KAZIMIERA H. BODEK

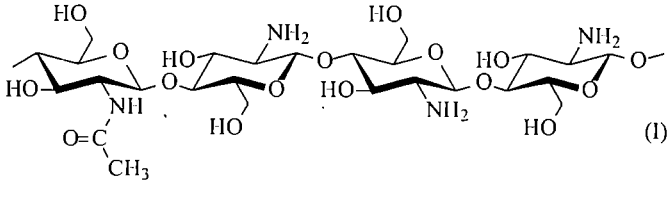
Medical University, Faculty of Pharmacy,
Department of Physical and Biocoordination Chemistry
ul. Muszyńskiego 1, 90-151 Łódź, Poland
e-mail: hbodek@pharm.am.lodz.pl

Study on the stability of microcrystalline chitosan systems with selected non-steroidal anti-inflammatory drugs

Summary — The stabilities of microcrystalline chitosan (MCCh) systems with selected non-steroidal anti-inflammatory drugs (NSAIDs) were investigated after storage at ambient temperature for 12 months or at 60 and 80 °C for 5 h. Diclofenac (DA) and ketoprofen (KTA) in free acid form were used as model drugs in this study. For both the MCCh-drug (DA, KTA) systems, the intensity of bands corresponding to chitosan and drug slightly decreased as the temperature increased. X-ray diffraction and differential scanning calorimetry (DSC) showed that KTA in the microcrystalline chitosan systems remained in the amorphous state in contrary to DA, which was present in crystalline state. The interactions between DA and MCCh are not as strong and develop with time. The interaction between KTA and polymer (decrease in drug crystallinity) in stored systems is similar to those in freshly prepared samples. The amorphic form of KTA is present throughout the whole storage time. Slight decrease in KTA release rate was observed for MCCh film stored at 80 °C. These results suggest that microcrystalline chitosan is a suitable carrier for drugs of different solubility.

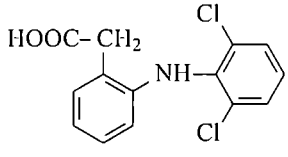
Key words: microcrystalline chitosan, non-steroidal anti-inflammatory drugs, drug carrier, storage time, temperature effect, release rate.

Chitosan — a copolymer consisting of 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glucopyranose [Formula (I)] [1] is widely used as pharmaceutical excipient [2, 3] for direct tablet compression [4], for the production of controlled release solid forms or for improving the dissolution properties and bioavailability of a number of poorly water-soluble drugs [5—7].



(I)

Many drugs in the presence of certain excipients undergo significant physico-chemical changes. A reduction in the degree of crystallinity, molecular complex formation, changes in the dissolution rate are of the utmost significance. Physical and chemical interactions occurring between the drug and the macromolecular excipient can substantially affect the bioavailability of the drug, and hence its therapeutic effect. Non-steroidal anti-inflammatory drugs (NSAIDs), such as diclofenac [DA, Formula (II)] and ketoprofen [KTA, Formula(III)], are widely used in the treatment of rheumatic diseases. Stability of NSAID is of utmost importance, as the products of its decomposition may enhance the undesirable effects.



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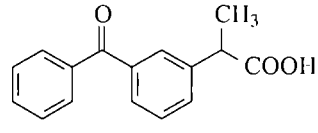

(III)

In our study chitosan was chosen as a drug carrier, because of its biocompatibility, biodegradability and non-toxicity, to achieve a modulation in drug release which in turn would lead to reduced irritation effects of NSAIDs [8—11].

Microcrystalline chitosan (MCCh) is a special multifunctional polymeric material prepared by the aggregation of glucosamine macromolecules from an aqueous solution of an organic acid [12]. It is the only form of

polyaminosaccharides characterised by the presence of free unbonded amine groups, which can be present in a liquid dispersion form with direct film-forming behavior. Quantitative studies [13, 14] have confirmed the superior properties of MCCh as a hydrogel better than its non-modified form, *i.e.* flakes.

In the available literature there are few reports on the application of MCCh in medicine, pharmacy, and other areas [15—18]. The results of recent study [19] indicate that MCCh in granules could offer advantages surpassing the non-modified chitosan, because the gels form more easily in acidic environments, *e.g.* in stomach, and drug (ibuprofen) release is more retarded.

