A crystalline acrylic-type polymerizable monomer of diclofenac (DF) was synthesized by linking DF molecule to 2-hydroxyethyl acrylate (HEA). Then, the monomer was polymerized in solid state by vibratory milling with plasma-irradiated lactose powders as postprocessing-free initiators. The characterization of the resulting polymeric prodrug powders by ESR and XRPD analysis confirmed their synthesis successfully. Hydrolysis study of the polymeric prodrugs was carried out in three different buffer solutions (pH 1.2, 6.8 and 8.5) at 37°C. The in vitro release profiles indicated that drug release from the polymeric prodrugs was in sustained manner and highly dependent on pH, in which ca. 9%, 25% and 48% of DF was released in 12 h at pH 1.2, pH 6.8 and pH 8.5, respectively. The results suggested that the studied polymeric prodrug systems in the present investigation can be applied to make a formulation for clinical administration of other drugs as carriers in controlled drug release.

**Keywords:** Plasma irradiation, Drug delivery system, Diclofenac, Polymeric prodrugs

1. Introduction

The development of a sustained- and controlled-release system for drug delivery is one of the most active areas in drug research. Among the various formulation techniques, the prodrug approach has been generally appreciated as one of the most useful means to minimize the undesirable drug properties such as limited bioavailability, lack of site specificity and chemical instability, while retaining the desirable therapeutic activity. Particularly, the polymeric prodrug, a chemical approach using reversible polymeric derivatives, can be useful in the improvement of the delivery to appropriate targets and the optimization of the clinical application of various drugs.

Actually, a number of studies of polymeric prodrugs which have been attached variety of drugs onto polymeric materials to impart desirable characteristics have been reported [1-3].

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed drugs worldwide that are generally used to treat pain, fever and inflammation [4]. Diclofenac (DF) is a kind of NSAIDs of the phenylacetic acid derivatives with anti-inflammatory, analgesic, and antipyretic properties. DF, however, is well known to cause serious gastrointestinal damage, like other NSAIDs. Long-term NSAIDs therapy has been associated with an increased risk of gastric erosion and bleeding [5]. It has been estimated that about 60% of all patients chronically treated with NSAIDs present dyspeptic symptoms and around 30% have gastroduodenal ulcers [6,7]. Recently, the discovery of new drugs to treat chronic inflammation...
without adverse effects is one of the major challenges to pharmaceutical industry.

Prodrugs can be utilized for a variety of purposes, including improvement of the bioavailability or pharmacokinetics of a drug, decreasing drug toxicity, facilitating administration of the drug, or delivering the drug effectively to specific cells or tissues [8]. By use of the prodrug approach for NSAIDs, one strategy that could be useful is to temporarily mask the carboxylic acid function of the NSAIDs for depressing the gastric toxicity.

Over the years, we have been working on the development of controlled-drug release systems by using plasma-assisted method, which was applicable to oral, topical and injectable formulations [9-16]. As part of our ongoing program to develop the polymeric prodrugs of NSAIDs with reduced gastrointestinal side-effects, herein we report a novel DF, one of the most commonly used NSAIDs, delivery system based on a polymeric prodrug by using plasma technique. It was proposed to synthesize a polymeric prodrug of DF for oral administration by coupling the drug to 2-hydroxyethyl acrylate (HEA) by ester linkages and polymerizing the resultant product to get polymeric prodrug. The prodrug should undergo hydrolysis of its ester linkages to cleave the parent drug in a sustained manner which may result in maintaining the plasma drug level within the therapeutic range. Therefore, the DF release from the system via chemical hydrolysis was also evaluated in buffer solutions mimicking the conditions of upper and lower gastrointestinal tract.

2. Experimental

2.1. Materials

Lactose monohydrate and diclofenac sodium salt were purchased from Wako Pure Chemical Industry Co. (Tokyo, Japan). The powdered lactose was screened through a 200–235 mesh sieve and dried at 60 °C for over 24 h in vacuo.

Diclofenac free acid was obtained by the dissolution of the sodium salt in water followed by acidification and extraction. An aqueous solution of diclofenac sodium was acidified with hydrochloric acid solution, up to pH 3. The white precipitate was isolated and washed with water to about neutral pH and dried at 60 °C for over 24 h in vacuo.

All other materials used were of analytical grade, and procured from commercial sources.

