Particle Design of Enoxacin by Spherical Crystallization Technique. I. Principle of Ammonia Diffusion System (ADS)

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Agglomerated crystals of enoxacin were prepared by a novel spherical crystallization technique using ammonia diffusion system (ADS). This technique made it possible to agglomerate amphoteric drugs like enoxacin which could not be agglomerated by the conventional means. When an ammonia water solution of enoxacin was poured into the mixture of acetone and a water-immiscible solvent such as dichloromethane under agitation, a small amount of ammonia water was liberated in the system. The ammonia water played a role both as a good solvent for enoxacin and a bridging liquid, which collected fine crystals precipitated into spherical agglomerates in one step. Moreover, agglomerates of the stable crystalline form were obtained by selecting the proper solvents. It was proved that the agglomeration mechanism follows these steps: acetone in the crystallization solvents enters into droplets of ammonia water and consequently enoxacin dissolved in the ammonia water is precipitated, while the droplets collect the crystals; simultaneously, a part of the ammonia in the agglomerates diffuses to the outer organic solvent phase.

Keywords enoxacin; spherical crystallization technique; agglomeration; ammonia water; pseudopolymorphism; phase separation

Introduction

We often experience that the quality of a solid pharmaceutical preparation is influenced by the primary micromeritic properties (shape and size of crystals, *etc.*) of a drug substance. Especially, when a large amount of poorly water soluble drugs are formulated, their micromeritic properties play an important role in achieving a successful formulation design. To improve their dissolution rates, they are usually comminuted, however, the size reduction makes the handling process of powder difficult. Accordingly, an appropriate pharmaceutical design to solve the above problem is desired.

Kawashima and others[1,2] established an agglomeration technique that transforms needle-like salicylic acid crystals into a spherical shape during crystallization. This technique, termed 'spherical crystallization' (SC), provides the agglomeration capability in the crystallization process for the control of primary micromeritic properties and can consequently control the massed properties at the same time. In this process, a partially miscible solvent system, *e.g.* water–ethanol–chloroform, is used for the crystallization. By selecting the proper ratio of solvents mentioned above, a small amount of water or chloroform which acts as a bridging liquid is liberated. It preferentially wets the resulting crystals and transforms them into a spherical form.

Enoxacin (Fig. 1) is a useful antibacterial agent of the new quinolone compounds, but it is slightly soluble in water and has other disadvantages such as poor compressibility, poor flowability, a bitter taste, *etc.* The authors tried to offset these disadvantages by using the SC technique. Enoxacin is soluble only in acidic and alkaline solution and is insoluble in neutral solution and all other organic solvents, it was therefore impossible to prepare the agglomerates by the conventional SC technique.

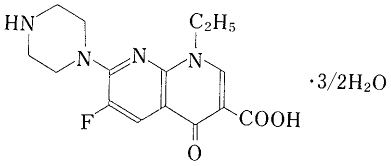

Fig. 1. Structural Formula of Enoxacin

In this study, the authors modified the SC technique and developed a new agglomeration system, *i.e.* ammonia diffusion system (ADS) which is applicable to amphoteric drug substances like enoxacin. A mixture of three partially immiscible solvents, *i.e.* acetone–ammonia water–dichloromethane, was selected as a crystallization solvent. The agglomeration mechanism in the new system was also analyzed for other aims.

Experimental

Liberation of Ammonia Water from the Crystallization Solvents Ammonia water (0.5—2 ml) and acetone (5—16 ml) were added to an organic solvent (1—10 ml) such as chloroform, dichloromethane or *n*-hexane in a glass stoppered test tube at 20 °C. The tube was vigorously shaken by a vortex mixer to induce the phase separation. Then, the points of phase change were determined by observation under an optical microscope (Olympus Optical Co., model VANOX). The concentration of ammonia in the ammonia water used was 25% (w/v). Other experiments were undertaken by replacing acetone with alcohols (ethanol, isopropanol or *n*-butanol).

