) Masaoka, M.; Michitaka, T.; Hashidzume, A. *Beilstein J. Org. Chem.* **2016**, *12*, 2663-2667.

(15) James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1910-1922.

(16) James, T. D. In *Boronic Acids. Preparation, Applications in Organic Synthesis and Medicine*; Hall, D. G., Ed.; WILEY-VCH: Weinheim, 2005, p 441-479.

(17) James, T. D.; Phillips, M. D.; Shinkai, S. *Boronic Acids in Saccharide Recognition*; The Royal Society of Chemistry: Cambridge, UK, 2006.

(18) Kubo, Y.; Nishiyabu, R.; James, T. D. *Chem. Commun.* **2015**, *51*, 2005-2020.

(19) Zhai, W.; Sun, X.; James, T. D.; Fossey, J. S. *Chem. Asian J.* **2015**, *10*, 1836-1848.

(20) Brooks, W. L. A.; Sumerlin, B. S. *Chem. Rev.* **2016**, *116*, 1375-1397.

(21) Matsuura, T.; Shigemasa, Y.; Sakazawa, C. *Chem. Lett.* **1974**, 713-714.

(22) Shigemasa, Y.; Shimao, M.; Sakazawa, C.; Matsuura, T. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 2099-2102.

(23) Shigemasa, Y.; Saimoto, H. *Trends Glycosci. Glycotechnol.* **1990**, *2*, 119-123.

(24) Imai, T.; Michitaka, T.; Hashidzume, A. *Beilstein J. Org. Chem.* **2016**, *12*, 2668-2672.

(25) http://www.maff.go.jp/nval/sosiki/kijyun/seizai/ST06130.PDF.

(26) Maruo, Y. Y.; Nakamura, J.; Uchiyama, M. *Talanta* **2008**, *74*, 1141-1147.

Chapter 4

Formose Reaction Controlled by a Copolymer of N,N-Dimethylacrylamide and 4-Vinylphenylboronic Acid

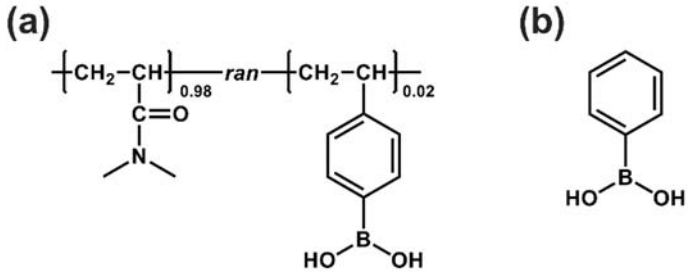
4-1. Introduction

Formose reaction provides a complicated mixture of monosaccharides and sugar alcohols from an aqueous solution of formaldehyde by heating under basic conditions.[1-3] Some research groups have devoted their efforts to selective formation of useful monosaccharides by formose reaction.[4-16]

In Chapter 3, the author described that boronic acid compounds decelerated formose reaction and provided selectivity of the product.[17] The polymer used in Chapter 3 was one based on poly(sodium styrenesulfonate) (pNaSS). The author observed that pNaSS retarded formose reaction by itself presumably because capture of calcium ions by sulfonate moieties. Thus the pNaSS-based copolymer may not be an appropriate polymer to elucidate the net effect of polymer carrying boronic acid residues. In this chapter, the author first reports water soluble polymers that have no effect on formose reaction and observe that a nonionic poly(*N*,*N*-dimethylacrylamide) (pDMA) has no or only a little effect on formose reaction. Then the author investigates formose reaction

in the presence pDMA carrying boronic acid moieties (pDMA/VPB, Scheme 4-1).[18]

Scheme 4-1. Chemical Structures of pDMA/VPB (a) and PBA (b).



4-2. Experimental Section

Materials. Methanol-d_4 (>99.8%, Merck, Darmstadt, Germany), deuterium oxide (D_2O, 99.9atom%D, Sigma Aldrich, St. Louis, Missouri, the United States), dimethyl sulfoxide-d_6 (DMSO-d_6, 99.5 atm%, Sigma-Aldrich), acetonitrile (>99.8%, HPLC grade, Kishida Chemical, Osaka, Japan), and dimethyl solfoxide (DMSO, >98.0%, Nacalai Tesque, Kyoto, Japan) were purchased and used as received. 4-Vinylbenzyltrimethylammonium chloride (VTAC, 99%, Sigma Aldrich), 4,4′-azobis(4-cyanovaleric acid) (ACVA, 98.0+%, Wako, Osaka, Japan), 4-vinylphenylboronic acid (VBA, >97%, TCI, Tokyo, Japan), calcium hydroxide ($Ca(OH)_2$, >95.0%, Sigma Aldrich), phenylboronic acid (PBA, >97.0%, TCI), lithium bromide (>98.0%, Nacalai Tesque), poly(sodium 4-styrenesulfonate) (pNaSS, average M_w~1,000,000, Sigma Aldrich), and

Amberlite® IR120 (hydrogen form, Sigma Aldrich) were purchased and used as received. Acetylacetone (99.0+%, Wako), acetic acid (>99.0%, Nacalai Tesque), ammonium acetate (>97.0%, Nacalai Tesque), and ethanol (>99.5%, Shinwa Alcohol) were used for the acetylacetone method as received. *N*,*N*-Dimethylformamide (DMF, >99.5%, Kanto Chemical) was purified using a Glass Contour solvent dispensing system. *N*,*N*-Dimethylacrylamide (DMA) (>99.0%, KJ Chemicals, Tokyo, Japan) was used after treatment with a short alumina column. Water was purified with a Millipore Elix 5 system. Formaldehyde solution (36.0–38.0%, Wako) was diluted with water to adjust 1/12 vol% and used for the formose reaction. Amberlite® IRA410 (chloride form, Sigma Aldrich) was used after treatment with 1 M NaOH, followed by washing with water. Spectra/Por 5 dialysis tubing, 12–14 kD MWCO (Spectrum Laboratories) was used for dialysis. Other reagents were used as received.

Measurements. Size exclusion chromatography (SEC) measurements were performed on a TOSOH HLC-8320GPC EcoSEC equipped with a TOSOH TSK gel α-M column using 1.05 $g \cdot L^{-1}$ lithium bromide in DMSO as the eluent at 40 °C, with a flow rate of 0.4 mL min^{-1}. The number of average molecular weights (M_n) and the ratios of the weigtht to the number average molecular weights (M_w/M_n) were calibrated with

polyacrylamide standards (American Polymer Standards).

UV-VIS spectra were recorded on a HITACHI U-4100 spectrophotometer equipped with a cell holder thermostated at 25 °C using a Yamato CF300 circulator for the acetylacetone method.

