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RAPID COMMUNICATION

Effect of ion exchange resin on increased surface area crystallization process for purification of vancomycin

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Abstract−We investigated the effects of ion exchange resins on the efficiency of crystallization with an increased surface area for the purification of vancomycin. As surface area-increasing materials, diverse types of ion exchange resin were used to increase the surface area per working volume (S/V). When the S/V was increased, in cation exchange resin Amberlite 200, Amberlite IR 120 (Na), and Amberlite IRC 50 and in anion exchange resin Amberlite IRA 400 (Cl) and Amberlite IRA 910 (Cl), vancomycin crystals were successfully generated. The yield of vancomycin increased (>97%), and the time necessary for crystallization was reduced dramatically (reduced from 24 hr to 12 hr). On the other hand, the purity of the vancomycin was approximately 95% and was not affected by increasing S/V. Use of an ion exchange resin also resulted in the production of smaller vancomycin crystals than in the absence of ion exchange resin.

Key words: Vancomycin, Crystallization, Purification, Surface Area per Working Volume (S/V), Ion Exchange Resin

INTRODUCTION

As the first glycopeptide antibiotic, vancomycin was discovered from *Amycolatopsis orientalis* (*Streptomyces orientalis*, *Nocardia orientalis*) separated from the soil of the Borneo area in 1956 by Eli Lilly [1,2]. Vancomycin inhibits cell wall synthesis in Gram positive bacteria, thereby causing cell death. It is widely used to treat methicillin resistant *Staphylococcus aureus* (MRSA) infection and endocarditis in patients who are allergic to penicillin and cephalosporin. In addition, vancomycin is the first therapeutic agent for MRSA infection to be widely used for preventive treatment during cardiac surgery involving an artificial implant, orthopedic surgery, and neurosurgery for the placement of a ventriculoperitoneal shunt [3].

The purification of vancomycin obtained from microbial fermentation requires several steps. For vancomycin now recorded in the United States and European pharmacopeia, the vancomycin content and the amount of total and individual impurities are strictly regulated. Using HPLC analysis suggested by the United States Pharmacopeia (USP), the vancomycin content must be greater than 88% and, among other materials that may be present, none may have a content exceeding 4% [4]. According to the European Pharmacopeia, the vancomycin content must be greater than 93% and the presence of any other material with a content exceeding 4% is restricted in the same manner as in the USP. Consequently, to satisfy such strict regulations, separation and purification processes in many steps are necessary. Generally, the crystallization process is often used as the final purification step in the process of producing high-purity drug such as antibiotics. Crystallization, which is the process of precipitating and producing a compound from a liquid or gas mixture [5,6], corresponds to a core technology for the isolation and purification of a material as well as the control of its physical properties and morphology. The minimum crystal particles initially formed from the solution are called nuclei, and nucleation is classified into homogeneous nucleation, where nuclei are generated in the liquid state due to supersaturation, and heterogeneous nucleation, where nuclei are generated with the assistance of external surfaces (external impurity particles, reactor walls, agitators, etc.) [7]. Crystallization not only improves the quality of final products but also produces high value-added products. It is a simple, energy efficient and environmentally friendly process that is widely applicable and has a low fixed investment cost [8]. In our previous study [9], most of the major process parameters (temperature, pH, conductivity, agitation, and initial vancomycin concentration) for the crystallization of vancomycin were optimized to obtain a highly pure (>97%) product with high yield (>95%). However, crystal formation required a long period of time (~24 hr), resulting in low productivity in the mass-production process. Improved crystallization processes were reported in 2011 [10] in which efficiency was enhanced by increasing the surface area per working volume (i.e., volume of reaction solution) (S/V) using glass beads or ion exchange resin (two types of ion exchange resins: Amberlite 200 and Amberlite IRA 400). However, not only are surface area-increasing materials very limited, but information on their effect on crystallization efficiency is also very inadequate. In this study, therefore, we attempted to systematically investigate the effects of various ion exchange resins on the efficiency and behavior (purity, yield, crystallization time, and shapes and sizes of the vancomycin crystals) of crystallization with an increased surface area for more wide application.