2.2. Synthetic route for preparation of DF-HEA

We designed a hydroxyethyl acrylate derivative of DF (DF-HEA) where DF was linked to HEA via hydrolysable ester linkage. DF-HEA was synthesized by direct esterification of DF with HEA in the presence of N,N-dicyclohexylcarbodiimide (DCC) in CH₂Cl₂ solution (Scheme 1). The hydroxyl group of HEA reacted with the carboxyl group of DF and the resulting water was absorbed by DCC to produce N,N-dicyclohexylurea as a white precipitate. The white precipitate was isolated and the solvent was evaporated in vacuum. The residue was recrystallized from ethyl acetate to give DF-HEA as a white crystalline powder in 47% yield (w/w).

The structure and physicochemical properties of the newly synthesized compound were confirmed by elemental analysis, IR, ¹H NMR, mass spectral data, and chemical methods. ¹H-NMR (500 MHz, Acetone-d₆) δ = 0 ppm (TMS), δ: 3.87 (2H, s, Ph-CH₂-CO), 4.32–4.49 (4H, m, -OCH₂CH₂CO⁻), 5.85 (1H, dd, J = 10.6, 1.4 Hz, =CH₂), 6.11 (1H, dd, J = 17.2, 10.3 Hz, COCH=), 6.32 (1H, dd, J = 17.5, 1.4 Hz, =CH₂), 6.46 (1H, d, J = 8.0 Hz, Ar-H), 6.93 (2H, m, Ar-H), 7.09–7.19 (2H, m, Ar-H), 7.27 (1H, s, -NH⁻), 7.47 (2H, d, J = 8.0 Hz, Ar-H). ESI-MS m/z: ([M-H]⁻): 394 (Calcd for C₁₉H₁₇Cl₂NO₄), found ([M-23]⁻): 416. Anal. Calcd for C₁₉H₁₇Cl₂NO₄: C, 57.88; H, 4.35; Cl, 17.98; N, 3.55; O, 16.23. Found: C, 57.79; H, 4.27; N 3.50.

Scheme 1. The synthesis route of vinyl ester type derivative of diclofenac (DF-HEA).

2.3. Analytical techniques

X-ray powder diffraction (XRPD) was carried out on a Rigaku RINT Ultima III (Rigaku, Tokyo, Japan). The XRPD patterns were recorded with Cu-Kα radiation at 40 kV and 40 mA in the range of 2θ range from 3° to 40° with a step size of 0.02°/min and counting time of 1 s per step.

Measurement of melting point and enthalpy of fusion of the crystalline compounds was performed with a Rigaku Thermo Plus DSC 8230 (RIGAKU, Japan) at a heating rate of 5 °C/min on 2–4 mg samples in aluminum pans with pierced lids under a flow of dry nitrogen. An empty pan was used as reference. Heat of fusion measurements were carried out for DF and DF-HEA monomer in triplicate. The temperature axis and the cell constant were calibrated with indium (ca. 3 mg, 99.99%, peak maximum at 156.6 °C and heat of fusion 28.4 J/g).
2.4. Plasma irradiation, plasma-induced polymerization and ESR spectral measurement

Powdered samples (30 mg) were placed in a specially designed Pyrex ampule (30-mm i.d., 100-mm long) connected with a thin-walled capillary tube (2-mm i.d.) at the uppermost part of the ampule. The ampule was filled with argon gas for plasmolysis (40.0 Pa) and sealed. Then the plasma state was sustained for the prescribed period of time with agitation of samples at room temperature by a radio frequency discharge of inductive coupling at a frequency of 13.56 MHz with the supplied power of 50 W. The ESR measurements were performed while turning the ampule upside down after plasma irradiation at appropriate intervals, which is fundamentally the same procedure as that reported earlier [17-19].