IR spectra were recorded on a JASCO FT/IR-6100 spectrometer equipped with an ATR PRO410-S carrying a diamond prism.

Liquid chromatography-mass spectroscopy (LC-MS) measurements were performed on a Bruker Daltonics micrOTOF-QIII compact connected with a Shimadzu Prominence UFLC system. In the LC system, Shimadzu LC-20AD pumps and a Shodex 5NH2P-50 4E column were equipped, and a mixed solvent of water and acetonitrile (25/75, *v*/*v*) containing 0.1 vol% formic acid was used as an eluent at a flow rate of 0.6 mL min^{-1}.

^{13}C NMR and distortionless enhancement of polarization transfer with a pulse delay adjusted for 3π/4 (DEPT-135) measurements were performed on a JEOL JNM ECA500 spectrometer using D_2O as a solvent at 25 °C. Two-dimensional heteronuclear single-quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC) spectra were recorded on a Bruker AVANCE700 spectrometer using D_2O as a solvent at 25 °C. Acetonitrile was used as an internal standard, and chemical shifts were

referenced to the signal due to the methyl carbon of acetonitrile (δ = 1.47 ppm).

Preparation of Water Soluble Polymers. VTAC (4.23 g, 20.0 mmol) and ACVA (57 mg, 0.21 mmol) were dissolved in a mixed solvent of DMSO and water (9/1, *v*/*v*, 10 mL). The solution was deoxygenated by purging with nitrogen gas for 30 min, and then heated with an oil bath thermostated at 60 °C with stirring for 7 h. Subsequently, the reaction mixture was treated with 1 M NaOH (100 mL) at room temperature overnight. The polymer obtained was purified by dialysis against water for 15 days. Yield 3.41 g, 87.0%.

DMA (5.1 mL, 50 mmol) and ACVA (140 mg, 0.498 mmol) were dissolved in water (50 mL). The solution was deoxygenated by purging with nitrogen gas for 30 min, and then heated with an oil bath thermostated at 70 °C with stirring for 18 h. The polymer obtained was purified by dialysis against water for six days. Yield 3.96 g, 77.8%.

Preparation of *N*,*N*-Dimethylacrylamide/4-Vinylphenylboronic Acid Copolymer. DMA (4.1 mL, 40 mmol), VBA (59 mg, 0.40 mmol), and ACVA (112 mg, 0.399 mmol) were dissolved in DMF (40 mL). The solution was deoxygenated by purging with argon gas for 1 h, and then heated with an oil bath thermostated at 70 °C

with stirring for 6 h. The polymer obtained was purified by dialysis against water for five days. Yield 3.01 g, 72.5%.

Formose Reaction in the Presence of Boronic Acid Compounds. A typical procedure of the formose reaction is described below. After pDMA/VPB (10 mg) was dissolved in an aqueous solution of formaldehyde (1 M, 2 mL), calcium hydroxide (18.8 mg, 0.240 mmol) was added to the mixture. The reaction mixture was warmed with a water bath thermostated at 60 °C with stirring. At predetermined reaction times, aliquots of the reaction mixture were taken to determine formaldehyde consumption by the acetylacetone method.[19,20] After a predetermined time, the reaction was quenched by adding 1 M HCl (0.24 mL). After the reaction mixture was treated with the ion exchenge resins for 30 min, the product was purified by dialysis against water (300 mL) for one day. The outer aqueous solution was concentrated under reduced pressure. After lyophilization of the solution, the product was obtained as pale brown solid. Yield 28 mg, 47%.

4-3. Results and Discussion

Effect of Water Soluble Polymers on the Formose Reaction. Before

investigation on the formose reaction in the presence of polymer-carrying boronic acid moieties, the author started this study with an examination on the effect of water soluble polymers. The author employed anionic poly(sodium 4-styrenesulfonate) (pNaSS), cationic poly(4-vinylbenzylammonium hydroxide) (pVTAOH), and nonionic poly(*N*,*N*-dimethylacrylamide) (pDMA) in this study. Figure 4-1 shows time–conversion plots for the formose reaction carried out using 0.2 M formaldehyde and 40 mM calcium hydroxide at 60 °C in the absence and presence of these polymers. In the absence of the polymers, the formaldehyde consumption increased up to a quantitative one within 30 min. However, the formose reaction was decelerated significantly in the presence of 10 $g \cdot L^{-1}$ pNaSS and pVTAOH, and the formaldehyde consumption became quantitative after ca. 40 and 50 min, respectively. In the presence of 10 $g \cdot L^{-1}$ pDMA, the formose reaction was slowed down only slightly, i.e., the formaldehyde consumption reached a quantitative one within 30 min. This observation indicates that pDMA does not considerably perturb the formose reaction.

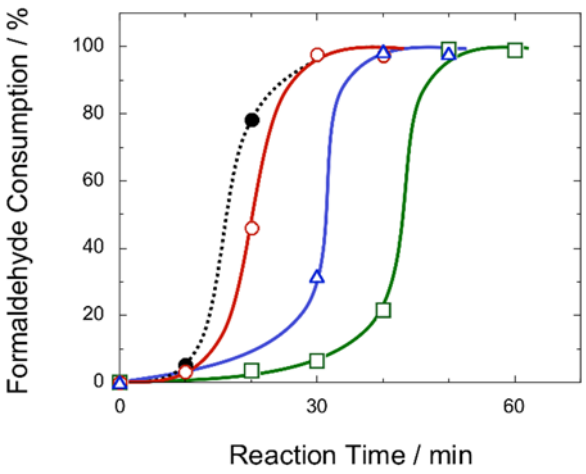


Figure 4-1. Time–conversion plots for the formose reaction carried out using 0.2 M formaldehyde and 40 mM calcium hydroxide at 60 °C in the absence (black circle, dotted line) and presence of 10 g L^{-1} pNaSS (blue triangle), pVTAOH (green square), and pDMA (red circle). The curves are drawn as a guide for the eye.

Preparation of *N*,*N*-Dimethylacrylamide/4-Vinylphenylboronic Acid Copolymer. On the basis of the observations described above, the author has chosen a copolymer of DMA and VBA (pDMA/VPB) to study the effect of polymer-carrying boronic acid moieties on formose reaction in this study. The copolymer was prepared by radical copolymerization of DMA and VBA in DMF using ACVA as initiator. The pDMA/VPB copolymer was purified by dialysis against water for five days. The VBA content in the copolymer was determined to be ca. 2 mol% by ^{1}H NMR. The M_n and M_w/M_n values were evaluated to be 3.58×10^5 and 1.58, respectively, by SEC. Thus, a

single chain carries ca. 70 boronic acid moieties in average. It is likely that the distribution of VBA units on the polymer chain is practically random.

