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ARTICLE

A low-viscous and flowable zwitterionic liquid

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Zwitterionic liquids have attracted significant attention in various fields due to their low-toxicity and the ability to tune their functional properties. However, despite being liquid in thermo-dynamic definition, zwitterionic liquids are not commonly recognized as "liquids" by the general public because of their extremely high viscosity, comparable to that of peanut butter, which prevents stirring even with a strong magnetic stirrer. In this study, we developed a flowable zwitterionic liquid, OE_2imOE_3C. Its viscosity was one-seventeenth that of traditional zwitterionic liquids. OE_2imOE_3C was able to stir and dissolve 11 wt% cellulose at 100 °C, which is a task unachievable by typical zwitterionic liquids due to their high viscosity. Furthermore, OE_2imOE_3C exhibited low toxicity to yeast, consistent with other standard zwitterionic liquids. This study successfully achieved a significant reduction in viscosity without compromising other properties.

Introduction

Ionic liquids have emerged as a subject of extensive research due to their potential as alternatives to water and organic solvents across various fields.[1, 2] Ionic liquids have been explored as electrolytes in electrochemistry,[3, 4] catalysts and solvents in chemical synthesis,[5, 6] extractants in chemical engineering,[7, 8] solvents for biomass in green chemistry,[9-12] and solvents for poorly soluble drugs and proteins in the life sciences[13-15].

On the other hand, typical ionic liquids are more toxic than traditional organic solvents.[16, 17] The toxicity of ionic liquids is linked to their cationic side chains, which can penetrate cell membranes through hydrophobic interactions, resulting in membrane disruption.[18, 19] This high toxicity raises concerns about potential human health risks and environmental leakage because of their widespread use. Especially in life sciences, the toxicity is problematic. For example, their use in biomass dissolution can lead to the death of fermenting microorganisms in biorefineries.[20, 21] Similarly, employing toxic ionic liquids to dissolve poorly soluble drugs does not make sense.

Zwitterions have attracted considerable interest recently. Zwitterions are intramolecular salts where cations and anions are connected by covalent bonds, which are called spacers. Zwitterions are low toxic, since they do not engage in hydrophobic interactions with cell membranes.[17, 22-24] Consequently, carboxylate-type zwitterions enabled dissolution of cellulose without inhibiting subsequent ethanol fermentation, achieving the critically efficient bioethanol production.[21] Zwitterions are also effective in dissolving poorly water-soluble drugs.[25, 26]

However, most zwitterions are solid at room temperature, which complicates their use as solvents, while their analogous ionic liquids are liquid. To address this, methods such as incorporating ethylene oxide oligomers or long-chain alkyl groups into the cationic side chain have been developed to reduce their melting points.[27-29] Despite these efforts, most zwitterions remain solids at room temperature. For example, a popular cellulose-dissolving zwitterion containing an ethylene oxide oligomer melts at 64 °C. To date, only around five zwitterions have been reported to be liquid at room temperature, defined here as room-temperature zwitterionic liquids.[23, 28-30] However, the reported room-temperature zwitterionic liquids have extraordinarily high viscosities, similar to that of peanut butter. For instance, OE_2imC_5C (Fig. 1) exhibits a viscosity of 14,000 mPa·s at 80 °C,[23] which is four orders of magnitude higher than that of analogous ionic liquids. This high viscosity not only significantly reduces the efficiency of solute dissolution but also hinders pipetting and stirring, even with strong magnetic stirrers (Movie S1). The viscosity critically makes practical applications of zwitterionic liquids challenging.

Although there are room-temperature zwitterionic “liquids” are in a thermodynamic definition, there are no flowable room-temperature zwitterionic “liquids” which is in generally acceptable concept. The development of flowable room-temperature zwitterionic liquids could significantly enhance the practical applications of zwitterions, particularly in the life sciences, where their low toxicity and functions are crucial.

