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Effects of conditions for preparing nanoparticles composed of aminoethylcarbamoyl-β-cyclodextrin and ethylene glycol diglycidyl ether on trap efficiency of a guest molecule

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Abstract

Nanoparticles comprising copolymers of aminoethylcarbamoyl-β-cyclodextrin (AEC-β-CD) and ethylene glycol diglycidyl ether (EGDGE) are prepared by an interfacial polyaddition reaction in a miniemulsion system. Polymers are formed in a W/O emulsion containing 0.25–10.0% (w/w) water and 5.0% (w/w) surfactant (MO-3S, tetraglycerin monoester, HLB 8.8), where simple particles are predominantly obtained when the water content is 1.0% and 5.0%. Notably, nano-size small particles (diameter: 0.3 μm) are formed under the condition of 5.0% water and 5.0% surfactant, which have the highest β-CD contents (75.5 wt.%) and the most positive ζ-potential (53.6 mV). The ζ-potential measurement indicates that the obtained particles have positive charge due to protonation of their amino groups below around pH 10. Actually, uptake of 8-anilino-1-naphthalenesulfonic acid (ANS) bearing negative charge (SO_3^-) and moderate hydrophobicity depends on the magnitude of ζ-potential of the particles; viz., the particles with ζ-potential of 53.6 mV show the highest efficiency of uptake. The diameter and the β-CD contents are closely related with the water/surfactant ratio, and the ζ-potentials are dependent on both the diameter and the β-CD contents. Inclusion of ANS into the CD cavity of EGDGE/AEC-β-CD particles can be controlled by electrostatic interaction between ANS (negatively charged) and the particle (positively charged). Namely, synergistic effect of cavity-inclusion and electrostatic interaction can dominate the uptake of guest molecules by the particles.
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Keywords: Cyclodextrin; Nanoparticle; Interfacial polyaddition reaction

1. Introduction

Biomaterials are developing in medical, dental and diagnosis fields. Especially, polymer particles were prepared for virus separation (Akashi et al., 1998), protein separation (Tomohiro et al., 2002), reactors (Demirel et al., 2004), drug carriers (Ito and Makino, 2004) and so on.

Cyclodextrins (CDs) are cyclic compounds consisting of six to eight glucose units, which are termed α-, β-, and γ-CD, respectively (Easton and Lincoln, 1999). To create functional polymer materials from CDs, cross-linking of CDs was carried out using various type of linkers; 1,2-ethanediol diglycidyl ether, 1,4-butanediol diglycidyl ether and 1,6-hexanediol diglycidyl ether (Komiyama and Hirai, 1987). Recently, microparticles based on CD were synthesized as pharmaceutical materials by cross-linking with terephthaloyl chloride (Pariot et al., 2000), epichlorohydrin (Fundueanu et al., 2004) and glutaraldehyde (Constantin et al., 2004).

The attractive property of CDs is not only inclusion complexation with guest molecules but also many hydroxyl groups of the glucose units, whose numbers are 18, 21 and 24 for α-, β-, and

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γ-CD, respectively. Modification of the hydroxyl groups of CD is expected to affect the capability of molecular recognition. In fact, methylation or hydroxyalkylation of the hydroxyl groups of CDs improved their solubility and stabilized their inclusion complexes with guest molecules (Uekama et al., 1998). Ooya et al. introduced carboxyl groups (Ooya et al., 2002) and amino groups (Ooya et al., 2004) via the hydroxyl groups of α-CD to functionalize polyrotaxane.

By combining these two technologies, namely, cross-linking and modification of hydroxyl groups, we have designed a novel functional nanoparticle based on β-CD, aiming at creating a new material of nanobiotechnology. Nanoparticles have been prepared by an interfacial polyaddition between aminoethylcarbamoyl-β-CD (AEC-β-CD) and ethylene glycol diglycidyl ether (EGDGE) (Eguchi et al., 2005). In the present study, we have analyzed the detailed correlation between the preparation conditions and the properties of EGDGE/AEC-β-CD particles. Furthermore, we have evaluated the importance of synergistic effect of cavity-inclusion and electrostatic interaction by using 8-anilino-1-naphthalenesulfonic acid (ANS) and we have found that electrostatic interaction can control the degree of cavity-inclusion depending on the ζ-potential of EGDGE/AEC-β-CD particle.

