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Direct preparation of gels from herbal medicinal plants by using a low toxicity liquid zwitterion

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RUNNING HEAD

Gels prepared from herbal medicinal plants

ABSTRACT

Gels containing medicinal ingredients of licorice were formed by dissolving into a biocompatible zwitterionic cellulose solvent and successive precipitation. The licorice gels gradually released glycyrrhizic acid, the main medicinal ingredient of licorice, within 3 h. Although the licorice gels were mechanically weak, gel strength was improved just by adding cellulose during the preparation of the gels.

KEYWORDS

Cellulose / Gel / Herbal medicine / Ionic liquid / Licorice / Liquid zwitterion / Toxicity

INTRODUCTION

Efficient extraction of physiologically active substances from medicinal plants is an active area of study. Conventional extraction techniques that use water or organic solvents, sometimes combined with Soxhlet extraction [1], are inefficient. Therefore, more efficient extraction methods are attracting attention [2]. Using cellulose-dissolving ionic liquids is one potential method [3,4]. Because these ionic liquids disrupt plant cell walls, the internal physiologically active substances of plant cells can be efficiently extracted [5]. However, typical ionic liquids are toxic [6,7].

Therefore, they are supposed to be excluded from lists of solvents applicable for pharmaceutical processing [8], although they are not classified at the present time because they are emerging solvents. Here we focused on a carboxylate-type liquid zwitterion, 3-(2-(2-methoxyethoxy)ethylimidazol-3-io)butane-1-carboxylate (OE_2imC_3C) that we developed (the structure is shown in Fig. 1) [9,10]. OE_2imC_3C can dissolve cellulose and exhibits excellent biocompatibility. For example, the toxicity of OE_2imC_3C to microorganisms is approximately 1/18 that of 1-ethyl-3-methylimidazolium acetate, a typical cellulose-dissolving ionic liquid. The toxicity of OE_2imC_3C is approximately half that of dimethyl sulfoxide, a solvent that is used in pharmaceutical manufacturing [8]. For these reasons, OE_2imC_3C is a promising solvent for efficient extraction of physiologically active substances from medicinal plants due to its ability to dissolve cellulose and its biocompatibility.

Furthermore, we also focused on gel formation when the cellulose solutions are dropped into poor solvents [11,12]. By exploiting the gel formation, we wondered whether the cellulose gels with extracted medicinal ingredients could be prepared simply by dropping the medicinal plant/OE_2imC_3C solutions into poor solvents. The direct production of the gels from medicinal plants is expected to not only prevent loss of the medicinal ingredients but also enable the gels to gradually release medicinal ingredients. In this study, OE_2imC_3C was used for efficient extraction of medicinal ingredients from licorice, a medicinal plant. Licorice is included in 70% of Chinese and Japanese herbal medicine mixtures called kampo. Licorice alleviates various symptoms and is effective for analgesia, antispasmodics, detoxification, etc. This is also a first report for gels directly prepared from whole herbal medicinal plants. The gels containing glycyrrhizic acid, the main medicinal ingredient of licorice, were made in this study. The gradual release of glycyrrhizic acid from the gels is expected and can contribute to improve quality of life of patients.

EXPERIMENTAL PROCEDURE

Extraction of glycyrrhizic acid from licorice

To extract glycyrrhizic acid from licorice, dry licorice (0.02 g) was added to water (0.48 g) or OE_2imC_3C (0.48 g), and the mixture was stirred at 95 °C for 2 h. The obtained solutions (0.3 g) were added dropwise to ultrapure water (15 mL), and the precipitates were vigorously stirred. The solution was analyzed using a high performance liquid chromatography/triple quadrupole mass spectrometer (LC/MS, LCMS-8030, Shimadzu Co., Ltd., Kyoto, Japan) equipped with an electrospray ionization interface to measure the concentration of glycyrrhizic acid. The extraction yield of glycyrrhizic acid (%) was derived from the following equation:

$$Extraction\ yield\ of\ glycyrrhizic\ acid\ (\%) = \frac{Concentraion\ of\ glycyrrhizic\ acid\ \left(\frac{\mu g}{mL}\right)\times 15(mL)}{The\ amount\ of\ licorice\ (\mu g)} \times 100 \quad (1).$$

The conditions of LC/MS are mentioned in the supplementary information. The spectra of glycyrrhizic acid and typical sample are shown in Fig. S1.