Microcrystalline chitosan in the form of hydrogel at neutral pH has not been used in the studies on NSAIDs so far. Our studies [20—23] were the first to evaluate the use of MCCh as NSAIDs carrier. The obtained preliminary results show that MCCh is useful for the preparation of semisolid and solid formulations. It was noted that the apparent viscosity of MCCh hydrogel was lower and that of the hydrogel containing a drug was slightly higher in a year's long storage time at 20 °C. The more stable hydrogel systems containing drugs were produced by using MCCh hydrogels of higher polymer content.

This study is the first one to evaluate the stability of solid state MCCh systems with selected NSAIDs, *i.e.* diclofenac or ketoprofen. In the solid state polymer affects the therapeutic substance crystalline structure. This interaction changes the solubility and drug release. Hence, the important aim was to determine how the storage time, temperature and these interactions affect the drug release from the MCCh film layer.

The solid state system was used to study the polymer-drug interactions by DSC, X-ray and IR methods. Hence, the solid state system was analysed to confirm the presence of these interactions in the semi-solid (hydrogel) state.

EXPERIMENTAL

Materials

Microcrystalline chitosan (MCCh) was prepared using the previously described method [12] at the Institute of Chemical Fibres, Łódź (Poland). It was obtained from non-modified chitosan (Chemopol Co., India) and had an average molecular weight $\overline{M}_w \approx 100$ kDa. The deacetylation degree (*DD*), equal to 92 %, was determined potentiometrically [24] in an anhydrous medium (anhydrous acetic acid and 1,4-dioxane) using a glass (indicator) electrode, calomel of modified type (reference) electrode and a salt bridge junction between the two.

The drugs: diclofenac sodium (DS) and KTA were purchased from Sigma Chemical Co. (St. Louis, MO), DA in free acid form was obtained from the Pharmaceutical Laboratory "BIOCOM" (Rzeszów, Poland).

All other reagents were of analytical grade.

Preparation of MCCh — NSAID systems

MCCh, as a hydrogel containing 4.0 wt. % of the polymer, was the precursor material for further sample preparation. The drugs (diclofenac and ketoprofen) were introduced into the MCCh hydrogel at 1.0 wt. % concentration.

The mode of preparation of MCCh hydrogel formulation was as follows. Drug powder was precisely triturated with a small amount (≈1 g) of the base (MCCh hydrogel) and mixed with the rest of the base at ambient temperature. In order to characterise the interacting systems in the solid phase, MCCh hydrogel samples containing a drug were freeze-dried using a laboratory freeze dryer or dried at ambient temperature (the sample was spread on a teflon plate and water evaporated). In the second case, a film was obtained. A physical mixture of MCCh (lyophilised powder) with a drug, at a 2:1 weight ratio was prepared by simple blending.

To determine the thermal stability, the samples were kept at 60 and 80 °C for about 5 h in dry air and the effects of these temperatures were analysed by instrumental analysis, including melting point determination, IR, DSC, X-ray, and determination of the release rate.

Methods

Melting points (*m.p.*) of drug alone and its physical mixture with MCCh were determined by standard capillary-tube method using electro-thermal melting point apparatus (LABO-PLUS IA 9200).

Infrared measurements were performed using KBr technique (1 mg of the investigated material was mixed with 300 mg of dry KBr powder) or as a thin film (5 μm thick). The IR spectra were recorded with Mattson FT-IR spectrometer at ambient temperature.

The DSC analysis was performed for a drug, a chitosan, their physical mixture, and the chitosan-drug system. Thermograms were obtained by use of a Shimadzu DSC-50 (diclofenac) or Mettler Toledo DSC-821 (ketoprofen) instruments using vented aluminum pans. The analyse were performed in nitrogen (DA) or argon (KTA) atmosphere. All samples were run at a scanning rate of 10 °C/min, from 20 to 400 °C.

Powder X-ray diffraction patterns of chitosan-drug system in the solid state were performed using a diffractometer (X-ray diffractometer TUR M 62) under the following conditions: X-ray, Ni-filtered Cu-Kα radiation (λ = 1.5418 Å). The voltage and current intensity were 30 kV and 30 mA, respectively. The chitosan-drug system was identified by comparing its X-ray patterns with those of pure chitosan, drug and their physical mixture.

