H_2O-H), 436 ($M^+-H_2O-HOAc$), 421 ($M^+-H_2O-HOAc-CH_3$), 300, 264 (c), 250 (a), 249, 246 (c-H_2O), 218 (c-COOH+H), 215, 189 (b, base peak), 175, 161, 147, 135, 121, 107. IR ν_{max}^{KBr} cm^{-1}: 3570 (OH), 1755 (γ-lactone), 1725, 1242 (ester). NMR: 0.86, 0.87, 0.91, 1.15, 1.30 and 1.52 (each 3H, s), 0.95 (3H, d, $J=4.0$ Hz), 2.04 (3H, s, $COCH_3$), 3.88 (1H, t, $J=1.4$ Hz, $CH_2\underline{C}HOH$), 4.58 (1H, t, $J=5.0$ Hz, $C\underline{H}OCOCH_3$). ^{13}C-NMR: 80.4 (C-3), 75.5 (C-16), 49.6 (C-17), 96.1 (C-19), 211.0 (C-28), 21.3 ($CO\underline{C}H_3$), 170.5 ($O\underline{C}OCH_3$). Chromic acid oxidation of VI in pyridine gave the ketone (VIII) as needles, mp 249—252°. IR ν_{max}^{KBr} cm^{-1}: 1770 (γ-lactone), 1730, 1725 (ester and cyclohexanone). *Anal.* Calcd for $C_{32}H_{48}O_5$: C, 74.96; H, 9.44. Found: C, 74.92; H, 9.59. VIII was hydrolyzed with 5% methanolic KOH to give the desacetyl compound (IX), amorphous. IR ν_{max}^{KBr} cm^{-1}: 1775 (γ-lactone), 1710 (C=O). MS *m/e*: 470 (M^+, 18%), 454, 426, 411, 300, 264, 262, 249, 248, 221, 218, 207 (100%), 205, 189, 175, 161, 149, 135, 121, 119, 107.

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Microencapsulation and Bioavailability in Beagle Dogs of Indomethacin[1,2]

YUTAKA TAKEDA, NAOKI NAMBU,* and TSUNEJI NAGAI

Hoshi Institute of Pharmaceutical Sciences, Ebara-2-4-41, Shinagawa-ku, Tokyo 142, Japan

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Indomethacin (IMC) suspended in soybean oil was microencapsulated in a gelatin-acacia system by a modified phase separation method from aqueous solution. The microcapsules were hardened with formaldehyde without the use of sodium hydroxide. Average particle diameter of the microcapsules was 111 μm, and the content of IMC in the microcapsules was more than 80% of the initial amount of IMC. The *in vitro* dissolution of IMC from the microcapsules was slower than that of intact IMC, and this indicated that the walls of the microcapsules affected the drug dissolution. The bioavailabilities in beagle dogs of the IMC microcapsules and of a soybean oil suspension of IMC administered in capsules were larger than that of intact IMC.

Keywords——indomethacin; microcapsule; soybean oil suspension; dissolution rate; bioavailability; beagle dogs

Microencapsulation of drugs offers several advantages, such as stabilization of the drug against light, moisture and oxygen, prevention of inactivation in the stomach, sustained release, prevention of adverse stomach reaction, and so on.[3] In the pharmaceutical field also, microencapsulation has been widely investigated for various drugs such as aspirin, phenacetin, salicylic acid, and so on.[4]

Indomethacin (IMC), a typical nonsteroidal antiinflammatory drug, is only very slightly soluble in water, and causes adverse reactions due to a stimulant effect on the stomach upon oral administration.

In the present study, we prepared microcapsules of IMC by a complex coacervation method using a gelatin-acacia system,[5] and investigated the dissolution properties and bioavailability of the microcapsules in comparison with a soybean oil suspension of IMC and intact IMC, aiming at a sustained-release preparation of IMC without adverse effects on the stomach.

Experimental

Materials——Indomethacin (IMC) was supplied by Sumitomo Chemical Co., Ltd. Gelatin of J.I.S. first grade was purchased from Nitta Gelatin Co., Ltd. Acacia, soybean oil and formaldehyde were of J.P.IX grade. Other chemicals used were all of reagent grade.

