resulting viscous liquid was placed in a refrigerator (−30°C) to crystallize. White crystal was collected and recrystallized from ethanol. 5.9g (45% yield): $^1$H NMR($CDCl_3$) $\delta$ 1.08–2.70 (*m*,4H), 3.70 (*t*,1H), 4.98 (*t*,1H), 7.35 (*s*,5H): IR (KBr) 2260 (C≡N) cm$^{-1}$.

**1,1-Dicyano-2-phenylcyclobutane (1b).** In a 250 m*l* round-bottomed (3-neck) flask fitted with a reflux condenser and a dropping funnel containing a dry benzene (10 m*l*) solution of triethylamine (12.0g, 0.12 mol) was placed a benzene (40 m*l*) solution of 1,1-dicyano-4-bromo-4-phenylbutane (26.3g, 0.1 mol). The reaction mixture was heated to reflux with stirring and the amine solution was added slowly during two hours. The reflux was continued for an additional three hours. The brown precipitate was removed by filtration. The resulting liquid was distilled under reduced pressure. 4.0g (22% yield): bp 50–52°C/0.1mmHg: $^1$H NMR($CDCl_3$) $\delta$ 2.05–2.95 (*m*,4H), 3.85–4.35 (*m*,1H), 7.30 (*s*,5H): IR (neat) 2240 (C≡N) cm$^{-1}$.

**Anionic polymerization of 1.** A represent anionic polymerization procedure were as follows: In a rubber stopper-capped glass ampoule were placed a solution of **1a** (1.5g, 0.01 mol) and 4 m*l* DMSO. Dry $N_2$ gas was passed through the solution by a syringe needle for 30 minutes. The solution was then cooled in a dry ice-acetone bath and it was added proper quantities of initiator solution by a microsyringe. The ampoule was then evacuated and sealed under vacuum. The sealed ampoule was taken out of dry ice-acetone and allowed to stand at room temperature. As polymerization proceeded, red color developed and viscosity of the solution increased. After three days the ampoule was opened and the polymer solution was poured into a large volume of cold methanol. Precipitated polymer was collected and reprecipitated from acetone into methanol. Thus obtained polymer was then dried in a vacuum oven at room temperature. 0.54g (36% yield); $\eta_{inh}$=0.03 dl/g (c: 1.0 g/dl in acetone at 25°C).

**Measurements.** IR spectra were taken on a Perkin-Elmer Model 283B spectrophotometer. $^1$H NMR spectra were obtained with a Varian Model T-60A spectrometer (60 MHz) at room temperature.

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# Preparation of Glutamic Acid-Leucine Copolymer Containing Indomethacin for Controlled Delivery

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A series of copolypeptides of glutamic acid and leucine have been synthesized by N-carboxy-α-amino acid anhydride procedure and cast to form injectable microparticulate monolithic devices in which indomethacin was physically dispersed. With these devices, various release properties and possible clinical application were studied. The release rate of the drug had a close relationship with the monomer composition of the copolymer matrix as well as the environmental pH condition. The monolithic device of glutamic acid/ leucine = 50/50 was found to be the most promising one as a ploymeric delivery system of indomethacin. The intrinsic viscosity of this copolymer was 4.35 dl/g and the release rate was 18.5 μg/g/day.

## Introduction

Among the many types of drug delivery systems, controlled delivery using wide varieties of polymers and drug entrapping method is one of the most efficient ways to deliver the drug to a specific body site over a desired time period.[1-3] Compared to the other polymeric delivery systems, those of the biodegradable polymers have a unique property in that the polymer matrices are degraded to nontoxic products in the body during or after the release of drugs so that they are

free from the problem of retrieval of the exhausted devices by surgical operation. Since it is possible to obtain an optimum drug release rate by changing the amino acid composition in the polymer, many researchers have placed their hopes to the development of biodegradable drug delivery systems using poly(amino acids) as a carrier.[4,5] Such poly(amino acids) can be prepared by several synthetic routes, but those with high molecular weight are usually obtained by the N-carboxy-$\alpha$-amino acid anhydride procedure.[6,7]

In the present investigation, we have prepared a series of copolypeptides of glutamic acid and leucine using the N-carboxy-$\alpha$-amino acid anhydride procedure. Indomethacin, which is a powerful nonsteroidal antnflammatory agent but has a limitation for use due to adverse effects,[8] was physically dispersed into this poly(amino acids). The resulting monolithic device can be a promising controlled delivery system for the long-term therapeutic application of indomethacin.