This study starts with the fundamental distinctions between high-viscosity zwitterions and low-viscosity ionic liquids: the alkyl chain spacers (Fig. 2). The spacers hinder the independent movement of cations and anions. The fixed anions/cations have large number of interaction and thus high

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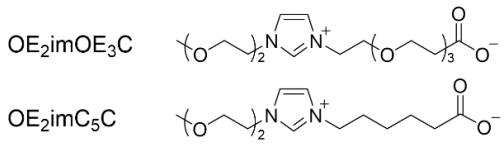

Fig. 1 Chemical structure of zwitterions used in this study.

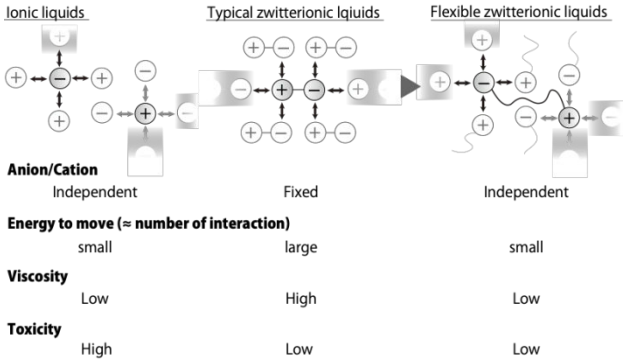

Fig. 2 Concept of this study.

activation energy to move. The high activation energy causes the high viscosity. If we can develop a zwitterionic liquids without the immobilizing spacers, it is expected to be low-viscosity. In response, we here propose the concept of a flexible spacer, while only alkyl chains have been utilized so far, to the best of our knowledge. Specifically, we synthesized a novel zwitterionic liquid featuring flexible oligoether spacers, OE_2imOE_3C (Fig. 1, Schemes S1 and S2). OE_2imOE_3C is both flowable and stirrable at room temperature.

Result and discussion

Thermal properties

The synthesis of OE_2imOE_3C was confirmed by using ^1H NMR, ^{13}C NMR, mass spectrometry, and elemental analysis. OE_2imOE_3C was liquid at room temperature (Table 1, Movie S2). Its glass transition temperature was approximately −30 °C, comparable to that of OE_2imC_5C (−38 °C). Notably, the viscosity of OE_2imOE_3C at 80 °C is 810 mPa·s, which is significantly lower than the 14,000 mPa·s recorded for OE_2imC_5C at the same temperature. The introduction of the oligoether spacer significantly reduced the viscosity as anticipated.

The decomposition temperature of OE_2imOE_3C was 253 °C. Its stability is adequate for various applications, including the dissolution of cellulose, which typically occurs below 150 °C. This thermal stability is comparable to that of OE_2imC_5C, suggesting that the introduction of the oligoether spacer did not compromise stability.

Table 1 Thermal properties and viscosity of OE_2imOE_3C and OE_2imC_5C.

	T_d [°C]	T_m [°C]	T_g [°C]	State at 25 °C	Viscosity at 80 °C [mPa·s]
OE_2imC_5C	252	–[a]	−38	liquid	14,000
OE_2imOE_3C	253	–[a]	–30	liquid	810

[a]Not detected.

We evaluated the stirrability of OE_2imOE_3C at room temperature (Movie S2). It could be magnetically stirred, unlike OE_2imC_5C. This notable reduction in viscosity presents a significant advantage for practical applications (details later).

Mechanism studies

To explore the mechanism behind this viscosity reduction, diffusion coefficients were measured using ^1H PFG-NMR (Fig. S3). Measurements in neat solutions were not possible due to equipment limitations, and thus were performed in 0.1 M solutions prepared with D_2O. The diffusion coefficients for the proton atoms of OE_2imOE_3C ranged from 2.04×10^{-9} to 2.47×10^{-9} m^2/s, while those for OE_2imC_5C ranged from 3.06×10^{-10} to 4.93×10^{-10} m^2/s. This increase in diffusion at the molecular level corresponds to a reduction in macroviscosity.

