Rotaxa-polymeric-gelation of acrylamides with vinyl-β-cyclodextrin

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Abstract

Previously, we have reported a single-step preparation method for a topological gel by copolymerizing isoprene and vinyl-modified β-cyclodextrin (V-β-CyD). In the prepared gel, the vinyl part of V-β-CyD consists a part of the copolymer chain, and the β-CyD part includes another copolymer chain, thus V-β-CyD works as a cross-linker. We call the procedure rotaxa-polymeric-gelation (RPG) because polymerization, rotaxane formation, and gelation proceed simultaneously. In this study, we applied acrylamide (AAm) instead of isoprene for RPG, and successfully obtained highly swellable hydrogel. By contrast, a combination of V-β-CyD and *N*-isopropylacrylamide (NIPAAm) was not suitable for RPG because NIPAAm and cavity of β-CyD were mismatched in size. In addition, we prepared three-component gels. Among of them, gels composed of NIPAAm, isoprene, and V-β-CyD showed thermo-responsiveness by reflecting the character of polyNIPAAm. These results provide an insight for usage of vinyl monomers for RPG strategy.

Keywords cyclodextrin, polyrotaxane, acrylamide, *N*-isopropylacrylamide, rotaxa-polymeric-gelation

Introduction

In the past, gels are classified in two types according to cross-linking mode; physically cross-linked gel and chemically cross-linked gel. Nowadays, topological gels have appeared as the third type of gels. They have attracted much attentions because of their special features originated from topological structures in which polymers are cross-linked by mechanically interlocked structures [1–10].

A representative topological gel has been developed by the Ito group [1–4]. Their gel used figure-of-8 cross-linker composed of two α-cyclodextrins (α-CyDs). As the first step of the preparation of the topological gel, inclusion complex of polyethylene glycol (PEG) and α-CyDs are formed, and it is called a pseudopolyrotaxane [11]. As the second step, the both side of terminals of PEG are chemically modified with bulky stoppers to prevent dethreading of α-CyDs, and the resulting structure is called a polyrotaxane [12–15]. And the final step, the α-CyDs on the polyrotaxane are cross-linked with a chemical cross-linker, which results in a formation of the figure-of-8 cross-linker which mechanically interlocks PEG chains by inclusion [1–4].

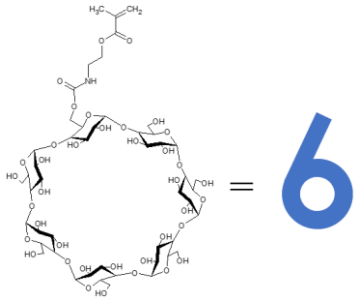

Fig. 1 The chemical structure and illustration of V-β-CyD.

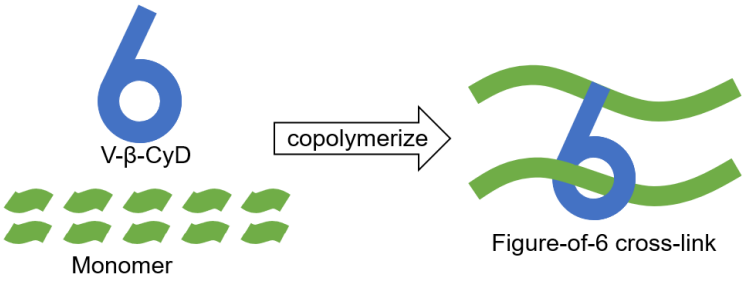

Fig. 2 The concept of RPG and figure-of-6 cross-linking.

Recently, we have reported another type of topological gel using vinyl-modified β-CyD (V-β-CyD in Fig. 1) [9]. When V-β-CyD and isoprene is copolymerized, processes of polymerization, rotaxane formation, and gelation occur simultaneously, thus, we call it rotaxa-polymeric-gelation (RPG). As shown in Fig. 2, the one side of the cross-linking points is a part of copolymer. On the other side of the cross-linking points, β-CyD includes a polymer chain to form a mechanically interlocked structure. As the image of shown in Fig. 2, we call it figure-of-6 cross-linking.

RPG was named after the concept of rotaxa-polymerization demonstrated by the Wenz group [6–8]. Their most important finding is that polymerization and rotaxanation can occur simultaneously. In rotaxa-polymerization, isoprene and styrene is copolymerized in the presence of methyl-β-CyD, which results in a formation of polyrotaxane composed of methyl-β-CyD and the copolymer of isoprene and styrene [6]. Isoprene part of the copolymer works as an axis of the polyrotaxane, and styrene part works as a stopper for methyl-β-CyD.