Drug release from the films (number of experiments = 3) into 1000 ml of distilled water at 37.0 ± 0.1 °C was studied by means of dissolution test using the modified paddle method [25] (paddle at 100 rev/min). Samples of 4 mL were periodically removed (5, 15, 30 min and 1, 2,

3, 4, 5, 6, 12, 24 h) and the drug release was assessed by measuring of the absorbance at 276 nm for DA, and at 260 nm for KTA, using UV/VIS ATI UNICAM spectrometer UV4.

Stabilities of MCCh systems were determined by comparison of release rates of the drugs from the fresh and stored samples.

RESULTS AND DISCUSSION

The interactions between acidic drugs (DA, KTA) and MCCh in stored systems are similar to those in freshly prepared samples (Fig. 1 and 2). IR spectra of MCCh-DA system showed all characteristic bands of the drug and polymer [21]. This fact indicates that weak interactions may appear in the studied formulation (DA was dispersed in the hydrogel rather than dissolved before freeze-drying). However, a spectral change with respect to drug alone in the IR spectra of MCCh-KTA system was observed [22]. The substantial decrease in absorbance at 1696 cm^{-1} may suggests the formation of charge transfer complex ($COO^-NH_3^+$) between -COOH group in KTA and $-NH_2$ group in chitosan (KTA was dissolved in the hydrogel before freeze-drying). IR Raman spectroscopy confirms the chemical interactions between KTA and MCCh molecules [26].

For both MCCh-drug (DA, KTA) systems, the intensities of the bands corresponding to chitosan and drug slightly decreased when the temperature increased (Fig. 1 and 2). The results of infrared absorption of MCCh and MCCh-DS system stored at various temperatures have been published before [27]. The absorbance corresponding to C-O-C band increases above the annealing temperature equal to 40 °C in MCCh-DS system. This may be due to crosslinking leading to increase in molecular weight. The above suggestion was confirmed by the investigations of molecular weight of MCCh-DS system.