2. Materials and methods

2.1. Materials

β-Cyclodextrin (β-CD), ethylenediamine, ethylene glycol diglycidyl ether (EGDGE), and 8-anilino-1-naphthalenesulfonic acid (ANS) were purchased from Wako Pure Chemical Co. Ltd. (Osaka, Japan). 1,1′-Carbonyldiimidazole was commercially available from Aldrich Co. Ltd. (St. Louis, MO, USA). MO-3S (tetraglycerin monoester, HLB 8.8) was purchased from Sakamoto Yakuhin Kogyo Co. Ltd. (Osaka, Japan). Other chemicals used were of the highest purity available.

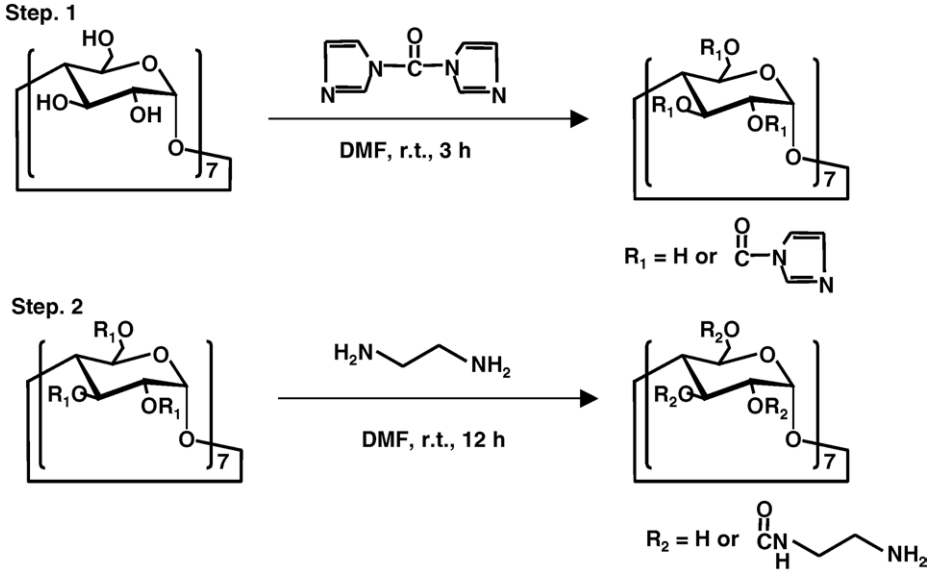
2.2. Synthesis of aminoethylcarbamoyl-β-cyclodextrin (AEC-β-CD) and preparation of EGDGE/AEC-β-CD particle via W/O emulsion

Synthetic routes of AEC-β-CD and EGDGE/AEC-β-CD particle are shown in Schemes 1 and 2. AEC-β-CD was synthesized according to the previously reported method (Eguchi et al., 2005). The resulting product was purified by GPC using a Sephadex G-15 column (18 cm × 3 cm) with water as an eluent (yield: 75.5%). The number of AEC groups in one β-CD unit was determined to be 8 by NMR measurement. ^{1}H NMR (D_2O, 500 MHz): δ 4.93 (s, 7H, H-1 of CD), 3.95–3.35 (2m, CD protons), 3.23–3.08 (m, 16H, CH_2 of AEC groups), 2.7–2.5 (m, 16H, CH_2 of AEC groups).