One of the strong points of rotaxa-polymerization is a wide acceptance in monomer choice. For example, hydrophilic vinyl monomers such as 2-hydroxyethyl methacrylate (HEMA) can be used to bring a good water solubility to polyrotaxane [7, 8]. We also consider that wide acceptance in monomer choice can be an advantage in RPG. In this, we examined the possibility to use acrylamide (AAm) because it is one of the most widely used monomer to make hydrogels. In fact, polyAAm is the

standard hydrogels for electrophoresis. In addition, we attempted to use *N*-isopropylacrylamide (NIPAAm) for RPG because thermo-responsive swelling and shrink behavior of NIPAAm-based gels has wide application in the biomedical fields [16–24].

In the previous reports of rotaxa-polymerization and RPG [7–9], isoprene was the only choice to make an axis polymer covered with β-CyD derivatives. Thus, at the beginning of this study, we investigated the possibility of AAm and NIPAAm to make an axis polymer. We demonstrated RPG with acrylamide monomers (AAm or NIPAAm) and V-β-CyD. As another reason for using AAm and NIPAAm, they have a common structure of vinyl amide; however, the bulkiness is different because of the absence and presence of the isopropyl residue, thereby, they are suitable to study the size balance between polymer axis and β-CyD cavity.

Furthermore, we mixed isoprene and acrylamides (AAm or NIPAAm) to implement RPG with V-β-CyD, and investigated their swelling behavior. Among of them, the NIPAAm-based gels were studied their thermo-responsive behavior.

Materials and Methods

Materials

β-CyD and methyl-β-CyD was obtained from Junsei Chemical Co. Ltd. (Tokyo, Japan). AAm, NIPAAm, ammonium peroxodisulfate (APS), 2-isocyanatoethyl methacrylate, and *N,N,N',N'*-tetramethylethylenediamine (TMEDA) were bought from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Isoprene (stabilized with 4-*tert*-butylcatechol: TBC), dibutyltin dilaurate, and 2,6-di-*tert*-butyl-*p*-cresol were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Isoprene was purified via distillation to remove the polymerization-inhibitor TBC. Other chemicals were used without further purification.

Preparation of V-β-CyD

V-β-CyD was prepared according to the previous report [9]. Briefly, 2-isocyanatoethyl methacrylate was reacted to hydroxy group of β-CyD in the presence of dibutyltin dilaurate. From a mixture containing unmodified β-CyD and mono-modified and di-modified derivatives, mono-modified derivative was purified with chromatography using a highly porous polystyrene gel (DiaionHP-20, Mitsubishi Chemical, Tokyo, Japan). The mono-modified derivative was confirmed with ^{1}H-NMR and FAB-MS. The detailed procedure were found in our previous report [9], and the spectra of ^{1}H-NMR and FAB-MS were in the supporting information (Fig. S1, S2) .

Preparation of gel

V-β-CyD (90.0 mg, 69.8 μmol) and APS (325 μg, 1.50 μmol) was dissolved in water (500 μL) and the solution was replaced in a screw vial or a well (9.75 mm diameter) of a 48 well-

microplate. The solution was bubbled with N_2 gas for 20 min. Then, AAm (16.0 mg, 225 μmol) was added to the solution, and it was gently shaken for 20 min. Subsequently, TMEDA (1.04 μL, 5.00 μmol) was added. After 24 h, the prepared gel was removed from the well, and it was immersed to water (30 mL) to remove unreacted species.

In a similar way, the kind and amount of monomers were changed to prepare various gels.

Swelling test

The gel was immersed to water, and the weight of swollen gel was measured with time. After the confirmation of no weight change, the weight was shown as $W_{swollen}$. After that, the gel was dried in vacuo at 40°C to measure the weight of dried gel (W_{dried}). The value of swelling degree was calculated with the following equation (1).

$$(W_{swollen} - W_{dried})/W_{dried} \times 100\ (\%) \qquad (1)$$

Results and discussion

Characterization of V-β-CyD

The details of the synthesis and purification procedure of V-β-CyD were described in our previous report [9]. Here, we discuss how many vinyl groups were modified to β-CyD using ^{1}H NMR and FAB-MS spectra (Fig. S1, S2). In the ^{1}H NMR spectrum of V-β-CyD (Fig. S1), a signal of the position 1 of glucose units of β-CyD was found at 4.87 ppm, and its integral value was set to 7.00 because β-CyD comprises seven glucose units. A signal of $-CH_3$ group of the modified group was found at 1.87 ppm, and its integral value was calculated as 3.04. The integral ratio of 7.00/3.04 proved that the ratio of β-CyD/modified group was 1/1.