Effect of the *N*,*N*-Dimethylacrylamide/4-Vinylphenylboronic Acid Copolymer. Since the formose reaction did not proceed in the presence of NaPB or pNaSS/NaVB under the reaction conditions in the author's previous study, a co-catalyst, i.e., fructose or glyceraldehyde, was necessary for conversion of formaldehyde. In this study, the author has used the reaction conditions under which the reaction proceeded without any co-catalyst. The author carried out the formose reaction using 1 M formaldehyde and 120 mM calcium hydroxide. Figure 4-2a indicates the time–conversion plots in the absence and presence of varying concentrations of pDMA/VPB. In the absence of pDMA/VPB, the formaldehyde consumption increased rapidly up to a quantitative one within 20 min. In the presence of 1 $g \cdot L^{-1}$ pDMA/VPB, the time–conversion plots were the same as those for the conventional formose reaction. In the presence of 5 $g \cdot L^{-1}$ pDMA/VPB, slight retardation of the reaction was observed, but the formaldehyde consumption became quantitative after 40 min. At 10 $g \cdot L^{-1}$ pDMA/VPB, significant retardation was not observed, but the formaldehyde consumption leveled off at ca. 80% after 60 min. These observations indicate that polymer-carrying boronic acid

moieties retard the formose reaction because DMA units have almost no effect. Similarly, in the case of 0.2 mM PBA (Figure 4-2b), the formose reaction was slightly decelerated, and the formaldehyde consumption increased up to a quantitative one after 40 min. In the presence of 0.5 mM PBA, the formaldehyde consumption leveled off at ca. 80% after 60 min. At 1 and 2 mM PBA, the formaldehyde consumption was saturated at ca. 40%. These observations confirm that PBA retards the formose reaction. A solution of 5 $g \cdot L^{-1}$ pDMA/VPB contains ca. 1 mM boronic acid moieties. It should be noted here that 1 mM PBA exhibits a stronger effect of retardation than does 5 $g \cdot L^{-1}$ pDMA/VPB. This observation indicates that low molecular weight boronic acid moieties have a stronger effect of retardation than do polymer-carrying ones presumably because of a lower effective concentration of polymer-carrying moieties.

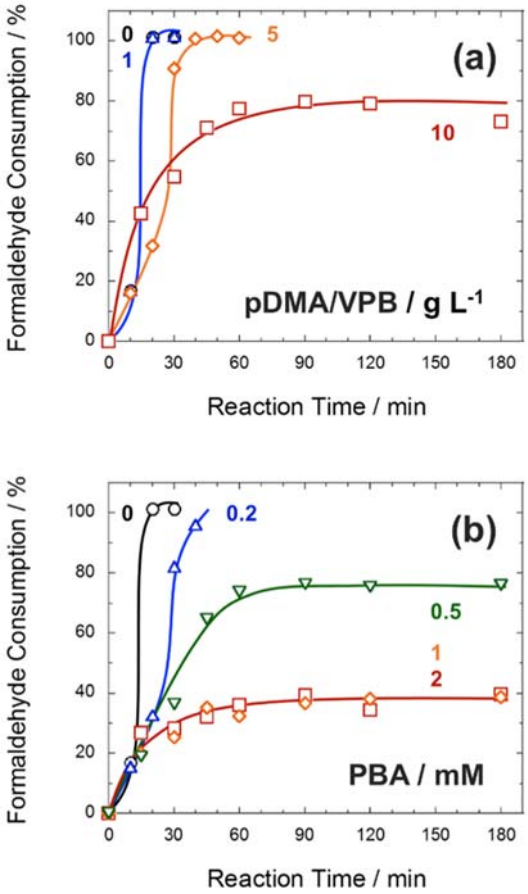


Figure 4-2. Time–conversion plots for the formose reaction carried out using 1 M formaldehyde and 120 mM calcium hydroxide at 60 °C in the presence of 0 (black circle), 1 (blue triangle), 5 (orange diamond), and 10 g L^{-1} (red square) pDMA/VPB (**a**) and 0 (black circle), 0.2 (blue triangle), 0.5 (green inverted triangle), 1 (orange diamond), and 2 mM (red square) PBA (**b**). The same colors indicate the same concentrations of boronic acid moieties. The curves are drawn as a guide for the eye.

Confirmation of the Formation of Boronic Acid Esters in Formose Reaction Mixtures. It is likely that the retardation effect of boronic acid moieties in formose

reaction is caused by the formation of boronic acid esters. To confirm the formation of boronic acid esters, IR spectra were recorded for mixtures before and after the reaction (Figure 4-3). The assignments were referred to IR spectra for the standard samples (Figure 4-4) and a computationally predicted IR spectrum (Figure 4-5). Figures 4-3a and b contain absorption bands ascribable to calcium hydroxide at 668, 710, 750, and 872 cm^{-1} (cf. Figure 4-4a). In Figure 4-3a, absorption bands at 1055, 1357, and 1379 cm^{-1} are assignable to the B–O–H deformation, B–O stretching, and B–O and B–C stretching vibrations, respectively[39,40]. Figure 4-3a also exhibits an absorption band due to the aromatic C–H deformation vibration at 789 cm^{-1} and a board absorption band assignable to formaldehyde in the region of 1000–1200 cm^{-1}. In the IR spectrum for the reaction mixture (Figure 4-3b), absorption bands at 787, 1353, and 1379 cm^{-1} are assignable to the aromatic C–H deformation, B–O stretching, and B–O and B–C stretching vibrations, respectively. It is noteworthy that Figure 4-3b does not exhibit any absorption band due to B–O–H deformation at ca. 1055 cm^{-1} whereas it contains a absorption band due to the B–O–H in quaternized boronic acid esters at 993 cm^{-1} (Figure 4-5)[41] and a broad absorption band due to the C–O stretching vibration in the region of 950–1100 cm^{-1}. These observations indicate that as the formose reaction proceeds in the presence of PBA, the free boronic acid is consumed to form esters.