MATERIALS AND METHODS

1. Preparation of Vancomycin Sample

Vancomycin used in this experiment was obtained through the fermentation of the microorganism *Norcardia orientalis* isolated from soil. Bacterial cells were removed from the fermentation solution containing vancomycin, which was then purified [10,11]. The solution was consecutively passed through cation exchange, anion ex-

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change and porous cation exchange resins and eluted with ammonia to obtain 88% pure vancomycin in the form of hydrochlorate. Impurities such as pigment and protein were removed using alumina and a weak acidic cation exchange resin. The resulting product was used for the crystallization process.

2. Vancomycin Analysis

An HPLC system (SCL-10AVP, Shimadzu, Japan) and Candenza CW-C18 column (4.6×100 mm, 3 μm, Imtakt, Japan) were used for analysis of vancomycin at 260 nm using a UV detector. Mobile phase A was prepared by mixing 1,000 mL of distilled water and 1 mL of formic acid. Mobile phase B was prepared by mixing 1,000 mL of acetonitrile and 1 mL of formic acid. The analysis was performed in gradient and isocratic mode for the 20 min. Elution was performed in a gradient using mobile phase A and mobile phase B mixture varying from 95 : 5 to 30 : 70 for the first 10 min. After then, the mixture of mobile phase A and mobile phase B (95 : 5) was isocratic from 10 min to 20 min. The flow rate was 0.8 mL/min and the injection volume was 20 μL. The concentration was calculated using the peak area acquired with the standard materials. Each sample was analyzed in triplicate.

3. Crystallization Method

Fig. 1 shows a schematic diagram of the crystallization with an increased surface area using a wider variety of ion exchange resins for the purification of vancomycin. The reactor size and experimental volume are 90 mL and 13.5 mL, respectively. First, samples (vancomycin purity: 88%) were dissolved in distilled water whose pH had been adjusted with 1 N hydrochloric acid to be 2.5 and whose conductivity had been adjusted with sodium chloride to be 20 ms/cm. Because sodium chloride does not affect H^+, the pH was adjusted first. Acetone, an organic solvent, was slowly dropped on the vancomycin solution, drop by drop and under agitation. In addition, at the storage temperature of 10 °C, the surface area increase effect was explored while changing the crystallization time (6 hr, 12 hr, 18 hr, and 24 hr). To increase the S/V, ion exchange resin was used. Four types of cation exchange resin (Amberlite 200, Amberlite IR 120 (Na), Amberlite IR 120 (H), Amberlite IRC 50, Rohm and Haas, USA) and five types of anion exchange resin (Amberlite IRA 400 (Cl), Amberlite IRA 400 (OH), Amberlite IRA 910 (Cl), Amberlite IRA 67, Amberlite IRA 96, Rohm and Haas, USA) were used. The ion exchange resin used in the experiments was dried at 60 °C for one day before use. When the S/V was greater than 0.428 mm^{-1}, controlling the crystallization process was extremely difficult. Therefore, the experiment was performed using an S/V of 0.428 mm^{-1}. The S/V was calculated for each ion exchange resin as follows:

$$S/V[mm^{-1}]=[\text{total surface area of resin } (mm^2)/\text{working volume } (mm^3)] \quad (1)$$

After crystallization, the parent solution including the solvent adhered to crystal surface, so it was removed. The vancomycin was then washed with acetone in order to obtain a clear, final crystal product. Impurities were removed from crystal surfaces by filtration through filter paper (150 mm, Whatman), and then the filtrate was dried under vacuum at 35 °C for 24 hr and analyzed by HPLC. Each experiment was performed in triplicate.

The vancomycin crystal was visualized during the crystallization process with an SV-35 Video Microscope System (Some Tech, Korea) at high magnification (×500). The size and shape of vancomycin crystals in dynamic images was verified with IT-Plus software (Some Tech, Korea).

4. XRD Analysis

The morphology of vancomycin was analyzed by X-ray diffractometer (SMD 3000, SCINCO, Italy). The measurements of XRD were performed in the 10 to 80° 2θ range at a rate of 2° 2θ/min using CuKα radiation (40 kV, 40 mA) as X-ray source. The amount of each sample was about 50 mg.