Polymerization of DF-HEA was carried out in accordance with the following procedure. After 180-s plasma irradiation of lactose, the powders were applied to mechanical vibration with DF-HEA powder (50% by weight) in a Teflon twin-shell blender (7.8 mm φ, 24 mm long) (Vibrating Mill, Shimadzu Co., Ltd.) containing an agate ball (7.0 mm φ, specific gravity 2.6 g/cm³) for the prescribed period of time at room temperature under strictly anaerobic conditions, and submitted to ESR measurement. All sample manipulations were carried out in a vacuum glove box (Sanplatec Corp., Japan). After the reaction, the residual monomer was analyzed using a HPLC system. ESR spectra were recorded with a JES-FA200 spectrometer (JEOL) with X-band and 100 kHz field modulation. Care was taken to ensure that no saturation occurred and that the line shape was not distorted by excessive modulation amplitude. The square root of the microwave power versus the signal peak height was plotted and a microwave power level of 0.01 mW was chosen. The ESR spectral intensity was determined by double integration. The radical concentration (spin numbers / g) was calculated from the spectral intensities with the aid of calibrated lines obtained from the spectral intensities of poly-methyl methacrylate sample impregnated with DPPH. Measurements of g values were made relative to the fourth signal from the lower magnetic field (g = 1.981) of Mn²⁺ in MgO.

2.5. In vitro release studies

The in vitro hydrolysis behavior of polymeric prodrugs was studied in physiological conditions (aqueous phosphate or hydrochloric acid buffers (900 mL), at 37 °C ± 0.5 °C), using a dissolution test apparatus with an auto sampling apparatus (Toyama Sangyo Co., Osaka, Japan) at 100 rpm. The DF release at different time intervals was measured by a Shimadzu UV-1800 spectrometer (Shimadzu Inc., Kyoto, Japan) at 276 nm using UV system ver. 3.2 software (Toyama Sangyo Co., Osaka, Japan). It was made clear that none of the ingredients used in the formulations interfered with the assay. The release studies were conducted in triplicate, and the mean values were plotted versus time.

3. Results and Discussion

3.1. Physicochemical properties of DF-HEA powders

Solid-state properties of DF-HEA powder was evaluated using XRPD and DSC. Figure 1 shows the XRPD profiles of the powder preparations. The XRPD pattern of the DF-HEA is significantly different from that of the DF because their crystal structures are not identical.

![Fig. 1. XRPD patterns of (a) DF and (b) DF-HEA monomer.](image)

The thermal profiles of DF and DF-HEA obtained by DSC experiments are exhibited in Fig. 2. The DSC curve of DF shows a sharp endothermic peak at around 180 °C, corresponding to fusion, which close to the value obtained by Giordano [20].

![Fig. 2. DSC thermograms of (a) DF (dotted line) and (b) DF-HEA (solid line).](image)
By contrast, melting point of DF-HEA was approximately 55 °C (ΔH = –20.3 kJ/mol), or 125 degrees lower when compared to that of the pure DF, suggested that DF-HEA had a decreased thermostability relative to the pure DF.

This result implies a potential for undesired melting of the material when stressed during the process of drug manufacturing such as milling and compaction.

3.2. ESR spectra of plasma-irradiated lactose powder

The progressive changes of the room-temperature ESR spectral pattern of plasma-irradiated powdered lactose with various plasma duration are shown in Fig. 3, together with those of cellulose for comparison purposes.

![Fig. 3. Progressive changes of observed ESR spectra of plasma-irradiated (a) lactose and (b) cellulose (cited from [21]) as a function of plasma duration.](image)

It is seen that the spectral features of lactose vary from those of cellulose, but remain nearly unchanged in the course of plasma irradiation. Figure 4 shows the progressive changes in the total spectral intensities on plasma duration at room temperature which were determined by double integration. It was shown that plasma-induced radical formation in lactose is much lower than those in cellulose reported before [21]. Note that the ESR spectrum of lactose after 180-s plasma irradiation remained unchanged in both spectral shape and intensity on standing for a long period of time at room temperature, even on exposure to air. It is apparent that oxygen under atmospheric pressure cannot diffuse through the tight hydrogen-bonding network of the crystalline state of lactose as in the case of the mono- and disaccharides reported earlier [22-24].

![Fig. 4. Progressive changes in spectral intensities of (a) lactose and (b) cellulose (cited from [21]) determined by double integration on plasma-duration.](image)

3.3. Preparation of polymeric prodrug (poly DF-HEA) by solid-state polymerization

Lactose is well recognized as one of the most commonly used safe excipients in pharmaceutical oral or inhalation formulations and is not likely to constitute any significant toxicological hazard to human. Figure 5 shows the progressive changes in the ESR spectra when the radical-containing lactose plasma-irradiated for 180 s was submitted to a vibratory mixing using a Teflon vessel and agate ball with or without DF-HEA monomer at room temperature for the prescribed period of time at 60 Hz under anaerobic conditions.