Furthermore, the FAB-MS spectrum demonstrated that the obtained V-β-CyD was a mono-modified derivative (Fig. S2). There was a peak at *m/z* 1312.4 that agreed with the expected *m/z* value of mono-modified derivative, whose expected *m/z* value for $[M+Na]^+$ was 1312.4, and there was no peak derived from di-modified derivative whose expected *m/z* value for $[M+Na]^+$was 1467.5.

RPG with AAm and V-β-CyD

We carried out polymerization in screw vials in some conditions. After polymerization, we turned the vials upside down (Fig. 3). In the case of vial (c) started with AAm and V-β-CyD, a gel was successfully formed, and it did not fall. All other comparative conditions show no gelation and no clouding, which demonstrated both of AAm and V-β-CyD are essential for gelation. This success for RPG supports the existence of the interaction between the axis polymer chain and the cavity of V-β-CyD. As far as we know, this is the first example of inclusion complex of AAm-based polymer chain

and CyD derivative. We postulate that there is an interaction between polyAAm and β-CyD in solution state; however, it is difficult to confirm it. In general, complexes of polymer and CyD are obtained as insoluble precipitate [11–16], thereby, studies about solution state complexes of polymer and CyD have not progressed enough. Our success in complexation of AAm-based polymer chain and the β-CyD cavity suggests that the RPG can be a new approach to investigate water-soluble inclusion complexes because even if the complex is water-soluble, it is trapped and fixed in the hydrogel.

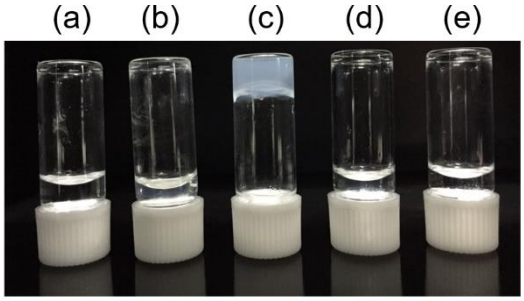

Fig. 3 Photographs of vials inverted after polymerization: (a) started with AAm (225 μmol); (b) started with V-β-CyD (69.8 μmol); (c) started with AAm (225 μmol) and V-β-CyD (69.8 μmol); (d) started with AAm (225 μmol) and native β-CyD (69.8 μmol); (e) started with AAm (225 μmol) and methyl-β-CyD (69.8 μmol).

RPG with NIPAAm and V-β-CyD

As shown in Fig. 4 (c), the result of copolymerization with NIPAAm and V-β-CyD showed a white viscous material instead of gel, and it flew down when the glass vial was turned upside down. The white color is because of Mie scattering, which means there are insoluble aggregates whose size is near the wavelength of visible light. From this result, there probably exists a certain interaction between the copolymer and the cavity of β-CyD to form an insoluble material; however, the interaction is not enough to achieve figure-of-6 cross-linking for RPG.

Around the same time as our previous report in 2018 [9], Malucelli et al. have reported a successful gelation by copolymerizing AAm-modified γ-CyD and NIPAAm [10]. Coincidentally, they and we share same concept of cross-linking. Their successful gelation proved that γ-CyD is more suitable than β-CyD for NIPAAm with the cross-linking concept. Wang et al have also reported a successful complexation of polyNIPAAm by γ-CyD but not by β-CyD [16].

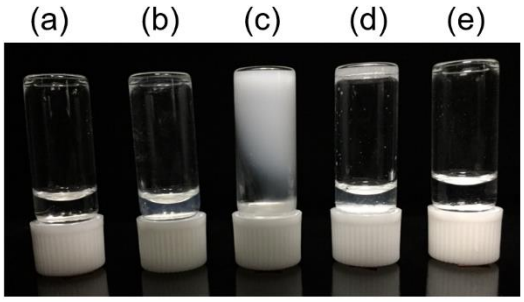

Fig. 4 Photographs of vials inverted after polymerization: (a) started with NIPAAm (225 μmol); (b) started with V-β-CyD (69.8 μmol); (c) started with NIPAAm (225 μmol) and V-β-CyD (69.8 μmol); (d) started with NIPAAm (225 μmol) and native β-CyD (69.8 μmol); (e) started with NIPAAm (225 μmol) and methyl-β-CyD (69.8 μmol).

