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Preparation and *In-Vitro* Evaluation of Gelatin Micropellets Containing Rifampicin

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리팜피신 마이크로펠렛의 제조에 관한 연구

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The sustained-release micropellets containing rifampicin were prepared by spray congealing micropelleting technique using gelatin as the embedding matrix, and hardened by treating with the formalin-isopropanol mixture. Dissolution of rifampicin from micropellets was significantly retarded, and greatly dependent on formalin concentration, hardening time and pH of the dissolution medium. It was found that this prolongation was more distinguished in pH 1.2 dissolution medium rather than pH 7.4, which might be attributed to the swelling characteristics of gelatin used in the dissolution medium. *In-vitro* dissolution kinetics indicated that the drug release followed the first-order process.

In the field of practical pharmacy, the sustained-release principle has universal application[1,2]. A variety of methods and techniques have been employed for the preparation of sustained-release dosage forms[3,4]. There have been many reports and patents concerning the potential application of microcapsules in sustained-release formulalutions[5-18]. Particularly, multiple units dosage forms have many merits such as uniform gastric emptying rate, regular blood drug concentration and no local toxicity, etc.

In this study, rifampicin sustained-release micropellets (RFP micropellets) were prepared by spray congealing micropelleting technique using gelatin as the embedding matrix, and hardened by treating with formalin-isopropanol mixture. Dissolution properpties of RFP micropellets prepared were tested in various dissolution media using the dissolution tester. The dissolution data were interpreted in terms of first-order, square-root and cube-root plots. The goodness of fit was evaluated by linear regression analysis.

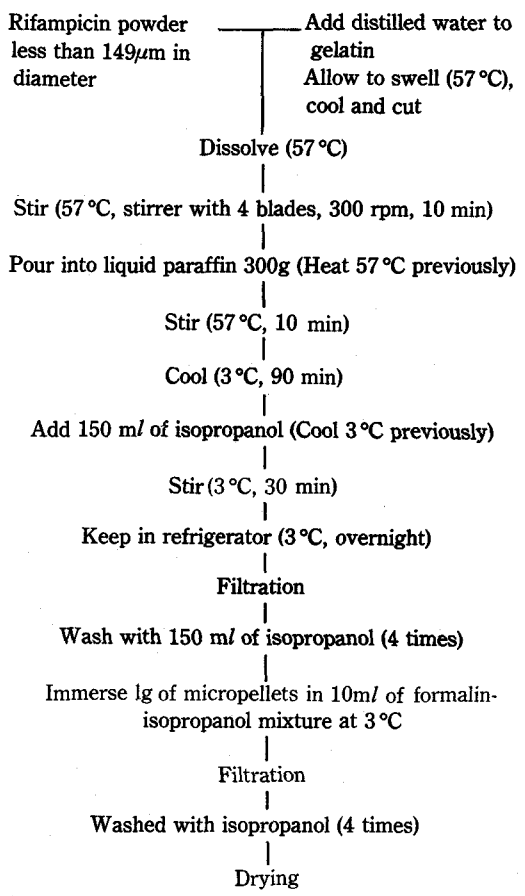
EXPERIMENTAL

Materials

Rifampicin was provided from Yuhan Corporation. Gelatin (B type, Am. Cyanamid Co.), liquid-paraffin (0.8cp. at 25°, Nippon Oil & Fats Co.), isopropanol (Duksan Co.) and formaldehyde (35%, Kanto Co.) were used. The raw materials used were of pharmaceutical grade. The reagents and solvents used in this work were of reagent grade.

Apparatus

Rotary mixer (RW 20 DZM, IKA-WERK Co.), water bath (K-W4, Dong Il Co.), dissolution tester (2760A, Hanson Res.), pH meter (PW 9410, Phillips Co.), K.P.-Sieve (Chung Ke Co.), scanning electron microscope (JSM-35, Jeol Co.), UV spectrophotometer (UV-260, Shimadzu Co.), test



Scheme 1—Preparation procedure of rifampicin micropellets.

screening machine and refrigerator were used.