RESULTS AND DISCUSSION

1. Effect of Increasing S/V of the Reacting Solution Using a Wider Variety of Ion Exchange Resins

In the conventional crystallization process, to obtain high-purity (>97%) and high-yield (>95%) vancomycin crystals, crystallization required 24 hr or more [9]. In this study, long crystallization time, which is the greatest disadvantage in the conventional crystallization process, was reduced to improve crystallization efficiency. Most of the crystallization occurs around the nucleus that is created with the help of the surface area (e.g., particulate impurities, reactor wall, and agitator surface) [7,12,13]. By taking advantage of this phenomenon, we intended to improve crystallization efficiency (purity and yield of vancomycin, crystallization time) by increasing S/V so as to increase the space for formation and growth of vancomycin crystals. As surface area-increasing materials, four most common cation exchange resins (Amberlite 200, Amberlite IR 120 (Na),

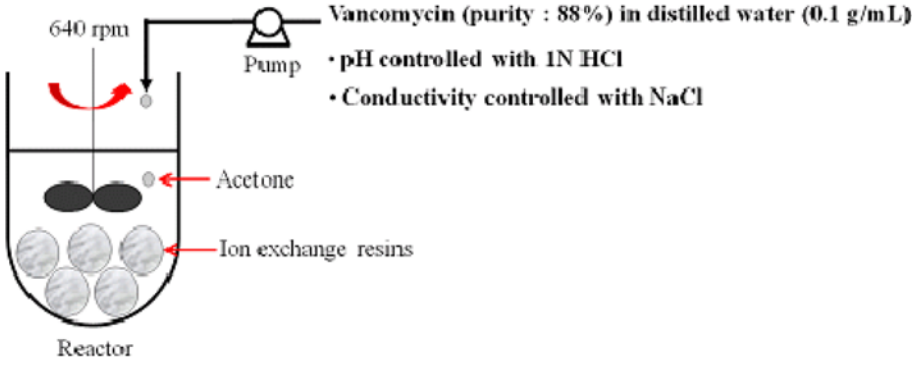

Fig. 1. Schematic diagram of crystallization with an increased surface area using a wider variety of ion exchange resins for the purification of vancomycin.