## Experimental

**Materials.** Indomethacin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). L-Glutamic acid was obtained from Wako Pure Chemicals (Japan). L-Leucine and carbobenzoxy chloride were the products of Pierce Chemical (Rockford, IL, USA) and Aldrich Chemical (Milwaukee, WI, USA), respectively. All other chemicals were of reagent grade.

**Assay of indomethacin.** Indomethacin was quantitatively measured using HPLC according to the method of Dusci and Hackette[9] by Waters chromatographic system equipped with Model 440 UV absorbance detector (254 nm) and $C_{18}$ $\mu$-Bondapak column (3.9×300 mm). The 60% acetonitrile solution in 45 mM potassium phosphate buffer, pH 3.0 was used as a mobile phase and the flow rate was set at 0.8 ml/min. The retention time of indomethacin was 8.5 min in this condition.

**Preparation of poly(glutamic acid-leucine).** $\gamma$-Methyl-N-carboxy-L-glutamate anyhydride ($\gamma$-OMe-L-Glu-NCA), m.p. 97-99°C (ref.[10] 96-97°C, ref.[11] 99-100°C) and N-carboxy-L-leucine anhydride (L-Leu-NCA), m.p. 75-77°C (ref.[12,13]76°C) were prepared and structurally indentified according to the method of Hanby *et al.*[11] Bergman *et al.*[14] and Becker *et al.*[15] As shown in Figure 1, the copolypeptides were obtained with varying ratio of $\gamma$-OMe-L-Glu-NCA and L-Leu-NCA. The NCA's were polymerized basically according to the method of Blout *et al.*[7] A typical polymerization procedure to obtain a copolymer of $\gamma$-OMe-L-Glu/L-Leu = 70/30 is as follows: in a round-bottom flask equipped with a calcium chloride tube, $\gamma$-OMe-L-Glu-NCA (2.45g) and L-Leu-NCA (0.88g) were dissolved by warming to make a final 2.5% solution in freshly distilled dry benzene. After cooling to room temperature, polymerization was initiated by adding $NaOCH_3$ (0.268 ml in benzene: methanol (3:1), anhydride/initiator = 200) with vigorous stirring. The reaction was allowed to proceed at room temperature with intermittent stirring until titration of the residual anhydride indicated the reaction to be completed more than 95%.[16] The product was precipitated by ethyl ether and dried *in vacuo* after suction-filtration [copolymer of $\gamma$-OMe-L-Glu and L-Leu]. The copolymers of various ratio ($\gamma$-OMe-L-Glu: L-Leu = 100:0, 50:50, 20:80 and 10:90) were also prepared in the same way. These copolymers were saponified to remove methyl ester group: to a solution of a polymer (1.5g) in pyridine (40 ml) was slowly added 0.85N EtOH-KOH (150 ml). Stirring was continued for 4 hrs. The white precipitate was collected by suction-filtration and dried *in vacuo* for 3 days [potassium salt of poly(DL-Glu-co-DL-Leu)]. The above salt was dissolved in water followed by acidification with 1N HCl to pH 1.5. The precipitate obtained was washed three times with cold water and ethanol, respectively. The product was finally dried over phosphorus pentoxide and stored in refrigerator until use [poly(DL-Glu-co-DL-Leu)].

