# Dissolution of celecoxib from mucoadhesive disks based on polyaspartamide derivatives

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# Dissolution of celecoxib from mucoadhesive disks based on polyaspartamide derivatives

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A series of mucoadhesive disks with celecoxib as a model drug of very low aqueous solubility were prepared and characterized. Two polymers of polyaspartamide type, poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)] (PHEA, **1**) and its thiolated analogue poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]-poly[α,β-(*N*-2-thioethyl-DL-aspartamide)] copolymer (PHTA, **2a,b**), and two commercially available polymers Carbopol 934P and hydroxypropylmethyl cellulose 4000 were used as excipients. Disks containing a mixture of equivalent amounts of thiomer **2b** and Carbopol 934P as an excipient exhibited the highest dissolution rate.

*Keywords:* celecoxib, disks, polyaspartamide, thiomer

Mucoadhesive drug delivery systems are very beneficial, since they can prolong the residence time of the drug at the site of absorption and increase drug bioavailability. Hydrogels, hydrophilic polymer networks, are often used as mucoadhesive drug delivery systems for their biocompatibility and similarity to a natural tissue (1) as well as for their ability to control drug release and to protect it from a hostile environment. The mucoadhesive polymers forming hydrogels are hydrophilic and swellable, containing numerous hydrogen bond forming groups, like hydroxyl, carboxyl or amine, which favor adhesion. When used in a dry form, they attract water from the mucosal surface and swell, leading to polymer/mucus interaction through hydrogen bonding, electrostatic, hydrophobic or van der Waals interactions.

Thiolated polymers (thiomers), are relatively new mucoadhesive excipients, which are capable of forming covalent bonds with the mucus layer covering mucosal tissues (2). Various thiomers, thiolated derivatives of polycarbophil, carboxymethyl cellulose, alginate and chitosan have been synthesized and evaluated in mucoadhesive drug delivery systems (see, for example, refs. 3–7). Several thiomers of polyaspartamide types were described in our previous papers. Chemical modification of poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)] (PHEA) with thiol bearing molecules led to thiomers of improved mucoadhesive properties. PHEA was first thiolated with thioglycolic acid and the resulting conjugate was successfully used in the preparation of microspheres with

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loratadine (8, 9), while two similar copolymers, poly[α,β-(*N*-2-aminoethyl-DL-aspartamide)]-poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]-poly[α,β-(*N*-2-thioethyl-DL-aspartamide)] copolymer (PAHTA) and poly[α,β-(*N*-2-aminoethyl-DL-aspartamide)]-poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]-poly[α,β-(*N*-3-mercapto-1-methoxycarbonyl-propyl-D,L-aspartamide)] copolymer (PAHMA) were prepared and used in the preparation of diclofenac and fenoprofen polymer-drug conjugates (10). Finally, poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]-poly[α,β-(*N*-2-thioethyl-DL-aspartamide)] copolymer (PHTA) was synthesized, characterized and screened as a protective polymer in quantum dots induced cell toxicity (11, 12).

In this paper, drug/polymer disks with PHEA, PHTA, Carbopol 934P (Cp 934P) and hydroxypropylmethyl cellulose (HPMC) are described. The effects of various ratios of these polymers on the rate of drug release were studied as well.

Celecoxib, a selective cyclooxygenase-2 (COX 2) inhibitor was used as a model drug. According to biopharmaceutical classification systems, celecoxib is classified as a low solubility and high permeability drug (13). By enhancing its dissolution rate, a faster onset of action and improved oral bioavailability could be achieved.

## EXPERIMENTAL

### *Material and methods*

IR and UV spectra were recorded on GX FT-IR (Perkin Elmer, UK) and Hewlett Packard 8452A (Hewlett Packard, Germany) spectrometers, respectively. The average molecular mass was determined by size exclusion chromatography (SEC) with UV detector (Series II, Hewlet Packard, USA). Dialysis was done with cellulose dialysis tubings with a molecular mass cut-off 8,000–12,000 (Sigma, USA).

