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Microcrystallization of indomethacin using a pH-shift method

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Abstract

This study developed a microcrystallization process for indomethacin, a nonsteroidal anti-inflammatory drug (NSAID), using a pH-shift method in aqueous solution. The physicochemical properties of the microcrystals produced were similar to those of the standard crystalline powder in X-ray diffraction (XRD), differential scanning calorimetry (DSC), and Fourier transform infrared spectroscopy (FT-IR) analyses, except for a lower XRD peak height and a slightly lower melting temperature (T_m) (1.5 °C). Phase contrast microscopy and scanning electron microscopy (SEM) showed that the indomethacin microcrystals were plate-like with a uniform size distribution (mean diameter $= 10.4 \pm 0.4\,\mu m$). In the initial phase, the dissolution rate of the indomethacin microcrystals was about 2.2 times higher than that of the standard crystalline powder. The biological activity of the indomethacin microcrystals was about 20% higher than that of the standard crystalline powder in their ability to inhibit the proliferation of colon cancer cells (HT-29).
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Keywords: Microcrystallization; Indomethacin; pH-shift; HT-29

1. Introduction

Many drugs are poorly soluble or insoluble in water, which results in poor bioavailability because the solubility of a drug is an important factor in determining the rate and extent of its absorption (Orienti et al., 2002). Hence, improving this property is of great importance in developing pharmaceuticals.

The rate-limiting step in the adsorption of hydrophobic drugs is usually the dissolution of the drug. One way to improve the dissolution rate is to reduce particle size, which increases the total surface area (Sarkari et al., 2002). Several methods of reducing particle size have been suggested. There are physical methods such as milling and grinding (Gibson, 2001), but it is difficult to obtain uniform fine particles and control the dose of the drug. To overcome these problems, hydrophobic drugs can be microcrystallized. Most of the crystallization processes that have been developed use organic solvents to crystallize hydrophobic drugs (Ying et al., 2001). However, the residual organic solvent remains a problem, even after evaporation. Therefore, in this study, an aqueous solution was used for crystallization instead of an organic solvent, and supersaturation was induced via a pH-shift to make use of pH-dependent solubility.

Indomethacin, 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid, is a nonsteroidal anti-inflammatory drug (NSAID) that is hydrophobic and shows pH-dependent solubility. NSAIDs such as indomethacin are used in the treatment of rheumatoid arthritis (Gabr, 1997), and work by blocking cyclooxygenase (COX 1 and 2) (Dannhardt and Kiefer,

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2001). Recently, this drug has been cited as a possible chemotherapeutic agent for treating colorectal cancer (Thun, 1994). Both epidemiological studies and animal models of colon carcinogenesis have indicated that NSAIDs have anti-colorectal cancer activity, although the precise mechanisms of this anti-neoplastic effect remain unclear (Smith et al., 2000), despite many studies evaluating this activity (Kubba, 1999). Therefore, this study also compared the biological activity of indomethacin microcrystals with standard crystalline powder in inhibiting the proliferation of colorectal cancer cells.

2. Materials and methods

2.1. Materials

Indomethacin crystalline powder and zinc acetate were purchased from Sigma (St. Louis, MO, USA). Cellulose nitrate filter (0.2 μm pore size) was purchased from Whatman Co. (Maidstone, England) and all other chemicals were analytical grade. A peristaltic pump was purchased from EYELA (Tokyo, Japan).

2.2. Microcrystallization of indomethacin

One gram of indomethacin was dissolved in 200 ml of double distilled water by adding 3 ml of 0.5 N NaOH. The aqueous suspension was filtered through 0.2 μm cellulose nitrate filter to prepared the saturated indomethacin solution. One milliliter of Zn-acetate (2.5 mg/ml) was added to adjust pH of the solution to about 7.3. The pH of the solution was increased up to about pH 8.3 by adding 0.5 N NaOH solution. Then, 0.5 N HCl was gradually added by a peristaltic pump at a given flow rate by pH 6. The supersaturated solution was stored at 20 °C for 24 h to form crystal. The indomethacin microcrystals suspension was recovered by filtration through 0.2 μm cellulose nitrate filter. Then, filter cake was dried at room temperature.

2.3. Morphology of indomethacin microcrystals

Indomethacin microcrystals produced, standard crystalline powder and various crystals were observed by a phase contrast microscope (400×, Olympus CK-2, Tokyo, Japan). Morphology of indomethacin microcrystals was observed by a scanning electron microscope (SEM, Hitachi S-450, Japan). Air dried microcrystals were mounted onto metal stubs using double-sided adhesive tape, vacuum-coated with gold and directly analyzed under SEM (8000×).