To verify the independent movement of cations and anions, diffusion coefficients of cationic and anionic protons were compared. Direct measurements were not feasible for the carboxylate anion due to the absence of protons and the low reliability of the imidazolium ring's measurements, attributed to facile proton exchange.[31, 32] Therefore, we compared the diffusion coefficients of the oligoether spacer to those of the cationic oligoether side chain in OE_2imOE_3C. The average diffusion coefficient of the oligoether spacer protons was 99% of that of the cationic oligoether side chain protons (Fig. S3),

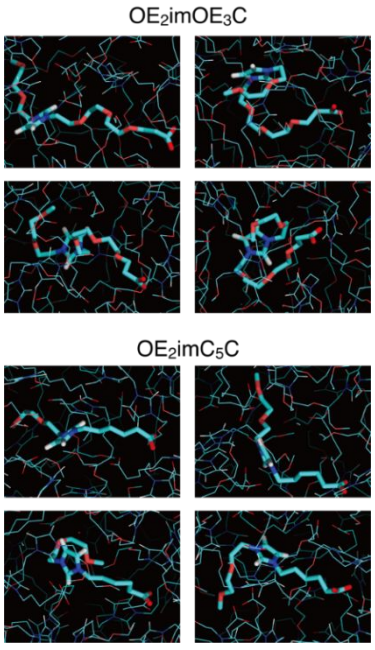

Fig. 3 Typical conformations of OE_2imOE_3C and OE_2imC_5C extracted by molecular dynamics trajectories. The movies are shown in Movies S3 (OE_2imOE_3C) and S4 (OE_2imC_5C).

suggesting comparable mobility. In contrast, the average diffusion coefficient of the alkyl spacer protons in OE_2imC_5C was only 83% of that of the cationic side chain, confirming the low mobility of the rigid alkyl spacer. These results support the hypothesis that the flexibility of the oligoether spacer facilitates the independent movement of the cation and anion, thereby reducing viscosity. The independent movement of cation and

anion was also confirmed by molecular dynamics simulations (Fig. 3, Fig. S4, and Movies S3 and S4).

Cellulose dissolution ability

To demonstrate the superior properties of OE_2imOE_3C, we assessed its cellulose dissolution ability (Table 2). Carboxylate-type zwitterions are known to dissolve cellulose and low toxicity and thus have enabled to successive bioethanol production procedures (i.e. dissolution, enzymatic hydrolysis, fermentation) in the single reaction vessel. However, the high viscosity of traditional zwitterions severely impedes cellulose dissolution. For instance, the highly viscous OE_2imC_5C dissolves only 1 wt% cellulose after 10 h at 100 °C.[33] In stark contrast, OE_2imOE_3C dissolved 11 wt% cellulose within 60 min at the same temperature. This result suggests that not only OE_2imOE_3C dissolves cellulose efficiently by the low viscosity but also the introduction of oligoether spacer does not induce defect in the functions of zwitterions. The latter is a noteworthy point because a property control in zwitterions/ionic liquids often result in functional losses. Furthermore, the hydrogen bonding basicity (β value of the Kamlet-Taft parameters), which is crucial for cellulose dissolution, was 1.07 for OE_2imOE_3C, similar to that of OE_2imC_5C (1.14). This similarity strongly supports that the oligoether spacer does not impair the cellulose dissolution ability of the zwitterion but effectively reduces its viscosity.

In this study, we were able to develop a low-viscosity zwitterion without any compromising the functions. The critical reason is that the spacers, typically simple 3-5 carbon alkyl chains, have never contributed to any specific function so far but merely served to tether the cations and anions. This study is the first to actively design zwitterionic spacers to enhance functionality without inducing defects.