Celecoxib was obtained from Cipla (India), Carbopol 934P from Noveon (Belgium) and hydroxypropylmethyl cellulose 4000 from Dow Chemical Company (USA). Gel filtration molecular mass standards were purchased from Bio Rad Laboratories CA (USA). Cysteamine was obtained from Sigma-Aldrich (Germany), L-aspartic acid and ethanolamine from Kemika (Croatia). Buffer solutions pH 6.6 were prepared from 0.2 mol $L^{-1}$ $KH_2PO_4$ and 0.2 mol $L^{-1}$ NaOH. The amine was distilled prior to use. All solvents used were of analytical grade and dry.

### *Syntheses of polymers*

*Poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)] (PHEA, **1**).* – PHEA was synthesized following the published procedure (14, 15). PSI was prepared by thermal polycondensation of L-aspartic acid in the presence of *o*-phosphoric acid (160 °C, reduced pressure).

*Poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]-poly[α,β-(*N*-2-thioethyl-DL-aspartamide)]-copolymers (PHTA, **2a**,**b**).* – The synthetic procedure for PHTA involved partial aminolysis of PSI with the thiol bearing compound cysteamine, followed by aminolysis of the remaining succinimide units with ethanolamine by the known procedure (12). Two products with different SH contents were prepared: **2a** (91 μmol $g^{-1}$) and **2b** (461 μmol $g^{-1}$).

*Molecular mass determination.* – The average molecular mass of polymers **1** and **2** was determined by size exclusion chromatography (UV detector, $\lambda$ = 200 ± 10 nm). The column set was composed of a precolumn and column BioSep-SEC-S 3000, 290 Å pore size (Phenomenex, USA). The experimental conditions were: mobile phase buffer solution pH 6.6, flow rate 0.35 mL $min^{-1}$ and injection volume 5 μL. The column was calibrated by protein molecular mass standards: thyroglobulin, γ globulin, ovalbumin, myoglobin and vitamin B-12. The column set, ionic strength and pH of the aqueous mobile phase were optimized prior to the molecular mass determination.

*Determination of thiol groups.* – The amount of free SH groups in thiomers **2a,b** was determined by iodimetric titration (11, 12). A solution of 15 mg thiomer in 1 mL distilled water acidified to pH 2 and 0.2 mL starch solution (0.5%, *m/V*) was titrated with 0.1 mmol $L^{-1}$ iodine solution until permanent light-blue colour. Each determination was performed in triplicate and the mean value was taken as final. The results were expressed as micromoles per gram of thiomers.

## *Preparation and characterization of disks*

*Preparation of disks.* – Celecoxib was used as a model drug in all formulations. Thiomers (**2a** and **2b**) were compressed alone (F2) or with Cp 934P or HPMC (formulations F3, F4, F6 and F8). For comparison, disks omitting thiomers were prepared with PHEA, Cp 934P or HPMC (F1, F5, F7, F9), as well as compressed celecoxib only (F10). Polymer or polymers mixture and celecoxib were dispersed in an appropriate amount of demineralized water. The obtained suspensions were lyophilized. Disks containing 100 mg of the drug were prepared using an IR hydraulic press (Perkin-Elmer, USA) by individual weighing and direct compression. The compaction pressure ($5 \times 10^3$ kg) and compression time (35 s) were kept constant during the preparation of all disks. The prepared disks were 13 mm in diameter and 1 mm thick. Mass of each disk was 200 ± 5 mg. Composition of the disks is shown in Table I.

In vitro *dissolution profile.* – Dissolution profiles were determined using USP apparatus II (16) at 37 °C, rotating speed of 50 rpm and phosphate buffer pH 6.6 with 0.5% sodium lauryl sulphate as media (500 mL). The samples were collected at predetermined time intervals and analyzed spectrophotometrically at 255 nm. After each withdrawall

*Table I. Composition of disks F1–F10*

| Component (mg) | Formulation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 |
| Celecoxib | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| PHEA (**1**) | 100 | – | – | – | 50 | – | – | – | – | – |
| PHTA (**2a**) | – | – | – | 50 | – | – | – | – | – | – |
| PHTA (**2b**) | – | 100 | 50 | – | – | 75 | – | 50 | – | – |
| Cp 934P | – | – | 50 | 50 | 50 | 25 | 100 | – | – | – |
| HPMC 4000 | – | – | – | – | – | – | – | 50 | 100 | – |

of a sample, an equal volume of fresh buffer was added to dissolution medium. Each determination was performed in triplicate and the mean value was taken as final.