Preparation of the licorice gels and their release of glycyrrhizic acid

To prepare licorice gels and measure the release of glycyrrhizic acid, phosphate buffer solutions with pH values of 5.0 or 7.4 and a Tris-HCl buffer with a pH value of 9.0 (150 mM) were prepared. Licorice (0.02 g) was added to OE_2imC_3C (0.48 g), and the mixture was stirred at 120 °C for 2 h. The OE_2imC_3C/licorice solution (0.3 g) was added dropwise to each buffer solution (15 mL) to prepare the gels and then agitated with shaking at 150 rpm at room temperature (Multi

Shaker MMS, EYELA, Tokyo Rikakikai Co., Ltd.). During shaking, 400 μL of solvent was collected at 0, 0.1, 0.2, 0.4, 0.7, 1, 2, 3, 6, and 24 h, and the released yield of glycyrrhizic acid was measured by LC/MS and calculated by the following equation:

$$Released\ yield\ of\ glycyrrhizic\ acid\ (\%) = \frac{concentration\ of\ glycyrrhizic\ acid\ \left(\frac{\mu g}{mL}\right) \times 15(mL)}{The\ amount\ of\ licorice\ (\mu g)} \times 100 \quad (2)\ .$$

In the case of licorice/microcrystalline cellulose (MCC) gels, licorice (0.02 g) was added to OE_2imC_3C (0.48 g) and stirred at 120 °C for 2 h. Then, 5 mg or 10 mg of MCC (corresponding to 1 or 2 wt%, respectively, against entire solutions) was added. The mixture was stirred at 120 °C for 2 h, and the MCC was completely dissolved in OE_2imC_3C. The gels were prepared with a buffer solution of pH 7.4, and the released yields were measured by LC/MS.

RESULTS AND DISCUSSION

Licorice (4 wt%) was mixed with either OE_2imC_3C or water, and glycyrrhizic acid was extracted at 95 °C. The extraction yield using water was 2.4% (calculated using the amount of licorice loaded as 100%). This value is reasonable because glycyrrhizic acid content is generally 2% or more in licorice. On the other hand, the extraction yield using OE_2imC_3C was 4.1%. Glycyrrhizic acid was efficiently extracted by OE_2imC_3C, a low toxicity cellulose solvent, as well as conventional cellulose-dissolving solvents.

Licorice gels were directly prepared from the solution obtained from the licorice/ OE_2imC_3C solution. Licorice of 4 wt% was dissolved in OE_2imC_3C (OE_2imC_3C:licorice = 96:4), and the solution was dropped into a buffer solution of pH 7.4 (a physiological condition) using a

micropipette. Because the viscosity of the solution was high, a micropipette for highly viscous conditions was used. When dropped into the buffer solutions, the polymer contained in the licorice precipitated and formed gel particles (Fig. 2). In addition, the gels formed in a similar manner when buffer solutions of pH 5.0 and 9.0, ultrapure water, and methanol were used as poor solvents.

The release of glycyrrhizic acid from the gels was estimated. Buffer solutions of pH 5.0, 7.4, and 9.0 were used as poor solvents to evaluate the effect of pH. The yield of glycyrrhizic acid released from the gels was calculated by measuring the concentration of the released glycyrrhizic acid in the buffer solutions (Fig. 3). With the pH 7.4 buffer solution, the released yield of glycyrrhizic acid at 0.2, 0.4, 1, and 3 h was 1.5%, 2.4%, 3.4%, and 4.2%, respectively (calculated using the loaded licorice as 100%), showing that glycyrrhizic acid was gradually released from the gels. The released yield of glycyrrhizic acid at 6 and 24 h was 4.2% and 4.0%, respectively. Because these percentages were similar to that at 3 h, this result suggests that the release of glycyrrhizic acid is completed within 3 h. The released yields after 3 h corresponds to the extraction yield with OE_2imC_3C. It suggests that all glycyrrhizic acid included in licorice was released from the gels after 3 h.