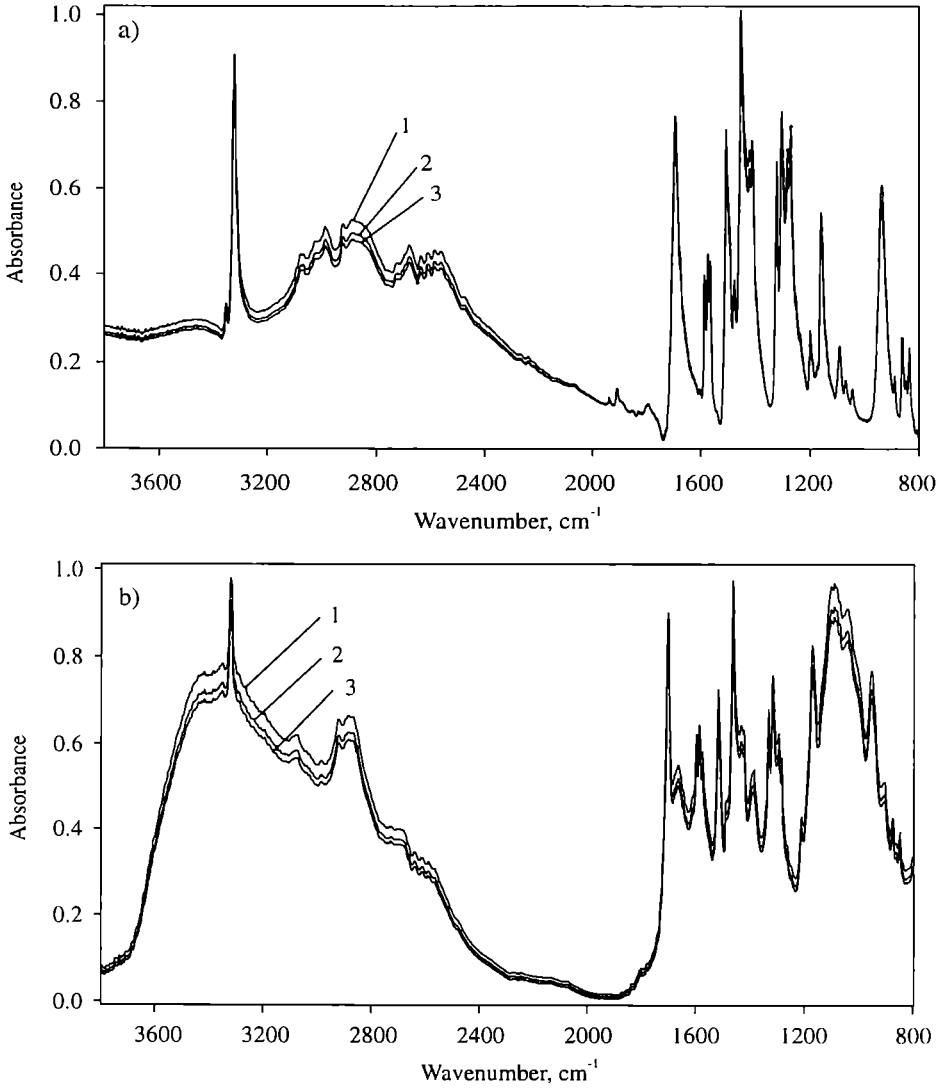

Fig. 1. IR spectra of: (a) DA itself (KBr) and (b) MCCh-DA system (film); 1 — freshly prepared, 2 — heated at 60 °C, 3 — heated at 80 °C

