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UNIVERSITY OF COPENHAGEN
DEPARTMENT OF PHARMACY

# Basic characteristics and kinetics of degradation in aqueous buffer of selected diclofenac prodrugs intended for joint injection

**Nina Mertz[1], Susan Weng Larsen[1], Claus Selch Larsen[1]**
**[1]Department of Pharmacy, Faculty of Health and Medical Science, University of Copenhagen, Denmark**

## Introduction

Recently, a novel ester prodrug approach for the accomplishment of local and sustained diclofenac action after injection into joints was reported (1). It is to be expected that both onset and duration of diclofenac action can be modified by variation of inherent ester prodrug properties including their pH dependent solubility and charge as well as their susceptibility to undergo esterase facilitated hydrolysis. In the present study, three diclofenac ester prodrugs differing with respect to the spacer carbon chain length (Fig. 1) were synthesized and evaluated *in vitro*.

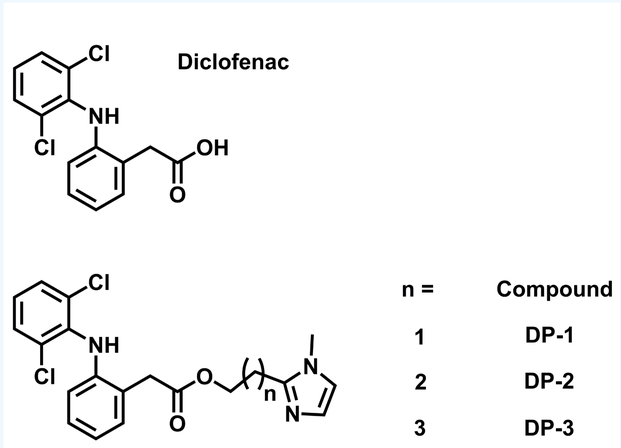


**Figure 1: Chemical structures of the diclofenac ester prodrug.**

Thus, the objectives of the present study were:

**(i)** to determine the effect of the spacer chain length on the $pK_a$ value and aqueous pH-dependent solubility of the prodrugs.

**(ii)** to investigate the kinetics and mechanism of degradation of the three prodrugs in aqueous solution in the pH-range 1-10 as well as in 80 %(v/v) human synovial fluid (SF) and 80 %(v/v) plasma at 37°C.

**(iii)** to characterize *in vitro* release of diclofenac from prodrug suspensions using the rotating dialysis cell model.

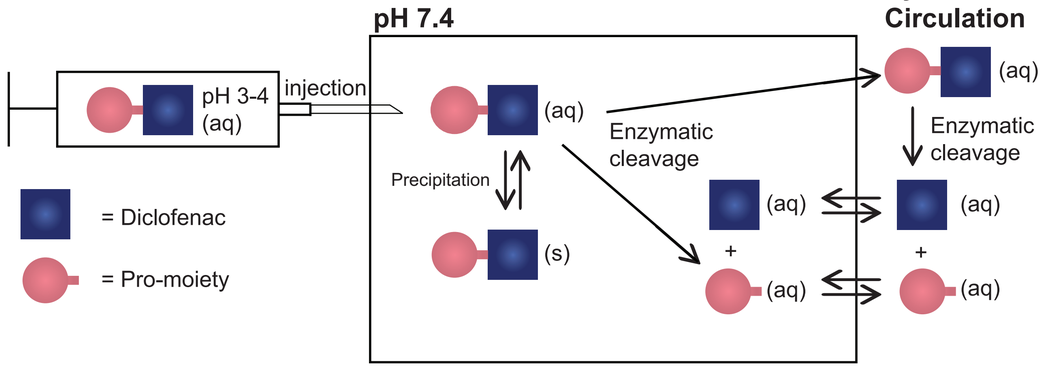


**Figure 2: Schematic representation of the *in situ* formulation principle**

## Prodrug concept

The prodrug approach involves the employment of pro-moieties that besides having an OH group (for diclofenac ester bond formation) comprises a weak base functionality with a $pK_a$ value of about 6-8 (2). Hence, this prodrug type experiences pH dependent solubility, i.e. a desired low solubility at physiological pH 7.4 in combination with a significantly higher solubility in slightly acidic solution.

It has been demonstrated that after injection of a concentrated slightly acidic prodrug solution (the preformulation) into SF, pH 7.4, *in situ* precipitation of the neutral prodrug species in this biological matrix occurs (1). Only the small amount of dissolved prodrug, in equilibrium with the precipitate, is subject to SF enzyme-mediated cleavage(Fig. 2).