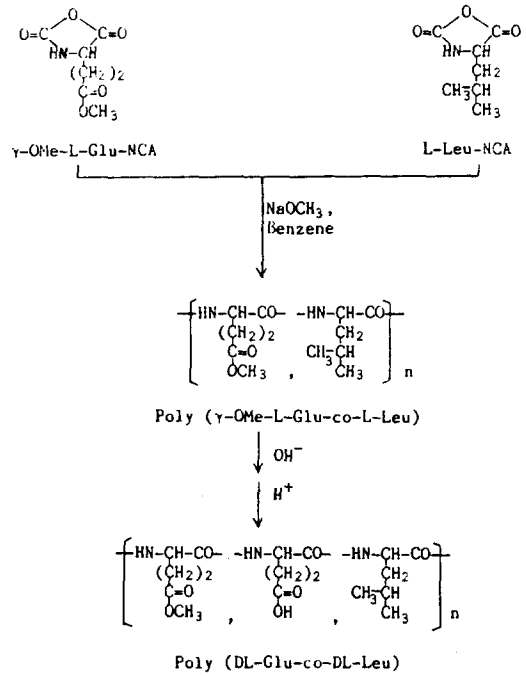


**Figure 1.** Preparation of copolypeptides of glutamic acid and leucine.

**Table 1. Physical properties of copolypeptides**

| Monomer feed[a] (%) | | Intrinsic viscosity[b] (dl/g) | Comparative density | Solubility | | | |
|---|---|---|---|---|---|---|---|
| | | | | Potassium salt | | Free acid | |
| L-Glu | L-Leu | | | Water | DMF | DMF | Pyridine |
| 100 | 0 | 0.725 | High | +[c] | + | + | + |
| 70 | 30 | 4.050 | | + | + | + | + |
| 50 | 50 | 4.350 | | + | + | + | + |
| 20 | 80 | 0.580 | | ±[d] | −[e] | − | + |
| 10 | 90 | 0.280 | Low | ± | − | − | ± |

[a]Anhydride/initiator ratio = 200. [b]Measured in pyridine at 20°C. [c]Readily soluble. [d]Relatively soluble or soluble with some difficulties. [e]Insoluble.

**Preparation of monolithic device containing indomethacin.** Indomethacin was added to a 3% (w/v) pyridine solution of the saponified copolypeptide, poly(DL-Glu-co-DL-Leu). The solution was shaken at 45°C for 1 day and cast into a film on a water-leveled plane. It was completely dried *in vacuo* at room temperature over 5 days. The film was ground into microparticles and the fraction between 80 and 165 mesh was collected for use in the release study.

***In vitro* release studies. A. Continuous elution system.** The monolithic device (250 mg) was suspended in normal saline solution (10 ml) and incubated at 37°C with shaking at 100 RPM. After 3 hrs, the device was packed into a small glass column (10.7 x 60 mm) and eluted continuously using pseudo-extracellular fluid (PECF)*[17] as a mobile phase at a flow rate of about 50 ml/day at 37°C.

**B. Batch elution system.** The monolithic device (100 mg) was suspended in PECF (12 ml) in a 50 ml centrifuge tube. The suspension was incubated at 37°C while shaking at 100 RPM. At every 24 hrs, thereafter, the suspension was centrifuged and the supernatant (10 ml) was replaced by equal volume of the fresh PECF. The supernatant collected was stored at −20°C until assayed for indomethacin content.

*Note: PECF was composed of 30 mM $NaHCO_3$, 2 mM $K_2HPO_4$, 115 mM NaCl and 1 mM KCl in distilled water. The solution was adjusted to desired pH with 5N HCl or 2N NaOH.

## Results and Discussion

**Preparation of copolypeptides.** As the polymerization reaction proceeded, extremely fibrous masses began to appear as products, and it took 1 to 4 days for 95% of the polymerization reaction to be completed, depending on the polymer composition. It was found that the reaction rate was greater in the system with greater content of L-glutamic acid residue (data not shown). The amount of the methyl ester group remaining after saponification has not been determined, but $^1$H-NMR revealed that a substantial portion was removed by the deesterification process. Table 1 summarizes the physical properties of the polymers. As expected, the potassium salt of the polymer showed good solubility in aqueous phase and the polymers with higher content of leucine residue (the more hydrophobic component) were shown to have poorer solubility in water than those with higher content of glutamic acid residue (the more hydrophilic component).