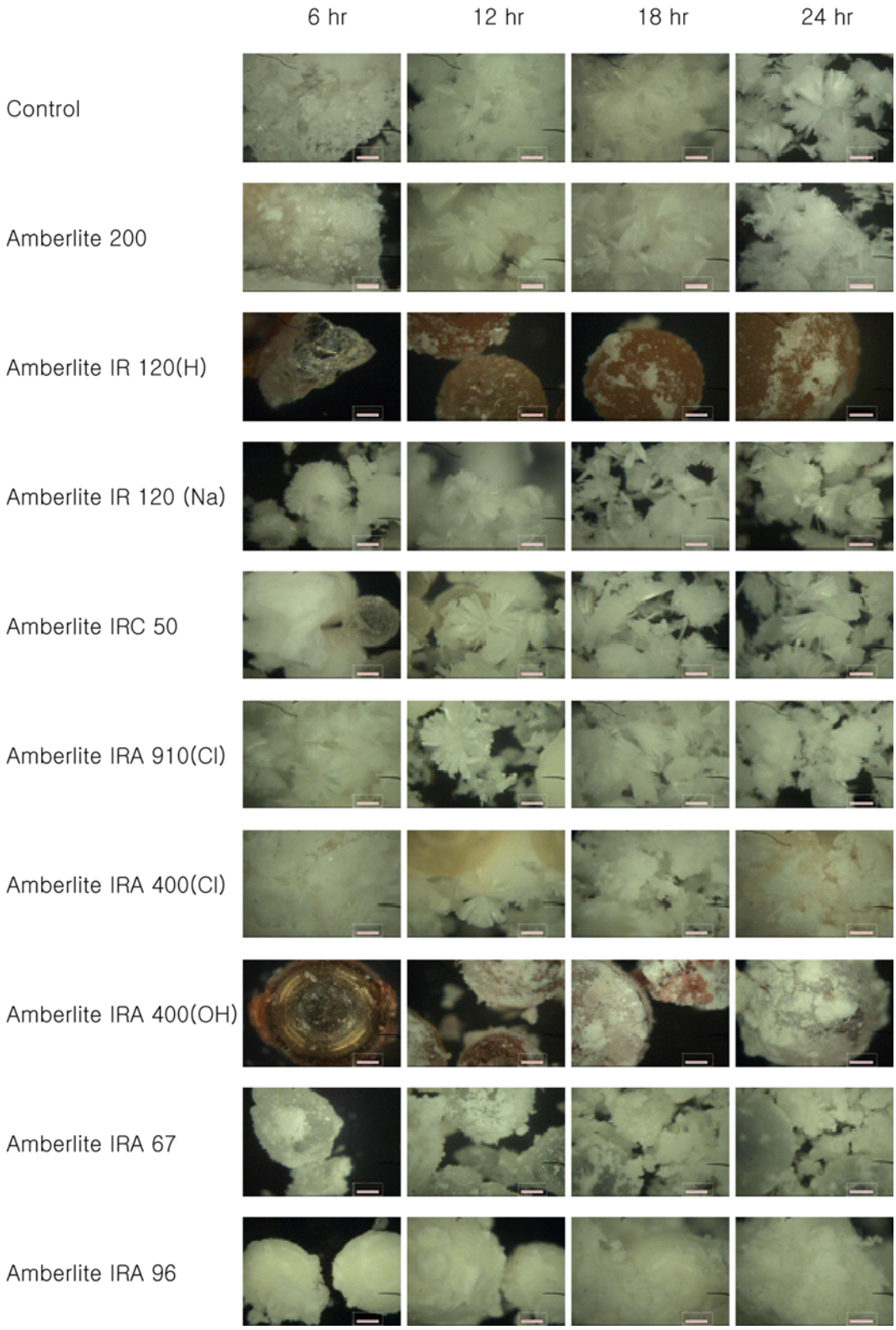

Fig. 2. Video microscope images of vancomycin crystals obtained at various ion exchange resins and crystallization times. Scale bar indicates 10 μm.

Amberlite IR 120 (H), Amberlite IRC 50) and five most common anion exchange resins (Amberlite IRA 400 (Cl), Amberlite IRA 400 (OH), Amberlite IRA 910 (Cl), Amberlite IRA 67, Amberlite IRA 96) were used to increase the S/V to 0.428 mm^{-1}. The morphology of vancomycin crystals obtained from the crystallization process was observed via video microscopy (Fig. 2). It was possible successfully to obtain vancomycin crystals from Amberlite 200, Amberlite IR 120 (Na), Amberlite IRC 50, Amberlite IRA 910 (Cl),

and Amberlite IRA 400 (Cl). On the other hand, vancomycin crystals could not be obtained from Amberlite IR 120 (H), Amberlite IRA 400 (OH), Amberlite IRA 67, and Amberlite IRA 96. These findings may be due to pH changes by the addition of ion exchange resins. Generally, the pH for crystallization of vancomycin is known as the pH range 2-7 [9]. However, with addition of these ion exchange resins (Amberlite IR 120 (H), Amberlite IRA 400 (OH), Amberlite IRA 67, and Amberlite IRA 96), the pH of the solution