2.4. Particle size distribution

The size distribution of microcrystals, standard crystalline powder and homogenized crystals homogenized by sonifier (Sonifier 450, Branson, USA) at various processing times (10, 20 and 30 min) was measured with a laser diffraction particle size analyzer (CILAS 1064, CILAS, France).

2.5. Analysis of physicochemical properties

2.5.1. X-ray diffractometry (XRD)

X-ray diffraction patterns of indomethacin microcrystals and standard crystalline powder were investigated using X-ray diffractometer (Philips XPERT MPD, Philips Analytical X-ray BV, The Netherlands) over the interval 5–30 °C/2θ. The profiles of two forms were compared.

2.5.2. Differential scanning calorimetry (DSC)

Differential scanning calorimeter (Suiko Instrument, DSC 6100, Chiba, Japan), equipped with a refrigerated cooling accessory using liquid nitrogen, was used to measure the melting temperature (T_m) of indomethacin microcrystals and standard crystalline powder at a heating rate of 5 °C/min, from 40 to 200 cm^{-1}. Temperature value was calibrated with indium (T_m = 156.6 °C). An empty pan was used as the reference.

2.5.3. Fourier transform infrared spectroscopy (FT-IR)

Microcrystals and standard crystalline powder were detected by FT-IR microscope spectrometer (FT/IR-430 Jasco, Japan) at range from 4000 to 400 cm^{-1} using the KBr disk method (Gibson, 2001).

2.6. Dissolution test

Two milligram of indomethacin standard crystalline powder and microcrystal samples were placed on a 0.2 μm pore microcentrifugal filter tube (Omron,

Japan). Then, 0.5 ml of phosphate-buffered saline (PBS) (pH 7.4), saline or double-distilled water (ddH_2O) was added into the tube and shaken for a given period of time in a 37 °C incubator. After dissolution, the solution was collected by centrifugation. The supernatant was sampled and equal volume of fresh medium was added. The concentration of indomethacin supernatant was determined by measuring the absorbance at 319 nm (Lim et al., 2001) using spectrophotometer (Shimadzu, Japan). The percent dissolution was determined by accumulating the amount of indomethacin dissolved up to the time period measured.

2.7. HT-29 cell culture

Colorectal cancer cells (HT-29), purchased from Korean Cell Line Bank (K30038), were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) (Gibco BRL, NY, USA). Cultures were incubated at 37 °C under a humidfied atmosphere of 95% air and 5% CO_2.

2.8. Biological activity of indomethacin

The viability of HT-29 cells was determined by a methyl thiazol tetrazolium (MTT) bromide assay

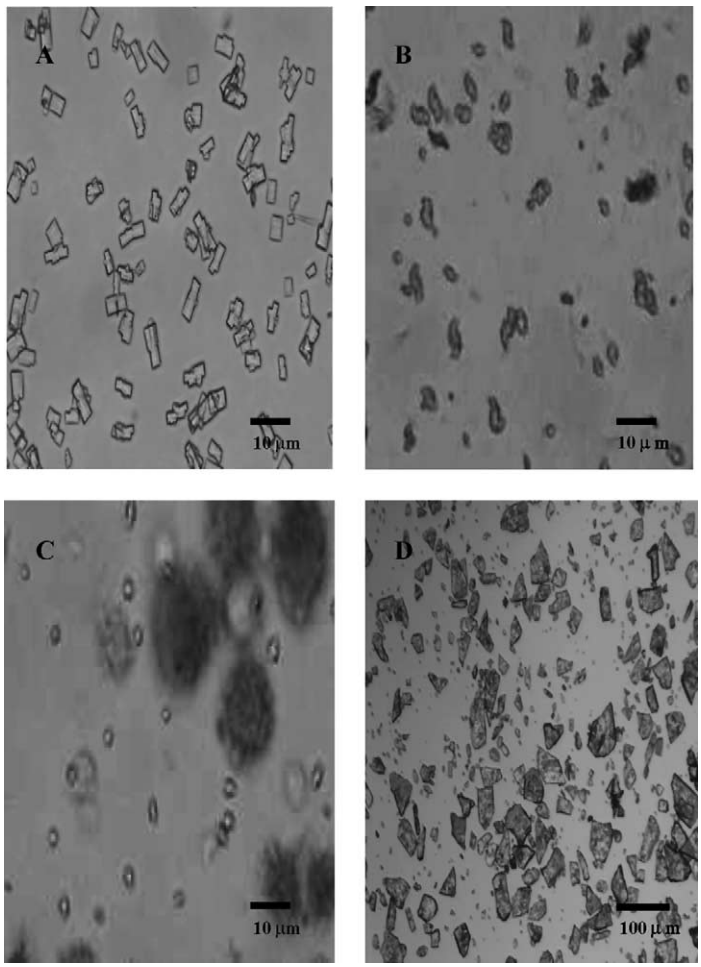

Fig. 1. Polymorphism of indomethacin crystals. Microcrystals (A–C) and standard crystalline powder (D) were observed by phase contrast microscope (original magnitude; A–C (400×) and D (40×)). In the microcrystallization, the rate of pH-shift was (A) 0.25, (B) 0.68 and (C) 1.04 ml/min.