Preparation of Rifampicin Micropellets

RFP micropellets were prepared according to modified Tanaka's method as shown in Scheme 1.[19-22] Rifampicin was passed through 100 mesh sieve (149 μm) before preparation. 385 ml of distilled water were added to 115g of gelatin and 57 °C with stirring. Gelatin was swollen completely after being allowed to stand at room temperature for 24 hrs. The swollen gelatin solution was refrigerated at 2-5 °C. The gel was cut into cubes using scissors before preparation.

The rifampicin powder was added to the gel cubes and the mixture was warmed in water bath at 57 °C with stirring for 10 min at 300 rpm, then poured into 300g of liquid paraffin previously heated at 57 °C. The mixture was stirred for 10 mins at 300 rpm, and then the vessel was placed in ice bath and cooled quickly to 3 °C within 5 min in order to prepare uniform size micropellets. Stirring at 300 rpm was continued for 90 min and the temperature was kept at 3 °C until the gelatin micropellets had gelatinized completely. Dehydration was carried out be adding 150 ml isopropanol previously cooled at 3 °C and the solution containing the gelatinized micropellets was stirred for 30 min at 300 rpm.

Thereafter, the solution was placed in refrigerator (2-5 °C) overnight. The gelatin micropellets were separated by filtration, washed four times with 150 ml of isopropanol previously cooled at 3 °C. Then, the sustained-release micropellets were obtained by immersing 1g of micropellets in 10 ml of formalin-isopropanol mixture in a covered glass vessel, followed by hardening in refrigerator at 2-5 °C. After filtrated and washed four times with isopropanol, RFP micropellets were dried at room temperature until the odour of formalin-isopropanol was unrecognized. According to this preparation procedure, the RFP micropellets with varing drug-gelatin ratio [1:9 (I), 3:7 (II) and 5:5 (III)] were prepared. The concentration of the formalin-isopropanol hardening mixture was varied in 1 (a), 5 (b) and 10% (c), and the hardening time was 1, 6 and 10hr.

Dissolution Test

The *in-vitro* release of rifampicin from the RFP micropellets was determined using the dissolution tester by U.S.P. XXI, paddle method. 900ml of the dissolution medium (pH 1.2, pH 7.4 U.S.P. XXI buffer solution or distilled water) was poured into the vessel, the paddle was rotated at the speed of 50 rpm, and the medium was allowed to come to 37 ± 0.5 °C. The accurate amount of RFP micropellets (200 mg as RFP) was placed in the basket (40 mesh steel basket), and dropped into the dissolution medium. Aliquots of 3ml of the dissolved solution were sampled at the prescribed time intervals, and the dissolved rifampicin from the RFP micropellets was measured by determining the absorbance of the suitably diluted sample

Table I—*Size Distribution of Rifampicin Micropellets.*

Size distribution (μm)	Weight percent of micropellets[a]		
	I	I	III
840-590	43.4±1.6[b]	37.4±5.5	49.1±3.0
590-420	38.8±3.6	33.9±3.8	21.8±4.0
420-297	16.2±7.4	21.0±7.5	20.1±4.6
297>	1.7±1.3	7.8±2.6	9.0±3.9

[a]Not hardened with formalin-isopropanol mixture.
[b]The average of three experiments.

solution at the wavelength of 329nm using spectrophotometer. Immediately, the sample volume taken was replaced by an equivalent volume of fresh buffer solution previously heated at 37 °C and the volume of medium in the vessel was kept constant. The dissolved amount was taken from the average of six experiments.

Particle Size Analysis

The size of RFP micropellets was determined mesh sieves. After 10g of RFP micropellets was vibrated for 10 min, the portions collected on each sieve were weighed accurately. The size distribution test was run in triplicated and a frequency distribution table was made.

RESULTS AND DISCUSSION

Preparation of Rifampicin Micropellets and Scanning Electron Microscopic Observation

In preparation process of RFP micropellets, isopropanol was used as dehydrating agent. The dehydrating effect of isopropanol is milder than other dehydrating agents (methanol, ethanol, etc.), and the solution capacity of isopropanol to rifampicin is lower than that of other agents. In consequence, isopropanol resulted in the production of very fine free-flowing micropellets in comparison with other recovery methods and no cluster occurred. Isopropanol was found to be superior to other agents in that it gave a product with no formation of agglomerates[17,23-25].