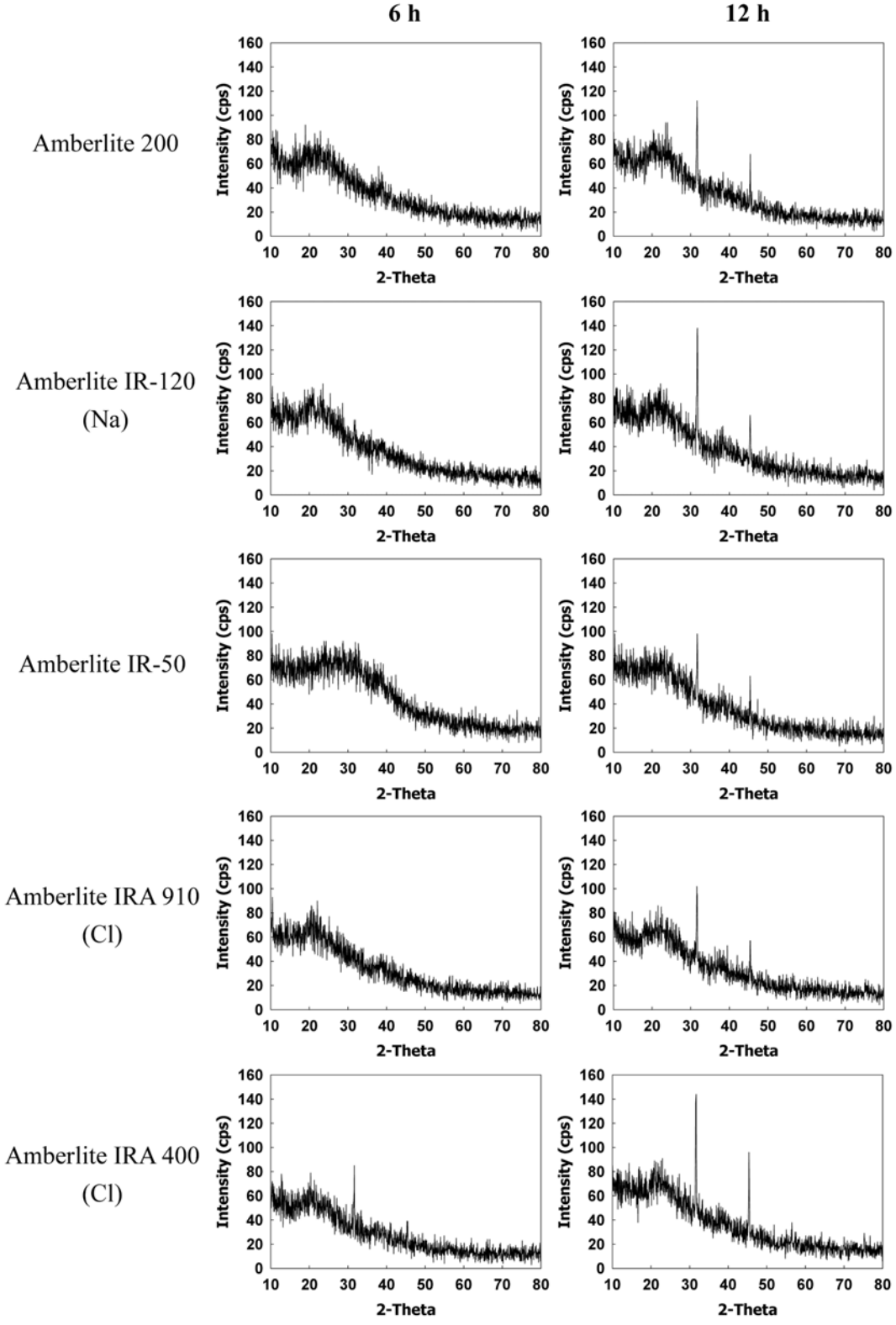

Fig. 3. XRD patterns of vancomycin crystals obtained from Amberlite 200, Amberlite IR 120 (Na), Amberlite IRC 50, Amberlite IRA 910 (Cl), and Amberlite IRA 400 (Cl) after 6 hr and 12 hr of crystallization.

out of this range was found (data not shown). When the S/V was increased using the five types of ion exchange resin (Amberlite 200, Amberlite IR 120 (Na), Amberlite IRC 50, Amberlite IRA 400 (Cl), and Amberlite IRA 910 (Cl)) where vancomycin crystals had been formed. These crystals were stably generated at 12 hr of crystallization (Fig. 2). To confirm the morphology of crystals obtained through the crystallization process with the surface area increased by Amberlite 200, Amberlite IR 120 (Na), Amberlite IRC 50, Amberlite IRA 910 (Cl), and Amberlite IRA 400 (Cl), XRD analysis was also conducted and compared with the XRD peaks of vancomycin in the previous study [9]. Vancomycin crystals were recovered at 6 hr, before crystal formation, and at 12 hr, after crystal formation, vacuum dried for 24 hr at 35 °C, and subjected to XRD analysis (Fig. 3). In the XRD patterns after 12 hr of crystallization, we could see meaningful peaks at 31.7° and 45.4° two theta degrees. On the other hand, we could see no meaningful peaks in the XRD patterns after 6 hr of crystallization. Therefore, vancomycin after 12 hr of crystallization was in a crystalline form. These results were consistent with those in the microscopic analysis (Fig. 2). With conventional crystallization methods, where the surface area had not been increased, crystals were generated at 24 hr of crystallization (Fig. 2, Control). Consequently, by increasing the S/V within crystallization devices, it was possible to reduce the time necessary for crystallization dramatically.