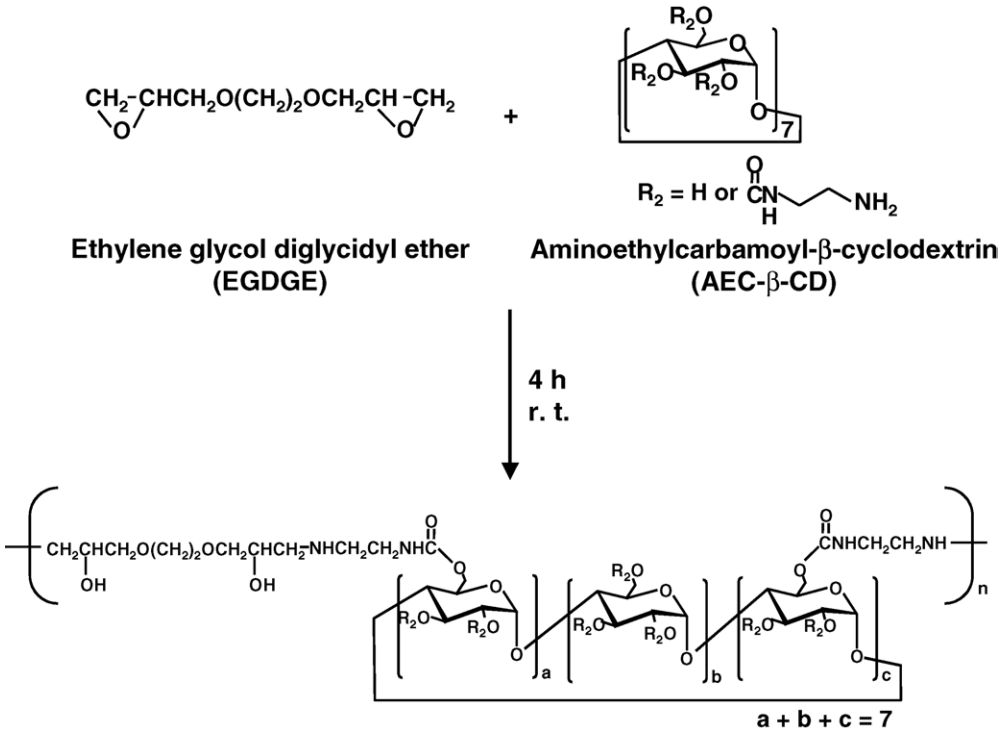
EGDGE/AEC-β-CD particles were also prepared according to the previously reported method (Eguchi et al., 2005). The recipe for preparing EGDGE/AEC-β-CD particle by a polyaddition reaction of EGDGE and AEC-β-CD is summarized in Table 1. The β-CD contents in the particle were evaluated by the anthrone–sulfonic acid assay (Trevelyan et al., 1952). Briefly, 1.0 mL of stock solution (0.16 g anthrone, 60 mL sulfonic acid, 20 mL DDI water) was mixed with 0.2 mL of sample solution. After heating at 90 °C for 10 min, the absorbance of the solution was measured at 620 nm by a UV–vis spectrophotometer (V-550, JASCO, Japan). A calibration curve was prepared by using β-CD solution.

2.3. Characterization

The EGDGE/AEC-β-CD particles were observed by a scanning electron microscope (SEM, S-4500 Hitachi, Japan). The IR spectrum (KBr method) was measured on a Fourier transform infrared spectrometer (FT/IR-7300, JASCO, Japan). The ζ-potential was measured by a zeta potential analyzer (ZEECOM®, MICROTEC Co. Ltd., Japan).



Scheme 1. Synthesis of AEC-β-CD.



Scheme 2. Preparation of EGDGE/AEC-β-CD particle (one of possible polymer structures is shown).

2.4. Uptake of ANS by EGDGE/AEC-β-CD particle

Solution (1.0 mL) of ANS (0.1 mM) and AEC-β-CD particles was mixed in a 1.5 mL tube for 30 s. After centrifugation, the concentration of ANS in the supernatant solution was determined from the absorbance at 400 nm by a UV–vis spectrophotometer (V-550, JASCO, Japan).

2.5. Measurement of circular dichroism spectrum

The circular dichroism spectrum was recorded by a J-720 WI spectropolarimeter (JASCO, Japan) at room temperature with a 1.0 cm path-length quartz cell. The sample solution was prepared by mixing 0.5 mL of ANS (0.2 mM in pH 7.4 phosphate buffer) and 0.5 mL of AEC-β-CD (2.0 mM in pH 7.4 phosphate buffer).

3. Results and discussion

3.1. Synthesis of aminoethylcarbamoyl-β-CD (AEC-β-CD)

From the ^{1}H NMR spectrum, the obtained compound was confirmed to be AEC-β-CD. Water solubility of AEC-β-CD was higher than unmodified β-CD, suggesting that hydrogen bonding between β-CD molecules was inhibited by the chemical modification. This result is well consistent with other studies about modification of CD (Uekama et al., 1998). The FT-IR

Table 1
Recipe for preparation of EGDGE/AEC-β-CD particle in W/O emulsion

Run no.	Water contents (wt.%)	Surfactant contents (wt.%)	Diameter[a] (μm)	C.V.[b] (%)	β-CD contents[c] (wt.%)	ζ-Potential[d] (mV)
1	0.10	5.0	–	–	–	–
2	0.25	5.0	17.5	42.9	38.7 ± 6.3	23.6
3	0.5	5.0	31.0	70.4	53.4 ± 2.1	20.1
4	1.0	5.0	1.8	84.5	51.6 ± 5.1	35.7
5	5.0	5.0	0.3	51.5	75.5 ± 6.7	53.6
6	10.0	5.0	Gel like	–	29.5 ± 4.3	33.3
7	20.0	5.0	–	–	–	
8	40.0	5.0	–	–	–	
9	5.0	1.0	Fiber like	–	–	26.4
10	5.0	10.0	2.0	37.7	66.5 ± 2.6	31.5
11	5.0	20.0	26.5	43.8	55.6 ± 3.5	27.3

[a] Diameters were determined from SEM photograph.
[b] Coefficient value % = standard deviation/average diameter × 100.
[c] β-CD contents were evaluated by the anthrone–sulfonic acid assay.
[d] ζ-Potentials were measured in distilled and deionized water.