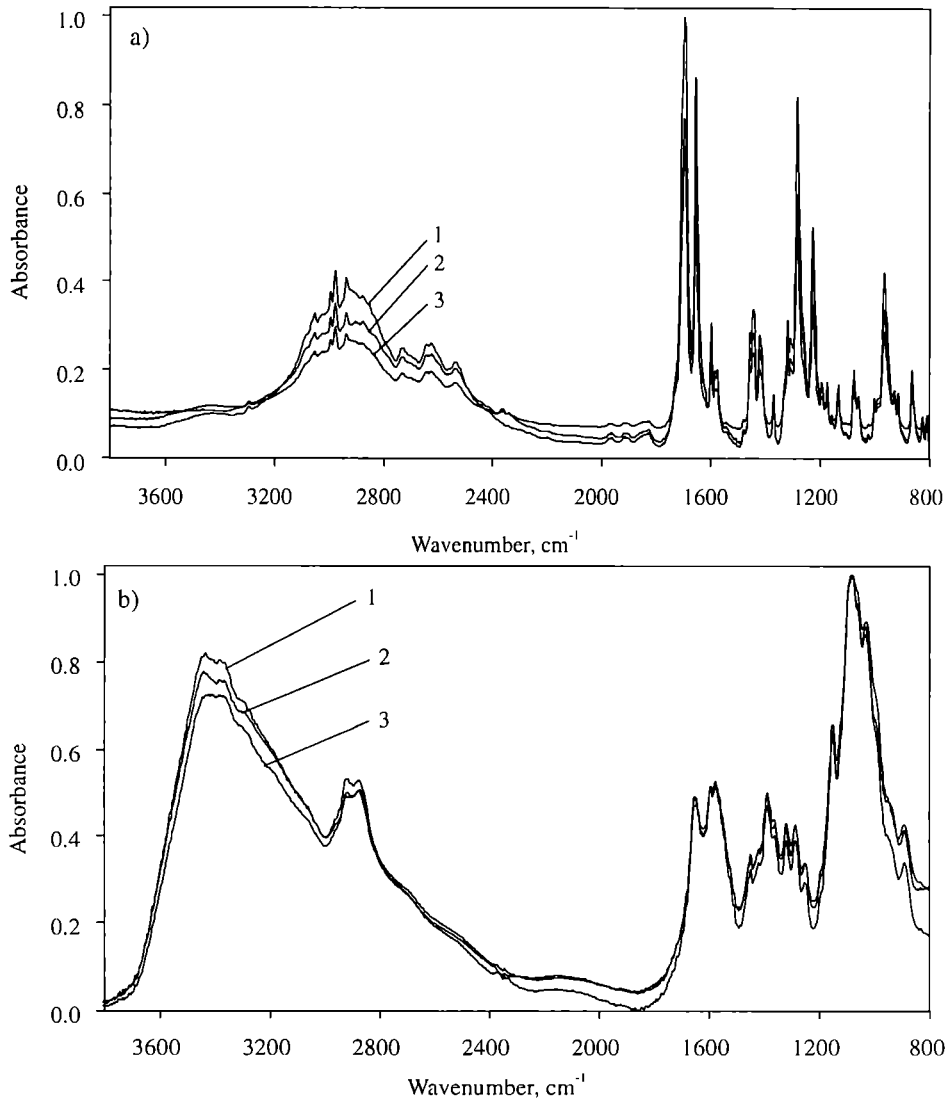

Fig. 2. IR spectra of: (a) KTA itself (KBr) and (b) MCCh-KTA system (KBr); 1 — freshly prepared, 2 — heated at 60 °C, 3 — heated at 80 °C

The intensity of a band in the range 3500—2500 cm^{-1} decreased with increasing temperature, which may suggest partial evacuation of water from MCCh and MCCh-drug systems. This conclusion is confirmed by direct measurements of water loss caused by heating. The weight loss of equals to 6—7% for heating at 60 °C and is close to 10 % for heating at 100 °C.

The DSC thermograms of MCCh-drug systems have been illustrated in Fig. 3. All the thermograms showed a broad endotherm in the range 40—70 °C, corresponding to the loss of residual water. The thermogram of MCCh (A) showed two peaks: endothermic at 66 °C and exothermic at 314 °C, the former corresponding to the loss of water, the latter one corresponding to the decomposition of the chitosan molecule. Samples of nothing but ketoprofen and ketoprofen in the physical mixture (Bd and Bm) showed a sharp melting endothermic peak at 94 °C. Melting points of fresh and stored at 80 °C samples of KTA itself and in a mixture with chitosan were nearly to the literature [28, 29] melting range of KTA itself (93—96 °C). In the thermogram of MCCh-KTA lyophilised system (Bs), this endothermic peak was found at a temperature lower by about 7 °C and had rounded profile. Lin et al. [30] explained that the shift of the endothermic peak of the melting of the active substance, in the case of mixtures containing warfarin ground with Eudragit® E or RL, could be attributed to the change in the drug state from the crystalline state to a partially amorphous one. In another case (mixture of piroxicam and Eudragit® S) the same phenomenon was attributed to the lower molecular weight and the higher brittleness of polymer [31]. In other studies on ketoprofen/Eudragit® S 100 mixture, the drug becomes almost completely amorphous and remains unchanged for at least six months. DSC data evidenced that ketoprofen inside the mixture was transformed into an amorphous state [32].

On the contrary, the endothermic peak at 290 °C corresponding to the melting point (283—290 °C) of alone

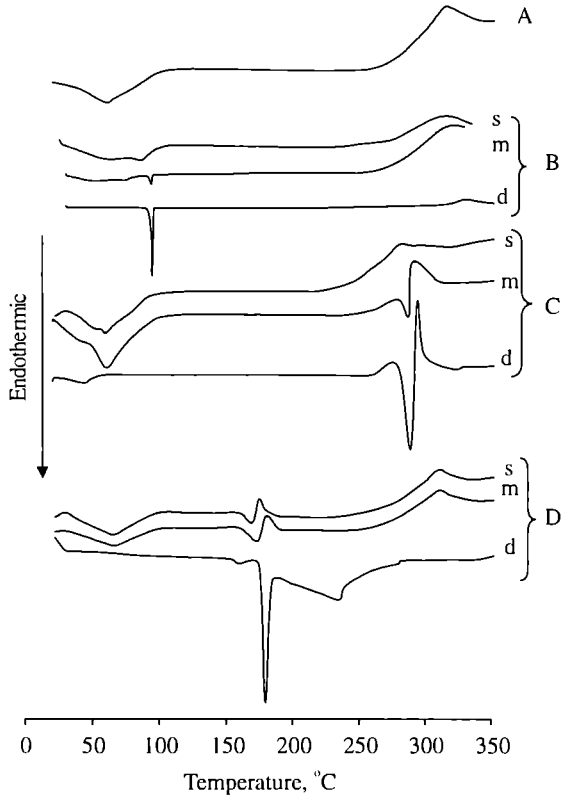

Fig. 3. Differential scanning calorimetry curves: (A) MCCh, (B) KTA, (C) DS, (D) DA; d — drug, m — physical mixture, s — system