![Fig. 5. Progressive changes of ESR spectra of 180-s plasma-irradiated lactose on vibratory mixing with or without DF-HEA using a Teflon vessel and agate ball under anaerobic conditions.](image)
vibratory milling due to the radical recombination reactions. In the case of vibratory milling of the lactose with DF-HEA monomer, however, it is immediately seen from Fig. 5(b) that the spectral pattern has changed to a different one, which is similar to that observed in mechanochemical polymerization of several acryloyl vinyl monomers derived from bioactive compounds. According to the earlier work [17, 25], it can be speculated that the ESR spectra can be unambiguously assigned to a single end-chain radical (equivalent to a propagating radical) of acrylic polymers.

XRPD analysis was used to compare the crystal phase of before and after vibratory-milled powder mixture of plasma-irradiated lactose and DF-HEA monomer. Figure 6 shows the XRPD patterns of lactose, physical mixture (PM) of lactose and DF-HEA in the ratio of 1:1 (w/w), and 30-min vibratory-milled DF-HEA monomer with plasma-irradiated lactose.

As shown in Fig. 6 (b), the XRPD pattern of PM contains peaks characteristic of both lactose and pristine crystalline DF-HEA. On the other hand, after 30-min vibratory milling of DF-HEA with plasma-irradiated lactose, only reflections corresponding to the lactose are slightly observed signifying the polymerization of DF-HEA.

HPLC was applied to measure the residual monomers. As the results, polymeric prodrugs that was vibratory-milled for 30 min had a very low content of unreacted monomer of less than 0.5% (data not shown). According to the amounts of residual monomers obtained, it can be concluded that the monomer conversion in the polymerization process is nearly complete.

Moreover, in the absence of plasma-irradiated lactose powder, no polymerization of DF-HEA occurred at powers during the course of the vibratory milling for 2 h.

These results suggested that the lactose radicals produced by plasma-irradiation initiated the polymerization of DF-HEA to form polymethacrylate-based polymeric prodrugs of DF derivatives.

3.4. Diclofenac release from poly DF-HEA powders

The DF release from poly DF-HEA was studied in three different buffer solutions (pH 1.2, 6.8 and 8.5) at 37 °C in order to evaluate the possible time of a prolonged action. As the polymers were not soluble in water, they were dispersed in buffer solution and the hydrolysis was performed in a heterogeneous system.

Figure 7 shows the degree of hydrolysis of the polymers containing DF as function of time in buffer solutions at 37 °C. The in vitro release studies showed that drug release from poly DF-HEA was highly dependent on pH, in which ca. 9%, 25% and 48% of DF was released in 12 h at pH 1.2, pH 6.8 and pH 8.5, respectively. By this way, we have achieved protection of the stomach and targeted controlled release of the drug to lower small intestine and large intestine. After an initially high rates of DF release, a very slow release rates were also observed in all test solutions. The physicochemical properties such as hydrophilicity and the number–average molecular weight of the polymeric prodrugs are very important parameters that affect the release rates and degrees of the bioactive agent. Therefore, further chemical optimization of the polymeric prodrugs might be needed in order to improve the drug release profiles.

Fig. 6. XRPD patterns of (a) lactose, (b) physical mixture of lactose and DF-HEA monomer (1:1), and (c) DF-HEA vibratory-milled for 30 min with plasma-irradiated lactose. The spectra are offset for clarity.

Fig. 7. DF release properties from polymeric prodrugs in physiological (aqueous phosphate or hydrochloric acid buffers, pHe 1.2–8.5) solutions at 37°C. Each value is mean of three readings.
4. Conclusion

In this study, we have successfully synthesized a novel crystalline DF-HEA, which was polymerizable acrylic monomer, from the reaction between HEA and DF by the esterification method. Then the resulted monomer was polymerized in solid state by vibratory milling with plasma-irradiated lactose powders as postprocessing-free initiators.

The release profiles of the polymeric prodrugs indicated that the hydrolytic behavior was in a sustained manner and the release rate was strongly based on the pH value of the hydrolysis solution. The obtained results suggested that the polymeric prodrugs could be useful for release of diclofenac in controlled release systems after oral administration.

However, during the whole period of release tests in this study, approximately only 60% of the total drug content was released from the polymeric prodrugs even in alkaline medium. Therefore, in the subsequent step, we are planning to introduce some hydrophilic units along the polymer chain in order to optimize both rates and degrees of the drug release by hydrolysis.”

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