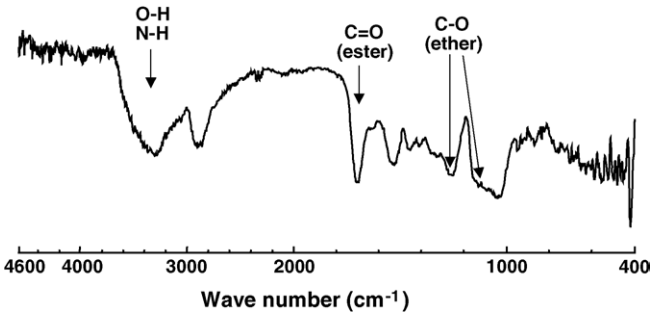


Fig. 1. IR spectrum of EGDGE/AEC-β-CD particle formed in run 5.

spectrum of the particle of run 5 (Fig. 1) suggests the existence of hydroxyl, amino, ester and ether groups in the particle.

3.2. Influence of reaction parameters on properties of polymer particles

By reference to the results of Landfester et al. (2000), the ratio of epoxy groups to amino groups was chosen to be 2:1. The preparation conditions and the properties of polymer particles are listed in Table 1, and the SEM images of the products are shown in Figs. 2 and 3. The water contents were changed from 0.1% to 40.0% with the surfactant contents being constant