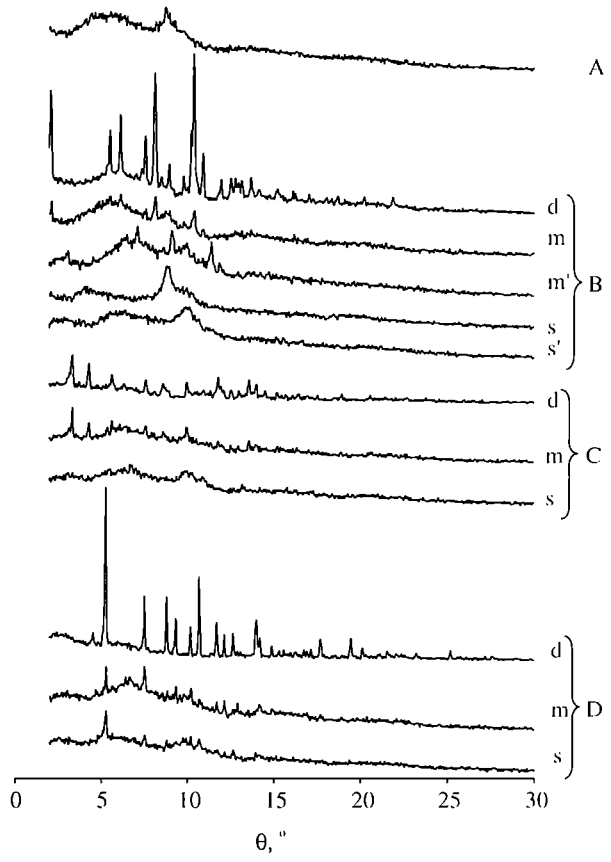

Fig. 4. Powder X-ray diffraction patterns: (A) MCCh, (B) KTA, (C) DS, (D) DA; d — drug, m — physical mixture, s — system, m' — physical mixture heated at 80 °C for 5 h, s' — system heated at 80 °C for 5 h

diclofenac sodium itself and in the physical mixture (Cd and Cm) was absent in MCCh-DS system (Cs) suggesting the formation of a new amorphous compound. The thermogram of diclofenac acid (Dd) showed an endothermic peak, at 180 °C due to the melting. In the thermogram of MCCh+DA physical mixture and MCCh-DA system (Dm and Ds) the endothermic peak characteristic for a drug was shifted at 174 and 170 °C suggesting a relative reduction in DA crystallinity. However, it was noticed that in the thermograms of both MCCh+DA physical mixture and MCCh-DA system a new exothermic peak at 180 °C and 175 °C respectively, was observed. The *m.p.* of DA itself and in the physical mixture is lower (155—156 °C and 146—148 °C) than that measured by DSC.

The powder X-ray diffraction patterns of MCCh-drug system in comparison with those of the physical mixture and the drug itself are presented in Fig. 4. KTA showed a characteristic X-ray diffraction pattern (Bd), which disappeared in its system with the amorphous polymer. Diffractometry patterns of MCCh-KTA system (Bs) are similar to those of a polymer (A). This indicated an important decrease in a crystallinity of ketoprofen. The analysis of X-ray data of the samples of MCCh-KTA system heated at 80 °C for 5 h (Bs') indicates that the peak of microcrystalline chitosan located at 8.8° was shifted to 10.0° Θ. In case of MCCh+KTA physical mixture heated at 80 °C for 5 h the peaks corresponding to ketoprofen crystals (Bm) were shifted to the right by about 1° Θ (Bm').

The spectrum of DS (Cd) when compared with those of KTA (Bd) or DA (Dd) showed a smaller number of reflections of lower intensity. X-ray diffraction pattern of MCCh-DS system (Cs) was poor in reflections, which indicated the reduction of crystallinity. DA itself presented a well defined crystalline X-ray pattern (Dd). The patterns of the MCCh+DA physical mixture (Dm) and MCCh-DA system (Ds) demonstrated the peaks of higher intensity located at 5.3° and 7.5° Θ characteristic for diclofenac. Their presence indicated that diclofenac is present in the crystalline state in this formulation.