Size balance consideration of monomer and β-CyD

So far, we succeeded in RPG with V-β-CyD in two kind of monomers: AAm and isoprene [9]. Then, we discuss the size balance of β-CyD cavity and monomers by using space filling model. As shown in Fig. 5, isoprene and AAm have very similar cross-sectional areas. Compared with these, NIPAAm is slightly larger. We have proposed that this difference is crucial to success in RPG.

Although RPG with NIPAAm and V-β-CyD was failed, the failure is a good evidence of the validity of RPG mechanism. The size mismatch between NIPAAm and V-β-CyD is the clear reason for the failure in RPG.

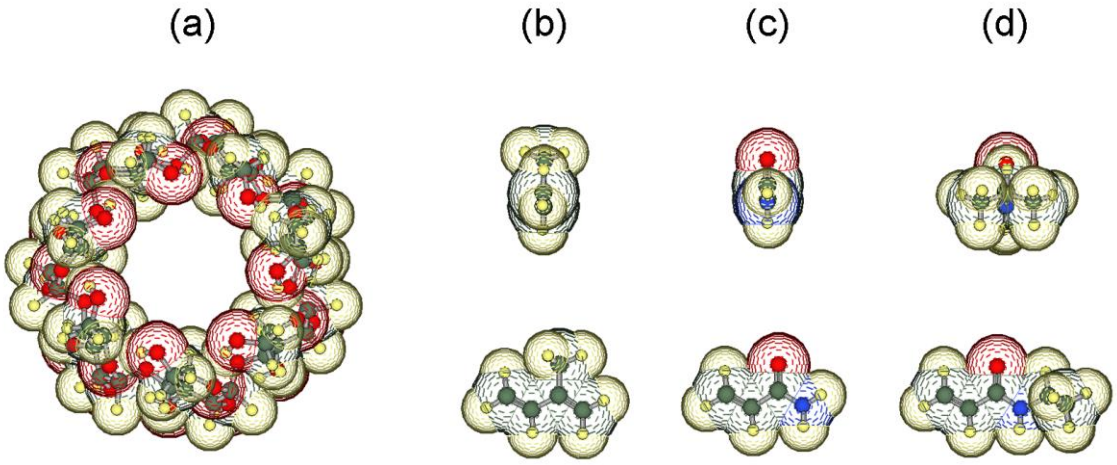

Fig. 5 Space-filling models: (a) β-CyD, (b) isoprene, (c) AAm, and (d) NIPAAm. (a) is based on a three dimensional arrangement file presented by Chaplin [25]. (b), (c), and (d) also show the models rotated 90º around the vertical axis.

Swelling behavior of AAm-based gels

The swelling degree of the gel made from isoprene and V-β-CyD was around 2000% (Fig. 6a). Then, we prepared three component gels (AAm, isoprene, V-β-CyD). The total amount of AAm and isoprene was fixed at 225 μmol, and their molar ratio of AAm/isoprene was changed. Fig. 6 (b–d) shows the swelling degree of each gels increased with the increase in the ratio of AAm. The two-component gel made from AAm and V-β-CyD was over 16000% (Fig. 6e). This tendency is because of the hydrophilicity of AAm. As shown in Fig. 3 (c), AAm-based gel was half-transparent, which suggests that insoluble aggregated part is not much in the gel. In contrast, polyisoprene is a hydrophobic polymer, and the isoprene based-gel showed white, which suggests that there exists insoluble aggregation in the gel [9]. These results show that swelling degree is controllable via changing the balance of hydrophilicity of monomers.