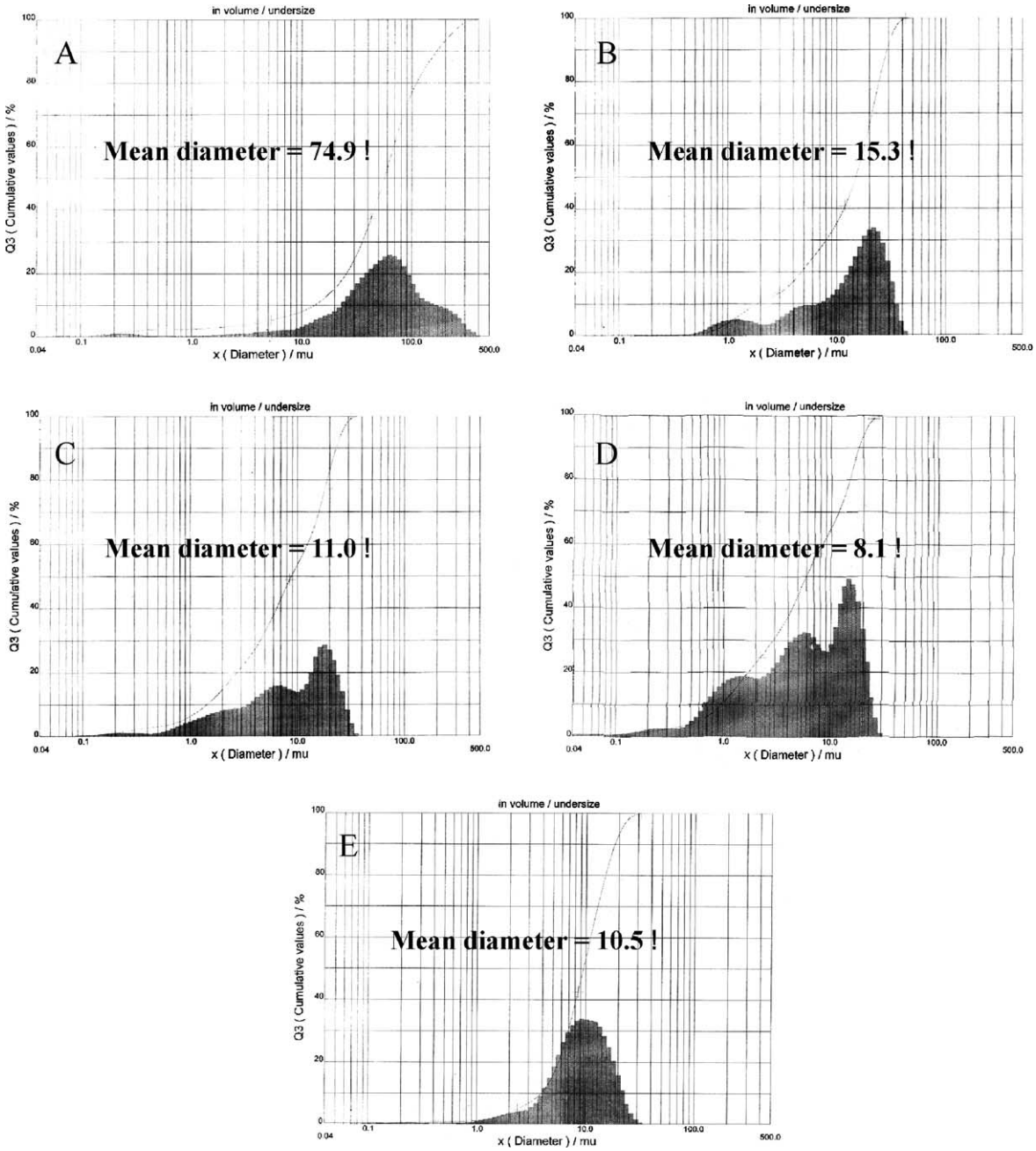

Fig. 2. Size distribution of standard crystalline powder, homogenized crystals and microcrystals: (A) standard crystalline powder; (B) homogenized crystals (10 min); (C) homogenized crystals (20 min); (D) homogenized crystals (30 min); and (E) microcrystals.

to measure the biological activity of indomethacin microcrystals and standard crystalline powder. Into 24-well plates 1×10^5 cells were seeded and cultured for 48 h, and the test medium containing indomethacin microcrystals and standard crystalline powder (100, 200, 400 and 800 μM) was added. After completion of incubation, 110 μl of 2 mg/ml MTT stock in phosphate-buffered saline was added to each well, and plate was incubated at 37 °C for 48 h. The water insoluble MTT–formazan crystals were dissolved in 0.05 N HCl in isopropanol. Absorbance of converted dye was measured in an ELISA plate reader at a wavelength of 595 nm.

