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# Oral delivery of diclofenac sodium using a novel solid-in-oil suspension

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**Abstract**

The present work reports on a new pharmaceutical formulation for oral delivery of diclofenac sodium (DFNa), a non-steroidal anti-inflammatory drug (NSAID). Although DFNa itself is water-soluble at neutral pH, it was readily suspended in soybean oil via complex formation with an edible lipophilic surfactant and a matrix protein. The resulting solid-in-oil (S/O) suspension containing stably encapsulated DFNa in an oil phase markedly reduced the risks for gastrointestinal ulcers upon oral administration even at the $LD_{50}$ level in rats (ca. 50 mg/kg DFNa). In addition, plasma concentration of DFNa upon administration of an S/O suspension was comparable with that of the aqueous counterpart at the same DFNa dose. These results indicate the potential use of S/O suspensions as novel oil-based pharmaceutical formulations for oral delivery of water-soluble drugs without causing severe mucitis.


*Keywords:* Diclofenac sodium; Gastric ulcer; NSAID; Oral administration; Solid-in-oil suspension

## 1. Introduction

Diclofenac sodium (DFNa) (Ku et al., 1975), the sodium salt of o-(2,6-dichlorophenylamino)-phenylacetic acid, is a representative non-steroidal anti-inflammatory drug (NSAID). The primary action of this drug is associated with inhibition of cyclo-oxygenase (COX) activity, which in turn prevents the production of prostaglandins, just like other NSAIDs, and is thus widely used for pain control and treatment of rheumatic diseases. Although DFNa is one of the best tolerated NSAIDs, severe side effects including gastrointestinal (GI) ulcers and renal damage upon administration limit its use (Sakamoto, 2003). Due to these adverse effects, development of a new drug, i.e., a COX-2-selective inhibitor which could eliminate such harmful side effects (Warner et al., 1999) is required. However, recent publicized withdrawal of a COX-2 inhibitor (Editorial, 2005) confirmed that approval of new drugs is difficult, and instead exploration of other routes to avoid risks for serious GI complications associated with NSAIDs, would be more relevant.

Although DFNa is a conventional NSAID, it could be fully utilized without harmful side effects if it was properly formulated. When it comes to oral administration of DFNa, at least two requirements should be considered: (a) perfect drug retention under gastric conditions, and (b) sufficient drug release during intestinal residence time. To achieve these requirements, a variety of controlled release formulations for DFNa have already been reported. In terms of pH-responsive matrices, water-soluble matrix tablets containing DFNa coated with hydroxypropyl methylcellulose phthalate (HPMCP) for delayed release of DFNa (Kim et al., 2003), and DFNa-loaded pH-sensitive microspheres comprising of poly(vinyl alcohol) and poly(acrylic acid) interpenetrating network for the delivery of DFNa to intestines (Kurkuri and Aminabhavi, 2004) were prepared and evaluated in vitro. Recently, Biju et al. reported novel enteric microcapsules, and in vivo evaluation of dosage forms showed successful pharmacodynamic activities (Biju et al., 2004).

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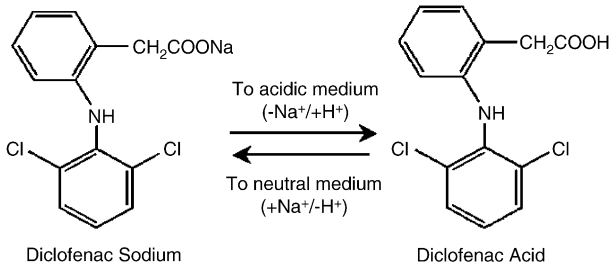


Fig. 1. Schematic diagram of pH-responsive conversion between water-soluble diclofenac sodium and water-insoluble diclofenac acid.