The polymers, glutamic acid/leucine = 70/30 and 50/50 were shown to have the highest intrinsic viscosities when compared to the others, as determined by extrapolating the reduced viscosity *vs* polymer concentration (Figure 2).

Since the purpose of this study is to prepare monolithic device for a promising controlled delivery system, the size distribution and amino acid composition of the products were not characterized. However, Waley *et al.*[18] reported that the size distribution of copolymer would be extremely sharp if it was synthesized by NCA method. Moreover, Becker *et al.*[6] revealed that the polypeptide preparations obtained by this method contained practically no free amino acid, suggesting that almost all of the amino acid monomers should have been incorporated into the polypeptide chains and, therefore, the variation in the amino acid composition between the monomer feed and the copolypeptide prepared by NCA procedure might

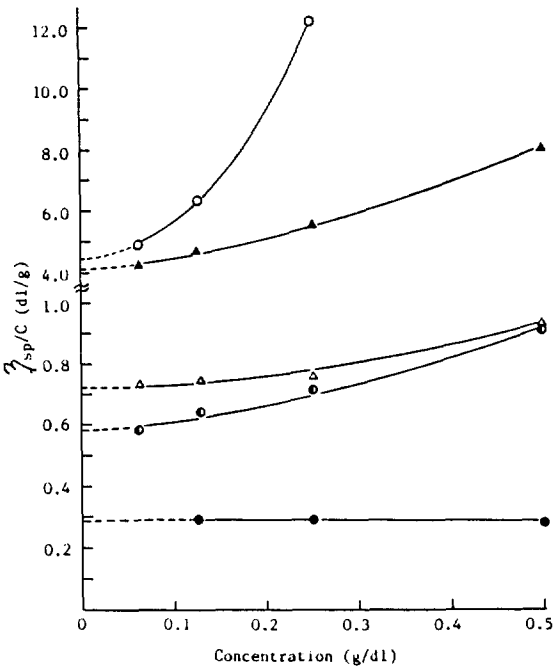


**Figure 2.** Reduced vicosity of copolypeptides. The viscosity was measured with Ubbelohode viscometer (capillary diameter; 0.3 mm) at 20°C. △—△;100:0 (glutamic acid: leucine), ▲—▲; 70:30, ○—○; 50:50, ◐—◐; 20:80, ●—●; 10:90.

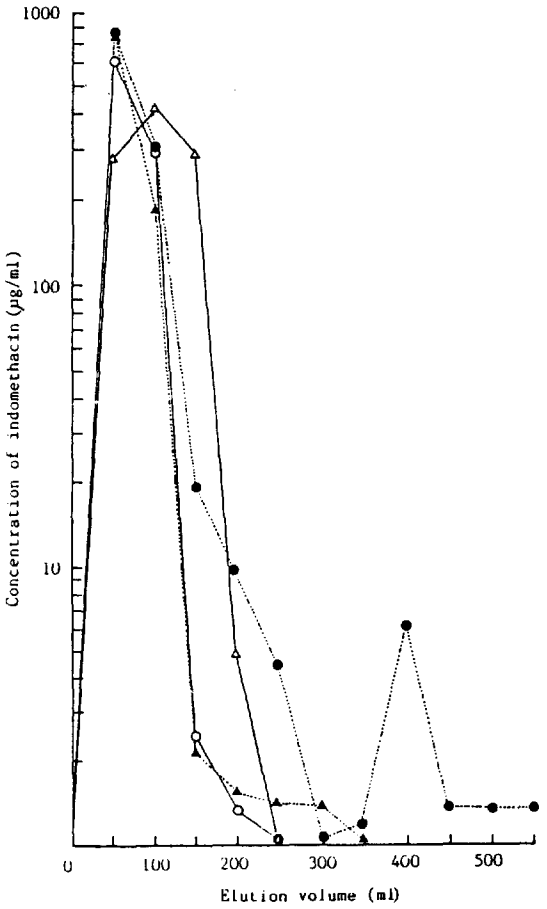


**Figure 3.** *In vitro* release of indomethacin from monolithic device during burst period in continuous elution system. △—△100:0 glutamic acid: leucine, ▲—▲; 70:30, ○—○; 50:50, ●—●; 10:90.

be insignificantly small.