Preparation of Microcapsules and Soybean Oil Suspension of Indomethacin——A modified phase separation method from aqueous solution, described by Miyano *et al.*,[6] was applied for the preparation of microcapsules of IMC. Procedures for the preparation of microcapsules are shown in Fig. 1. A soybean oil suspension of IMC was prepared by suspending 10% IMC in soybean oil, packed in capsules and sealed with 20% gelatin aqueous solution. IMC in the microcapsules was determined by an ultraviolet (UV) absorption method at 318 nm after vigorous agitation with a magnetic stirrer in methanol.

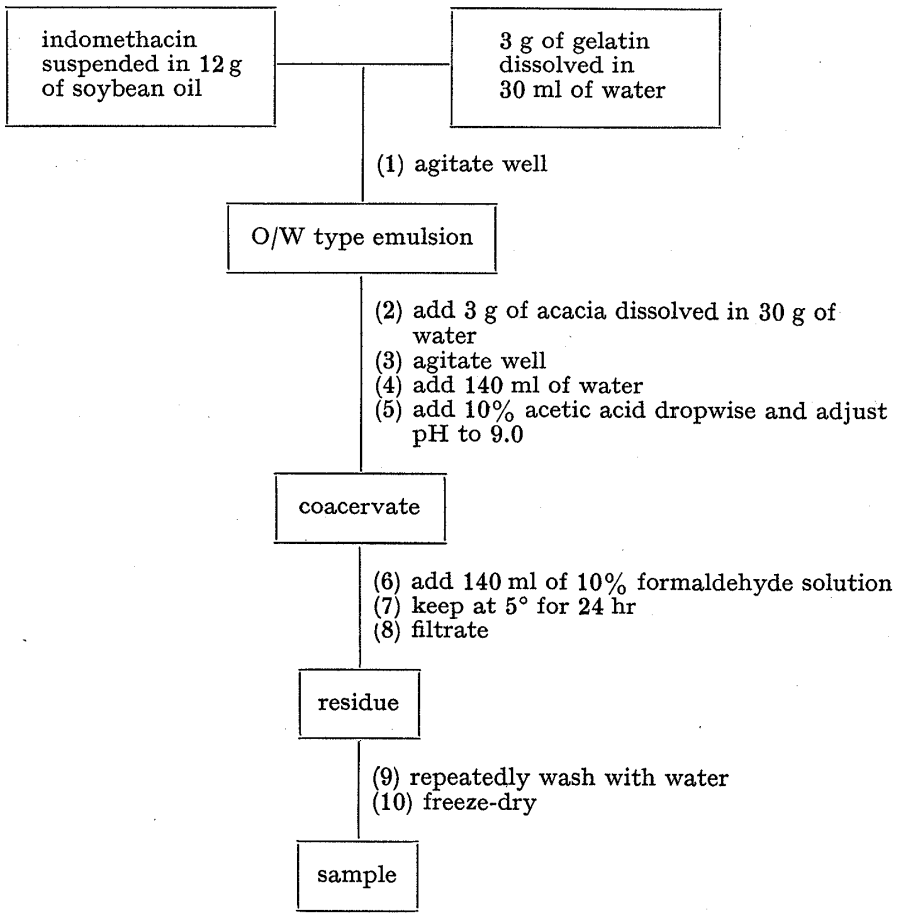

Fig. 1. Preparation of Microcapsules of Indomethacin

***In Vitro* Dissolution of Indomethacin from Microcapsules**——*In vitro* dissolution of IMC from microcapsules and intact IMC was tested according to a modified beaker method reported by Levy[7] with stirring at 100 rpm at 37°. The diameter of microcapsules used was 177—250 μm. The test solution was 900 ml of a mixture of ethanol and the first medium of the J.P.IX disintegration test (30: 70% (v/v)). The dissolution of a soybean oil suspension of IMC enclosed in capsules could not be tested because an appropriate test method was not found.

Bioavailability of Indomethacin in Beagle Dogs——Six healthy male dogs weighing about 13 kg were used after fasting for 24 hr. They were randomly divided into three groups for a cross-over experiment according to a Latin-square design at intervals of one week over three weeks. Microcapsules and intact IMC were administered orally at a dosage of 150 mg of IMC in the form of powders wrapped in two pieces of oblate. The particle diameter of microcapsules used was 177—250 μm. A soybean oil suspension of IMC (150 mg IMC) was administered in a capsule. Five ml blood samples were collected from the cephalic veins centrifuged to obtain the serum sample, and stored in a refrigerator at −10°.