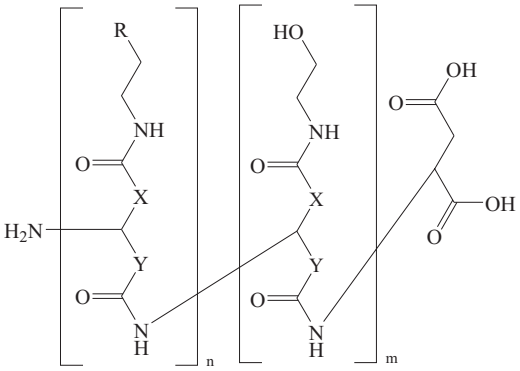
*Statistical analysis.* – Statistical significance for the comparison of release profiles was tested by one-way ANOVA. Statistically significant differences were defined as $P$ values of less than 0.05. Calculations were performed with the GraphPad Prism program (GraphPad Software, Inc., San Diego, USA; www.graphpad.com).

## RESULTS AND DISCUSSION

PHEA (**1**) and PHTA (**2**) are hydrophilic polymers of polyaspartamide type bearing hydroxy or both hydroxy and thiol groups. The starting polymer for their synthesis, poly-DL-(2,5-dioxo-1,3-pyrrolidinediyl) (PSI), was synthesized by thermal polycondensation of L-aspartic acid (14, 15). PHEA was prepared by total aminolysis of PSI with ethanolamine, while the synthetic procedure for PHTA involved partial aminolysis of PSI with cysteamine, followed by aminolysis of the remaining succinimide units with ethanolamine (12). During the reaction, succinimide rings of PSI were opened and amide bonds with both amines were formed. Structural formulas of polymers **1** and **2** are presented in Scheme 1.

By varying the PSI/cysteamine molar ratio, two batches of PHTA **2a** and **2b**, differing in the thiol content, total sulphur and mucoadhesive properties, were prepared. The amount of thiol groups was 91 in **2a** and 461 μmol per gram of polymer in **2b**. Average molecular mass of polymers **2a**,**b** was between 63 and 65 kDa.

Our previous investigations revealed that total work of adhesion (TWA) of thiolated polymers PHTA and PHEA conjugate with thioglycolic acid was increased 1.5 to 7.5 times,




α unit X = $CH_2$, Y = 0; β unit X = 0, Y = $CH_2$

PHEA (**1**): R = OH
PHTA (**2**): R = SH

Scheme 1

compared to the similar nonthiolated polymer PHEA (8, 12). A clear correlation between the amount of thiol groups and mucoadhesive properties was observed: the more thiol groups were attached to the polymer, the higher was the TWA value. Increased bioadhe-