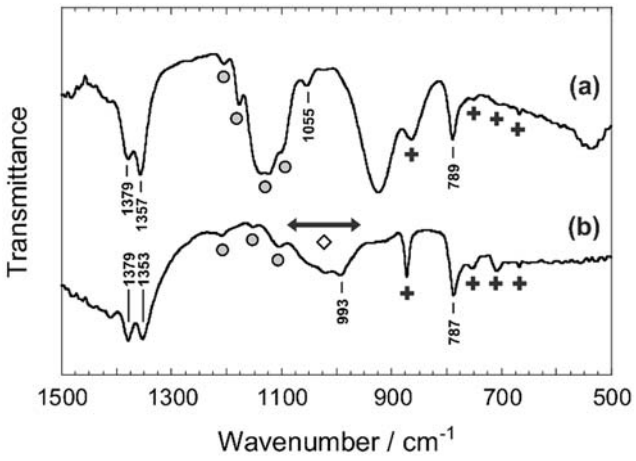


Figure 4-3. IR spectra for the reaction mixture containing 1 M formaldehyde, 120 mM calcium hydroxide, and 2 mM PBA before (a) and after heating at 60 °C for 60 min (b). The measurements were carried out after evaporation of the solvent by air blowing on a diamond prism loaded in the ATR attachment. Symbols (cross and circle) denote the absorption bands assignable to calcium hydroxide and formaldehyde, respectively.

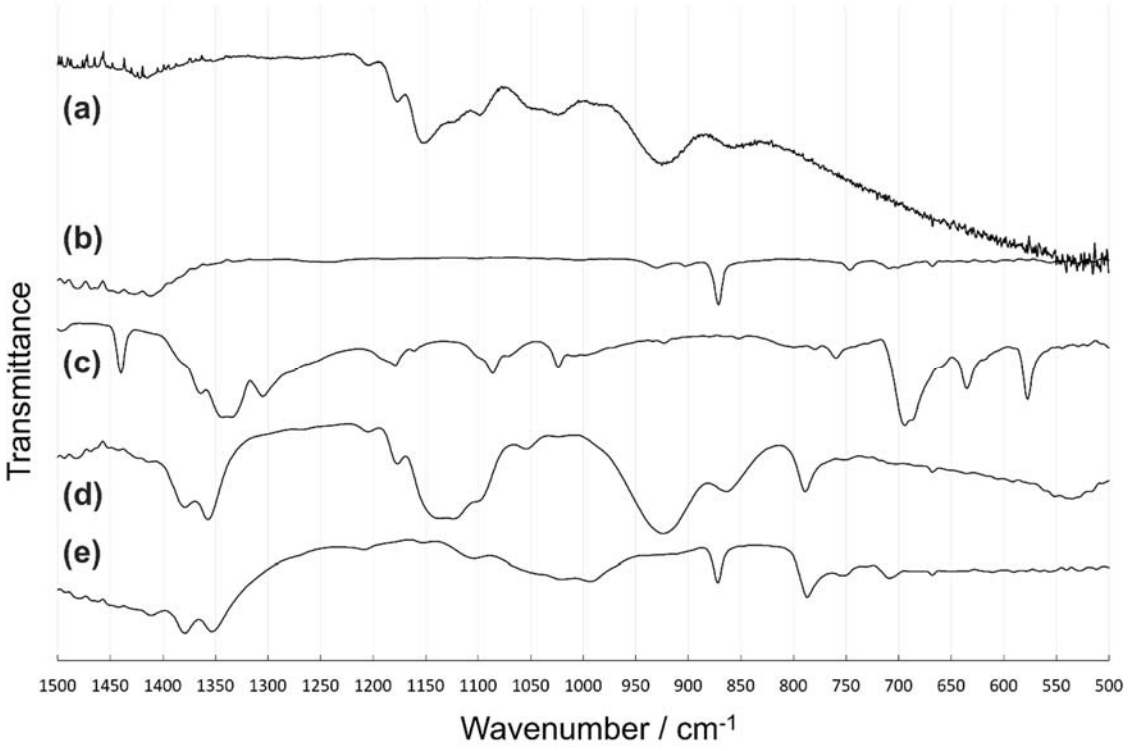
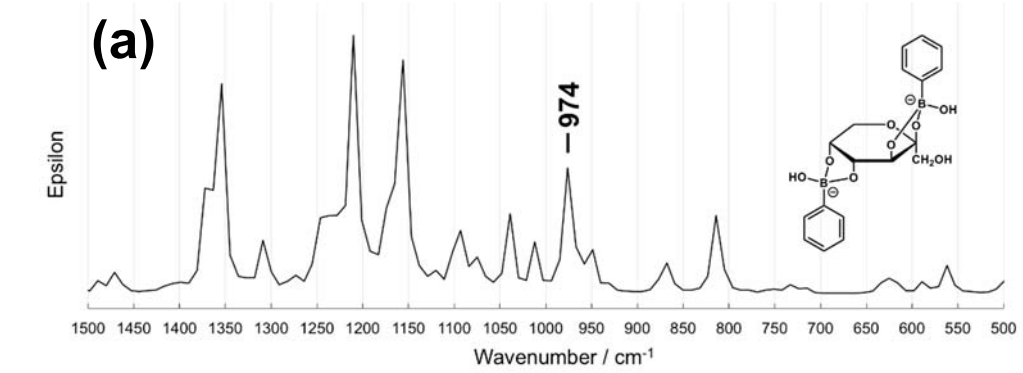


Figure 4-4. IR spectra for the standard samples and the reaction mixtures: A mixture of 1 M formaldehyde and 120 mM calcium hydroxide in water (a), a mixture of 120 mM calcium hydroxide and 2 mM PBA in water (b), PBA (c), the mixtures containing 1 M formaldehyde, 120 mM calcium hydroxide, and 2 mM PBA before (d) and after formose reaction at 60 °C for 60 min (e). The measurements were carried out after evaporation of the solvent by air blowing on a diamond prism loaded in the ATR attachment.