Recently, lipid-based formulations have attracted increasing attention for improvement of bioavailability of hydrophobic drugs in comparison with solid dosage forms (Pouton, 2000). In fact, lipid microspheres composed of lecithin and soybean oil were tested as carriers for hydrophobic NSAIDs (Ohmukai, 1996). Unlike many of NSAIDs, DFNa is basically water-soluble at neutral pH (Fig. 1), making it difficult to exist in an oil-based formulation. Although self-emulsifying drug delivery system (SEDDS) composed of goat fat and Tween 65 was also applied to diclofenac, the protonated water-insoluble form of DFNa, in vivo evaluation was not conducted (Attama et al., 2003). Therefore, little is known about the potential of oil-based formulations to be used as oral delivery systems of DFNa. To this end, we have recently reported the preparation of a solid-in-oil-in-water (S/O/W) emulsion in which insulin, a water-soluble peptide drug, was stably entrapped in the oil phase by complex formation with a lipophilic surfactant (Toorisaka et al., 2003). The S/O/W emulsion showed hypoglycemic activity after oral administration to rats, suggesting prevention of insulin degradation in stomach concomitant with absorption of active insulin through intestines.

In the present study, preparation of the novel oil-based formulation of DFNa is reported. After complex formation with a lipophilic surfactant and a matrix protein, DFNa readily became suspended in soybean oil to yield a solid-in-oil (S/O) suspension (Fig. 2). Basic characterization and in vivo evaluation of the S/O suspension are demonstrated. Results indicated potentials for oral delivery of DFNa without serious GI injuries.

## 2. Materials and methods

### 2.1. Materials

DFNa was supplied by ASPION Co. (Tokushima, Japan). Sucrose erucate (commercial name: ER290) was kindly provided by Mitsubishi-Kagaku Foods Co. (Tokyo, Japan). Porcine pancreas lipase (PpL) was purchased from Sigma–Aldrich (USA). Bovine serum albumin (BSA), sodium taurocholate, and soybean oil were purchased from Wako Pure Chemicals Industris, Ltd. (Osaka, Japan).

### 2.2. Preparation of S/O suspension

A 10 ml PBS solution (pH 8.0) containing both 10 mg/ml DFNa and BSA, and a 20 ml toluene solution containing 5 wt.% ER290 were poured into a round-bottom flask (100 ml) and mixed with a homogenizer at 26,000 rpm for 2 min to form stable W/O emulsions. The resulting emulsions were frozen rapidly in liquid nitrogen, and lyophilized using a freeze-drying machine (EYELA-FD5N, Japan) for 24 h. Soybean oil (5 ml) was added to the resulting viscous solid, and dispersed thoroughly by ultrasonication (140 W for about 30 min). The resulting suspension was employed as S/O suspension.

Fig. 2. Photograph of solid-in-oil (S/O) suspension prepared in this study.

### 2.3. In vitro drug release studies

JP disintegration no.1 fluid (pH 1.2) and no.2 fluid (pH 6.8) were used as simulated gastric and intestinal solutions, respectively. Drug release studies were carried out at 37 °C, with gentle stirring (100 rpm). In the case of simulated intestinal solution, effects of addition of 20 mM sodium taurocholate and 500 U/ml PpL were also tested. S/O suspension (100 mg) was added to each solution, and release of DFNa was assessed using HPLC (Shimadzu LC-10AT, Japan) at appropriate time intervals. Experimental conditions for HPLC analysis were: column, Shiseido CAPCELL PAK C18 MG (4.6 mm × 250 mm); mobile phase, 50 mM acetic acid:methanol = 25:75; flow rate, 0.7 ml/min. Detection of DFNa was done at 270 nm.

### 2.4. Ulcerogenicity studies

Male Wistar rats (140–150 g) were fasted for 18 h prior to drug administration, but were allowed to drink water ad libitum. S/O suspension (15 mg/ml DFNa) was administered orally (equivalent to 50 mg/kg DFNa) to ten rats using a sonde. As a