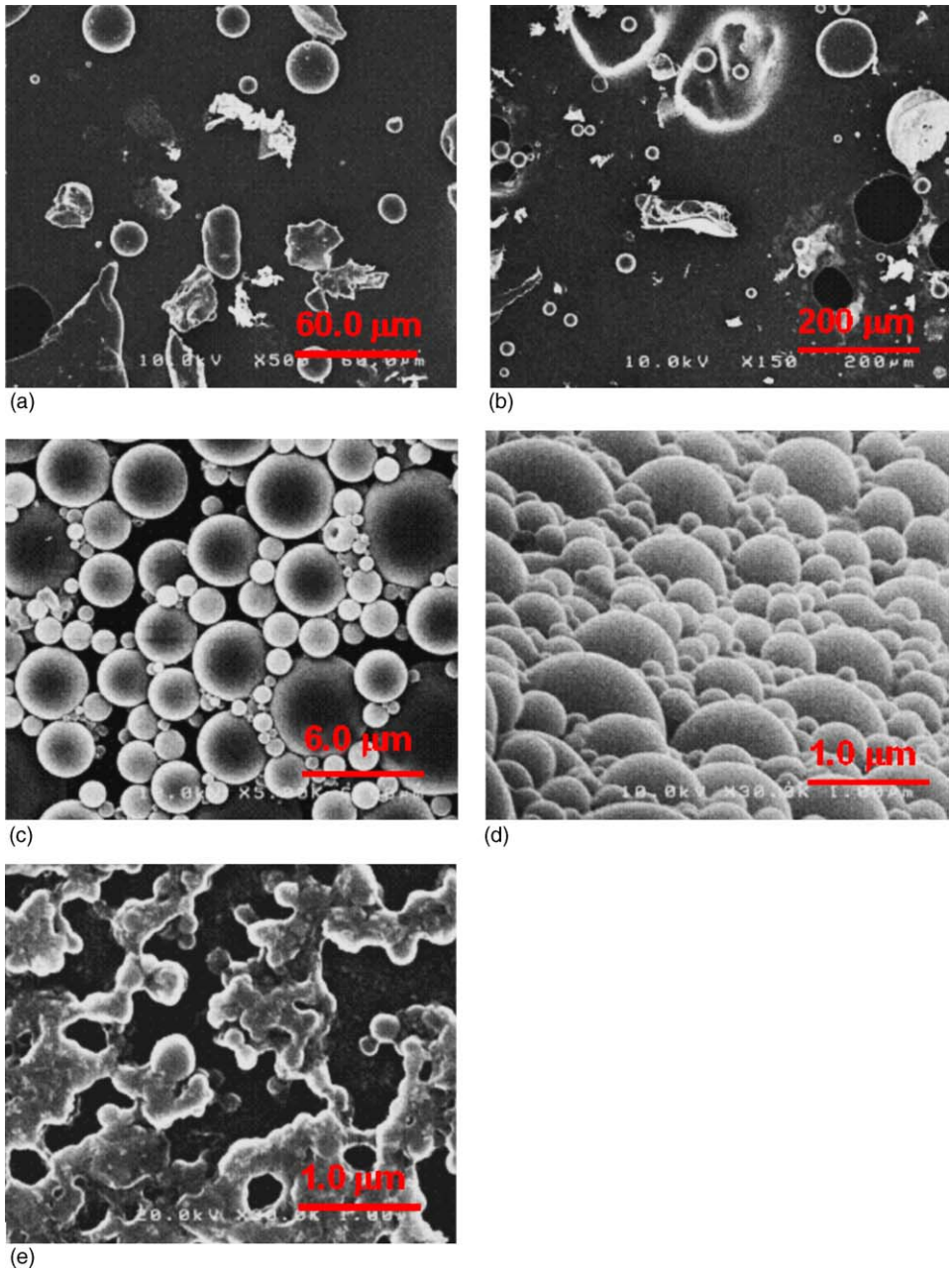


Fig. 2. SEM photographs of EGDGE/AEC-β-CD particles. Water contents: (a) 0.25%, (b) 0.5%, (c) 1.0%, (d) 5.0% and (e) 10.0%.

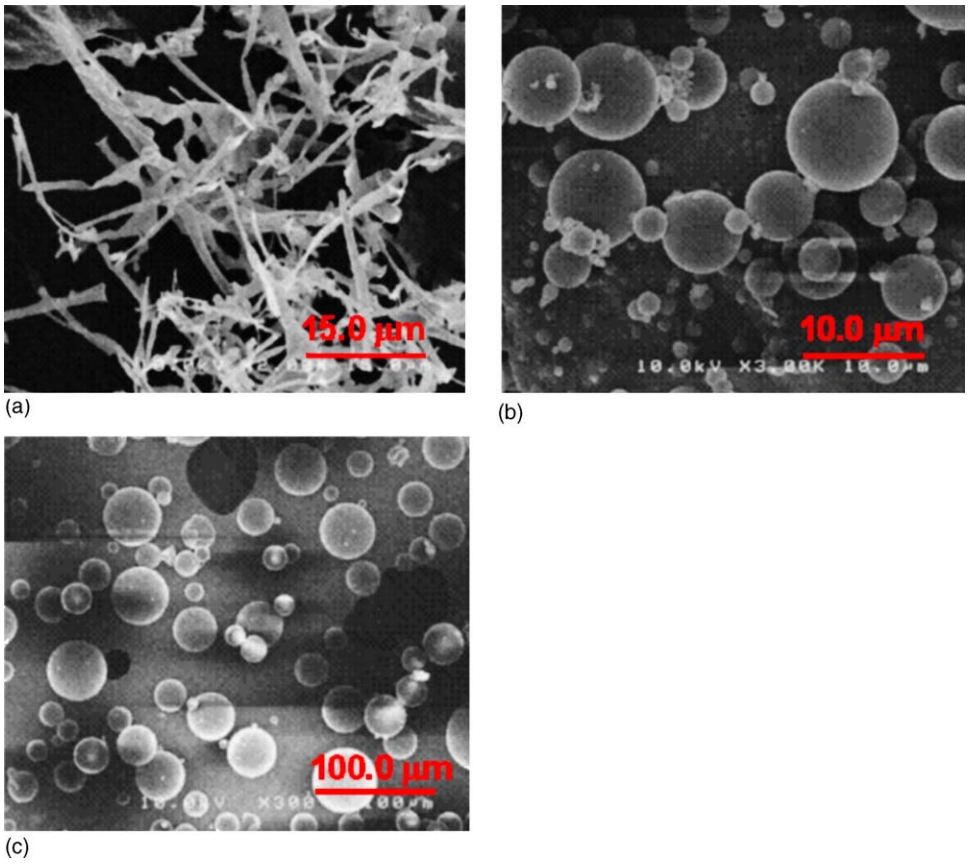


Fig. 3. SEM photographs of EGDGE/AEC-β-CD particle. Surfactant contents: (a) 1.0%, (b) 10.0% and (c) 20.0%.