The influence of the ageing phenomena on release of drug from the discussed systems is important from the practical point of view and that is why it has become the subject of present investigations. The release rate of drugs from MCCh-hydrogel and -film depends on the physical and chemical interactions between an excipient and a drug. For example, the release of soluble drugs (KTA and DS) in vehicle was higher in comparison with the dispersed drug (DA) [33].

The release of DA from MCCh-hydrogel system increases during the storage because of the interactions between the drug and the MCCh-hydrogel, probably oc-

curring after a long storage time. Increased solubility of DA in MCCh hydrogel had been confirmed by earlier studies [21]. In that study the solubility of DA increased from 0.020 mg/ml to 0.120 mg/ml after 3 months of storage. Diclofenac shows a high partition coefficient ($\log P = 4.0$), but very low water solubility (0.0178 mg/l) in its unionized form. Because of this characteristics, it is often administered in salt form [34]. The enhanced dissolution rate can be attributed to an increase in solubility and a decrease in crystallinity of the drug [7]. In other studies on selected NSAIDs poorly soluble in water, the solubility increased in the presence of *N*-methylglucamine. The authors attribute this phenomenon to additional hydrogen bonding [35].

The enhanced pharmaceutical availability of another acidic drug (KTA) can be attributed to charge transfer complex (CT) formation ($COO^-NH_3^+$) between carboxylic group of drug and free amine group of MCCh [26]. It must be emphasized that Raman investigations show the existence of CT complex both in virgin and aged samples. The occurrence of interaction between polymer and a drug has been confirmed by X-ray diffraction. In MCCh-KTA system, the crystallinity of a drug was found decreasing to a considerable extent as a result of its interaction with the chitosan in amorphous form. The release rate of KTA from MCCh film was not greatly influenced by storage at ambient temperature for 12 months. This fact indicated that the amorphous state did not change over a period of 12 months at ambient temperature. The comparison of ketoprofen release from chitosan film proves the influence of storage time and temperature on the drug release profile (Fig. 5). The amount of a drug released from the film heated at 80 °C for 5 h

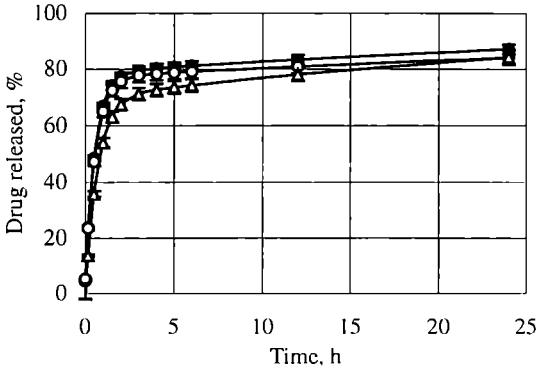

Fig. 5. Profiles of release of KTA from chitosan film when fresh (●), stored at room temperature for 12 months (o), and annealed at 80 °C for 5 h (△)

was decreased by approx. 6 % if compared with the results obtained directly after preparation or after 12 month storage at ambient temperature. However, the entire amount of ketoprofen released after 24 h from a heated film was nearly the same as from an unheated sample.

CONCLUSIONS

From the results of this study it was concluded that MCCh, as a hydrogel at neutral pH is a suitable vehicle for drugs of different solubility. Specific interactions between acidic drugs and MCCh containing free amine groups concern the formation of the compounds, which are better soluble in water. For ketoprofen, which is better soluble in water than diclofenac, storage time does not affect the amount of released drug: the release from a fresh sample and from the one after 12 months of storage was similar. In this case, chitosan changes the crystalline structure of ketoprofen towards the amorphic form, which is present throughout the whole storage time. However, diclofenac release does not significantly increase during the storage. Microcrystalline chitosan improves the solubility, but does not significantly change the crystalline form of diclofenac. Thus, the interactions between a polymer and a drug are not as strong and develop with time. As reported for nonmodified chitosan, its microcrystalline form is a suitable carrier for NSAIDs. The decreasing crystallinity of the drugs influences the physical properties of MCCh-drug systems. The physical properties (solubility and release) of these systems are better than those of the drugs themselves.