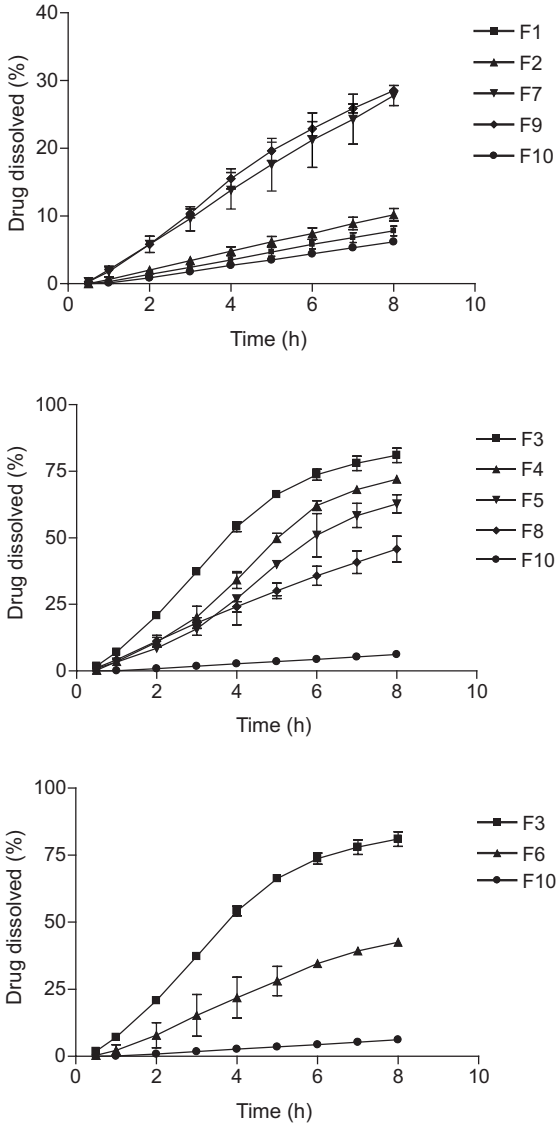



Fig. 1. Dissolution profiles of pure celecoxib disk (F10) and disks made of: a) PHEA (F1), PHTA **2b** (F2), Cp 934P (F7) and HPMC (F9); b) Cp 934P/ PHTA **2b** (F3), Cp 934P/PHTA **2a** (F4), Cp 934P/ PHEA (F5) and HPMC/PHTA **2b** (F8); c) Cp 934P/ PHTA **2b** 1:1 (*m/m*) (F3) and Cp 934P/PHTA **2b** 1:3 (*m/m*) (F6) (mean ± SD, $n = 3$).

sive properties make PHTA an interesting polymer in drug formulations. A series of disks with celecoxib as a lipophilic model drug were prepared and characterized. Disks F1–F10 were prepared by direct compression and their composition is shown in Table I.

Dissolution data were analyzed using the Ritger and Peppas equation (17, 18) to describe the mechanism of drug release from matrices [$(M_t/M_\infty) = K\,t^n$, $M_t/M_\infty$ is the drug fraction released in time $t$, $K$ is a constant and the diffusional exponent, $n$, is an important indicator of the mechanism of drug transport through the polymer]. For disks (tablets), and depending on the aspect ratio, *i.e.*, the diameter to thickness ratio, the Fickian diffusion mechanism is described by $0.43 < n < 0.50$ and the drug release rate is time-dependent. When $n$ is between 0.5 and 1.0, the drug release rate is time-dependent, but other factors such as polymer relaxation and swelling control the solute transport. Case-II type diffusion occurs when $n$ is equal to 1.0, which indicates that the release rate is time-independent. Super-case II transport occurs when $n$ is greater than 1.0; in that case, the release rate is time-dependent.

The influence of PHTA **2a** and **2b** alone or in combination with Cp 934P and HPMC on dissolution properties of celecoxib from disks was studied. Regarding the dissolution profiles (Fig. 1), there is no significant difference between the dissolution of pure celecoxib (F10) and dissolution of celecoxib from disks made of PHEA (F1) or PHTA **2b** (F2) alone. Up to 10% of the drug was dissolved from disks after 8 hours. The drug dissolution from disks made of Cp 934P (F7) or HPMC (F9) alone reached 28% in 8 hours (Fig. 1a).

The dissolution profiles of celecoxib from disks F3, F4, F5 and F8 composed of polymer mixtures are presented in Fig. 1b. Dissolution efficiency of each formulation achieved in 8 hours (45–81%) was significantly different ($p = 0.002$) from the dissolution efficiency of the pure drug (6%). The best dissolution was achieved from the disk composed of PHTA **2b** and Cp 934P in equal mass ratios (F3). Disks F3 and F4 differ in the content of SH groups from PHTA, while disk F5 contains the parent polymer PHEA (without SH groups). It can be seen from the dissolution profiles of these disks that the content of SH groups of polymers affected the dissolution profile in such a way that the best dissolution was achieved from disk F3 with the thiomer containing the highest amount of SH groups. The lowest dissolution efficiency (45% in 8 hours) was achieved from disk F8 made of PHTA **2b** and HPMC in equal mass ratios.