at 5.0% (runs 1–8 in Table 1). Polymers were not formed below 0.25% and above 10% water contents. Simple particles were abundantly formed at 1.0% and 5.0% water contents (Fig. 2(c) and (d)), while gel-like polymers were produced at 10% water contents (Fig. 2(e)). The average diameter of EGDGE/AEC-β-CD particle was the smallest at 5.0% water contents (0.3 μm, Fig. 2(d)). Runs 2 and 3 (0.25% and 0.5% water contents) did not give simple spherical particles (Fig. 2(a) and (b)). The product in run 6 appears gel-like polymer holding a lot of water (Fig. 2(e)). As shown in runs 9–11, surfactant (MO-3S) contents were changed from 1.0% to 20.0% under the constant water contents (5.0%), where polymer particles were formed in runs 10 and 11 (Fig. 3(b) and (c)). The diameter of EGDGE/AEC-β-CD particle was increased from 0.3 to 26.5 μm as the surfactant contents were increased from 5.0% to 20.0% (runs 5, 10 and 11). Fiber-like polymer (Fig. 3(a)) was formed at very low surfactant contents (1.0%, run 9), which might be correlated to the observation that MO-3S forms tubule-like micelles at ca. 1.0% concentration (data not shown). The β-CD contents evaluated by the anthrone–sulfonic acid assay were located in the range between 29.5 and 75.5 wt.%. The ζ-potential measurements showed that all the EGDGE/ACE-β-CD particles had positive charge and run 5 gave the highest value (53.6 mV). The β-CD contents tend to increase as the water/surfactant ratio is increased (Fig. 4). Concerning the average diameter, the dependence on the water/surfactant ratio is not monotonous; that is, there exists the maximum in the average diameter (Fig. 5).

3.3. pH-dependence of ζ-potential

The ζ-potential of EGDGE/AEC-β-CD particle of run 5 was measured in the wide pH range (Fig. 6). The ζ-potential was shifted from 43 to −5 mV with increasing pH from 2.3 to 11.3. The positive ζ-potential in the pH range between 2 and 10 means that EGDGE/AEC-β-CD particle is positively charged, which is compatible with the FT-IR spectrum (Fig. 1) showing that

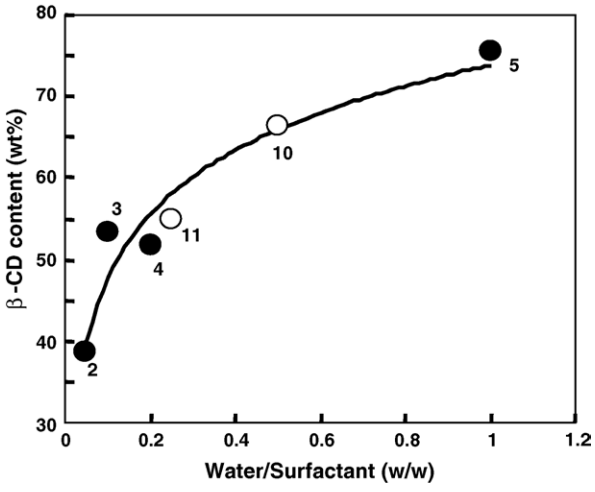


Fig. 4. Relationship between β-CD content and water/surfactant ratio. The numbers represent the runs in Table 1.

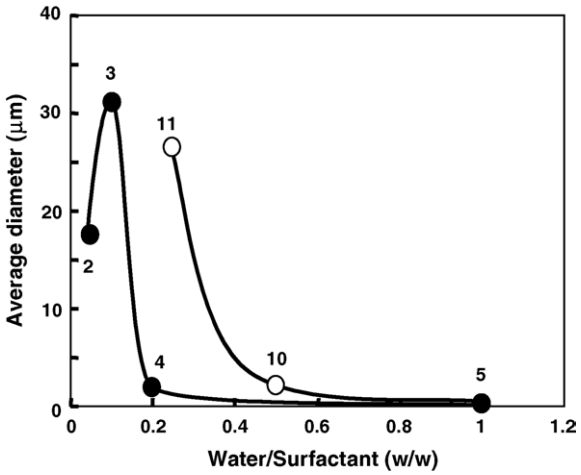


Fig. 5. Relationship between average diameter and water/surfactant ratio. The numbers represent the runs in Table 1.

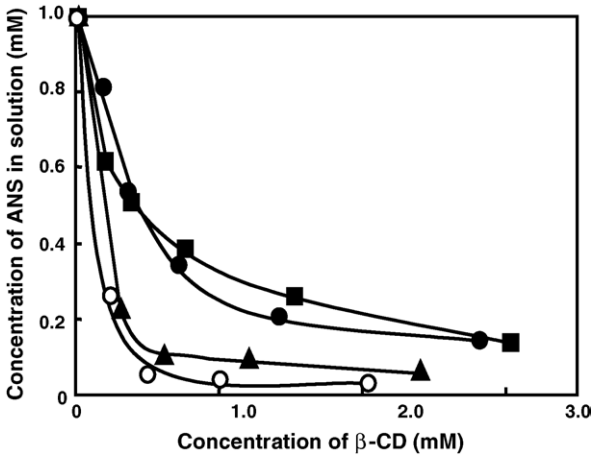


Fig. 7. Relation between concentration of unbound free ANS and total concentration of β-CD units in various particles (phosphate buffer, pH 7.4); (●) run 2, (■) run 3, (▲) run 4, and (◯) run 5; [ANS] = 0.1 mM.