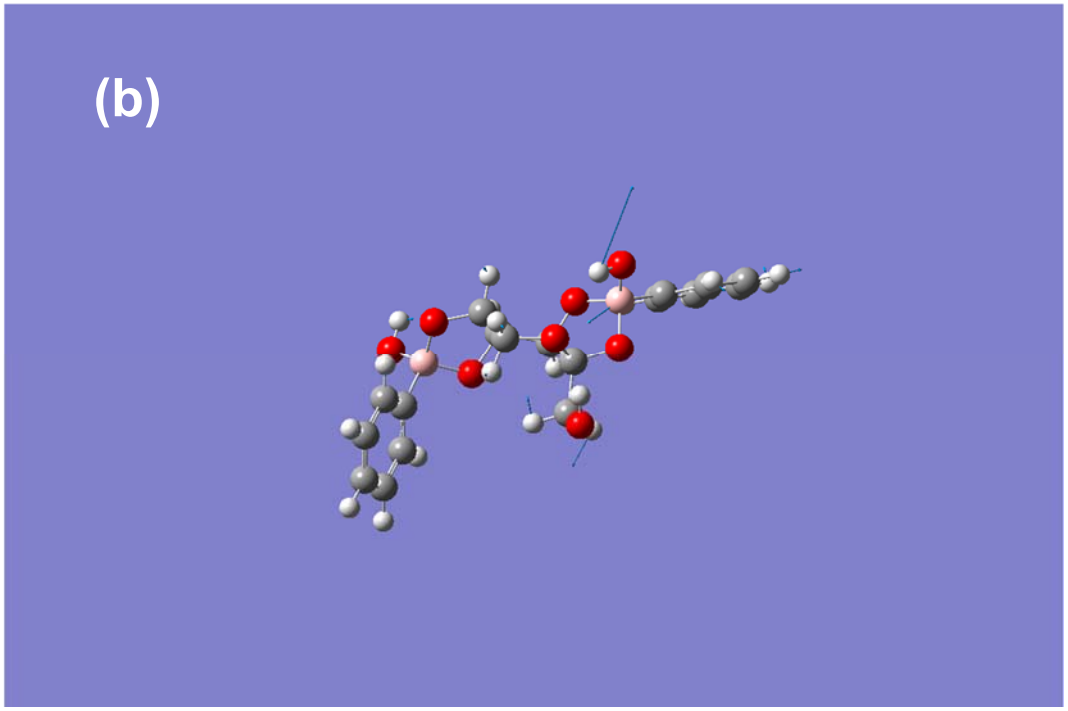


Figure 4-5. IR spectrum computationally calculated for the ester of quaternized diphenylboronic acid with fructose using a Gaussian 09W software with the following settings: Calculation type, Frequency; calculation method, RHF; basis set, 3–21G (a) and a snapshot of the three-dimensional vibrational structure responsible for the absorption band at 974 nm^{-1} (b).

Characterization of Formose Reaction Products. The formose reaction products were characterized by LC-MS. The products were purified by treatment of the reaction mixtures with the ion-exchange resins followed by dialysis against pure water. Figures 4-6a and b display mass chromatograms for the products of the formose reaction carried out in the presence of pDMA/VPB and PBA, respectively. For reference, the mass chromatogram for the product without any boronic acid compound is indicated in Figure 4-6c. This figure indicates a number of signals in the elution time region of 7–14 min, indicative of the formation of a complicated mixture in the conventional formose reaction. On the other hand, as can be seen in Figures 4-6a and b, the mass chromatograms for the products of formose reaction in the presence of pDMA/VPB and PBA exhibit four major signals at 7.7, 8.7, 9.3, and 9.6 min. These chromatograms do not contain any signals in the elution time region of 10–14 min, indicating that the selectivity of the formose reaction is somehow enhanced.

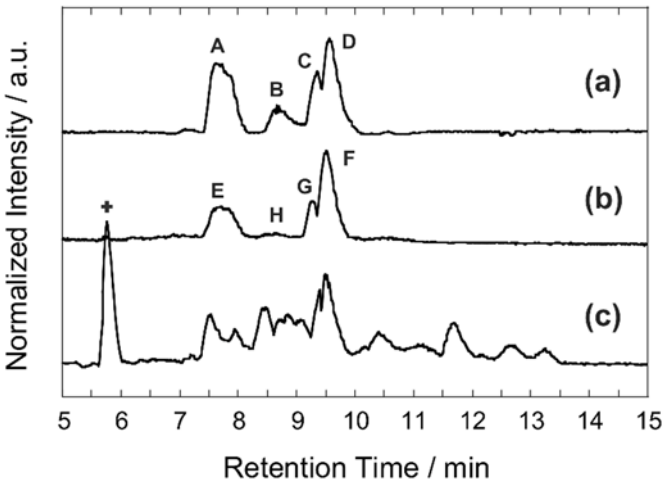


Figure 4-6. Mass chromatograms for the products of the formose reaction carried out using 1 M formaldehyde and 120 mM calcium hydroxide at 60 °C in the presence of 10 g L^{-1} pDMA/VPB (a) and 0.5 mM PBA (b), and in their absence (c). The reaction time was 180 (a and b) or 20 min (c). The *m*/*z* range of 60–250 was detected.

Figure 4-7 compares the mass spectra for the four major signals in Figures 4-6a and b. As a reference, the mass spectrum for the product of the conventional formose reaction is shown in Figure 4-8. The spectrum indicates a number of signals at *m*/*z* = 145.06, 153.09, 175.07, 183.10, 203.10, 213.12, and 232.08 assignable to four- to seven-carbon sugar alcohols, as well as six-carbon monosaccharides. These signals are indicative of the formation of a complicated mixture. Figures 4-7a and b show the mass spectra at the maxima of four major signals in Figures 4-6a and b for the products of the formose reaction in the presence of pDMA/VPB and PBA, respectively. Figures 4-7a

and b indicate that the formose reaction yields, selectively, six- and seven-carbon monosaccharides and sugar alcohols in the presence of pDMA/VPB and PBA.

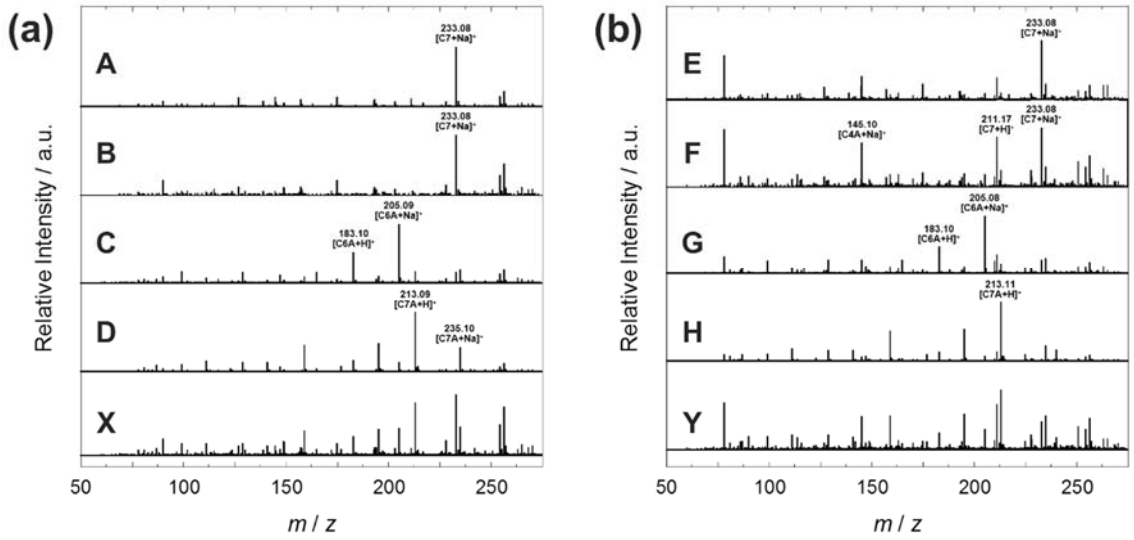


Figure 4-7. Mass spectra obtained in the LC-MS measurements for the products of the formose reaction carried out using 1 M formaldehyde and 120 mM calcium hydroxide at 60 °C for 180 min in the presence of 10 g L^{-1} pDMA/VPB (a) and 0.5 mM PBA (b). Spectra A – H were recorded at the maxima of signals A – H in Figure 4-6. Spectra X and Y are the total mass spectra recorded in the region of 7 – 14 min in Figures 4-6a and b, respectively. C*n* and C*n*A denote *n*-carbon monosaccharide and sugar alcohol, respectively.