As shown in Table I, RFP micropellets with a particle size of 297-840 μm accounted for about 90 percent of the total product prepared by this procedure. Also, the size distribution of micropellets was not related to the drug-gelatin ratios. On the cooling step (57→3 °C) in the preparation process, the shorter cooling time, the more uniform size distribution of RFP micropellets.

The reaction between formaldehyde and rifampicin in the hardening step produced a soluble compound whose removal and gelatin shrinkage (intra- or inter-molecular crosslink) by formaldehyde might cause a diminution in micropellet size, but the overall size distribution proportion was not influenced by hardening.[26-29]

Fig. 1. shows the scanning electron micrographs of RFP micropellets before and after dissolution test. As shown in the Fig. 1, the whole shape of micropellets was round before and after dissolution (A,C). Particularly, the spherical shape was maintained even after dissolution test. But, the appearance of micropellets after dissolution was wrinkled in comparison with that of intact micropellets (B,D). Also, the surface of micropellets after dissolution was more porous and curved laxly. From this observation, it was suggested that the rifampicin was released through the pore and diffused through the gelatin matrix.

***In-Vitro* Release of Rifampicin from Micropellets**

Fig. 2 shows the dissolution profiles of rifampicin in distilled water from the micropellets prepared with various drug-gelatin ratios and hardened with 5% formalin-isopropanol solution for 6 hr. As shown in Fig. 2, the dissolution profile was not influenced by drug-gelatin ratio.

Fig. 3 shows the dissolution profiles of rifampicin in the distilled water from micropellets (III) hardened with different formalin concentration for 6 hr. The release rate of rifampicin was decreased with increasing concentration of formalin. Also, the effects of hardening time on drug release are shown in Fig. 4.

Therefore, the release of rifampicin from the RFP micropellets was not influenced by the drug-gelatin ratio, but by the concentration of formalin and hardening time.

