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Dimethyl sulfoxide enhances both cellulose dissolution ability and biocompatibility of a carboxylate-type liquid zwitterion

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LETTER

Dimethyl sulfoxide enhances both cellulose dissolution ability and biocompatibility of a carboxylate-type liquid zwitterion

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Cellulose dissolution ability of a liquid zwitterion, the most biocompatible cellulose solvent, was improved by adding a co-solvent, dimethylsulfoxide. Moreover, biocompatibility of the liquid zwitterion was also improved by adding dimethylsulfoxide although it is toxic relative to the liquid zwitterion. The mixture is the efficient and extremely biocompatible cellulose solvent.

Despite being the most abundant biopolymer on earth, cellulose faces challenges in biorefinery applications because of its poor solubility. The recalcitrance of cellulose is due to its highly crystalline structure. Therefore, efficient solvents are necessary to convert cellulose into biofuels or the other highly valuable chemical compounds.¹ Some solvents or solvent systems, e.g. *N*-methylmorpholine oxide², *N,N*-dimethylacetamide/lithium chloride³, 1,3-dimethyl-2-imidazolidinone/lithium chloride⁴, and dimethyl sulfoxide (DMSO)/tetrabutylammonium fluoride⁵, can dissolve cellulose directly. Recently, ionic liquids (ILs) which are liquid salts below 100 °C, have been highlighted for their ability to dissolve cellulose. Swatloski *et al.* have reported that 1-butyl-3-methylimidazolium chloride can dissolve 10 wt% of cellulose at 100 °C.⁶ ILs containing carboxylate, dialkylphosphate, or alkylphosphonate anions have also been reported to have superior cellulose solubility.⁷⁻¹² Currently, ILs are recognized as one of the most effective solvents for dissolving cellulose.

However, ILs must overcome some critical challenges before they can be practically applied in biorefinery.^{13,14} One of their problematic characteristics is their toxicity to microorganisms when bioconversion is used in biorefinery. ILs show toxicity towards microorganisms by destructing their cell membranes via a two-step mechanism.¹⁵ First, cations of ILs

are electrostatically attracted to anionic phospholipids of cell membranes. Then, the ILs insert the hydrophobic alkyl chain of their cations (called the cation tail) into the microorganism's cell membrane via hydrophobic interactions.

To overcome the problem of toxicity, our group has previously developed a biocompatible and cellulose-dissolving zwitterion¹⁶, carboxylate-type liquid zwitterion (OE₂imC₃C, Fig. 1) as an analogue of cellulose dissolving ILs. The structure has no hydrophobic cation tail, which contributes to the toxicity, and has a similar polarity to other ILs capable of dissolving cellulose. Consequently, OE₂imC₃C has showed cellulose dissolution ability and the highest biocompatibility among all cellulose solvents. However, OE₂imC₃C has high viscosity, which limits its capability to dissolve cellulose. For example, the solubility of cellulose in OE₂imC₃C is 6 wt% at 100 °C due to its high viscosity (details later), which is lower than 1-butyl-3-methylimidazolium chloride (10 wt%).⁶ It is the critical problem to overcome before practical use.

DMSO is a relatively polar aprotic solvent and has been often used as a co-solvent for the dissolution of cellulose with ILs. It is reported that DMSO can reduce the viscosity of ILs without hindering their ability to dissolve cellulose.¹⁷⁻²² In this study, we investigated the solubility of cellulose in OE₂imC₃C/DMSO mixtures. In addition, the toxicity to *Escherichia coli* (*E. coli*) growth was also investigated and surprisingly improved by addition of DMSO, although DMSO is less biocompatible than pure OE₂imC₃C (but even DMSO is generally considered biocompatible).

Figure 2a shows cellulose solubility in OE₂imC₃C/DMSO mixtures at 100 °C. Pure OE₂imC₃C was capable dissolving up to 6 wt% of cellulose. However, at this concentration, the solution became too viscous to stir. Thus, we could not confirm whether 6 wt% was the true maximum solubility in pure OE₂imC₃C. We found that the addition of DMSO accelerated the dissolution of cellulose. The OE₂imC₃C/DMSO

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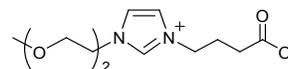


Fig. 1 A structure of OE₂imC₃C.