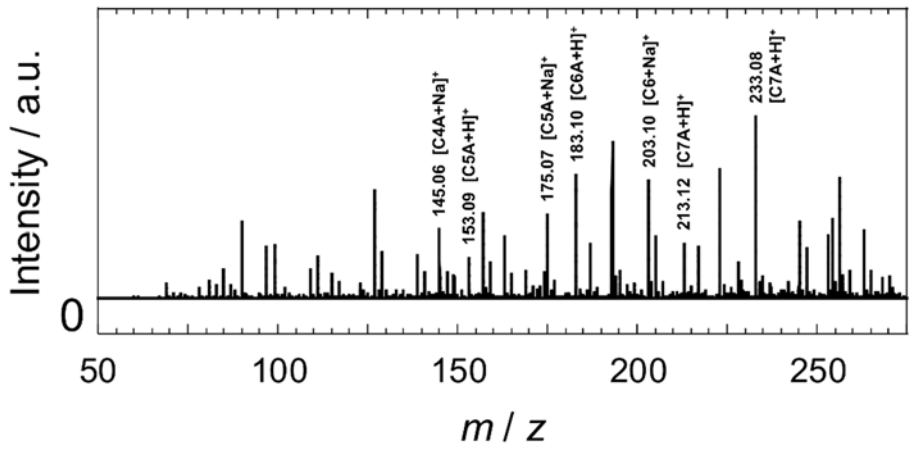


Figure 4-8. Mass spectrum recorded in LC-MS measurements for the region of 7 – 14 min in Figure 4-6 (c), corresponding to the products of formose reaction carried out using 1 M formaldehyde and 120 mM calcium hydroxide at 60 °C for 20 min in the absence of the boronic acid compounds.

The structure of products was also characterized by NMR spectroscopy. As an example, Figure 4-9 shows ^{13}C NMR and DEPT-135 spectra for the product of formose reaction in the presence of pDMA/VPB. The ^{13}C NMR spectrum shows a number of signals, indicating that the product contains some isomers. The DEPT-135 spectrum contains positive and negative signals ascribed to methine and methylene carbons, respectively. It is noteworthy that there are significant signals ascribable to quaternary carbons in the region of 70–90 ppm in the ^{13}C NMR spectrum, which are not observed in the DEPT-135 spectrum. This observation indicates that the products contain branched

monosaccharides and sugar alcohols. The formation of branched products was confirmed by HSQC and HMBC spectra (Figures 4-10 and 4-11). Although in the HSQC spectrum we can see no correlation with any signals in the ^{1}H NMR spectrum (x-axis) in the 70 – 90 ppm region of the ^{13}C NMR spectrum (y-axis) except for the correlation at 82 ppm assignable to paraformaldehyde, many correlation peaks were observed in the same regions in the HMBC spectrum. These 2D NMR spectra obviously demonstrate the peaks at 70 – 90 ppm in the ^{13}C NMR spectrum are assignable to quaternary carbons.

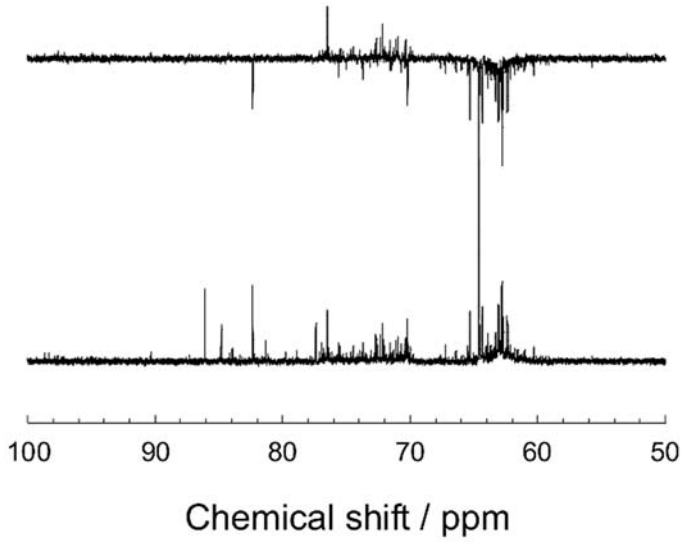


Figure 4-9. ^{13}C NMR (lower) and DEPT-135 spectra (upper) for the product of the formose reaction carried out using 1 M formaldehyde and 120 mM calcium hydroxide at 60 °C for 60 min in the presence of 5 g L^{-1} pDMA/VPB.