We noted that the released yield of glycyrrhizic acid decreased slightly at 24 h. We investigated whether glycyrrhizic acid was hydrolyzed to a sugar and glycyrrhetinic acid. Therefore, we assayed for the presence of glycyrrhetinic acid in the sample solution at 24 h by LC/MS, but it was not detected. This result indicates that glycyrrhizic acid either decomposed to different compounds or the value itself might have been in error.

With the pH 9.0 buffer solution, the released yield of glycyrrhizic acid from precipitated gels at 0.2, 0.4, 1, 3, 6, and 24 h is 0.8%, 1.9%, 2.9%, 3.9%, 4.0%, and 4.1%, respectively.

Glycyrrhizic acid is gradually released and is completed within 3 h, which is similar to release kinetics in the pH 7.4 buffer solution. With the pH 5.0 buffer solution, the released yield of glycyrrhizic acid at 0.2, 0.4, 1, and 3 h is 0.5%, 1.2%, 2.1%, and 3.4%, respectively, also showing a gradual emission of glycyrrhizic acid. However, the released yield at pH 5.0 is lower than the yields from pH 7.4 and 9.0 buffer solutions. Even at 6 and 24 h, the released yield of glycyrrhizic acid was 3.5% and 3.4%, respectively, at pH 5.0. They are approximately the same as the yield at 3 h and still less than the yields at pH 7.4 and 9.0. These results suggest that the upper limit of the amount of glycyrrhizic acid released is lower at pH 5.0 and that the lower releasing yield is not a problem with the release rate.

The solubility of glycyrrhizic acid in each buffer solution was investigated. Glycyrrhizic acid was added at 25, 27, 30, and 40 μg/mL to buffer solutions of pH 5.0, 7.4, or 9.0. Each solubilization was checked by observing the absence of crystals with a microscope (ECLIPSE Ts2, Nikon, Co., Ltd. Tokyo, Japan). The saturated solubility of glycyrrhizic acid in pH 7.4 and 9.0 buffers is between 30 and 40 μg/mL; i.e., glycyrrhizic acid in the 30 μg/mL samples is dissolved, but crystals are observed in the 40 μg/mL samples. In Fig. 3, the concentrations of glycyrrhizic acid released at 24 h in pH 7.0 and 9.0 buffers are 34 and 32 μg/mL, respectively, and are approximately equivalent to the saturated solubility. On the other hand, the saturated solubility of glycyrrhizic acid in the pH 5.0 buffer solution is between 27 and 30 μg/mL. In Fig. 3, the concentration of glycyrrhizic acid at 24 h in the pH 5.0 buffer solution is 26 μg/mL, which is consistent with the solubility. These results show that the low released yield is not attributable to the gel structures, but rather to the solubility of glycyrrhizic acid. Glycyrrhizic acid has carboxyl groups whose protons are prone to associate with carboxylates in a pH 5.0 buffer solution (e.g., the pK_a of acetic acid is 4.76 [13]). Therefore, several tens of percent of carboxylic acid is neutral

in charge, having weaker interaction with water, resulting in low solubility. From these results, glycyrrhizic acid was released from the licorice gels, and the released yield of glycyrrhizic acid varied depending on the pH of the buffer solutions. This also indicates that the release is controllable by shifting pH; e.g., we would expect less emission of glycyrrhizic acid in lower pH environments, such as the stomach, and more release in neutral or higher pH environments, such as the gut.