The dissolution data were treated by following

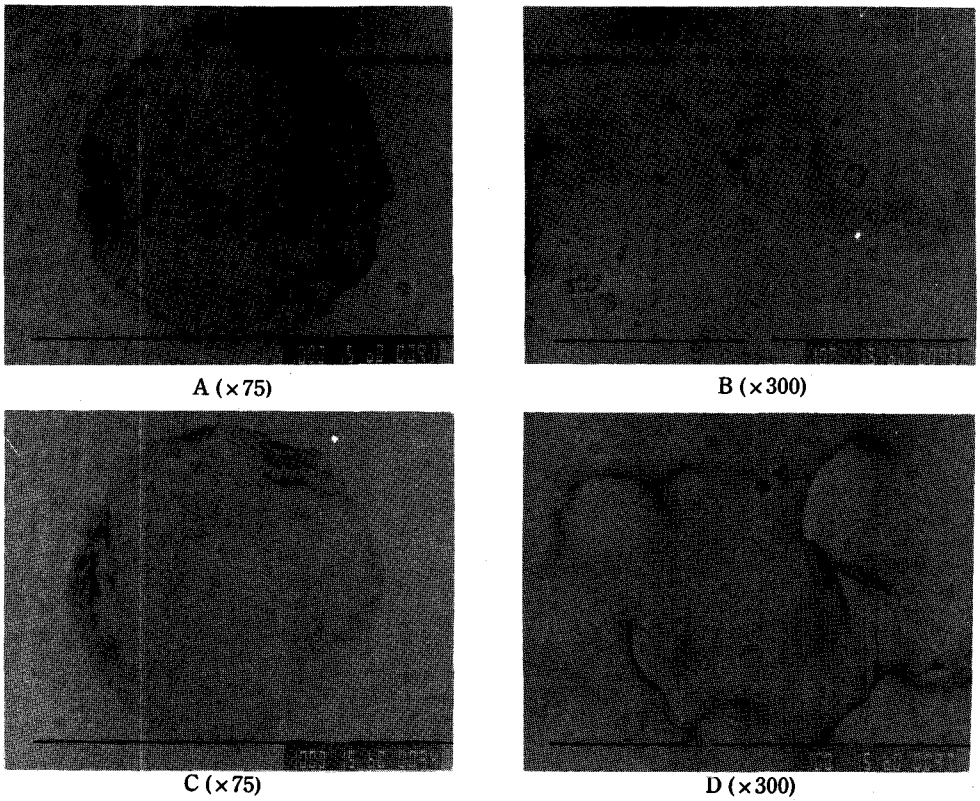


Figure 1—Scanning electron micrographs of rifampicin micropellets (II) before (A,B) and after dissolution test (C,D).

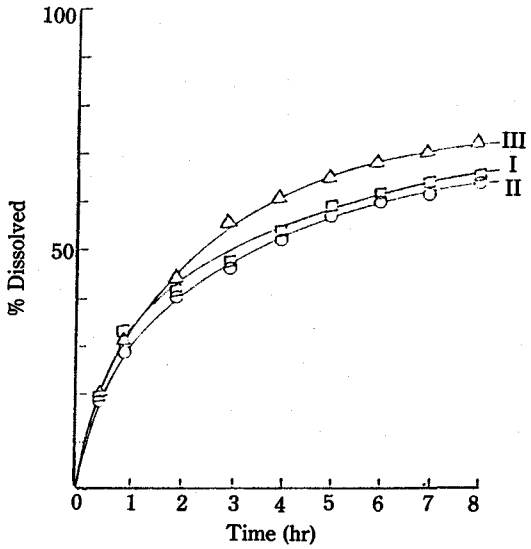


Figure 2—Dissolution profiles of rifampicin from micropellets prepared at various rifampicin to gelatin ratios and hardened with 5% formalin-isopropanol mixture for 6 hr in distilled water.
Key: △,5:5; □, 1:9; ○, 3:7

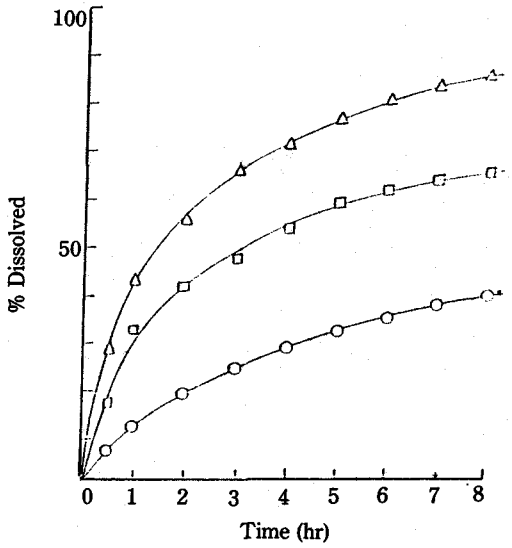


Figure 3—Dissolution profiles of rifampicin from micropellets (III) hardened with different formalin concentration for 6 hr in distilled water.
Key: △, 1%; □, 5%; ○, 10%

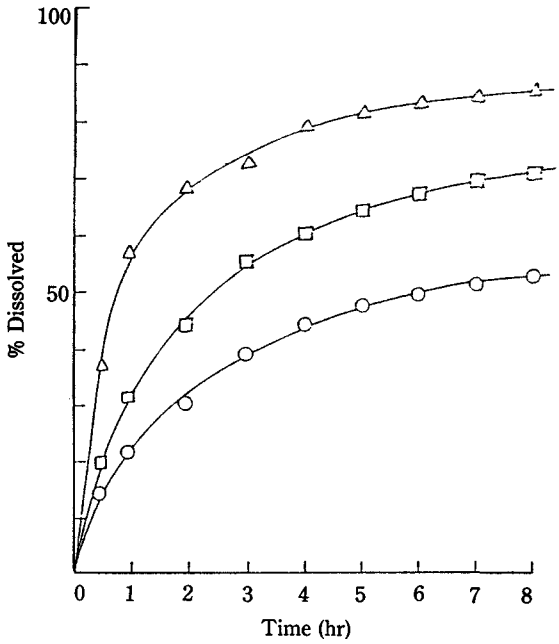


Figure 4—Dissolution profiles of rifampicin from micropellets (I) hardened with 5% formalin-isopropanol mixture for different hardening times in distilled water.
Key: △, 1hr; □, 6hr ; ○, 20hr

mechanical equations.