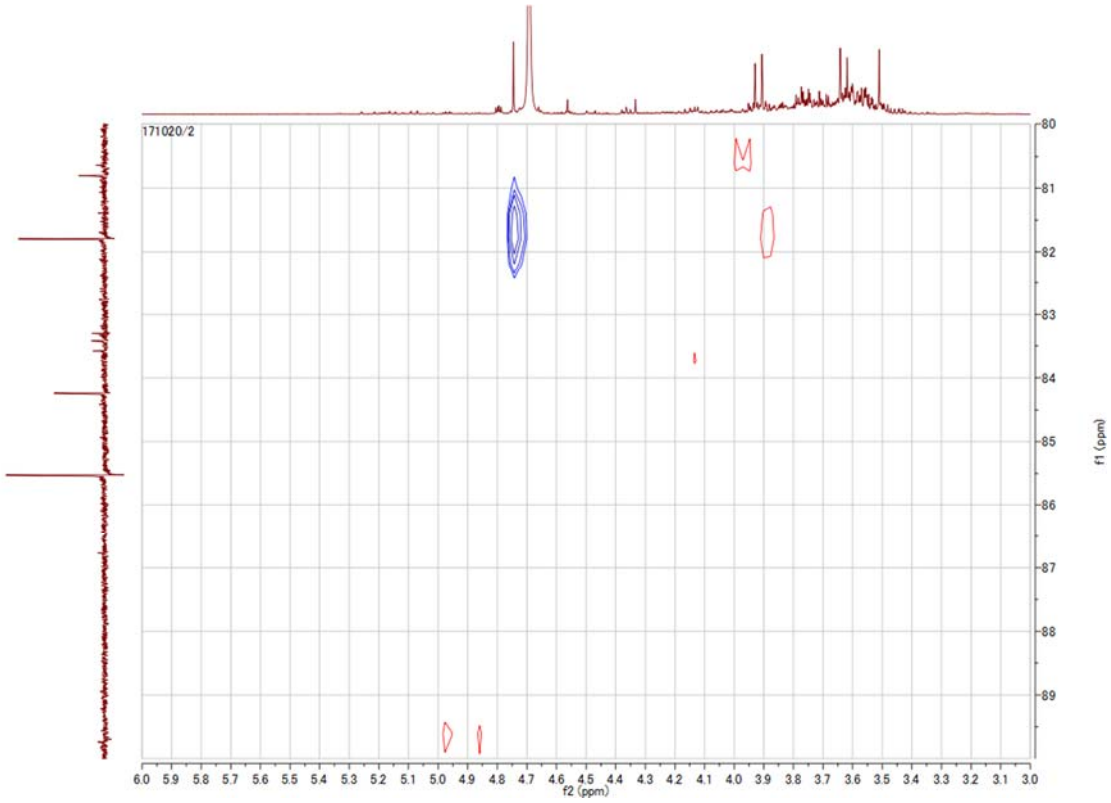


Figure 4-10. HSQC spectrum for the product of formose reaction carried out using 1 M formaldehyde and 120 mM calcium hydroxide at 60 °C for 60 min in the presence of 5 g L^{-1} pDMA/VPB. The signals due to quaternary carbons in the ppm range of 80 – 86 ppm do not show any correlations with ^{1}H signals.

Figure 4-11. HMBC spectrum for the product of formose reaction carried out using 1 M formaldehyde and 120 mM calcium hydroxide at 60 °C for 60 min in the presence of 5 g L^{-1} pDMA/VPB. The signals due to quaternary carbons in the ppm range of 80 – 86 ppm show correlations with ^{1}H signals.

Discussion. Here the author discusses why boronic acid compounds somehow enhance the selectivity of formose reaction products. As described in the introduction part, formose reaction proceeds via some elemental steps, including acyloin condensation, aldol and retro-aldol reactions, aldose–ketose isomerization, and the Cannizzaro reaction. Thus, it is known that formose reaction provides favorably branched monosaccharides

and sugar alcohols.[4-6] On the other hand, boronic acid compounds form esters with the formose reaction products. Since added boronic acid compounds significantly retard the formose reaction, the formation of boronic acid esters protect the products against further reactions. The stability of boronic acid esters depends on the relative orientation of two hydroxy groups, i.e., the species of monosaccharides and sugar alcohols. It is also known that boronic acid esters of a five- or six-membered ring are rather stable.[21] It is, thus, likely that formose reaction in the boronic acid compounds produces favorably six- and seven-carbon monosaccharides and the corresponding sugar alcohols, which can form rather stable esters of a five- or six-membered ring (Figure 4-12).

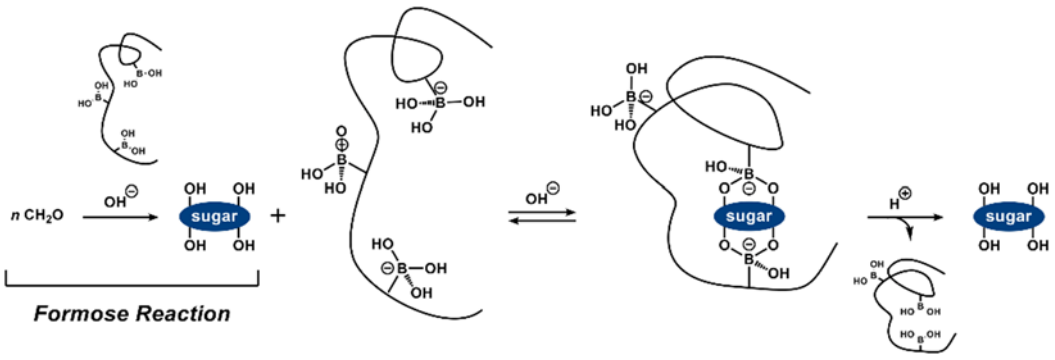


Figure 4-12. Conceptual illustration of formose reaction in the presence of a boronic acid polymer, in which the polymer stabilizes the product via ester formation, leading to an enhanced selectivity.

It has been reported that two boronic acid moieties of a specific relative location form ester with monosaccharides showing an enhanced selectivity.[22] Thus, molecularly imprinted polymers[23,24] or cross-linked dendrimers,[25] in which two boronic acid moieties are located appropriately, are promising to give a higher selectivity of the formose reaction product.

4-4. Conclusion

In summary, the author has investigated formose reaction in the presence of pDMA/VPB and PBA. pDMA/VPB and PBA decelerated the formose reaction because of the formation of boronic acid esters with products, judged from the time-conversion data and IR study. The formose reaction in the presence of pDMA/VPB and PBA formed favorably six- and seven-carbon branched monosaccharides and sugar alcohols, judged from LC-MS and NMR spectroscopy. This study has demonstrated that the boronic acid compounds can enhance selectivity of formose reaction through controlling the formaldehyde-converting behavior.

4-5. References and Notes

(1) Butlerow, A. *Ann. Chem.* **1861**, *120*, 295-298.

(2) Mizuno, T.; Weiss, A. H. *Adv. Carbohydr. Chem. Biochem.* **1974**, *29*, 173-227.

(3) Weiss, A. H.; Socha, R. F.; Likholobov, V. A.; Sakharov, M. M. *Appl. Cat.* **1981**, *1*, 237-246.

(4) Shigemasa, Y.; Nagae, O.; Sakazawa, C.; Nakashima, R.; Matsuura, T. *J. Am. Chem. Soc.* **1978**, *100*, 1309-1310.

(5) Shigemasa, Y.; Kawahara, M.; Sakazawa, C.; Nakashima, R.; Matsuura, T. *J. Cat.* **1980**, *62*, 107-116.

(6) Shigemasa, Y.; Akagi, S.; Nakashima, R.; Saito, S. *Carbohydr. Res.* **1980**, *80*, C1-C3.