Measurement of Solubility of Enoxacin in Various Solvents An excess amount of enoxacin (obtained from Dainippon Pharmaceutical Co.) was added to 40 ml of various solvents such as ethanol, isopropanol, acetone, dichloromethane, chloroform or *n*-hexane in a flask. The suspensions were shaken at 25 °C for 24 h. Samples were centrifuged at 25 °C, aliquots of the supernatant were evaporated and the residue was diluted with 0.1 N hydrochloric acid. The enoxacin contents in the medium were assayed spectrophotometrically at 268 nm (Shimadzu, UV-240).

Solubilities of enoxacin in a mixture (20 ml) of ammonia water and solvents such as ethanol, isopropanol, acetone or distilled water were also determined in the same way. Moreover, the crystalline forms of enoxacin particles after filtration of suspension in each of the above solvents were determined by X-ray diffractometry (Rigaku Denki, Geigerflex).

SC Process Enoxacin was dissolved in ammonia water to 16% (w/v) at about 40 °C. This solution (20 ml) was poured into a mixture of acetone (140 ml) and dichloromethane (40 ml) under agitation at 600 rpm using a screw type agitator in a cylindrical vessel (500 ml). The system was thermally controlled at 20 °C during the process. After 20 min, the agglomerated crystals were separated, washed with dichloromethane and dried at 50 °C. In a system composed of an ammonia water solution of enoxacin (16% (w/v), 20 ml), isopropanol (72 ml) and dichloromethane (108 ml), SC was also performed in the same way.

Rates of SC During the processing, a 3 ml aliquot portion was sampled from the system of acetone–ammonia water–dichloromethane or isopropanol–ammonia water–dichloromethane at suitable intervals. The sample was filtered through a membrane filter with a pore size of 0.45 μm (Sumitomo Electric Co., Fluoropore). The filtrate was evaporated and the residue was diluted with 0.1 N hydrochloric acid. The enoxacin content remaining in the medium without crystallization was determined spectrophotometrically as described above. As a reference, ordinary crystallization without the agglomeration of enoxacin was carried out by introducing acetone or isopropanol (270 ml) into an ammonia water solution of enoxacin (16% (w/v), 30 ml).

Measurement of the Crystalline Form and Surface Topography of the Agglomerates The shape and surface topography of the resultant agglomerated crystals were investigated with an optical microscope and a scanning electron microscope (Nihon Densi, Model JSM-T20). The crystalline forms of the agglomerates were analyzed by X-ray diffractometry, infrared (IR) spectroscopy (Nippon Bunko, IR-810) and differential scanning calorimetry (Rigaku Denki, TAS 100 System).

Diffusion of Ammonia from Ammonia Water Droplets to Organic Phase Ammonia water (5—15 ml) and mixtures (15 ml) of acetone and dichloromethane in various ratios (acetone/dichloromethane = 0 to 4) were placed in a 50 ml-centrifuge tube. The tube was shaken for 25 min at 20 °C and centrifuged for 5 min at 3000 rpm. One milliliter of the aqueous phase was sampled and diluted with distilled water. Contents of each organic solvent in the aqueous phase were assayed by gas chromatography (Shimadzu, GX-RIA column; PEG 600 25% Shimalite (BT)1301) equipped with a flame-ionization detector. Contents of ammonia in the aqueous phase were determined spectrophotometrically by the indophenol method.

Results and Discussion

Solubilities and Pseudopolymorphism of Enoxacin in Various Solvents Enoxacin with the quinolone skeleton exists in the form of a sesquihydrate as shown in Fig. 1. This quinolone skeleton has two other pseudopolymorphs, anhydrate and trihydrate. This sesquihydrate (enoxacin) is most stable under certain conditions of heat, humidity and light.[3)]

The solubilities and pseudopolymorphism of enoxacin in the various solvents suspending the sesquihydrate are tabulated in Table I. Enoxacin retained a sesquihydrous form in acetone, where the solubility was 3.0×10^{-3} g/ml at 25 °C, but it changed to an anhydrous form in alcohols, *e.g.* ethanol or isopropanol, where the solubilities were lower than those in acetone.