Using cation exchange resin (Amberlite 200, Amberlite IR 120 (Na), and Amberlite IRC 50) and anion exchange resin (Amberlite IRA 400 (Cl) and Amberlite IRA 910 (Cl)), where crystal formation had been successful, the S/V was increased to 0.428 mm^{-1}, and the yield and purity of vancomycin according to the crystallization time (6 hr, 12 hr, 18 hr, and 24 hr) were observed (Fig. 4). When the S/V was increased, the vancomycin yield generally increased in comparison with the case where the surface area had not been increased (control). In the cases of Amberlite IRA 910 (Cl), whose yield was higher than that of the control at 24 hr of crystallization. With the exception of Amberlite IRC 50, vancomycin purity remained approximately 96% regardless of increase in the surface area, thus showing that increase in the S/V hardly affected vancomycin purity. Different types of ion exchange resins gave different crystallization times, yields, and purities in the above results, which implied that these are not caused only from effect of surface area change, and might involve like ionic interactions between ion exchanger and cores of crystal [10].

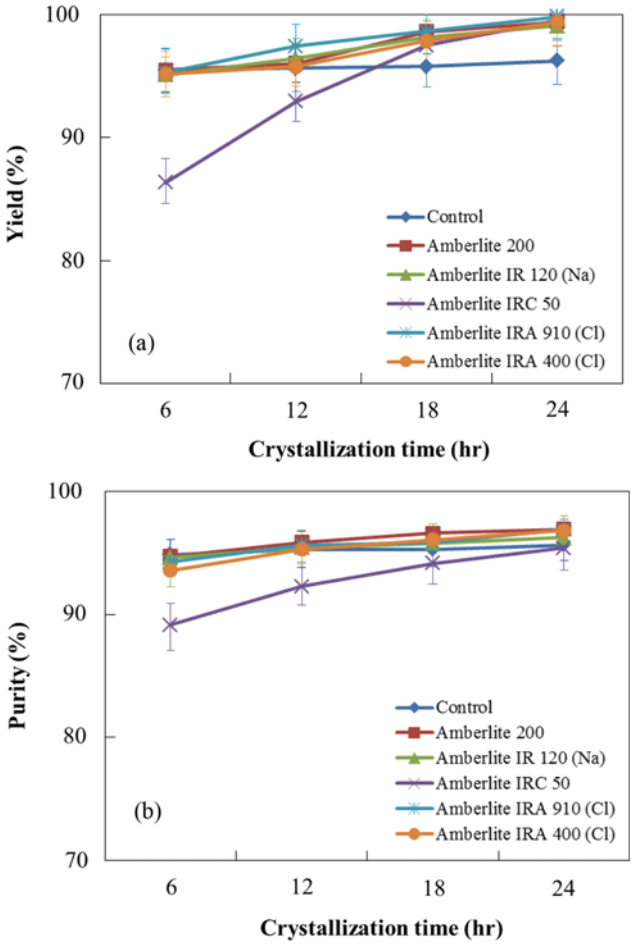

Fig. 4. Effect of ion exchange resin used to increase surface area per working volume (S/V: 0.428 mm^{-1}) on the yield (a) and purity (b) of vancomycin during crystallization.