## pH-rate profile

The degradation rate of the prodrugs was studied over the pH range 1-10 in aqueous buffer solutions at 37°C. Based on the shape of the pH-rate profiles (Fig. 3) an expression for the overall apparent first-order rate constant for the degradation of the individual diclofenac ester prodrug in the pH range 1-10 was proposed:

$$k_{obs} = \left(k_{H^+} \cdot a_{H^+} + k_{H_2O} \cdot [H_2O] + k_{OH} \cdot \frac{K_w}{a_{H^+}}\right) \cdot f_{DPX\text{-}H^+} + k'_{OH} \cdot \frac{K_w}{a_{H^+}} \cdot f_{DPX} \quad (1)$$

where $k_{obs}$ refers to the pseudo-first-order rate constant for prodrug degradation in buffer free solution (pH 2-10 (n = 3-4) and in dilute HCl, pH 1-1.5 (n = 1)). The full lines in Fig. 3 where obtained by fitting the data to Eq. 1 using a $K_w$ of $1.297 \cdot 10^{-14}$ (37°C, I = 0.3 M) (3).
As apparent from Fig. 3, good agreement between the solid curves and the experimental determined rate constants was achieved ($R^2 > 0.997$).

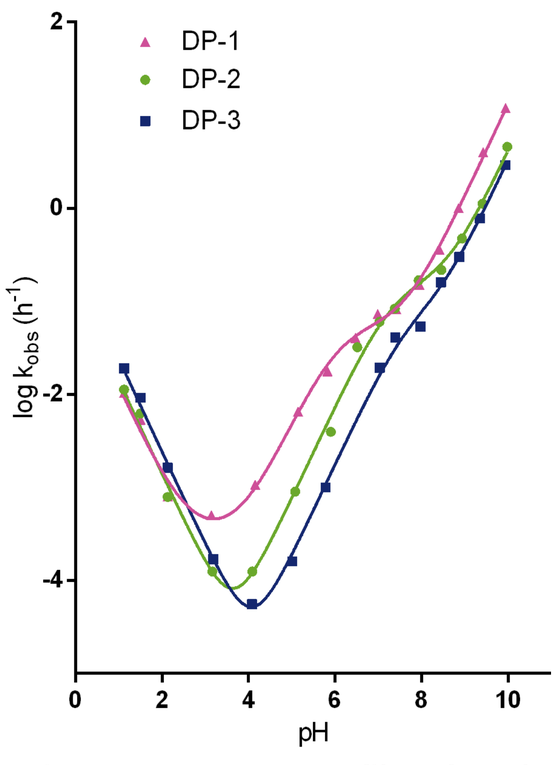


**Figure 3: pH-rate profiles for the degradation of the diclofenac prodrugs in aqueous solution at 37°C (I = 0.3 M).**

## Mechanism of degradation of the diclofenac ester prodrugs in aqueous solution pH 1-10

The experimental data suggested that below pH 5 formation of diclofenac and the indolinone 1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one (ILO) took place in a consecutive manner (Fig. 4A). In the pH range 6-10, HPLC analyses revealed that the prodrugs were subject to parallel degradation to yield diclofenac and ILO (Fig. 4B).

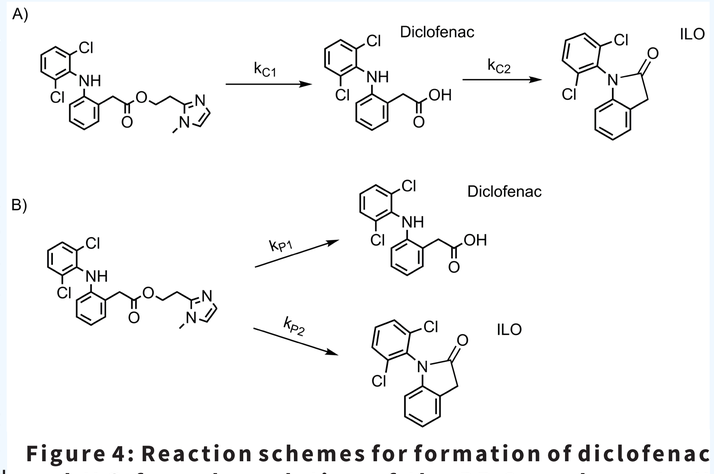


**Figure 4: Reaction schemes for formation of diclofenac and ILO from degradation of the DP-2 prodrug at pH below 2 (A) and at 5.8 ≤ pH ≤ 10 (B) and 37°C.**

## Stability in human plasma and synovial fluid

The stability of the diclofenac ester prodrugs after incubation in human 80 %(v/v) SF and 80 %(v/v) plasma was studied at 37°C. Compared to the stability in 67 mM phosphate buffer solution at pH 7.4, the prodrugs underwent much faster degradation in the biological media indicating the involvement of enzyme-mediated prodrug conversion (Table 1) to yield the active diclofenac.