Selection of Solvents In order to obtain the agglomerates of enoxacin using the SC technique, a proper solvent was selected. The only good solvents were acidic or alkaline solution, and the others were found to be poor. The system of the agglomeration of enoxacin, therefore, did not coincide with the three solvent system which Kawashima and others[4)] established, consisting of (1) good solvents, (2) poor solvents for a drug substance, and (3) a small amount of a third solvent. Solvent (3) has an affinity for some drug substances and is immiscible with poor solvents. Moreover, trihydrous crystals of enoxacin were obtained without agglomeration in the system such as the neutralization[5)] method applied for phenytoin.

Thus, the mechanism of the modified system is as follows: ammonia water acted as both a bridging liquid and a good solvent for enoxacin. Since ammonia water was miscible with acetone or alcohols (poor solvents), enoxacin was precipitated by the solvent change without forming ammonium salt. Water-immiscible solvents such as hydrocarbons or halogenated hydrocarbons induced liberation of the ammonia water.

Table I. Solubilities and Pseudopolymorphs of Enoxacin in Various Solvents

Solvent	Solubility (g/ml), 25 °C	
	Pseudopolymorphs[a)]	
	Sesquihydrate	Anhydrate
Acetone	3.0×10^{-3}	—
Ethanol	—	4.7×10^{-4}
Isopropanol	—	2.6×10^{-4}
Dichloromethane	5.9×10^{-3}	—
Chloroform	4.6×10^{-3}	—
n-Hexane	3.5×10^{-7}	—

a) Pseudopolymorphs of enoxacin suspended in solvents.

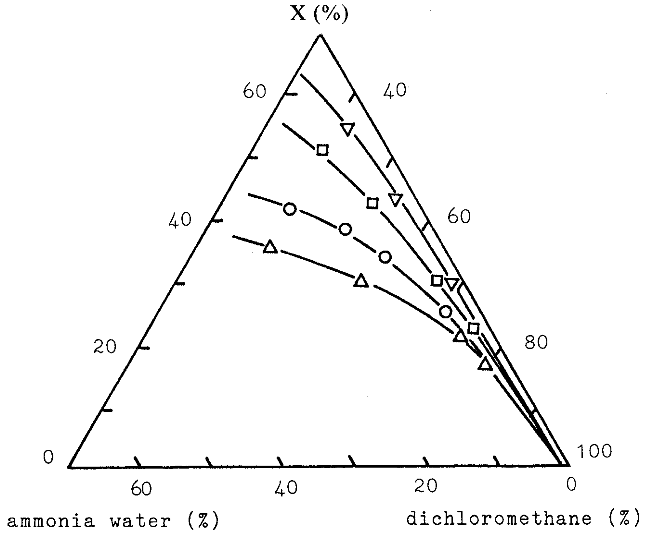

Fig. 2. Triangular Phase Diagram of the Ammonia Water–Dichloromethane–X System

X: △, ethanol; ○, isopropanol; □, *n*-butanol; ▽, acetone.

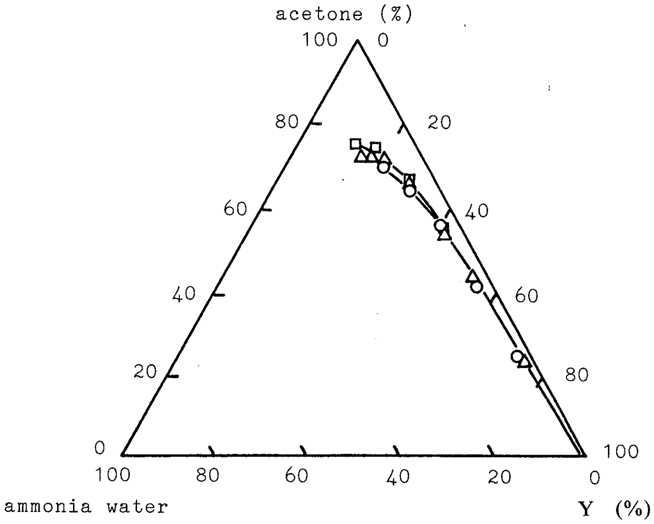

Fig. 3. Triangular Phase Diagram of the Acetone–Ammonia Water–Y System

Y: ○, chloroform; △, dichloromethane; □, *n*-hexane.