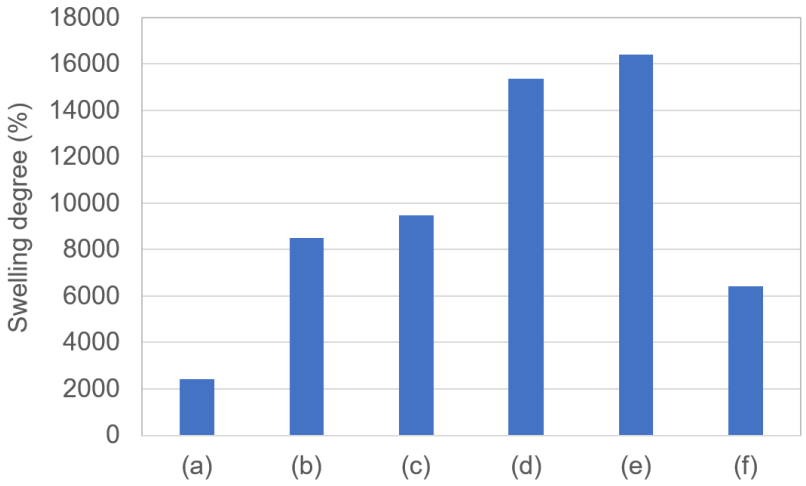

Fig. 6 Swelling degree in water of gels prepared with V-β-CyD and other monomers (225 μmol). (a) V-β-CyD (69.8 μmol) and isoprene (225 μmol); (b) V-β-CyD (69.8 μmol), AAm (106 μmol), and isoprene (119 μmol); (c) V-β-CyD (69.8 μmol), AAm (159 μmol), and isoprene (66 μmol); (d) V-β-CyD (69.8 μmol), AAm (212 μmol), and isoprene (13 μmol); (e) V-β-CyD (69.8 μmol) and AAm (225 μmol); (f) V-β-CyD (140 μmol) and AAm (225 μmol).

As another factor for the swelling degree, the effect of the amount of V-β-CyD was investigated. We regarded the gel prepared with V-β-CyD (69.8 μmol) and AAm (225 μmol) as a standard gel (Fig. 6e), and we attempted to prepare a gel with V-β-CyD (34.9 μmol, half the amount) and AAm (225 μmol); however, the gelation was not observed in this condition. On the other hand, the use of V-β-CyD (140 μmol, twice the amount) and AAm (225 μmol) resulted in gelation, and its swelling degree was lower than that of the standard gel (Fig. 6e, f). These results suggest that the cross-linking points are created through V-β-CyD and the amount of cross-linking points affect the swelling degree.

Thermo-responsive behavior of a three-component gel (NIPAAm, isoprene, V-β-CyD)

NIPAAm-based gel is widely investigated in the biomedical filed because of thermo-responsiveness [20] and its potential for expansion such as sensors [21], drug delivery systems [22, 23] and tissue engineering [24]. As mentioned above, copolymerization of NIPAAm and V-β-CyD did not form a gel. Next, we attempted to prepare a gel by combining NIPAAm and isoprene because the isoprene part in the prepared copolymer can be included in the β-CyD cavity to form figure-of-6 cross-linking. Total amount of NIPAAm and isoprene was set to 225 μmol, and the molar ratio of NIPAAm/isoprene was changed. All three conditions were successful in RPG.

The lower critical solution temperature (LCST) of the polyNIPAAm is 32ºC [23]; therefore, we monitored the swelling degree of gels at 7ºC and 60ºC because they were sufficiently far from the LCST. In addition, we chose the physiological temperature to be 37ºC because we were interested in the biomedical application of the gel. Fig. 7 (a) shows the results in the swelling degree of the two-component gel (V-β-CyD, isoprene). The swelling degree increased according to temperature increase, which is common feature of ordinary gels. The gel prepared in the NIPAAm/isoprene ratio of 106/119 also showed the same tendency (Fig. 7b). In contrast, the gels prepared in the NIPAAm/isoprene ratio of 159/66 and 212/13 showed shrinking in high temperature (Fig. 7c,d). This result shows that a certain degree of NIPAAm is needed to show thermo-responsiveness of NIPAAm.

This three-component strategy offers a solution for how to introduce relatively large monomers like NIPAAm in the RPG. For another example, we prepared a three-component gel polymerized with V-β-CyD (69.8 μmol), NIPAAm (212 μmol), and AAm (13 μmol). The gel was successfully obtained and showed the thermoresponsiveness of NIPAAm. The swelling degree of the gel using AAm (Fig. 7e) was much higher than that of the gel using isoprene (Fig. 7d). This result is probably attributed to the hydrophilic character of AAm; however, we need more information about the obtained gel to explain the swelling degree, which will be a part of our future work.