2. Change in the Sizes of Vancomycin Crystals through the Crystallization Time

To find the effect of surface area-increasing materials on the sizes of vancomycin crystals, the sizes of vancomycin crystals were meas-

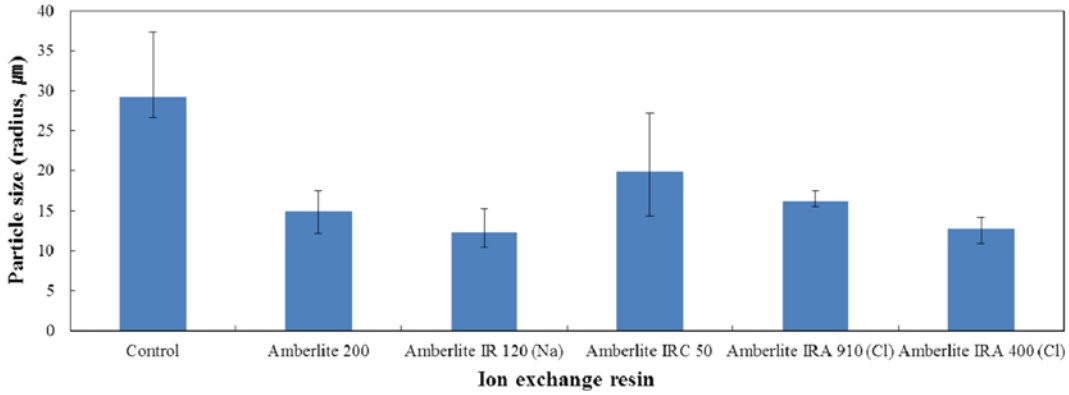

Fig. 5. Effect of ion exchange resin used to increase surface area per working volume (S/V: 0.428 mm^{-1}) on the size of vancomycin crystal at the time of crystal formation. The time of crystal formation in the absence and presence of an ion exchange resin was 24 hr and 12 hr, respectively.

ured at the time of crystal formation (12 hr) with electron microscopy. In the case of the control, the size of vancomycin crystal was measured at 24 hr, the time of crystal formation. When crystallized with the addition of ion exchange resin, a surface area-increasing material, it was possible to obtain vancomycin crystals smaller than in the case of the control (Fig. 5). Also, crystal size differed depending on the type of surface area-increasing agent used. This is likely due to the difference in the affinity between the surface area-increasing agent and vancomycin particles. As the affinity between surface area-increasing materials and vancomycin particles increases, it serves as a more effective steric barrier, which inhibits the growth of vancomycin crystals [14-16]. In particular, in the case of cation exchange resin Amberlite IR 120 (Na), it was possible to obtain vancomycin particles that were smaller than those for the control by 2-3 times. In the case of active pharmaceutical ingredients (API), their particle sizes are generally manipulated to be smaller during the crystallization process in order to enhance their usability. This is because a better dissolution rate, uniformity of drug dispersion, and oral bioavailability can be achieved with smaller particle size during formulation [14,17]. Furthermore, smaller particle size facilitates the removal of residual water and solvent during the drying process after purification [18]. From this point of view, vancomycin with reduced particle size due to the addition of surface area-increasing materials during the crystallization process is believed to be useful in respect of the usability of the drug.

CONCLUSIONS

This study investigated the effects of ion exchange resins on the efficiency of crystallization with an increased surface area for the purification of vancomycin. As the surface area-increasing materials, cation exchange resin (Amberlite 200, Amberlite IR 120 (Na), Amberlite IR 120 (H), and Amberlite IRC 50) and anion exchange resin (Amberlite IRA 400 (Cl), Amberlite IRA 400 (OH), Amberlite IRA 910 (Cl), Amberlite IRA 67, and Amberlite IRA 96) were used to increase the S/V to 0.428 mm^{-1}. When the surface area inside the reactor was increased, vancomycin crystals were successfully generated in cation exchange resin Amberlite 200, Amberlite IR 120 (Na), and Amberlite IRC 50 and anion exchange resin Amberlite IRA 400 (Cl), and Amberlite IRA 910 (Cl). The yield of vancomycin increased (>97%), and the time necessary for crystallization was confirmed to be reduced dramatically (from 24 hr to 12 hr). On the other hand, the purity of the vancomycin was approximately 95% and was not affected by increasing S/V. Use of an ion exchange resin also resulted in the production of smaller vancomycin crystals than in the absence of ion exchange resin. According to the results of XRD analysis, vancomycin (12 hr lapsed after crystallization) obtained from the crystallization process with the surface area increased had a crystalline form.

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