Solubility Diagram of Ammonia Water In order to perform the SC, a small amount of bridging liquid must be liberated from the solvent system. A solubility diagram of ammonia water in the three solvent systems was therefore constructed. The diagrams in the dichloromethane–X (= acetone or alcohols) system, and the acetone–Y

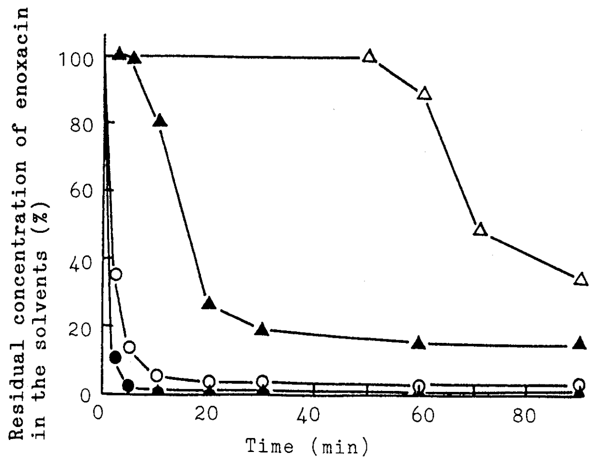

Fig. 4. Residual Concentration of Enoxacin in the Solvents as a Process of Time

Composition of solvents: ordinary crystallization ●, acetone : ammonia water (90 : 10); ▲, isopropanol : ammonia water (90 : 10), spherical crystallization ○, acetone : ammonia water : dichloromethane (70 : 10 : 20); △, isopropanol : ammonia water : dichloromethane (36 : 10 : 54).

TABLE II. Solubility and Pseudopolymorphs of Enoxacin in Mixtures of Ammonia Water and Various Solvents

Mixing ratio Ammonia water : solvent	Solubility (g/ml), 25 °C	Pseudopolymorphs[a]
1 : 9		
Solvent		
Acetone	4.0×10^{-4}	Sesqui- + anhydrate
Ethanol	6.3×10^{-3}	Sesquihydrate
Isopropanol	2.2×10^{-3}	Sesqui- + anhydrate
Distilled water	3.2×10^{-2}	Trihydrate
1 : 1		
Acetone	2.6×10^{-2}	Sesquihydrate
Ethanol	8.2×10^{-2}	Sesquihydrate
Isopropanol	5.7×10^{-2}	Sesquihydrate
Distilled water	8.8×10^{-2}	Trihydrate

a) Pseudopolymorphs of enoxacin suspended in a mixture of solvents.

(= chloroform or *n*-hexane) system are displayed in Figs. 2, 3, respectively. The solid lines in each figure represent the phase separation curves. In the region above the lines, the solvents were miscible, but in the lower region, phase separation occurred and some amount of ammonia water was liberated.

In the ethanol system, ammonia water was more miscible than the other systems. In addition, the longer the hydrocarbon chain of alcohols was, the lower were the miscibilities of ammonia water in the system observed. Acetone has good miscibility with water as well as with ethanol or isopropanol, but the miscibility of ammonia water in an acetone system was lower than in a system of *n*-butanol, which is partly miscible with water.

On the other hand, in the systems containing various water-immiscible solvents, no significant difference in miscibilities of ammonia water was recognized.

Rates of SC The crystallization rates of enoxacin from the solvent system using acetone or isopropanol for ammonia water solution are shown in Fig. 4. This figure shows the decrease in the residual concentration of enoxacin dissolved in these crystallization solvents. In the ordinary crystallization of enoxacin with the addition of acetone for the ammonia water solution of enoxacin without agglomeration, the residual concentration decreased rapidly and was in an equilibrium state after 5—10 min. The recovery rate of enoxacin was high, *i.e.* about 98%. When isopropanol was used instead of acetone, the residual concentration decreased gradually to an equilibrium state after a longer induction period, and the crystallized particles were a mixture of sesquihydrates and trihydrates. Although the case of ethanol alone is not shown here, enoxacin was not crystallized even after 3 h. In SC with agglomeration in an acetone system, on the other hand, equilibrium concentration was achieved within 20 min