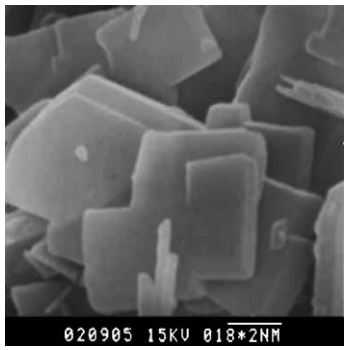

Fig. 3. The observation of indomethacin microcrystals with scanning electron microscope (SEM). Microcrystals were observed at various magnification (8000×). The indomethacin microcrystals were produced at 0.25 ml/min of pH-shift rate.

3. Results and discussion

3.1. Microcrystallization of indomethacin

The solubility of indomethacin in water depends on the pH (Budavari, 1996). With increasing pH, the solubility of indomethacin increased dramatically (data not shown). Consequently, indomethacin solution can be supersaturated easily by lowering the pH (Ducruix and Giege, 1999). Details of the rate of supersaturation, the driving force for nucleation and crystal growth, can be predicted from the solubility profile. The supersaturation value (β), the ratio of the actual and saturation concentrations (Ducruix and Giege, 1999), of indomethacin is about 2 when the pH is lowered from 8.3 to 6. As shown in Fig. 1, the resulting crystals

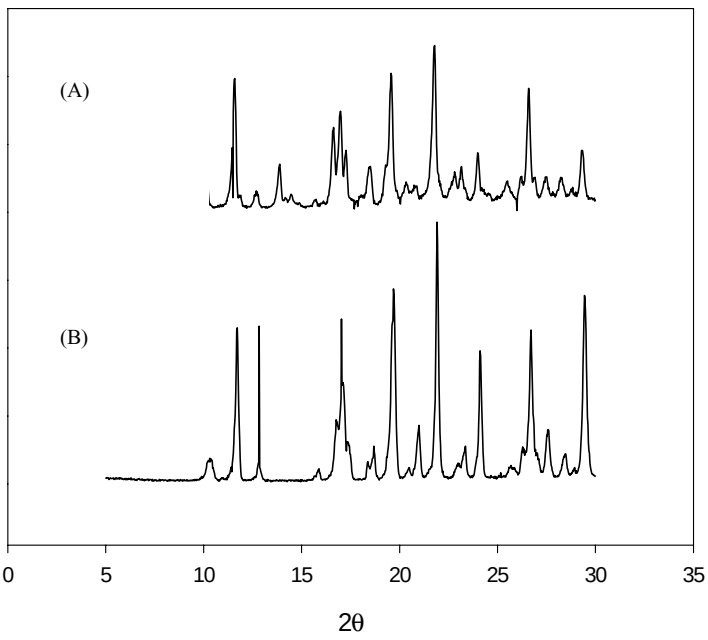

Fig. 4. X-ray diffractograms (XRD). Indomethacin microcrystals (A) and standard crystalline powder (B) were compared. The peaks were measured over the interval 5–30 °C/2θ.

have different shapes depending on the rate of the decrease in pH, even at the same β-value. At a flow rate of 0.25 ml/min, tetragonal crystals were formed (Fig. 1A), whereas the crystals formed clumps at flow rates of 0.68 and 1.04 ml/min (Fig. 1B and C). The greater the rate of pH decrease, the more clumped or needle-like the crystals became. Crystallization at 0.25 ml/min was optimal and the yield under optimal conditions was about 30%. This relatively low yield is not a problem because the indomethacin remaining in solution can be reused.

3.2. Size distribution of microcrystals

The size distributions of standard indomethacin crystalline powder, homogenized crystals, and microcrystals are shown in Fig. 2. The crystals were the largest in standard indomethacin crystalline powder. The mean diameter was 74.9 μm and the size distribution of the particles was broad. For crystals homogenized using an ultrasonic homogenizer, the size of the particles decreased with increasing homogenization (Fig. 2B–D). Nevertheless, although the average particle size was reduced, the size distribution remained broad, which might make it difficult to control the drug dosage using these crystals. By contrast, the size distribution of the microcrystals was narrow and uniform, and the crystals were uniformly shaped (tetragonal, Fig. 1A) with a mean diameter of 10.5 μm (Fig. 2E). The indomethacin microcrystals were also observed using scanning electron microscopy (8000×). As shown in Fig. 3, the microcrystals were plate-like and about 10 μm in size.