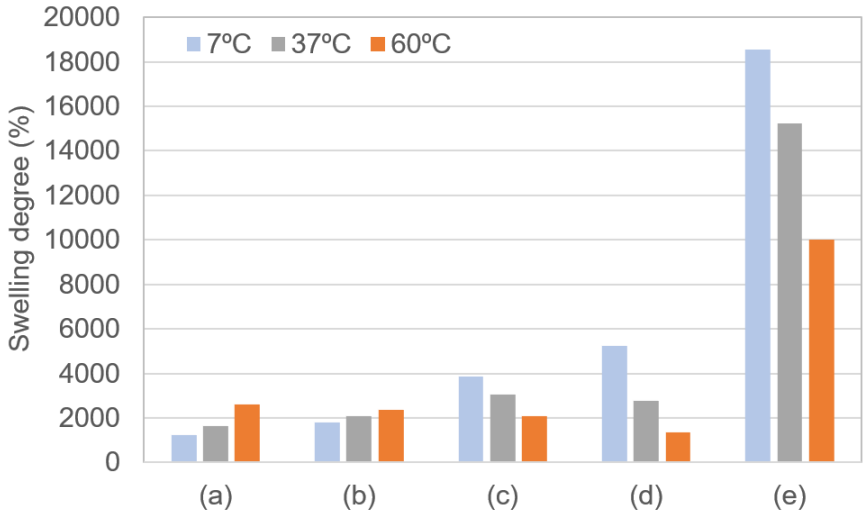

Fig. 7 Swelling degree of gels prepared with V-β-CyD (69.8 μmol) and other monomers (225 μmol) in water at each temperature. (a) isoprene (225 μmol); (b) NIPAAm (106 μmol) and isoprene (119 μmol); (c) NIPAAm (159 μmol) and isoprene (66 μmol); (d) NIPAAm (212 μmol) and isoprene (13 μmol); (e) NIPAAm (212 μmol) and AAm (13 μmol).

Conclusion

In this study, we carried out RPG with AAm and V-β-CyD. The gel prepared well swelled in water thanks to hydrophilic feature of AAm. In contrast, we could not obtain a gel using NIPAAm and V-β-CyD. From these results and consideration using space-filling molecular models, we have concluded that RPG needs a proper size monomer which can fit the cavity of CyD. The failure with NIPAAm and V-β-CyD shows a limitation of monomer choice for RPG. However, it can be overcome by mixing monomers. We copolymerized NIPAAm, isoprene, and V-β-CyD, which result in a gel formation. The three-component gel showed thermo-responsiveness of polyNIPAAm. The three-component strategy can widen the applicability of monomers for RPG.