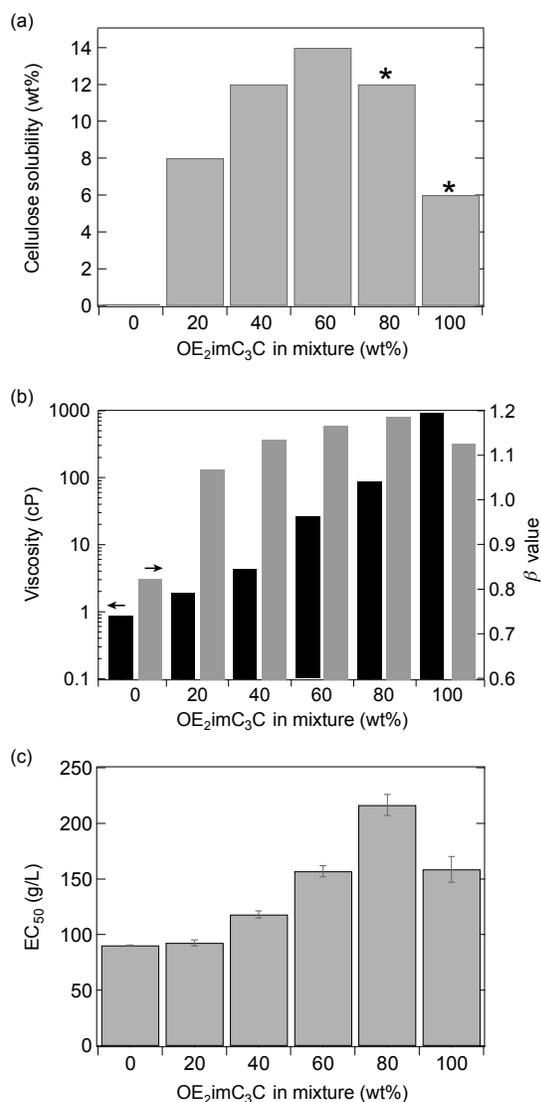


Fig. 2 (a) Cellulose solubility, (b) viscosity and β value, and (c) EC₅₀ of OE₂imC₃C/DMSO. *Solubility of cellulose could not be evaluated anymore because the mixture could not be stirred due to the high viscosity. The viscosity was measured at 80 °C.

mixture (80/20) dissolved up to 12 wt% while the mixture faced a similar problem with pure OE₂imC₃C. The OE₂imC₃C/DMSO mixture (60/40) achieved the highest solubility of cellulose at 14 wt%. When 15 wt% of cellulose was added, the mixture remained stirrable but did not dissolve the cellulose. The solubility was much improved by addition of DMSO and intermediate between chloride-type ILs^{6,7} (cf. 1-butyl-3-methylimidazolium chloride: 10 wt%; 1-allyl-3-methylimidazolium chloride: 11 wt% at the same temperature) and carboxylate-type ILs⁷ (1-ethyl-3-methylimidazolium formate: more than 20 wt%). This result indicates that OE₂imC₃C/DMSO mixture is ready to use. The dissolution ability of the OE₂imC₃C/DMSO mixture decreased when the OE₂imC₃C concentration is less than 60 wt%. The solubility was 12 and 8 wt% in the OE₂imC₃C/DMSO mixtures (40/60 and

20/80), respectively. This trend is similar to that of previously reported cellulose-dissolving ILs.²¹

To clarify the reason for the increase of cellulose solubility, we measured the viscosity of each mixture (Figure 2b). We measured the viscosity at 80 °C due to the high viscosity of OE₂imC₃C. Addition of DMSO caused the viscosity of the mixture to decrease almost exponentially. The OE₂imC₃C/DMSO mixture (60/40), which showed the highest solubility, had a much lower viscosity (26.2 cP at 80 °C) compared to that of pure OE₂imC₃C (935.2 cP at 80 °C), suggesting that low viscosity is related to cellulose solubility. We here would like to stress that OE₂imC₃C/DMSO solutions show similar viscosity to general carboxylate-type ILs²³ (cf. 1-ethyl-3-methylimidazolium acetate: 18 cP at 70 °C). It is noted that the viscosity of OE₂imC₃C/DMSO (60/40) at 30 °C was 175.8 cP while that of pure OE₂imC₃C is too high to be measured at the same temperature.