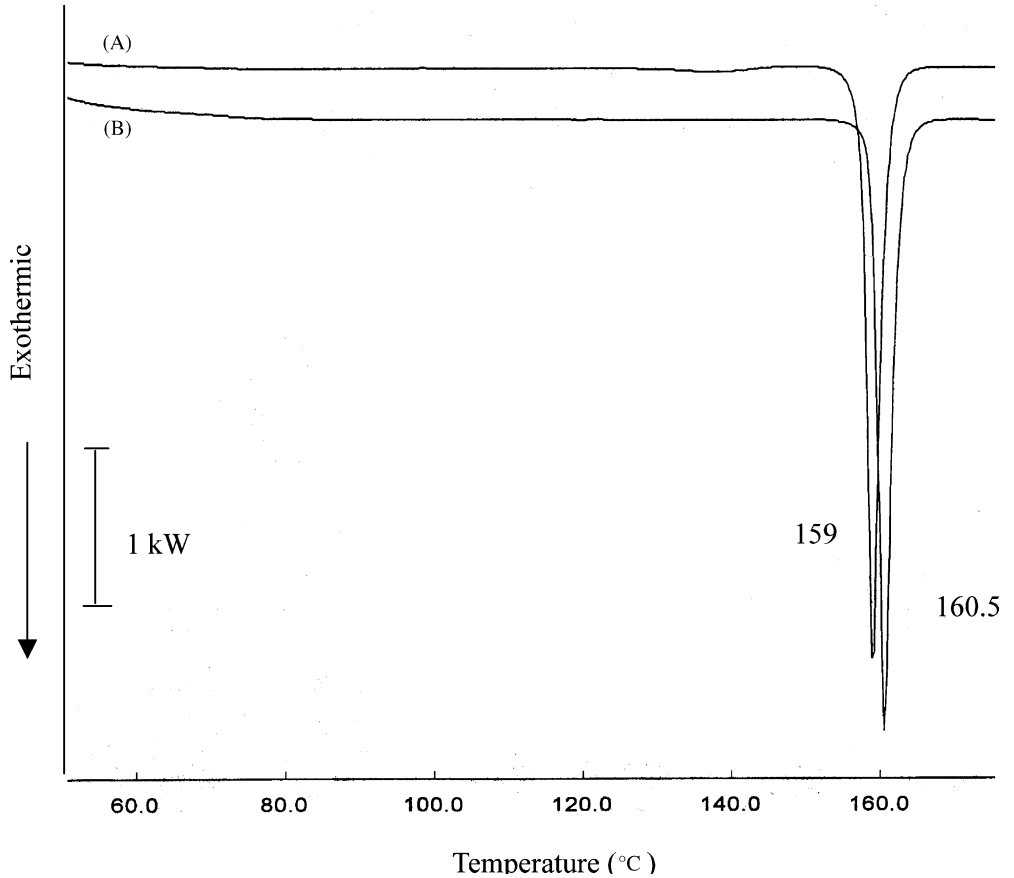

Fig. 5. Differential scanning calorimeter (DSC) thermograms. Indomethacin microcrystals (A) and standard crystalline powder (B) were compared. DSC curves were measured at a heating rate of 5 °C/min, from 40 to 200 °C. Each value of melting temperature was indicated.

3.3. Analysis of physicochemical properties

3.3.1. X-ray diffraction studies

As shown in Fig. 4, the XRD pattern of the indomethacin microcrystals was very similar to that of standard crystalline powder. The peak position was similar, although the peaks for the standard indomethacin crystalline powder were higher than those of the indomethacin microcrystals (Fig. 4A). The peak height is affected by crystal size and crystallinity (Gibson, 2001). Therefore, the peak height of the microcrystals might be slightly smaller than that of the standard crystalline powder because the microcrystals were about seven times smaller. Moreover,