We noted that the mechanical strength of the gels was insufficient. For example, the gels partly broke by shaking for long time (e.g., 24 h) or touching them with a micro spatula. Therefore, the compressive strength of the licorice gels prepared from a licorice/OE_2imC_3C solution (OE_2imC_3C:licorice = 96:4 in the precursor solution) was measured using a desk-type tensile compression tester (MCT-2150, A & D Co., Ltd. Tokyo, Japan) (Table 1). The compressive strength of the gels was below the lower limit of detection ($< 3.2\times10^{-2}$ MPa), confirming that the gels were very weak. We then added 1 wt% MCC (OE_2imC_3C:licorice:MCC = 96:4:1) during the step of dissolving licorice in OE_2imC_3C and prepared the gels in the same manner (Fig. 4 (a)). The compressive strength of gels prepared with 1 wt% MCC was 6.7 MPa, showing that the strength of the gels significantly increased by adding cellulose. We also measured the strength of gels made in the same manner with 2 wt% MCC (the picture is shown in Fig. 4 (b)). The compressive strength of gels prepared with 2 wt% MCC was 6.7 MPa, which is the same as gels prepared with 1 wt% MCC. The addition of 1 wt% MCC was sufficient to improve the compressive strength. The gels did not break by shaking for long time and even pushing them with a micro spatula.

The main macromolecules in plants are cellulose, hemicellulose, and lignin, but hemicellulose and lignin have relatively small molecular weights and are assumed to make a small

contribution to the formation of the gels. When 1 wt% MCC (MCC:licorice = 1:4) was added, the cellulose content of the gels increased to around 120% compared with the original gels (the cellulose content in licorice is 48%, detected by NREL method [14]), resulting in an improvement in strength. The reason why the loaded cellulose amount did not affect the strength might be related to the molecular weight of cellulose. Since the molecular weight of cellulose contained in plants is several to several tens of times that of the added MCC [15], the effect of the added MCC may not be significant.

The release of glycyrrhizic acid from gels with MCC was also measured. Gels with 1 wt% MCC were prepared using the pH 7.4 buffer. The released yield of glycyrrhizic acid from the gels at 0.2, 0.4, 1, 3, 6, and 24 h is 1.7%, 2.2%, 3.4%, 4.1%, 4.5%, and 4.1%, respectively (Fig. 5). The released yield of glycyrrhizic acid from the gels with 2 wt% MCC at 0.2, 0.4, 1, 3, 6, and 24 h was 1.6%, 2.8%, 3.5%, 4.1%, 4.3%, and 4.0%, respectively. Glycyrrhizic acid was gradually released from both gels, and the release was considered to be complete at 3 h. These results are approximately the same as those from gels without MCC. The addition of MCC improved the strength of the gels without affecting the release of glycyrrhizic acid.

To investigate why the release did not change despite the addition of cellulose, the structures of the outer and inner surfaces of the gels were observed by scanning electron microscopy after freeze drying [16] (Fig. S2). The outer surface of the gels without MCC is smooth and does not contain conspicuous pores (Fig. S2 (a)). The outer surface of the gels with 1 or 2 wt% MCC is also smooth, and the addition of MCC does not affect the structure of the gels (Fig. S2 (c) (e)). The inner surface of the gels without MCC is rough, and there are pores (Fig. S2 (b)). The inner surface of the gels with 1 or 2 wt% MCC also shows pores (Fig. S2 (d) (f)). These results suggest that the structure of the gels does not change regardless of the presence or amount of

cellulose, and this may be the reason for the consistent release behavior. The improvement in strength despite no significant difference in gel structure may be related to differences in more minute structures and density of the gels, but further investigation is required. In addition, the surface of the gels is smooth and has no pores, but glycyrrhizic acid continued releasing from the gels for 3 h. This is due to the pores at the molecular level or changes in the pore structures during vacuum drying. Careful discussion is necessary at this point.

In this study, gels were prepared from licorice, an herbal medicinal plant. On the other hand, various kinds of ingredients are used in kampo and most of them are plants. The proposed process in this study is thus applicable to make various gels from various herbal medicinal plants. Additionally, kampo is often used in combination with several kinds of herbal medicines to achieve synergistic effects and suppress side effects, and the proposed process in this study is also applicable to such mixtures.