*Table II. Fitting of dissolution data of celecoxib from different formulations*[a]

| Formulation | $K$ ($h^{-n}$) | $n$ | $R^2$ |
|---|---|---|---|
| F3 | 0.0771 | 1.413 | 0.9987 |
| F4 | 0.0639 | 1.206 | 0.9752 |
| F5 | 0.0361 | 1.452 | 0.9928 |
| F6 | 0.0448 | 1.108 | 0.9880 |
| F7 | 0.0285 | 1.105 | 0.9951 |
| F8 | 0.0578 | 1.007 | 0.9957 |
| F9 | 0.0334 | 1.053 | 0.9881 |

[a] *Ritger and Peppas equation* $M_t/M_\infty = K\,t^n$ *(17, 18)*
$M_t/M_\infty$ – drug fraction released; $t$ – time; $K$ – constant; $n$ – diffusional exponent.

The dissolution of celecoxib from the disk made of Cp 934P and PHTA **2b** in 1:3 (*m/m*) ratio (F6) was significantly lower than that from the disks made of the same polymers in 1:1 (*m/m*) ratio (Fig. 1c), though it was significantly higher than the dissolution of pure drug.

The fitting of dissolution profiles according to the Ritger and Peppas equation is reported in Table II. Exponents *n*, related to drug release kinetics, were in all cases higher than 1, indicating that drug release was time dependent and controlled by the relaxational process due to the swelling of the polymeric network. Differences observed in *n* and *K* values, *K* being a constant incorporating structural and geometric characteristics of the device, could be explained by the difference in the swelling behaviour depending on the polymer composition of the disks. The drug/polymer systems with the highest values of *K* were F3 and F4, composed of Cp 934P and PHTA in 1:1 (*m/m*) ratio, which showed the highest release rate (F3 82% and F4 71% after 8 h). The disks composed of Cp 934P (F7) or HPMC (F9) alone gained the lowest *K* and lesser ability of promoting drug release, though both polymers are very hydrophilic and swellable. Therefore, it seems that blending of these polymers with PHTA **2b** produced more swellable systems with a promoted dissolution rate of celecoxib.

## CONCLUSIONS

Hydrophilic, mucoadhesive PHTA copolymer, a polyaspartamide thiomer bearing both hydroxy and thiol groups, was successfully used in celecoxib disk formulations alone or in combination with Cp 934P and HPMC. Enhanced dissolution properties of the PHTA containing disks suggest the possible use of this new polymer as an excipient in drug delivery.

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*Acronyms and codes.* – COX – cyclooxygenase; Cp 934P – Carbopol 934P; HPMC – hydroxypropylmethyl cellulose 4000; PAHMA – poly[α,β-(*N*-2-aminoethyl-DL-aspartamide)]-poly[α,β-(*N*-2--hydroxyethyl-DL-aspartamide)]-poly[α,β-(*N*-3-mercapto-1-methoxycarbonyl-propyl-D,L-aspartamide)] copolymer; PAHTA – poly[α,β-(*N*-2-aminoethyl-DL-aspartamide)]-poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]-poly[α,β-(*N*-2-thioethyl-DL-aspartamide)] copolymer; PHEA – poly[α,β-(*N*--2-hydroxyethyl-DL-aspartamide)]; PHTA – poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]-poly -[α,β-(*N*-2-thioethyl-DL-aspartamide)] copolymer; SEC – size exclusion chromatography.

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*S A Ž E T A K*

## Oslobađanje celekoksiba iz mukoadhezivnih diskova s polimerima poliaspartamidnog tipa

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Pripravljeni su i karakterizirani mukoadhezivni diskovi s celekoksibom kao lipofilnom modelnom supstancijom. Kao ekscipiensi uporabljena su dva polimera poliaspartamidnog tipa, poli[α,β-(*N*-2-hidroksietil-DL-aspartamid)] (PHEA, **1**) i njegov tiolirani analog poli[α,β-(*N*-2-hidroksietil-DL-aspartamid)]-poli[α,β-(*N*-2-tioetil-DL-aspartamid)] kopolimer (PHTA, **2a,b**), te dva komercijalno dostupna polimera Carbopol 934P i hidroksipropilmetilceluloza 4000. Najbolji profil oslobađanja celekoksiba postignut je iz diskova sastavljenih od tiomera **2b** i Carbopola 934P u masenom omjeru 1:1.

*Ključne riječi:* celekoksib, diskovi, poliaspartamid, tiomer

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