$$\log m = k_1 t + C \qquad \text{Eq. 1.}$$

$$100 - m = k_2 t^{1/2} \qquad \text{Eq. 2.}$$

$$100^{1/3} - m^{1/3} = k_3 t \qquad \text{Eq. 3.}$$

where m represents the percentage of drug undissolved at time t, k_1 is the apparent first-order rate constant, k_2 is the square-root rate constant, k_3 is the cube-root rate constant and C is a constant.

The goodness of fit was evaluated by linear regression analysis and summarized in Table II. From the Table II, the release of RFP from the micropellets mainly followed the first-order kinetic process (Eq. 1), and it was suggested that the RFP release rate was proportional to the amount of drug remaining in the micropellets. The swelling might lengthen the path of a drug molecule in the matrix appreciably. After the dissolution of about 60—70% of the drug, the surface area of the undissolved drug may decrease rapidly. So, the release rate was more retarded.

Table II—*Comparative Linear Regression Analysis of Data Using Different Drug Release Models for Rifampicin Micropellets.*

Formulations	Correlation coefficients (r)		
	First-order	Square-root	Cube-root
I-a-6*	0.990	0.976	0.940
I-a-20	0.990	0.985	0.947
II-b-6	0.984	0.975	0.949
II-b-20	0.998	0.995	0.965
III-c-6	0.998	0.990	0.960
III-c-20	0.999	0.980	0.945

*I-a-6 means that drug-gelatin was 1:9 and hardened with 1% formalin-isopropanol mixture for 6hrs.

Table III—*First-Order Release Rate Constants (k, hr^{-1}) of Rifampicin from Micropellets in Distilled Water.*

Formulations	Hardening time (hr)		
	1	6	20
I-a	0.693±0.033	0.409±0.026	0.363±0.045
I-b	0.606±0.047	0.308±0.025	0.202±0.031
I-c	0.280±0.012	0.069±0.009	0.063±0.010
II-a	0.709±0.013	0.421±0.018	0.343±0.021
II-b	0.595±0.024	0.298±0.016	0.288±0.025
II-c	0.169±0.015	0.088±0.020	0.080±0.007
III-a	0.539±0.016	0.454±0.020	0.421±0.016
III-b	0.478±0.014	0.354±0.010	0.286±0.005
III-c	0.091±0.013	0.085±0.008	0.081±0.007

The effect of gelation was not pronounced during the dissolution procedure. This could be explained by the fact that the dissolution temperature was far below the gelation temperature of the gelatin.

In the preparation procedure of micropellets, the main step affecting the drug release behaviour was the hardening process. Table III shows the first-order release rate constants (hr^{-1}) of RFP in the distilled water. From the Figs. 3, 4 and Table III, the release rate constants were influenced by the formalin concentration and hardening time. The tortuosity in the gelatin matrix was increased