REFERENCES

1. Struszczyk M. H.: *Polimery* 2002, **47**, 316.
2. Illum L.: *Pharm. Res.* 1998, **15**, 1326.
3. Singla A. K., Chawla M.: *J. Pharm Pharmacol.* 2001, **53**, 1047.
4. Nagai T., Sawayanagi Y., Nambu N., in: "Chitin, Chitosan and Related Enzymes" (Ed. Zikakis J. P.), Academic, New York 1984, p. 21—39.
5. Sawayanagi Y., Nambu N., Nagai T.: *Chem. Pharm. Bull.* 1982, **30**, 4464.
6. Hou W. M., Miyazaki S., Takada M., Komai T.: *Chem. Pharm. Bull.* 1985, **33**, 3986.
7. Acartürk F., Şencan A., Çelebi N.: *Pharmazie* 1993, **48**, 605.
8. Struszczyk M. H.: *Polimery* 2002, **47**, 619.
9. Açikgöz M., Kaş H. S., Hasçelik Z., Milli Ü., Hincal A. A.: *Pharmazie* 1995, **50**, 275.
10. Sezer A. D., Akbuğa J.: *Int. J. Pharm.* 1995, **121**, 113.
11. Tarimci N., Ermiş D.: *Int. J. Pharm.* 1997, **147**, 71.
12. Struszczyk H.: *J. Appl. Polym. Sci.* 1987, **33**, 177.
13. Bodek K. H.: *Chem. Anal.* 1996, **41**, 339.
14. Bodek K. H., in: "Progress on Chemistry and Application of Chitin and Its Derivatives", Monograph Vol. II (Ed. Struszczyk H.), Polish Chitin Society, Łódź 1996, p. 59—69.
15. Zięba J., Knapczyk J.: *Acta. Pharm. Technol.* 1988, **34**, 84.
16. Struszczyk H., Kivekäs O.: *Brit. Polym. J.* 1990, **23**, 261.

17. Hoekstra A., Struszczyk H., Kivekäs O.: *Biomaterials* 1998, **19**, 1467.
18. Struszczyk M. H.: *Polimery* 2002, 47, 396.
19. Säkkinen M., Seppälä U., Heinänen P., Marvola M.: *Eur. J. Pharm. Biopharm.* 2002, **54**, 33.
20. Bodek K. H.: *Polimery* 2000, **45**, 821.
21. Bodek K. H.: *Acta Polon. Pharm. - Drug Res.* 2000, **57**, 431.
22. Bodek K. H.: *Acta Polon. Pharm. - Drug Res.* 2001, **58**, 185.
23. Bodek K. H.: *Acta Polon. Pharm. - Drug Res.* 2002, **59**, 105.
24. Bodek K. H.: *Acta Polon. Pharm. - Drug Res.* 1995, **52**, 337.
25. European Pharmacopoeia, 1997. Pharmaceutical Technical Procedures. Council of Europe, Strasbourg, Codex, p. 127—135.
26. Bąk G.W., Bodek K. H., Hilczer B., Pawłowski T.: *IEEE Trans. Dielectr. Electr. Insul.* 2001, **8**, 555.
27. Bodek K. H., Bąk G. W.: *Eur. J. Pharm. Biopharm.* 1999, **48**, 141.
28. Fini A., Fazio G., Feroci G.: *Int. J. Pharm.* 1995, **126**, 95.
29. Abd-El-Bary A., Geneidy A., Osman A., Shalaby S., Morshan A.: *Pharmazie* 1999, **54**, 202.
30. Lin S. Y., Cheng C. L., Perng R. I.: *Eur. J. Pharm. Sci.* 1994. **1**, 313.
31. Lin S. Y., Perng R. I., Cheng C. L.: *Eur. J. Pharm. Biopharm.* 1996. **42**, 62.
32. Sancin P., Caputo O., Cavallari C., Passerini N., Rodriguez L., Cini M., Fini A.: *Eur. J. Pharm. Sci.* 1999, **7**, 207.
33. Bodek K. H., in: "Progress on Chemistry and Application of Chitin and Its Derivatives", Monograph Vol. V (Ed. Struszczyk H.), Polish Chitin Society, Łódź 1999, p. 103—113.
34. Fini A., Fazio G., Gonzalez-Rodriguez M., Cavallari C., Passerini N., Rodriguez L.: *Int. J. Pharm.* 1999, **187**, 163.
35. Villiers M. M., Liebenberg S., Malan S. F., Gerber J. J.: *Pharmazie* 2000, **55**, 544.

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