To determine the reason for decreased cellulose solubility in the mixtures (40/60 and 20/80), β value of Kamlet-Taft parameters²⁴ of each mixture was measured as reported²⁵ (Figure 2b). The β value describes hydrogen bond basicity, and it is known as a key factor in disrupting the hydrogen bond networks between cellulose chains.^{7, 26} β value somewhat decreased in the mixtures (40/60 and 20/80). The β value is reported to have a rough correlation with cellulose solubility²⁶, and it may be responsible for low solubility of cellulose in the OE₂imC₃C/DMSO mixtures (20/80 and 40/60). Another hypothesis regarding the low solubility in this region is the molar ratio of OE₂imC₃C to the OH groups of cellulose. In the mixture exhibiting maximum solubility, 14 wt% cellulose in OE₂imC₃C/DMSO (60/40), the molar ratio of OE₂imC₃C/OH is 1.00. In contrast, the molar ratios of OE₂imC₃C/OH in the cellulose-saturated OE₂imC₃C/DMSO mixtures (12 and 8 wt% cellulose in 40/60 and 20/80) are only 0.60 and 0.45, respectively. In the case of cellulose dissolved in excess of pure 1-ethyl-3-methylimidazolium acetate, a popular carboxylate-type IL, it is reported that one OH group makes a hydrogen bond with 0.92 ILs.²⁷ Therefore, while a ratio of 1.00 is sufficient to solubilize cellulose, ratios of 0.60 and 0.45 seem to be relatively low. Regarding the difference in the molar ratio of OE₂imC₃C/OH between the mixtures (60/40 and 20/80), the β values and cluster structure²⁸ of OE₂imC₃C/DMSO may also be involved, but further investigation is required.

We investigated the toxicity of the mixtures to *E. coli* growth (Figure 2c), by means of EC₅₀, which is the critical concentration of chemical compounds required for inhibiting the growth of microorganisms (details in Experimental section). The EC₅₀ of pure OE₂imC₃C was 159 g/L was almost 1.7-fold higher than that of DMSO (90 g/L). Therefore, it was confirmed that the toxicity of OE₂imC₃C was even lower than that of DMSO, a known biocompatible organic compound often used as a solvent for adding hydrophobic compounds to cultures. Remarkably, the EC₅₀ value increased to 217 g/L in the mixture (80/20): a lower toxicity than that of either pure OE₂imC₃C, although the addition of DMSO was expected to decrease the EC₅₀. It is noted that this value is extremely high because EC₅₀ of 1-ethyl-3-methylimidazolium acetate is only 9

g/L¹⁶. In the mixture (60/40), the EC₅₀ decreased to 157 g/L, which is close to that of the pure OE₂imC₃C. Further, addition of high concentration of DMSO to OE₂imC₃C (40/60 and 20/80) caused the EC₅₀ of the mixtures to decline to 118 and 93 g/L respectively. As expected, the EC₅₀ of the solutions with high concentration DMSO became nearly equal to that of pure DMSO.

In order to explain the trend of the EC₅₀ in Figure 2c, contribution of each solvent to the total EC₅₀ was separately calculated. The contribution of OE₂imC₃C to the total EC₅₀ in the OE₂imC₃C/DMSO (80/20) was 173 g/L (namely, that of DMSO and total EC₅₀ were 44 and 217 g/L, respectively). Because this calculated contribution is higher than the EC₅₀ of pure OE₂imC₃C (159 g/L), there may be positive synergistic effect. It may be caused by strong interaction of cations with DMSO²⁸ although further investigation is required to clarify. We think that there also seems to be another possibility—it is not synergistic effect—because 159 and 173 g/L is not so different and could be in error (see error bars in Figure 2c, and details are in discussed in ESI, the text for Fig. S1). In contrast, in the mixtures (60/40, 40/60, and 20/80), the EC₅₀ values of each component were 94/62, 47/70, and 19/74 g/L (OE₂imC₃C/DMSO), respectively; it appears that the toxicity does not come from only one of the components, because the values are not similar to the EC₅₀ of either of the pure OE₂imC₃C or pure DMSO (159 or 90 g/L). This observation may indicate a negative synergetic effect between OE₂imC₃C and DMSO when the DMSO concentration is over 20 wt%. The reason of positive/negative synergistic effect depending on the DMSO concentration is presumably due to forming ion clusters in DMSO at higher concentration.²⁸ In conclusion, the capability of OE₂imC₃C and DMSO mixtures to dissolve cellulose and their toxicity towards *E. coli* were evaluated. The addition of DMSO significantly increased the cellulose solubility. Notably, the mixtures with 20-60 wt% DMSO showed two fold higher cellulose solubility compared to that of pure OE₂imC₃C. Regarding the toxicity of the mixtures to *E. coli*, addition of 20 wt% DMSO unexpectedly improved the biocompatibility, despite DMSO having higher toxicity than that of OE₂imC₃C. From all results, OE₂imC₃C/DMSO (80/20) is the first solvent satisfying both efficient cellulose dissolution and utilization of microorganisms: the mixture is a promising solvent for biomass via bioconversion.