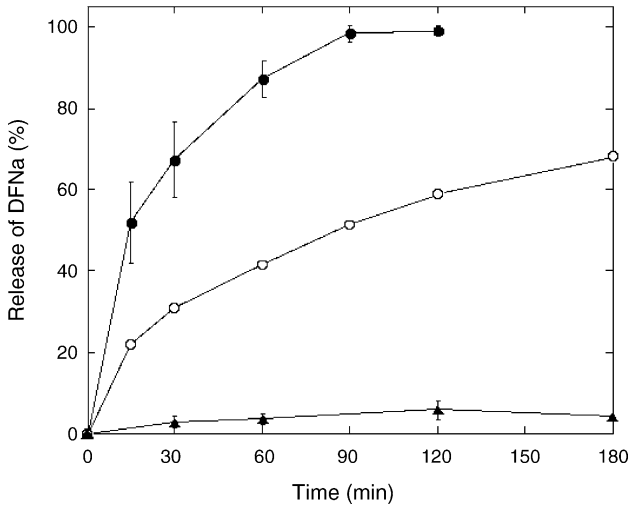


Fig. 3. DFNa release characteristics of the S/O suspension in simulated gastric (▲) and intestinal solutions (◯), and in a simulated intestinal solution with lipase (●).

control, an aqueous DFNa solution in phosphate buffered saline (PBS, pH 7.4) was administered orally to six rats. In both cases, DFNa dose was adjusted to 50 mg DFNa per kg of body weight. Blood samples were withdrawn into heparinized vaccutainer tubes 3.5 h after oral administration. Rats were subsequently sacrificed, and the stomach was excised. The stomach was incised, and then examined for ulcer development. The stomach was stained with hematoxylin and eosin (H&E), and immersed under a 10% formalin buffer solution for one night to prepare pathological samples.

## 3. Results and discussion

### 3.1. In vitro characterization of S/O suspension

To obtain an S/O suspension containing DFNa, complexed DFNa with ER290, an edible lipophilic surfactant, was first prepared via the formation of water-in-oil (W/O) emulsion, and subsequent lyophilization of the resulting W/O emulsion (Toorisaka et al., 2003). In the preparation, we added BSA because its addition prevented from the release of DFNa compared to that without BSA under low pH conditions (data not shown). The viscous solid obtained was readily and highly dispersed in soybean oil with a concentration of 15 mg DFNa/ml suspension. Fig. 2 represents the physical appearance of the novel S/O suspension.

The first requirement for an S/O suspension is to be able to retain DFNa in the oil phase under gastric conditions. Fig. 3 shows release behavior of DFNa from an S/O suspension in simulated gastric and intestinal solutions. Release of DFNa at pH 1.2 was below 5%, suggesting the possibility of reducing GI damages caused by DFNa. In fact, water-solubility of DFNa in an acidic medium is limited, and the highly lipophilic property of diclofenac acid (Fig. 1) makes it easier for DFNa to distribute into the oil phase. The second requirement involves the complete release of encapsulated DFNa from the oil phase of the suspension under intestinal conditions. As opposed to gastric conditions, about 60% of encapsulated DFNa were gradually released over 3 h in a simulated intestinal solution at pH 6.8. On the other hand, release rate significantly increased in the presence of PpL. Gradual release of DFNa from the suspension at neutral pH was basically due to diffusion of ionized DFNa, and enhancement in release rate upon addition of lipase was due to enzymatic degradation of soybean oil. The basic performance of S/O suspension prompted us to test if it could retain DFNa under gastric conditions while releasing DFNa under intestinal conditions in vivo.

### 3.2. In vivo evaluation of S/O suspension

Excess amounts of DFNa, which were close to the lethal dose in rats, were orally administered using an S/O suspension or aqueous PBS solution. Results are summarized in Table 1. To our surprise, biological examination showed that no gastric damage was observed in eight out of ten rats upon oral administration of the suspension. On the contrary, severe gastric erosions or gastric ulcers were observed in all control rats (aqueous PBS solution, Table 1). To gain further insights into GI damage caused by S/O suspensions, characteristic samples of incised stomach were compared. Fig. 4 illustrates the mucous surface of the stomach, and shows pathological photos of a stomach section. In Fig. 4A, many coagulation clots were observed on the mucous surface, after administration of the aqueous control solution. Histopathological diagnosis showed deep gastric ulcer (in lower half of Fig. 4A). On the other hand, the mucous surface of the stomach was stained with bright pink color, and no histological alterations on the mucous surface and submucosa were seen upon oral administration of the S/O suspension (Fig. 4B). These results clearly indicate that the S/O suspension can induce reduction of DFNa-induced GI toxicity in vivo.