(7) Shigemasa, Y.; Hamada, T.; Hirabayashi, M.; Waki, E.; Nakashima, R.; Harada, K.; Takeda, N.; Suzuki, M. *Chem. Lett.* **1981**, 899-902.

(8) Shigemasa, Y.; Oogaki, K.; Ueda, N.; Nakashima, R.; Harada, K.; Takeda, N.; Suzuki, M.; Saito, S. *J. Carbohydr. Chem.* **1983**, *1*, 325-329.

(9) Shigemasa, Y.; Sasaki, Y.; Ueda, N.; Nakashima, R. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 2761-2767.

(10) Shigemasa, Y.; Ueda, T.; Saimoto, H. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 389-394.

(11) Matsumoto, T.; Komiyama, M.; Inoue, S. *Chem. Lett.* **1980**, 839-842.

(12) Matsumoto, T.; Inoue, S. *J. Chem. Soc., Chem. Commun.* **1983**, 171-172.

(13) Matsumoto, T.; Yamamoto, H.; Inoue, S. *J. Am. Chem. Soc.* **1984**, *106*, 4829-4832.

(14) Ricardo, A.; Carrigan, M. A.; Olcott, A. N.; Benner, S. A. *Science* **2004**, *303*, 196.

(15) Lambert, J. B.; Gurusamy-Thangavelu, S. A.; Ma, K. *Science* **2010**, *327*, 984-986.

(16) Masaoka, M.; Michitaka, T.; Hashidzume, A. *Beilstein J. Org. Chem.* **2016**, *12*, 2663-2667.

(17) Imai, T.; Michitaka, T.; Hashidzume, A. *Beilstein J. Org. Chem.* **2016**, *12*, 2668-2672.

(18) Michitaka, T.; Imai, T.; Hashidzume, A. *Polymers* **2017**, *9*, 549.

(19) http://www.maff.go.jp/nval/sosiki/kijyun/seizai/ST06130.PDF.

(20) Maruo, Y. Y.; Nakamura, J.; Uchiyama, M. *Talanta* **2008**, *74*, 1141-1147.

(21) *Boronic Acids. Preparation, Application in Organic Synthesis and Medicine*; 1 st ed.; Hall, D. G., Ed.; Wiley-VCH: Weinheim, 2005.

(22) Zhai, W.; Sun, X.; James, T. D.; Fossey, J. S. *Chem. Asian J.* **2015**, *10*, 1836-1848.

(23) Wulff, G.; Schauhoff, S. *J. Org. Chem.* **1991**, *56*, 395-400.

(24) Wulff, G. *Chem. Rev.* **2002**, *102*, 1-27.

(25) Hashidzume, A.; Zimmerman, S. C. *Tetrahedron Lett.* **2009**, *50*, 2204-2207.

Chapter 5

Summary

In this thesis, the author has developed controlled formose reaction toward selective sugar synthesis. To obtain a narrow product distribution, the author has focused on suppression of the rapid proceedings of the propagation and decomposition step in formose reaction. To suppress the rapid proceedings, the author has investigated formose reaction through the novel approaches to use nanometer-scale reactors in Chapter 2, and to use boronic acid compounds in Chapters 3 and 4.

In Chapter 2, the author has studied formose reaction in reverse micelles as nanometer-scale reaction media to control the propagation step. The author observed a significant acceleration of the induction period of formose reaction, i.e., dimerization of formaldehyde to yield glycolaldehyde, in aerosol-OT (AOT) reverse micelles. Time-conversion data has indicated that the acceleration is caused by the interfacial water layer of AOT reverse micelles. Although the product analysis was quite hard because of the non-negligible amount of residual surfactants after the product recovery, ^{13}C NMR study using formaldehyde-^{13}C as the starting material has indicated that the major product was

ethylene glycol.

In Chapter 3, the author has studied formose reaction in the presence of sodium phenylboronate (SPB) and a random copolymer of sodium 4-vinylphenylboronate and sodium 4-styrenesulfonate (pNaSS/VPB) to suppress the propagation and degradation steps. The author observed marked retardation of formose reaction in the presence of the boronic acid compounds, indicative of stabilization of sugars. The high-performance liquid chromatography (HPLC) and NMR measurements indicated narrow product distributions in the formose reaction. Mass analysis revealed that SPB favorably provided sugars of a smaller carbon number, whereas pNaSS/VPB preferably provided sugars of a larger carbon number.

In Chapter 4, the author has studied formose reaction in the presence of a copolymer of *N*,*N*-dimethylacrylamide and 4-vinylphenylboronic acid (pDMA/VPB) and phenylboronic acid (PBA). The author also observed retardation of formose reaction in the presence of the boronic acid compounds, which was also shown in Chapter 3. Infrared measurements demonstrated the formation of boronate ester during formose reaction. Liquid chromatography-mass spectroscopy (LC-MS) and NMR

measurements revealed that pDMA/VPB and PBA selectively provided six- and seven-carbon branched monosaccharides and sugar alcohols.

In conclusion, the author has successfully developed controlled formose reaction toward selective sugar synthesis. Chapter 2 has demonstrated that nanometer-scale reaction media show unique behavior of formose reaction. Chapters 3 and 4 have provided new aspects of formose reaction inspired from boronic acid chemistry. These studies can be applied into more highly-organized reaction systems for formose reaction like artificial enzymes, which ultimately can encapsulate a controlled number of formaldehyde molecules and stabilizes a certain sugar with high efficiency. Throughout this study, the author has dedicated to work on controlled formose reaction, which will hopefully contribute to advanced science of sugar synthesis deeply and widely in the future.

List of Publications

Formose Reaction Accelerated in Aerosol-OT Reverse Micelle
Makoto Masaoka, Tomohiro Michitaka, and Akihito Hashidzume
Beilstein J. Org. Chem. **2016,** *12,* 2663-2667.

Formose Reaction Controlled by Boronic Acid Compounds
Toru Imai, Tomohiro Michitaka and Akihito Hashidzume
Beilstein J. Org. Chem. **2016**, *12*, 2668-2672.

Formose Reaction Controlled by a Copolymer of *N*,*N*-Dimethylacrylamide and 4-Vinylphenylboronic Acid
Tomohiro Michitaka, Toru Imai and Akihito Hashidzume
Polymers **2017**, *9*, 549.

Other Paper

Aggregation Behavior of Polystyrene-Based Amphiphilic Diblock Copolymers in Organic Media
Tomoe Arai, Makoto Masaoka, Tomohiro Michitaka, Yosuke Watanabe, Akihito Hashidzume and Takahiro Sato
Polymer Journal **2014**, *46*, 189-194.