CONCLUSION

OE_2imC_3C efficiently extracted glycyrrhizic acid from licorice compared with water (water, 2.4% vs. OE_2imC_3C, 4.1%). We also successfully prepared gels directly by merely dropping the licorice/OE_2imC_3C solutions into buffers. The licorice gels released glycyrrhizic acid over time. Furthermore, the compressive strength of the licorice gels was improved by adding MCC (1 or 2 wt%) without affecting the release of glycyrrhizic acid from the gels.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 De Castro MDL, García-Ayuso LE. Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. Anal Chim Acta. 1998;369:1-10.

2 Bubalo MC, Vidović S, Redovniković IR, Jokić S. New perspective in extraction of plant biologically active compounds by green solvents. Food. Bioprod Process. 2018;109:52-73.

3 Swatloski RP, Spear SK, Holbrey JD, Rogers RD. Dissolution of cellose with ionic liquids. J Am Chem Soc. 2002;124:4974-75.

4 Zhao H, Baker GA, Song ZY, Olubajo O, Crittle T, Peters D. Designing enzyme-compatible ionic liquids that can dissolve carbohydrates. Green Chem. 2008;10:696-705.

5 Usuki T, Yasuda N, Yoshizawa-Fujita M, Rikukawa M. Extraction and isolation of shikimic acid

from ginkgo biloba leaves utilizing an ionic liquid that dissolves cellulose. Chem Commun. 2011;47:10560-62.

6 Zhao DB, Liao YC, Zhang ZD. Toxicity of ionic liquids. Clean. 2007;35:42-48.

7 Mikkola SK, Robciuc A, Lokajová J, Holding AJ, Lämmerhofer M, Kilpeläinen I, Holopainen JM, King AWT, Wiedmer SK. Impact of amphiphilic biomass-dissolving ionic liquids on biological cells and liposomes. Environ Sci Technol. 2015;49:1870-78.

8 Impurities: Guideline for residual solvents; The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, 2011.

9 Kuroda K, Satria H, Miyamura K, Tsuge Y, Ninomiya K, Takahashi K. Design of wall-destructive but membrane-compatible solvents. J Am Chem Soc. 2017;139:16052-55.

10 Satria H, Kuroda K, Tsuge Y, Ninomiya K, Takahashi K. Dimethyl sulfoxide enhances both the cellulose dissolution ability and biocompatibility of a carboxylate-type liquid zwitterion. New J Chem. 2018;42:13225-28.

11 Kadokawa JI, Murakami MA, Kaneko Y. A facile preparation of gel materials from a solution of cellulose in ionic liquid. Carbohyd Res. 2008;343:769-72.

12 Kimura M, Shinohara Y, Takizawa J, Ren S, Sagisaka K, Lin YD, Hattori Y, Hinestroza JP. Versatile molding process for tough cellulose hydrogel materials. Sci Res. 2015;5:16266-73.

13 International Baccalaureate Organization, Chemistry data booklet, 2014. Cardiff: International Baccalaureate Organization. 2016. https://www.ibchem.com/root_pdf/data_booklet_2016.pdf.

14 A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton and D. Crocker; “Determination of Structural Carbohydrates and Lig- nin in Biomass,” Technical Report, NREL/TP-510-42618, National Renewable Energy Laboratory, US Department of Energy, Golden, U.S.A. 2012.

15 Kuroda K, Fukaya Y, Yamada T, Ohno H. Molecular weight distributions of polysaccharides and lignin extracted from plant biomass with a polar ionic liquid analysed without a derivatisation process. Anal Methods. 2015;7:1719-26.

16 Yang Y-J, Shin J-M, Kang TH, Kimura S, Wada M, Kim U-J. Cellulose dissolution in aqueous

lithium bromide solutions. Cellulose. 2014;21:1175-81.

Titles and legends to figures

Fig. 1 Structures of OE_2imC_3C and glycyrrhizic acid.