the particle has unreacted primary amino groups and secondary amino groups (Scheme 2). The negative ζ-potential at pH 11.3 suggests that the hydroxyl groups in this particle (Scheme 2) were ionized at this pH. The pK_a of the amino groups is equal to pH at which half of the amino groups are positively charged. As show in Fig. 6, the pK_a of the amino groups is approximately estimated to be 8, since the ζ-potential becomes half (15 mV) at pH 8 compared to the value (30 mV) at acidic pH.

3.4. Uptake of ANS by EGDGE/AEC-β-CD particle

Previously, we confirmed that ANS was included in the cavity of EGDGE/AEC-β-CD particle by the competitive inhibition experiment using 1-adamantane carboxylic acid (Eguchi et al., 2005). Namely, ANS was excluded from the CD cavity of EGDGE/AEC-β-CD particle in the presence of excess 1-adamantane carboxylic acid. Contrary to EGDGE/AEC-β-CD particle, uptake of ANS was negligibly small in the presence of EGDGE/hexamethylenediamine (HMDA) particle containing HMDA instead of AEC-β-CD (Eguchi et al., 2005), since there is no host cavity capable of including ANS in the EGDGE/hexamethylenediamine (HMDA) particle. This shows that inclusion in CD cavity greatly contributes to the uptake of ANS in the particle. Figs. 7 and 8 illustrate the dependence of the ANS uptake by various EGDGE/AEC-β-CD particles prepared in different conditions (Table 1) on the concentration of β-CD unit. In run 5 ANS is efficiently trapped by EGDGE/AEC-β-CD particles even when the concentration of β-CD unit is low, while in run 3 the trapping ability is smaller. These results clearly indicate that the ζ-potential influences the uptake of ANS. In fact, the uptake efficiency obviously depends on the ζ-potential as shown in Fig. 9 ([β-CD unit] = 1.0 mM). The tendency that ANS is more trapped as ζ-potential is increased suggests that electrostatic attraction is one of driving forces in the uptake of ANS.

Fig. 10 shows the induced circular dichrosim (ICD) spectrum of ANS in the presence of AEC-β-CD molecule. The ICD spectrum exhibits a positive Cotton effect at around 350 nm,

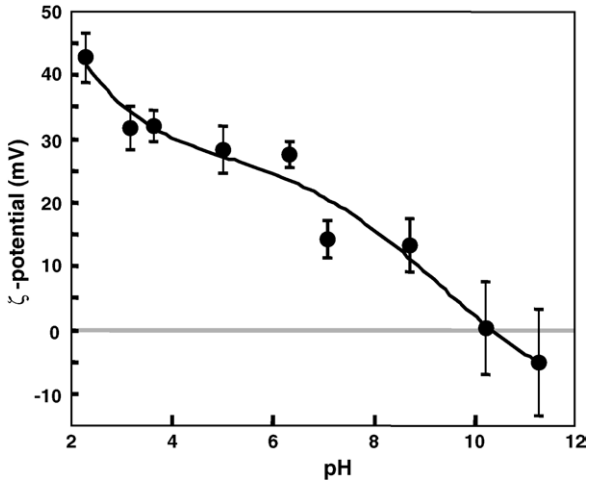


Fig. 6. Relation between ζ-potential and pH for EGDGE/AEC-β-CD particles (run 5).

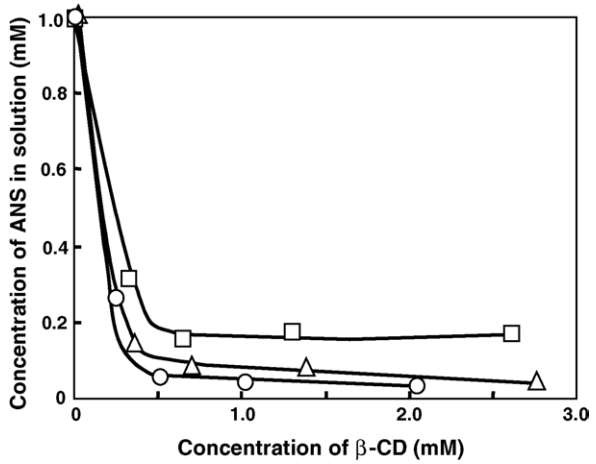


Fig. 8. Relation between concentration of unbound free ANS and total concentration of β-CD units in various particles (phosphate buffer, pH 7.4); (◯) run 5, (△) run 10, and (□) run 11; [ANS] = 0.1 mM.