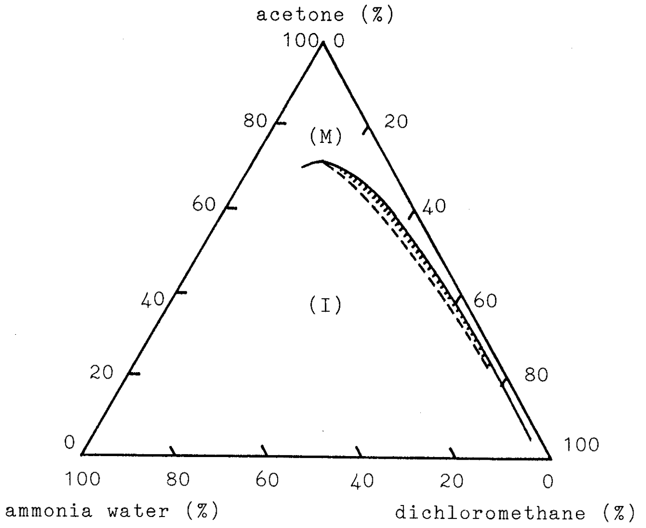

Fig. 5. Triangular Phase Diagram for Agglomeration of Enoxacin in the Acetone–Ammonia Water–Dichloromethane System

Shaded area shows the region capable of agglomeration.

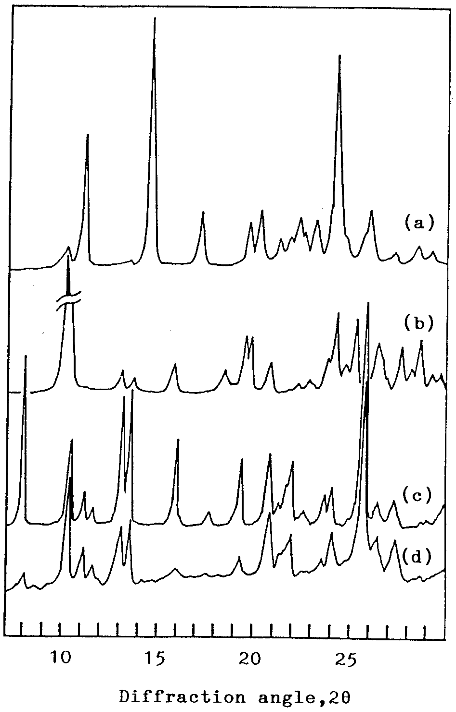

Fig. 6. X-Ray Diffraction Patterns of the Spherical Agglomerates, Enoxacin (Sesquihydrate), Its Anhydrate and Its Trihydrate

(a) anhydrate; (b) trihydrate; (c) sesquihydrate; (d) spherical agglomerates.