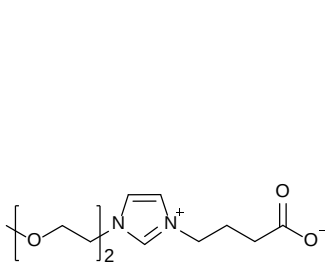
Fig. 2 Licorice gels prepared by OE_2imC_3C (the vial diameter: 3 cm).

Fig. 3 Relationship between the extraction yield of glycyrrhizic acid and the time that the gels are immersed in pH 5.0, 7.4, and 9.0 buffer solutions. *Calculated using the amount of licorice loaded as 100%.

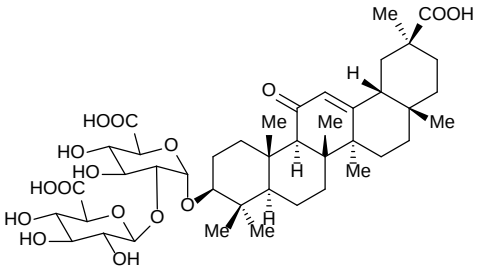
Fig. 4 The licorice gels with (a) 1 wt% and (b) 2 wt% of MCC (the vial diameter: 3 cm).

Fig. 5 Relationship between the extraction yield of glycyrrhizic acid and the immersion time of licorice gels prepared with 0, 1, or 2 wt% MCC in a pH 7.4 buffer. *Calculated using the amount of licorice loaded as 100%.

DISPLAY ITEMS (Figures)



OE_2imC_3C



Glycyrrhizic acid

Fig. 1

Fig. 2

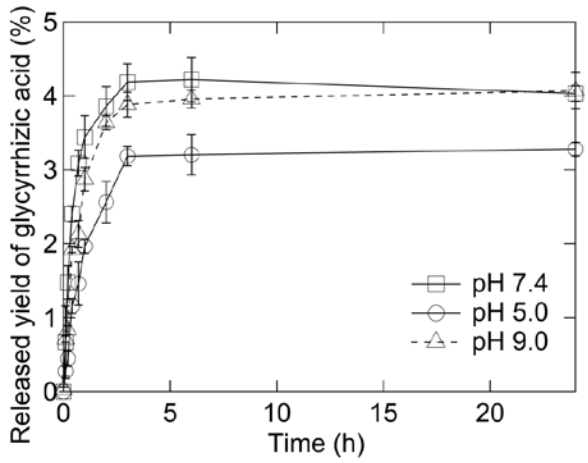

Released yield of glycyrrhizic acid (%)
Time (h)
pH 7.4
pH 5.0
pH 9.0
0
1
2
3
4
5
0
5
10
15
20

Fig. 3


(a)


(b)

Fig. 4

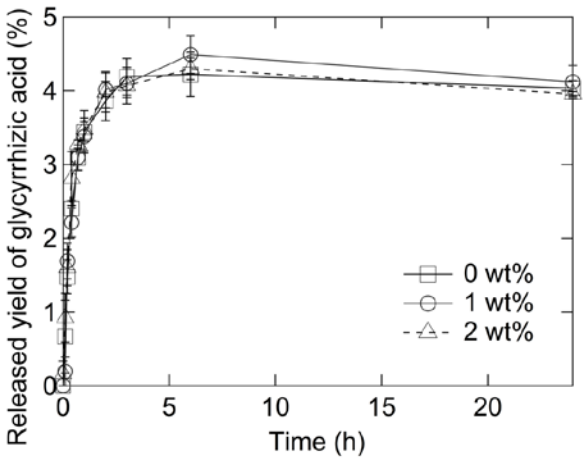

Released yield of glycyrrhizic acid (%)
Time (h)
0 wt%
1 wt%
2 wt%
0
1
2
3
4
5
0
5
10
15
20

Fig. 5

Titles and legends to Table

Table 1 Compressive strength of licorice gels containing 0, 1, or 2 wt% MCC.

DISPLAY ITEMS (Table)

Table 1

	Strength (MPa)
0 wt%	$< 3.2\times10^{-2}$
1 wt%	6.7
2 wt%	6.7