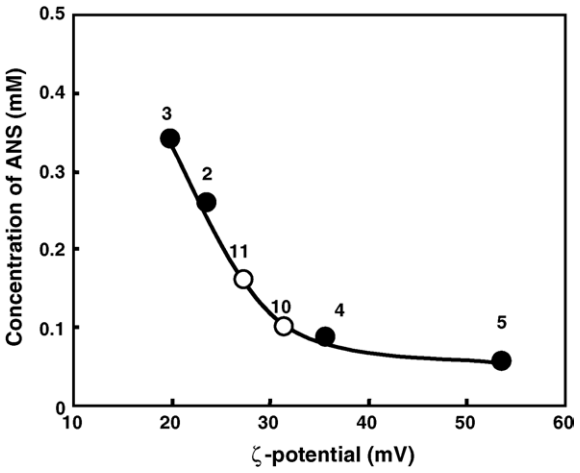


Fig. 9. Relationship between concentration of unbound free ANS and ζ-potential under constant concentration (1.0 mM) of β-CD units in particles. The numbers represent the runs in Table 1.

which corresponds to the absorption band (λ_{max} = 350 nm) of ANS. According to Kodaka's rule about circular dichroism induced by interaction between an achiral guest molecule and a chiral cyclic host molecule (Kodaka, 1991, 1993, 1998), ANS should be included in the cavity of AEC-β-CD. This conclusion is well compatible with the experimental results. Concerning unmodified β-CD, inclusion of ANS in β-CD cavity was proved by NMR measurement (Nishijo et al., 1992). In the previous study, we reported that fluorescence of ANS was greatly enhanced in the presence of EGDGE/AEC-β-CD particles and that the fluorescence was decreased by adding excess adamantane carboxylic acid (Eguchi et al., 2005). It is generally known that fluorescence of ANS is enhanced in a hydrophobic environment and that adamantane carboxylic acid is easily included in CD cavity. All these results suggest that ANS is included in the hydrophobic cavity of the β-CD unit in EGDGE/AEC-β-CD particle. It should be noted furthermore that electrostatic interaction could control the degree of cavity-inclusion depending on the ζ-potential of EGDGE/AEC-β-CD particle.

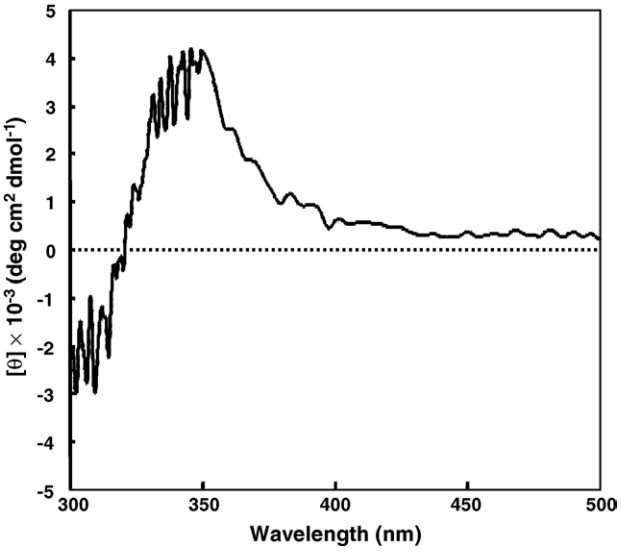


Fig. 10. CD spectrum of ANS (0.1 mM) in the presence of AEC-β-CD (1.0 mM) in phosphate buffer (pH 7.4).

4. Conclusion

We succeeded in preparing the new functional polymer particles based on AEC-β-CD by an interfacial polyaddition reaction of EGDGE and AEC-β-CD in the W/O miniemulsion system. The most effective particle for trapping hydrophobic and anionic compounds was obtained when the water content was 5.0% and the surfactant content was 5.0%. Actually, this particle had the highest ζ-potential, the highest β-CD content, and the smallest diameter. It should be noted that synergistic effect of cavity-inclusion and electrostatic interaction resulted in higher trapping efficiency than cavity-inclusion alone or electrostatic interaction alone. This particle would be useful for a variety of affinity-based molecular selection and extraction. Furthermore, addition of various functional groups to EGDGE/AEC-β-CD particle would lead to wide application of this new material in nanobiotechnology.

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