Experimental

Materials

OE₂imC₃C was synthesised as reported.⁽¹⁾ Avicel PH-101 was purchased from Sigma-Aldrich.Co., Llc. DMSO was purchased from Nacalai Tesque Inc. The solvatochromic dyes, 4-nitroaniline was purchased from Tokyo Chemical Industries Co., Ltd. and *N,N*-diethyl-4-nitroaniline was purchased from Kanto Chemical Co., Inc. *E.coli* was purchased from ATCC. Tryptone, NaCl (Nacalai Tesque Inc.) and yeast extract (Becton, Dickinson and Company) were purchased and used for preparing lysogeny broth (LB) without purification. Viscometer

(Brookfield DV-II+ Pro) was used for measurement of viscosity of OE₂imC₃C/DMSO.

Dissolution of cellulose

OE₂imC₃C/DMSO mixtures were prepared by mixing dry OE₂imC₃C and DMSO. Cellulose (1 wt%) was added into mixtures and the resulting solutions were stirred gently at 100 °C in an oil bath for 10 minutes. When cellulose was solubilised in the mixtures, the procedure was repeated until the maximum solubility of cellulose was achieved.

Measurement of β value of Kamlet-Taft parameters

Stock solutions of each solvatochromic dye, 4-nitroaniline (1 mg/mL) and *N,N*-diethyl-4-nitroaniline (1 mg/mL) were made with methanol. The solutions of 4-nitroaniline (30 μ L) and *N,N*-diethyl-4-nitroaniline (30 μ L) were taken into vials respectively, and were dried carefully under vacuum pressure. OE₂imC₃C/DMSO (200 μ L) mixtures then were mixed into each dried dye. The homogenous mixtures were placed into quartz cells with 0.1 mm light-path length. The maximum absorption (λ_{max}) of the mixtures was determined to calculate the β value as following equations.

$$\nu(\text{dye}) = 1/(\lambda_{\max(\text{dye})}10^{-4})$$

$$\beta = (1.035 \nu_{(N,N\text{-diethyl-4-nitroaniline})} + 2.64 - \nu_{(4\text{-nitroaniline})})/2.80$$

Assay of inhibition to growth of *E. coli* by OE₂imC₃C/DMSO mixtures

LB was made by mixing 10 g of tryptone, 5 g of yeast extract, 10 g of NaCl, and 1 liter of ultra pure water. The OE₂imC₃C/DMSO mixture (5.0 g) was diluted by the LB (10 mL) to obtain a stock solution. OE₂imC₃C/DMSO/LB mixture solutions with various concentrations were prepared by dilution the stock solution with the LB. *E. coli* was pre-cultured aerobically at 37 °C in the test tube containing 2 mL of the LB. After pre-cultured, the *E. coli* cells were collected by centrifugation and inoculated into the OE₂imC₃C/DMSO/LB mixtures (2mL each tube) as to be an initial optical density at 600 nm (OD₆₀₀) of 0.1. The inoculated media were incubated at 37 °C for 24 h using a reciprocal shaker at 160 rpm, and the OD₆₀₀ of solutions were measured. The median effective concentration (EC₅₀) concerning growth of *E. coli* was determined as concentration of the OE₂imC₃C/DMSO mixture at which the relative growth was reduced to a half of the value in pure medium.

Conflicts of interest

There are no conflicts to declare.

Acknowledgement

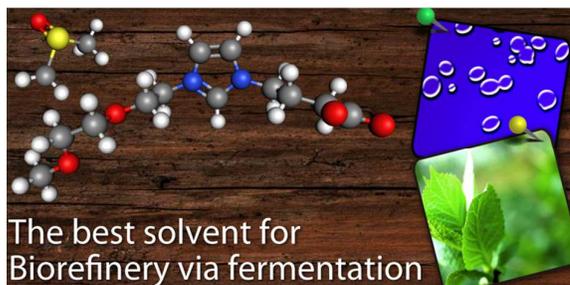
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- 25 K. Kuroda, H. Kunimura, Y. Fukaya and H. Ohno, *Cellulose*, 2014, **21**, 2199-2206.
- 26 A. Brandt, J. Gräsvik, J. P. Hallett and T. Welton, *Green Chem.*, 2013, **15**, 550.
- 27 B. D. Rabideau, A. Agarwal and A. E. Ismail, *J. Phys. Chem. B*, 2013, **117**, 3469-3479.
- 28 C. Zhang, H. Kang, P. Li, Z. Liu, Y. Zhang, R. Liu, J.-f. Xiang and Y. Huang, *Cellulose*, 2016, **23**, 1165-1175.