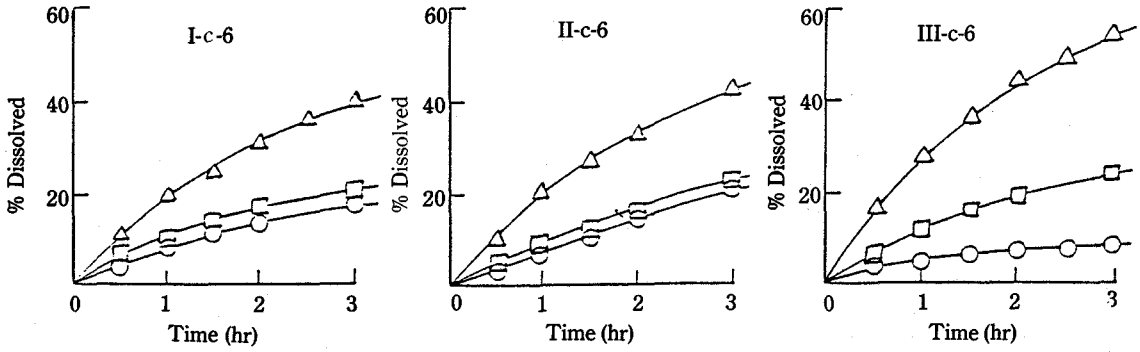


Figure 5—Dissolution profiles of rifampicin from micropellets hardened with 10% formalin-isopropanol mixture for 6 hr in various dissolution media.
Key: △, pH 7.4 buffer; □, distilled water; ○, pH 1.2 buffer

Table IV—*First-Order Release Rate Constants (k, hr^{-1}) of Rifampicin from Micropellets Hardened with 5% Formalin-Isopropanol Mixture for 6 hr in Various Dissolution Media.*

Formulation	Dissolution medium		
	pH 1.2 buffer	Distilled water	pH 7.4 buffer
I-b-6	0.138±0.016	0.308±0.025	0.860±0.025
II-b-6	0.243±0.010	0.298±0.016	0.871±0.027
III-b-6	0.128±0.012	0.354±0.010	0.827±0.030

in compliance with increasing the formalin concentration and hardening time. Also, the more increasing the concentration and time, the more crosslinking in the gelatin matrix. Hence, the release rate could be controlled by the hardening process.

Effect of pH on Drug Release

Fig. 5. and Table IV show that the release pattern of rifampicin was varied with the change of the pH of dissolution medium. Although the solubility of rifampicin in the pH 1.2 solution is by far higher than those in the distilled water and pH 7.4 solution[24], the release rate in the pH 1.2 solution was inferior to that in the other media. This is attributed to the fact that the exposure of gelatin to formaldehyde make it acid-resistant, which results in the formation of crosslinked gelatin (methylene or dimethylene ether bridge formation) that is completely insoluble in the gastric juice[5,17]. Because the swelling of gelatin matrix in the alkaline medium is by far superior to that in the acidic

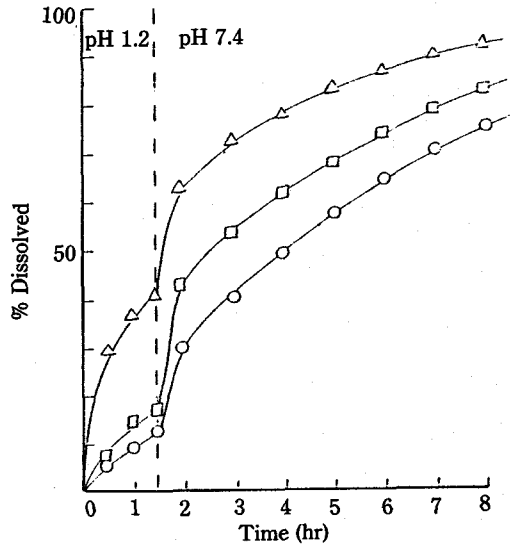


Figure 6—Dissolution profiles of rifampicin from the pooled micropellets with varing ratios of III-c-6 to III -a-1.
Key; △, 1:3; □, 2:2; ○, 3:1

medium, the drug release rate in the alkaline medium was more distinguished in consequence of increased penetration of medium, regardless of solubility difference. Consequently, the effect of the pH of dissolution medium on the drug release was more pronounced than that on RFP solubility.

After the various micropellets releasing at different rates were mixed, the release of the pooled micropellets were tested in the pH 1.2 buffer solution for 1.5 hr and then in the pH 7.4 solution for 6.5 hr. As shown in the Fig. 6, the RFP release

pattern of the pooled micropellets was supposed to meet the promising pattern of the controlled release preparations. Therefore, it is thought that the gelatin micropellet system with drug would be a good candidate for the controlled-release drug delivery system, which contained priming and loading doses.

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