Toxicity to microorganisms

The toxicity of OE_2imOE_3C to microorganisms was assessed to ensure the preservation of another zwitterion function. The ethanol-fermenting yeast *Kluyveromyces marxianus* (*K. marxianus*) was employed due to its capacity to ferment at approximately 50 °C,[34, 35] which aligns with the optimal temperature for cellulase. This study utilized the growth inhibition of *K. marxianus* as a metric, with OD_{600} after 6 h of culture serving as the indicator. The relative OD_{600}, calculated by normalizing against the value in a pure medium, was used to assess inhibition. A 0.1 M solution of OE_2imOE_3C did not inhibit the growth of *K. marxianus*, similar to OE_2imC_3C, which is recognized as one of the least toxic zwitterions (Fig. 4).[21, 23] In contrast, $[C_2mim]OAc$, the most famous cellulose-dissolving ionic liquid, significantly inhibited the growth of *K. marxianus* (Fig. 4). OE_2imOE_3C is potentially less toxic than even OE_2imC_3C, particularly evident when examining the OD_{600} in 0.5 M solutions (Fig. 4). The strategic use of unfunctionalized spacers has successfully rendered the zwitterion less viscous without compromising all other functional properties.

Table 2 Cellulose dissolution ability and Kamlet-Taft parameters.

	Cellulose solubility at 100 °C [wt%]	Kamlet-Taft parameters		
		α	β	π^*
OE_2imC_5C	< 1	0.38	1.14	1.02
OE_2imOE_3C	11	0.30	1.07	1.03

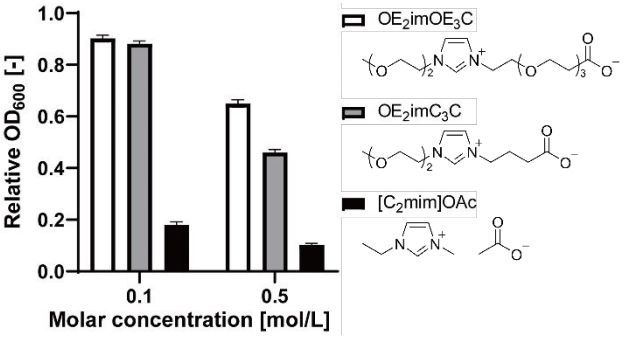

Fig. 4 Relative OD_{600} for K. marxianus after 6 h of culture in media supplemented with OE_2imOE_3C, OE_2imC_3C, and $[C_2mim]OAc$.

Conclusions

In Conclusion, a novel zwitterionic liquid, OE_2imOE_3C, was synthesized by incorporating an oligoether chain into the spacer. This modification resulted in OE_2imOE_3C being a liquid at room temperature. Notably, its viscosity at 80 °C was 810 mPa·s, merely one-seventeenth that of OE_2imC_5C. The mechanism behind the viscosity reduction was explored using ^{1}H PFG NMR, which suggested that the flexible spacer facilitated the free movement of cations and anions. OE_2imOE_3C exhibited outstanding performance as a cellulose solvent, dissolving 11 wt% cellulose at 100 °C within 60 min, in stark contrast to OE_2imC_5C, which only dissolved 1 wt% after 10 h. Additionally, OE_2imOE_3C displayed low toxicity to yeast, comparable to another low-toxicity zwitterion. These findings demonstrate that the intro-duction of an oligoether chain into the spacer effectively reduces viscosity, while preserving the inherent benefits of zwitterionic liquids. This method is expected to enhance numerous applications, particularly in life sciences, including the use of zwitterions as solvents for bioethanol production and dissolution of poorly soluble drugs.

Author contributions

M. Shimizu: data curation, investigation, methodology, visualization, writing – original draft, writing – review & editing. T. Fujie: investigation, methodology, supervision, writing – review & editing. M. Shibata: data curation, investigation. T. Komori: data curation. K. Ninomiya: resources. K. Takahashi: resources. T. Uto: data curation, formal analysis, investigation, methodology, resources, supervision, software, visualization, writing – review & editing. K. Kuroda:

conceptualization, funding acquisition, project administration, resources, writing – original draft, writing – review & editing.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the Supplementary Information.

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Data Availability

All data are available in the main text or the supplementary materials.