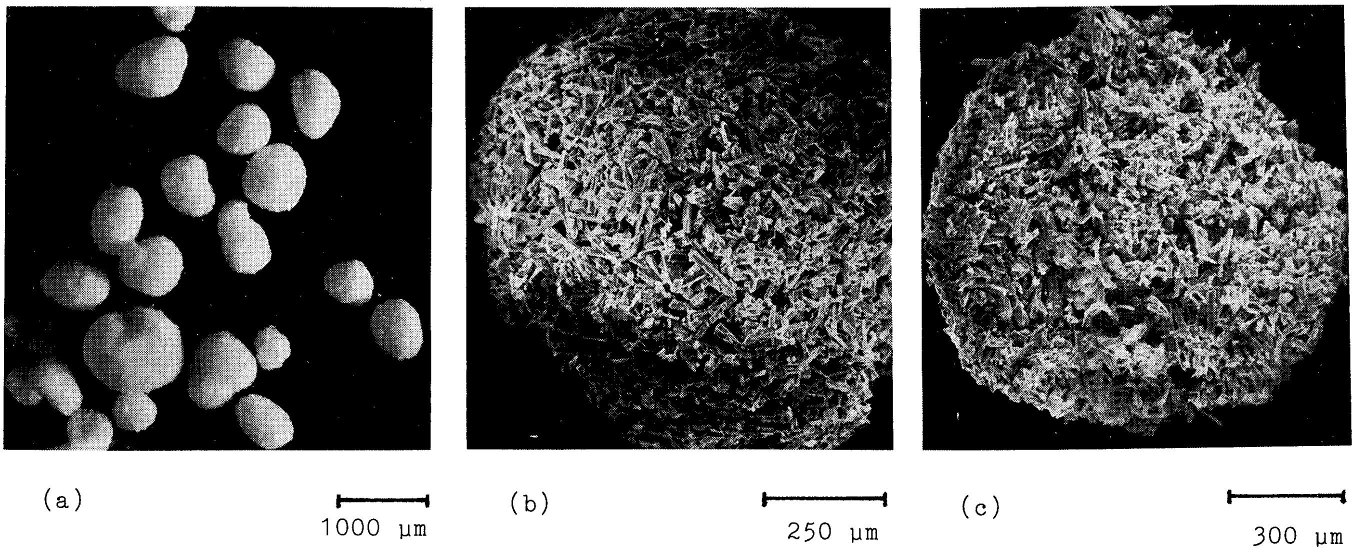

Fig. 7. Optical and Scanning Electron Microscopic Photographs of Spherical Agglomerated Crystals

Optical microscopic photograph: a) spherically agglomerated crystals. Scanning microscopic photographs: b) surface topography of agglomerated crystals, c) cross-section of agglomerated crystals.

and the recovery of enoxacin was about 96%, as shown in Fig. 4.

These results do not correspond with those of solubilities of enoxacin in the various organic solvents in Table I, but correspond well with the solubilities in the system containing ammonia water and the organic solvents in Table II, which shows that enoxacin is less soluble in the system containing acetone than in other solvent systems. This finding is considered to indicate the following: alcohols have the ability of hydrogen bonding with ammonia water as well as with water, so the solubility of enoxacin in the mixture of alcohols and ammonia water increased more than that in alcohols alone.

Considering the crystallization rate, the recovery and the pseudopolymorphism (sesquihydrates), the authors decided to perform the agglomeration mainly in the acetone–ammonia water–dichloromethane system.

Agglomerated Crystals and the Constitutive Particles The region capable of agglomeration is shown by a shaded area in the triangular phase diagram for the acetone–ammonia water–dichloromethane system (Fig. 5). In the region above the phase separation curve (solid line), enoxacin crystals were dispersed without agglomeration, whereas, in the region below the shaded area (broken line), the resultant agglomerates formed a large solid mass or a paste.

X-Ray diffraction patterns of enoxacin (sesquihydrate), its anhydrate, its trihydrate and the resultant spherical agglomerates are shown in Fig. 6. Although the strong intensities of the diffraction peaks of the agglomerates differed partly from those of the sesquihydrate, the characteristic peaks of the sesquihydrate were detected at diffraction angles of 7.9 and 25.6. These results were confirmed by IR spectra and thermal analyses.

Representative photographs of the agglomerates are shown in Fig. 7. The agglomerates were spherical in shape making them suitable for pharmaceutical processing, *e.g.* the coating process. The original primary particles of sesquihydrous forms were rectangular platen crystals which were collected and closely packed on the surface of the agglomerates. The cross-section of the agglomerates was also closely packed.