Notes and references

- 1 D. Klemm, B. Heublein, H. P. Fink and A. Bohn, *Angew. Chem. Int. Ed. Engl.*, 2005, **44**, 3358-3393.
- 2 H. P. Fink, P. Weigel, H. J. Purz and J. Ganster, *Prog. Polym. Sci.*, 2001, **26**, 1473-1524.
- 3 C. L. McCormick and T. R. Dawsey, *Macromolecules*, 1990, **23**, 3606-3610.
- 4 N. Tamai, D. Tatsumi and T. Matsumoto, *Biomacromolecules*, 2004, **5**, 422-432.
- 5 G. T. Ciacco, T. F. Liebert, E. Frollini and T. J. Heinze, *Cellulose*, 2003, **10**, 125-132.
- 6 R. P. Swatloski, S. K. Spear, J. D. Holbrey and R. D. Rogers, *JACS*, 2002, **124**, 4974-4975.
- 7 Y. Fukaya, A. Sugimoto and H. Ohno, *Biomacromolecules*, 2006, **7**, 3295-3297.
- 8 I. Kilpelainen, H. Xie, A. King, M. Granstrom, S. Heikkinen and D. S. Argyropoulos, *J. Agric. Food Chem.*, 2007, **55**, 9142-9148.
- 9 N. Sun, H. Rodriguez, M. Rahman and R. D. Rogers, *Chem. Commun.*, 2011, **47**, 1405-1421.
- 10 Y. Fukaya, K. Hayashi, M. Wada and H. Ohno, *Green Chem.*, 2008, **10**, 44-46.
- 11 H. Ohno and Y. Fukaya, *Chem. Lett.*, 2009, **38**, 2-7.
- 12 B. Zhao, L. Greiner and W. Leitner, *Rsc Advances*, 2012, **2**, 2476-2479.
- 13 F. Ganske and U. T. Bornscheuer, *Biotechnol. Lett.*, 2006, **28**, 465-469.
- 14 M. Ouellet, S. Datta, D. C. Dibble, P. R. Tamrakar, P. I. Benke, C. L. Li, S. Singh, K. L. Sale, P. D. Adams, J. D. Keasling, B. A. Simmons, B. M. Holmes and A. Mukhopadhyay, *Green Chem.*, 2011, **13**, 2743-2749.
- 15 G. S. Lim, J. Zidar, D. W. Cheong, S. Jaenicke and M. Klahn, *J. Phys. Chem. B*, 2014, **118**, 10444-10459.
- 16 K. Kuroda, H. Satria, K. Miyamura, Y. Tsuge, K. Ninomiya and K. Takahashi, *J. Am. Chem. Soc.*, 2017, **139**, 16052-16055.
- 17 L. Wu, S. H. Lee and T. Endo, *Bioresour. Technol.*, 2013, **140**, 90-96.
- 18 X. Zhang, W. W. Zhao, Y. J. Li, C. Li, Q. P. Yuan and G. Cheng, *Rsc Advances*, 2016, **6**, 62278-62285.
- 19 X. D. Li, Y. Zhang, J. W. Tang, A. Lan, Y. P. Yang, M. Gibril and M. H. Yu, *J Polym Res*, 2016, **23**.
- 20 D. A. Fort, R. C. Remsing, R. P. Swatloski, P. Moyna, G. Moyna and R. D. Rogers, *Green Chem.*, 2007, **9**, 63.
- 21 K. Ohira, K. Yoshida, S. Hayase and T. Itoh, *Chem. Lett.*, 2012, **41**, 987-989.
- 22 R. Rinaldi, *Chem Commun (Camb)*, 2011, **47**, 511-513.
- 23 E. Quijada-Maldonado, S. van der Boogaart, J. H. Lijbers, G. W. Meindersma and A. B. de Haan, *The Journal of Chemical Thermodynamics*, 2012, **51**, 51-58.
- 24 M. J. Kamlet and R. W. Taft, *JACS*, 1976, **98**, 377-383.

Graphical Abstract



Cellulose dissolution ability and biocompatibility of a liquid zwitterion, the most biocompatible cellulose solvent, were further improved by adding dimethylsulfoxide.