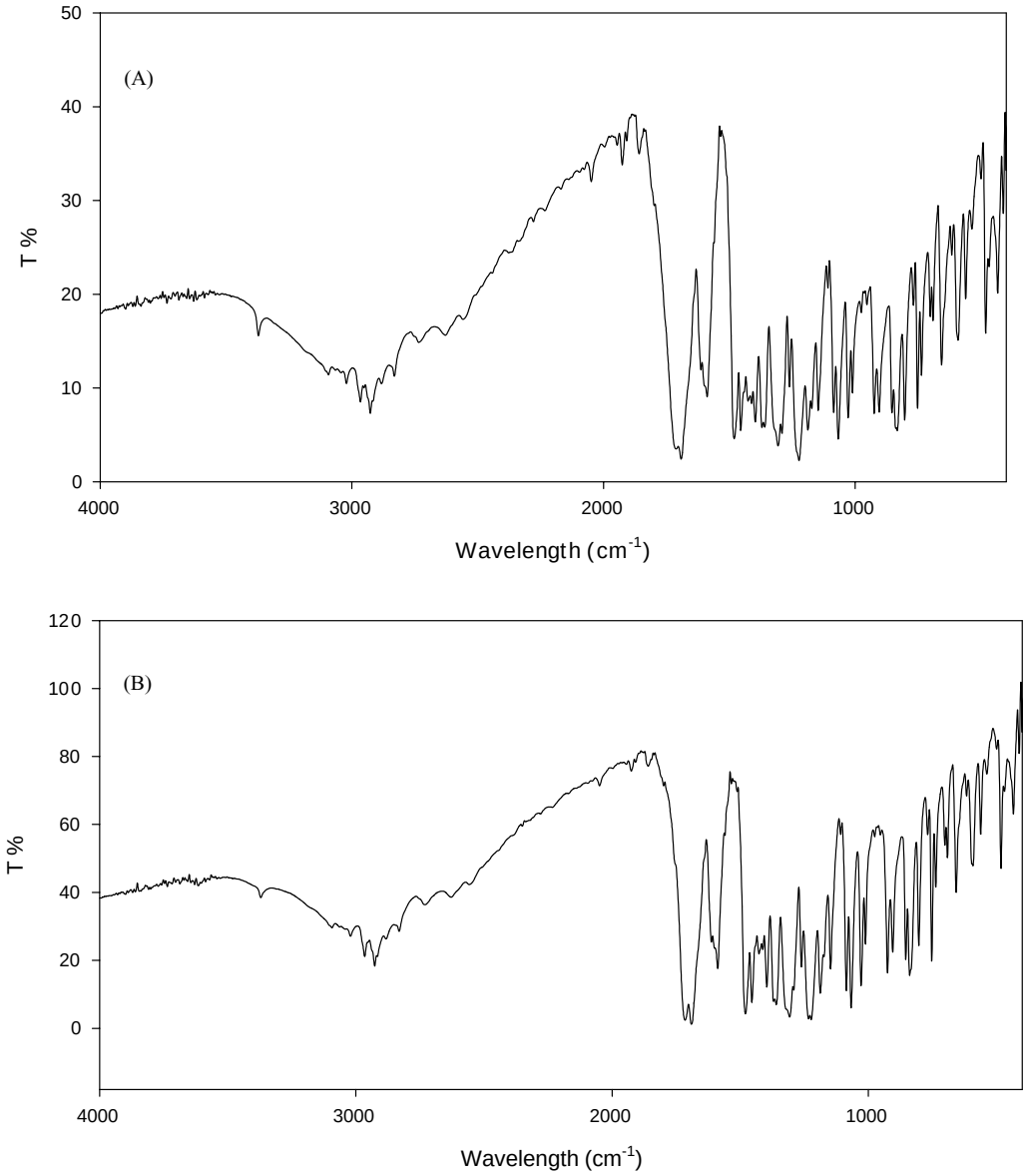

Fig. 6. Fourier transform infrared spectroscopy (FT-IR) profiles. Standard crystalline powder (A) and microcrystals (B) were measured, ranging from 4000 to 400 cm^{-1}.

the crystallinity of microcrystals produced using the pH-shift method might be slightly lower than that of the standard crystalline powder.

3.3.2. Differential scanning calorimetry

The melting temperature of the indomethacin crystals was measured using differential scanning calorimetry. Both the microcrystals and standard crystalline powder showed a single exothermic peak (Fig. 5). The T_m was 159 °C for the microcrystals and 160.5 °C for the standard crystalline powder. This agrees with the result of the X-ray diffraction study. The slightly lower T_m suggests that the microcrystals are not perfect crystals, although they are somewhat similar to the γ-form of indomethacin (Singla and Wadhwa, 1995).

3.3.3. Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy was used to analyze the microcrystals and standard crystalline powder in detail, to characterize the drug. The spectra of the two forms are shown in Fig. 6. The standard crystalline powder and microcrystals had similar patterns in the region of C=O absorption around 1700 cm^{-1}. Similarly, the COOH peaks at 3300 cm^{-1} were not shifted (Fessenden et al., 1998). The microcrystals produced in this study were almost identical to the crystals produced by other methods.

3.4. Dissolution studies

As shown in Fig. 7, the percent dissolution of standard indomethacin crystalline powder and the microcrystals in double-distilled water, saline, and PBS (pH 7.4) were determined. The dissolution of indomethacin crystals was higher in PBS (pH 7.4) than in ddH_2O or saline; indomethacin is more soluble in basic solutions than in acidic solutions.