**Release studies.** Indomethacin was eluted by PECF (pH 7.4) from the glass column packed with monolithic device. The content of indomethacin in the eluate was determined by HPLC analysis. The daily release rate was calculated by normalizing the apparent release rate against elution volume to compensate for the fluctuations in release rate due to the variation in the flow rate of the eluent. As shown in Figure 3, high degree of burst effect was noticed in all cases. Most of the systems reached steady-state of release in 10 days. Once the system reached the steady-state, the amount of indomethacin released from the device was almost constant, while the average release rate varied linearly according to the amino acid composition of the monolithic device (Figure 4). Monolithic devices with higher content of glutamic acid residue released indomethacin faster than those with higher content of leucine residue. However, the device with the highest content of leucine residue (glutamic acid: leucine = 10:90) released the drug at the highest rate over the longest period of time during the steady-state period. Moreover, the life span of the polymer matrix was much longer in the system with higher content of leucine residue (data not shown). Such tendencies became more obvious in batch elution system (Figure 5). These observations obviously reflect the findings that the release rate in aqueous system of a drug from a polymer matrix is greatly influenced by the hydrophilicity of the polymer which is, in turn, determined largely by the nature of the constituent monomers and their composition in the polymer.[19-21] Since it is essential for a drug delivery system that it should reach a steady-state of release as soon as possible, and that it should release fairly constant amount of drug over desired time period, the copolypeptide, glutamic acid/leucine = 50/50 was thought to be the most promising as the polymeric support for the delivery of indomethacin, which requires relatively long-term administration in the clinical therapy of pertinent diseases (Figures 4 and 5). The average concentration of indomethacin released from this system during the steady-state was calculated to be 0.09 μg/ml (Figure 4), yielding an average release rate of 18.5 μg/day for 1 g of the monolithic device (average flow rate of PECF = 51.4 ml/day).

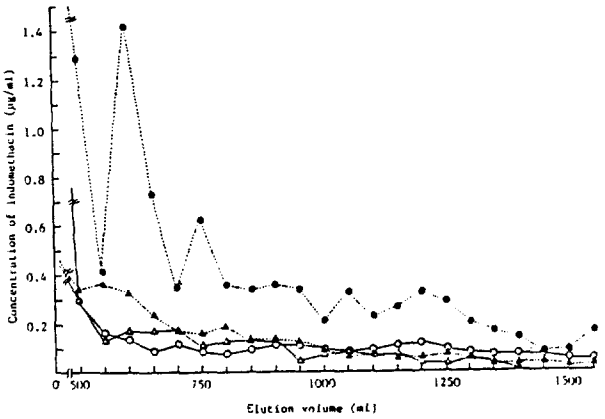


**Figure 4.** *In vitro* release of indomethacin from monolithic device after burst period. Δ---Δ; 100:0 glutamic acid: leucine, ▲—▲; 70:30, O---O; 50:50, ●---● 10:90.

Since the conformation of a polypeptide is greatly influenced by the environmental pH, it is very interesting to study the effect of pH on the release rate of indomethacin. The monolithic device of glutamic acid/leucine = 50/50 was chosen for this study. The release rate of indomethacin was studied at three differet pH conditions and the results are given in Figure 6. Great burst effects were observed at higher pH conditions, while no significant burst effect was detectable at pH 3.0. At pH 11.0, the overall amount of indomethacin released was quite small and the system was shut off much faster. As