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Supporting Information

Rotaxa-polymeric-gelation of acrylamides with vinyl-β-cyclodextrin

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Contents

^{1}H NMR spectrum of V-β-CyD

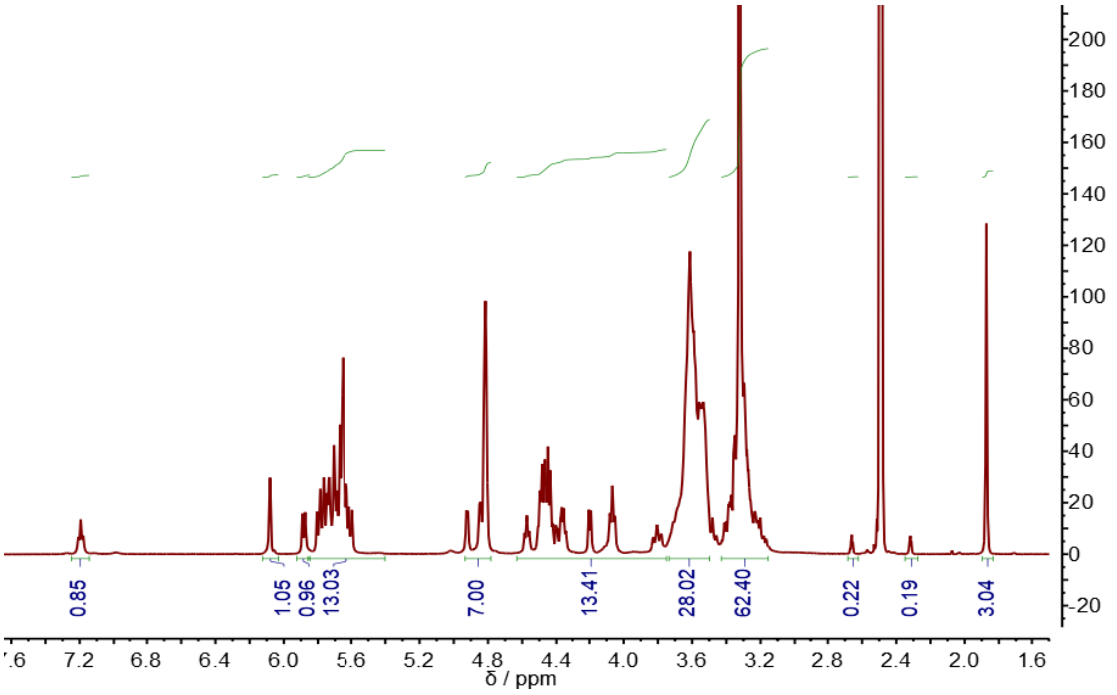

Fig. S1 ^{1}H NMR (400 MHz) spectrum of V-β-CyD in DMSO-d_6. Reprinted with permission from [9]. Copyright 2018 Springer Nature.

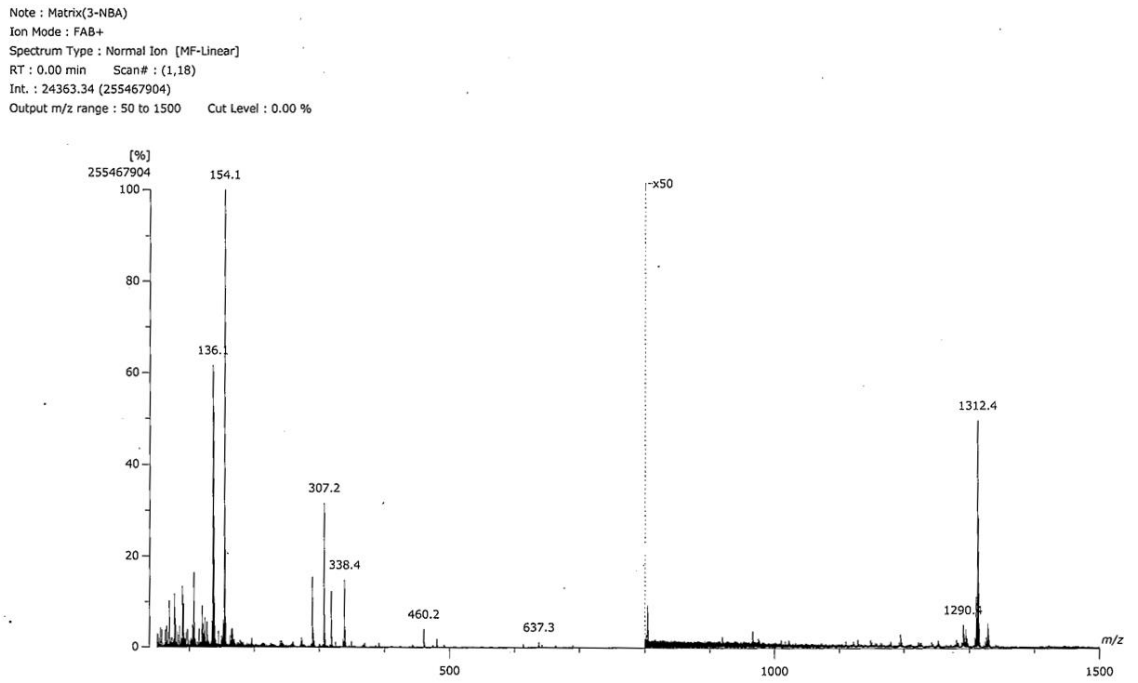

Fig. S2 Mass spectrum of V-β-CyD (FAB, positive mode, matrix: 3-NBA) *m/z*: 1312.4 (expected *m/z* of $[M+Na]^+$: 1312.4). Reprinted with permission from [9]. Copyright 2018 Springer Nature.

Reference [9]

Kobayashi, Y., Kojima, Y., Miki, R., Seki, T., Fujihara, T., Ishimaru, Y., Egawa, Y.: Single-step preparation of topological gels using vinyl-modified β-cyclodextrin as a figure-of-six cross-linker. J. Incl. Phenom. Macrocycl. Chem. 92, 311–317 (2018).