Determination of Blood Level of Indomethacin——IMC in serum samples was determined by a modification of the gas chromatographic method reported by Aoyama *et al.*[8] A Shimadzu GC-4CM gas chromatograph equipped with a hydrogen ionization detector and packed with 3% OV-17 on Chromosorb 750 was used.

Results and Discussion

Preparation of Microcapsules of Indomethacin

It has been thought that sodium hydroxide is required in the process of hardening of microcapsule walls with formaldehyde.[3] However, IMC is easily degradated by alkali,[9] so a hardening method for IMC microcapsule walls with formaldehyde was investigated without the use of sodium hydroxide. The method as shown in Fig. 1 was effective. Formaldehyde was not detected in microcapsules by the method described in J.P.IX.

The average particle diameter of the microcapsules calculated from Green's diameter by optical microscopy for 242 capsules which passes through an 80 mesh seive and remained on a 100 mesh seive was 111 μm. The content of IMC in the microcapsules was always more than 80% of the initial amount of IMC.

In Vitro Dissolution of Indomethacin

As shown in Table I, the dissolution of IMC from the microcapsules was slower than that of intact IMC, being statistically significant at the 1% level (*t*-test) at the initial stage. To compare the dissolution curve of the microcapsules with that of intact IMC, log normal distribution analysis was applied and the 50% dissolution time, t_{50}, was obtained. The t_{50} values were 20.1 and 10.1 min for the microcapsules and intact IMC, respectively. There was a significant difference between the microcapsules and intact IMC in t_{50}, which suggests that the walls of the microcapsules affect the release of IMC.

Bioavailability of Indomethacin in Beagle Dogs

Table II summarizes the average serum concentrations with standard errors after oral administration of IMC to beagle dogs. The microcapsules and soybean oil suspension of IMC

Table I. Dissolution Testing of Indomethacin (Average ± Standard error)

Time (min)	Microcapsules of indomethacin	Intact indomethacin	*t*-Value
5	29.0±0.6	38.3±0.4	13.69[a]
10	38.6±0.8	49.5±0.5	11.87[a]
20	47.8±0.9	60.9±0.7	11.17[a]
30	54.5±1.1	67.3±1.0	8.82[a]
60	68.0±2.3	76.8±1.0	3.47[a]
120	79.0±1.9	83.2±1.2	1.84[b]
180	83.3±1.6	85.0±1.0	0.85[b]

a) Statistically significant at the 0.01 level.
b) Not statistically significant at the 0.01 level.

TABLE II. Serum Concentration of Indomethacin (μg/ml) (Average ± Standard error)

Time (hr)	Microcapsules of indomethacin	Intact indomethacin	Soybean oil suspension of indomethacin
0.5	11.27±3.13	7.41±2.85	11.84±2.40
1	16.43±7.35	14.57±3.92	22.31±1.89
2	22.83±3.62	14.83±3.27	26.19±3.24
3	13.58±1.75	12.69±2.89	11.89±1.55
4	8.15±0.67	7.59±2.18	7.92±1.13
6	5.49±0.27	4.57±0.95	4.85±1.13
8	5.93±1.11	3.10±0.45	3.54±0.85
12	0.00	0.00	0.00
Peak of the average serum concentration-time curve (μg/ml)			
	22.83	14.83	26.19
Time of the peak of the average serum concentration-time curve (hr)			
	2.0	2.0	2.0
Average of the individual peak serum concentration (C_{max}) (μg/m)			
	27.10±5.40	18.80±3.15	28.82±2.41
Average of the individual peak time (t_{max}) (hr)			
	1.58±0.27	1.92±0.42	1.50±0.22
Average of the area under the individual serum concentration-time curves (AUC) ((μg/100 ml) × hr)			
	47.60±6.39	36.08±5.53	49.63±4.19

gave higher serum concentrations than intact IMC. The reason for this is not clear, but oily substances may enhance the absorption of IMC from the GI tract.

A double maxima phenomenon was observed in the serum concentration curve of the microcapsules of IMC, but not in those of soybean oil suspension and intact IMC. Further investigations are required to elucidate the mechanism of this phenomenon by means of studies of absorption, distribution, metabolism and excretion.

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