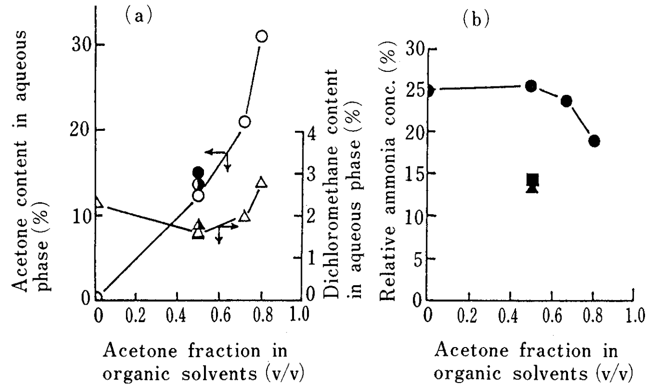

Fig. 8. The Transfers of Acetone, Dichloromethane and Ammonia to Ammonia Water Phase in the Acetone–Ammonia Water–Dichloromethane System

Composition of solvents; ammonia water : acetone–dichloromethane; (a) ○ △, 1 : 1; ◑ ▲, 2 : 3; ● ▲, 1 : 3, (b) ●, 1 : 1; ▲, 2 : 3; ■, 1 : 3.

Mechanism of Agglomeration In order to investigate the mechanism of agglomeration, the transfer of ammonia from the aqueous phase to the organic phase was investigated. Figure 8 shows contents of ammonia, acetone and dichloromethane in aqueous phase after shaking the mixture of the three components. The transfer of ammonia was determined as the relative ammonia concentration (*RAC*), which is expressed by Eq. 1

$$RAC=(A_a \times V_a)/V_i \tag{1}$$

where A_a is ammonia concentration in aqueous phase after shaking and V_i and V_a are the initial ammonia water volume and aqueous volume after shaking, respectively. When the ammonia water fraction was equal to the fraction in organic solvents, with an increase of acetone fraction the content of acetone in aqueous phase increased, but that of dichloromethane increased very slightly. When the ammo-

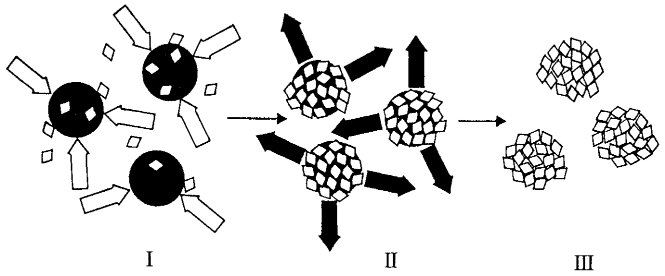

Fig. 9. Mechanism for Spherical Crystallization Using ADS (I) Invasion of Acetone into Ammonia Water Droplets; (II) Diffusion of Ammonia in the Agglomerates to the Outer Solvent; (III) Ending of Agglomeration

⇧, invasion of acetone; ⬆, diffusion of ammonia; ◇, crystals of enoxacin; ●, ammonia water.

nia water fraction to organic solvents was smaller, *i.e.* 2/3—1/3, the content of acetone in aqueous phase increased slightly (Fig. 8a). The *RAC* decreased slightly in the acetone fraction, being more than 0.5 in the system where the ratio of ammonia water fraction to organic solvents was 1 : 1 (Fig. 8b). In a smaller ammonia water fraction, the *RAC* decreased more remarkably. In the spherical agglomeration the ammonia water fraction is even smaller than under the above experimental conditions, so it is assumed that a great deal of ammonia transfers to the organic phase.

Considering the above results, the mechanism of agglomeration was explained using the model shown in Fig. 9. It was assumed that acetone in the solvents enters into droplets of ammonia water which are liberated from the acetone–ammonia water–dichloromethane system, and, consequently, enoxacin dissolved in ammonia water is precipitated, while the droplets collect the crystals (I). Simultaneously, ammonia in the agglomerates diffuses to the outer organic solvent phase and its ability as a bridging liquid becomes weaker (II), then the size of the agglomerates is determined (III). This technique was termed 'ADS.' ADS could be applied for other amphoteric drugs which have the same properties as enoxacin.

Conclusion

It was proved that the novel spherical crystallization technique using ammonia water which acts as both a bridging liquid and a good solvent, is a useful agglomeration method for enoxacin, which can be soluble only in an acidic or an alkaline solution, *i.e.* is amphoteric. Moreover, the agglomerates consisting of desirable pseudopolymorphs were obtained by selecting the proper solvents. Further studies on the characteristics of the agglomerates will be reported in a later paper.

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