**Table 1: pKa, half-lives and solubility (mean ± S.D., n=3) of prodrugs in aqueous solution at pH 7.4, 80 %(v/v) SF and 80 %(v/v) plasma at 37°C.**

| | Solubility pH 7.4 (µg/mL) | $pK_a$ [a] (25°C) | $pK_a$ [b] (37°C) | Chemical hydrolysis pH 7.4[b] (min) | 80% SF (min) | 80% plasma (min) |
|---|---|---|---|---|---|---|
| DP-1 | 0.84 ± 0.03 | 5.72 | 6.04 | $4.97 \cdot 10^2$ | 10.5 ± 0.9 | 1.7 ± 0.1 |
| DP-2 | 4.3 ± 0.4 | 7.66 | 7.31 | $4.55 \cdot 10^2$ | 115 ± 6.9 | 16.5 ± 0.7 |
| DP-3 | 4.6 ± 1.7 | 7.43 | 7.64 | $1.32 \cdot 10^3$ | 18.9 ± 1.2 | 3.0 ± 0.1 |

[a] Determined by potentiometric titration
[b] Calculated using Eq.1

## Release studies

*In vitro* release studies performed on pre-formed suspensions of the prodrugs revealed significantly different release behaviour among the prodrugs and indicate a complex relationship between solubility, dissolution and prodrug cleavage rate.

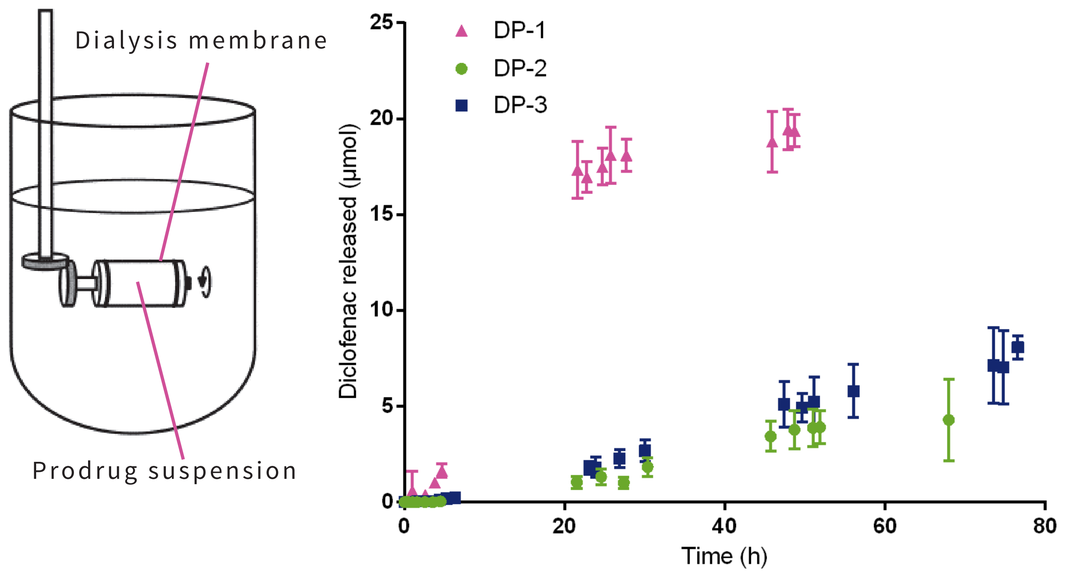


**Figure 5: Release profiles of diclofenac from prodrug suspensions of DP-1, DP-2, and DP-3 in 80 %(v/v) human synovial fluid at 37°C in the rotating dialysis model (n=2-3). A total dose of approximately 25 µmol prodrug was added to the donor compartment. Bars represent standard deviations.**

## Conclusion

The rate of degradation of the prodrugs was about 6-fold faster in 80 %(v/v) human plasma than in 80 %(v/v) human SF, which might reflect the higher protein content of human plasma relative to SF or the presence of different esterases in the biological media. *In vitro* release studies performed on pre-formed suspensions of the prodrugs revealed significantly different release behaviour among the prodrugs and indicate a complex relationship between solubility, dissolution and prodrug cleavage rate. Overall the results from the study indicated that design of prodrugs suited for local pain management through joint injection may be feasible by tuning of the physicochemical properties.

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