In the initial dissolution profile, the dissolution of the microcrystals was 2.2 times greater than that of

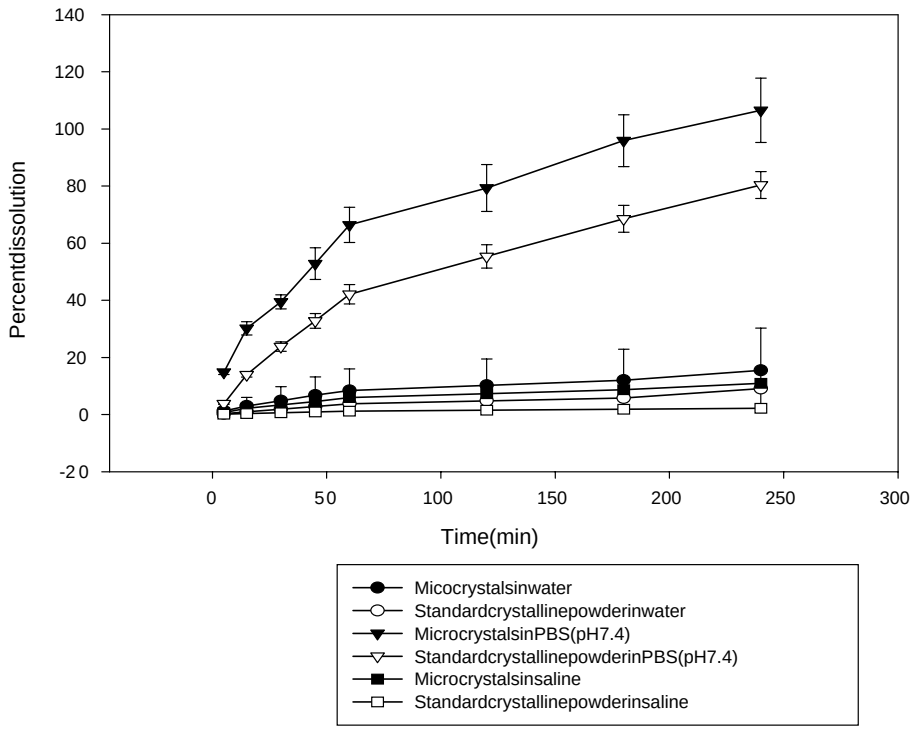

Fig. 7. Dissolution profiles. Microrystals and standard crystalline powder are dissolved in double distilled water, PBS (pH 7.4) or saline on a 0.2 μm filter tube in a rotating shaking incubator for 4 h. The percent dissolution is the percent dissolved fraction of the total indomethacin added (4 mg/ml).

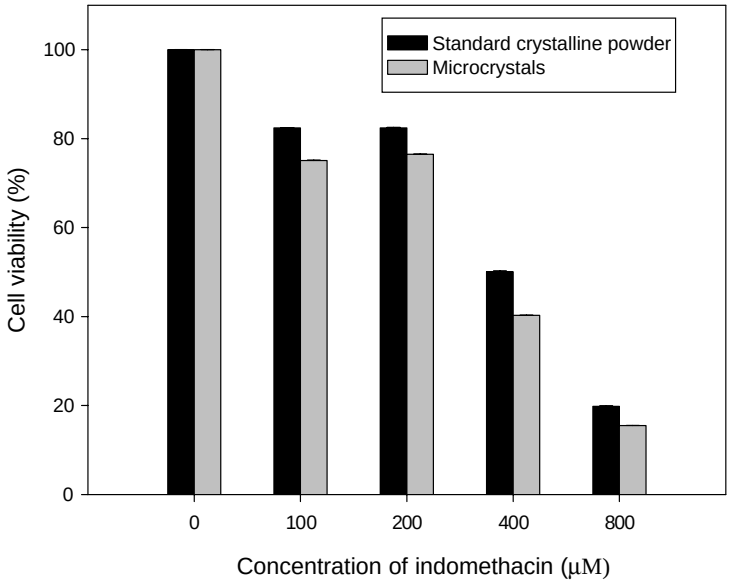

Fig. 8. Inhibition of HT-29 cell proliferation. HT-29 cells were incubated with either indomethacin microcrystals or standard crystalline powder at various concentrations (100, 200, 400 and 800 μM). The cell viability was determined by the MTT assay after 2 days of incubation with indomethacin.

the standard crystalline powder at 15 min in PBS. The major factor that determines the dissolution rate is the solubility of a compound in aqueous solution; however, other factors can affect the rate, such as particle size, crystalline state, pH, and buffer concentration (Gibson, 2001). A reduction in particle size is an important factor because a reduction in particle size increases the surface-to-volume ratio. Since the microcrystals were smaller than the crystalline powder (mean diameter 10.5 μm versus 74.9 μm), they dissolved better than the standard crystalline powder, as shown in Fig. 7. In addition, the difference in the crystalline state may affect the dissolution rate (Longuemard et al., 1998) as shown in Fig. 7. The crystalline state of the microcrystals and standard crystalline powder differed slightly as shown in the differences in T_m and peak height on XRD.