It was also found that both S/O suspension and the aqueous control solution showed similar plasma concentrations of DFNa (Table 1), suggesting that release of DFNa from S/O suspensions under in vivo intestinal conditions was as anticipated, and shown in Fig. 3. Together with results from Fig. 4, DFNa encapsulated in S/O suspension seemed to mainly be absorbed after entering the small intestines where degradation of soybean oil by GI

Table 1
In vivo evaluation of the S/O suspension in comparison with the aqueous PBS solution of DFNa[a]

| Samples | Gastric disturbance | | | | |
|---|---|---|---|---|---|
| | Ulcer | Severe erosion | Mild–moderate erosion | Normal | Plasma concentration (μg/ml) |
| PBS control | 2 | 4 | – | – | 6.20 ± 2.03 |
| S/O suspension | – | 1 | 1 | 8 | 6.32 ± 1.69 |

[a] The same amount of DFNa (50 mg/kg) was orally administered in both cases.

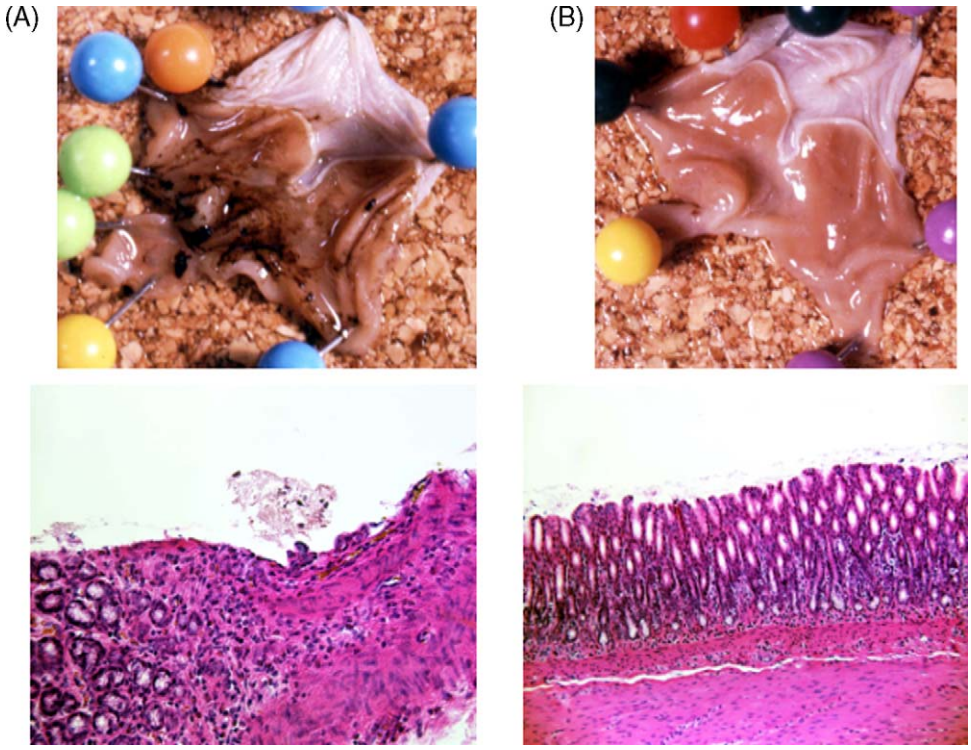


Fig. 4. Photographs of surface morphology of the stomach (upper half), and typical characteristics of a stomach section stained with H&E (lower half) after oral administration of DFNa using aqueous PBS solution (A) or S/O suspension (B).

lipases and ionization of the drug simultaneously occur. Basic characteristics observed during animal experiments with rats clearly demonstrated potentials of the present S/O suspension for oral DFNa delivery.

## 4. Conclusion

Development and validation of an S/O suspension of DFNa for oral administration, that can significantly reduce GI injuries are reported here. Although the pH-dependent change in the solubility of DFNa may benefit the present formulation for oral delivery, the novel strategy is basically applicable to any water-soluble drugs (e.g. peptides (Toorisaka et al., 2003)) because of its unique preparation procedure (Okazaki et al., 1997). Further research on S/O suspensions is currently underway in our laboratory.

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