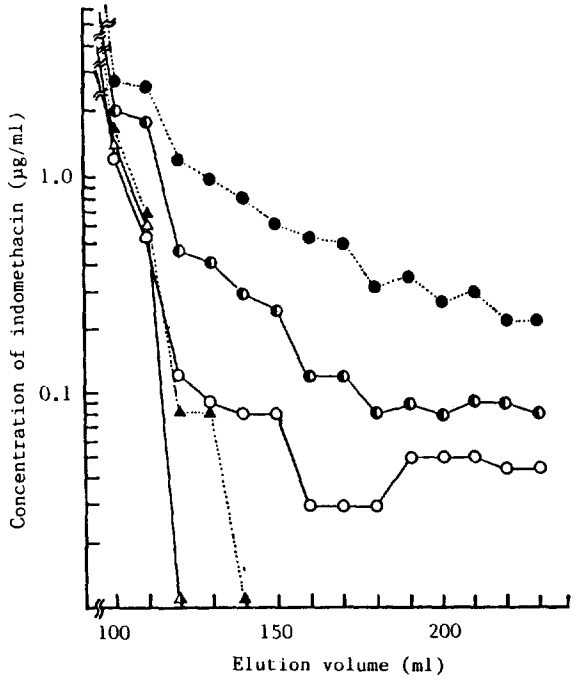


**Figure 5.** *In vitro* release of indomethacin from monolithic device after burst period in batch elution system. Δ—Δ; 100:0 glutamic acid: leucine, ▲—▲; 70:30, O—O; 50:50 ◐—◐; 20:80 ●—●; 10:90.

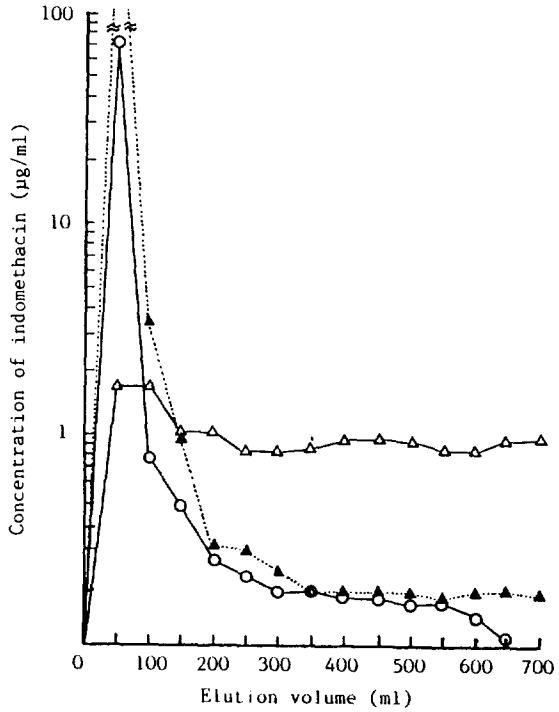


**Figure 6.** Effect of pH on the release pattern of indomethacin. Δ—Δ; pH 3.0, ▲---▲; pH 7.4, O—O; pH 11.0.

a whole, the time needed for each system to be exhausted seemed to be shorter at higher pH condition, while the daily release rate of indomethacin, after the burst period, increased with the decrease of pH. There might be several explanations for these observations. Firstly, the conformation of the copolypeptide molecules at different pH conditions possibly affected the release pattern. It is well known that acidic homopolypeptide assumes $\alpha$-helical conformation at low pH conditions, while it is unfolded to form a random coil at high pH conditions.[22] The rather compact $\alpha$-helix portion of the copolypeptide may have formed a diffusion barrier against the solute or solvent molecules at lower pH conditions, thus causing the solute to be released more slowly. Secondly, the solubility property of indomethacin would also have acted as a factor affecting the release pattern of the drug. Indomethacin, being an acidic compound, shows poor solubility at low pH conditions, but its solubility increases with the increase of pH.[23] Therefore, indomethacin might be eluted more slowly at pH 3.0 without any significant burst effect, while the drug being exhausted relatively faster at pH 11.0. Thirdly, indomethacin is known to be unstable at alkaline pH conditions.[24,25] At pH 11.0, the drug seems to be destroyed or transformed to a form which shows much reduced absorbance at 254 nm. This should be a major reason for the small amount of indomethacin released at pH 11.0.

In conclusion, the monolithic copolypeptide device of glutamic acid/leucine = 50/50 appeared to be the best system for delivery of indomethacin and the release pattern of this system was greatly affected by environmental pH.

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