3.5. Biological activity study

To compare the compounds' biological activity, colon cancer cells (HT-29) were cultured in media containing different concentrations of 100, 200, 400 and 800 μM of indomethacin using either indomethacin standard crystalline powder or microcrystals.

With the microcrystals, cell viability decreased by about 20% and they were more effective than the standard crystalline powder, as shown in Fig. 8. At concentrations over 400 μM, indomethacin dose-dependently inhibited the proliferation of HT-29 cells. In comparison with the control, cell viability decreased by about 50% at 400 μM. At the same concentration, the cell viability was about 20% lower with the microcrystals than with the standard crystalline powder. The results imply that both the standard crystalline powder and microcrystals have dose-dependent anti-colon cancer activity, and that the microcrystals are more effective than the standard crystalline powder. This clearly has implications for improving the efficiency of chemotherapy in treating patients with malignant neoplasms.

4. Conclusions

A process of producing indomethacin microcrystals was developed using a pH-shift method. The

microcrystals were plate-like and about 10 μm in diameter. In XRD, DSC, and FT-IR analyses, the physicochemical properties of the microcrystals were similar to those of standard crystalline powder, except for lower XRD peaks and a 1.5 °C lower T_m. In the dissolution experiment, the microcrystals dissolved about twice as well as the standard crystalline powder. In the biological activity test, the anti-colon cancer cell activity of the microcrystals was about 20% greater than that of the standard crystalline powder.

References

Budavari, S., 1996. The Merk Index, 20th ed. Merk & Co. Inc., NJ, USA.

Dannhardt, G., Kiefer, W., 2001. Cyclooxygenase inhibitor-current status and future prospects. Eur. J. Med. Chem. 36, 109–126.

Ducruix, A., Giege, R., 1999. Crystallization of Nucleic Acids and Proteins. Oxford University Press, New York.

Fessenden, R.J., Fessenden, J.S., Logue, M.W., 1998. Session 9.4. The Infrared Spectrum, Organic Chemistry—Fessenden Fessenden, sixth ed. Brooks/Cole Publishing Company, New York.

Gabr, K.E., 1997. Influence of indomethacin-mastic combinations on dissolution, absorption and gastrointestinal mucosal damage in rats. Int. J. Pharm. 158, 137–145.

Gibson, M., 2001. Pharmaceutical Preformulation and Formulation—A Practical Guide from Candidate Drug Selection to Commercial Dosage Form. HIS Health Group, Englewood.

Kubba, A.K., 1999. Nonsteroidal anti-inflammatory drugs and colorectal cancer: is there a way forward? Eur. J. Cancer 35, 892–901.

Lim, S., Lee, K.Y., Lee, M.S., Choi, C.N., Kim, Y.D., 2001. Controlled release of indomethacin using biodegradable polymer microspheres. Korean J. Biotech. Bioeng. 16, 505–510.

Longuemard, P., Jbilou, M., Guyot-Hermann, A.M., Guyot, J.C., 1998. Ground and native crystals: comparison of compression capacity and dissolution rate. Int. J. Pharm. 170, 51–61.

Orienti, I., Bigucci, F., Luppi, B., Cerchiara, T., Zuccari, G., Giunchedi, P., Zecchi, V., 2002. Polyvinylalcohol substituted with triethyleneglycolmonoethylether as a new material for preparation of solid dispersion of hydrophobic drugs. Eur. J. Pharm. Biopharm. 54, 229–233.

Sarkari, M., Brown, J., Chen, X., Swinnea, S., William, R.O., Johnston, K.P., 2002. Enhanced drug dissolution using evaporative precipitation into aqueous solution. Int. J. Pharm. 243, 17–31.

Singla, A.K., Wadhwa, H., 1995. Zinc-indoemthacin complex: synthesis, physicochemical and biological evaluation in the rat. Int. J. Pharm. 120, 145–155.

Smith, M.L., Hawcroft, G., Hull, M.A., 2000. The effect of non-steroidal anti-inflammatory drugs on human colorectal cancer cells: evidence of different mechanisms of action. Eur. J. Cancer 36, 664–674.

Thun, M.J., 1994. Aspirin, NSAIDs and digestive tract cancers. Cancer Metastasis Rev. 13, 269–277.

Ying, R.M., Turner, P., Kennedy, B.J., Hambley, T.W., Lay, P.A., Briffin, J.R., Regtop, H.L., Waraick, B., 2001. Preparation and characterization of dinuclear copper-indomethacin anti-inflammatorydrugs. Inorg. Chem. Acta 324, 150–161.