11. MICROCRYSTALLINE CHITOSAN AS PHARMACEUTICAL PREPARATION

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1. Introduction

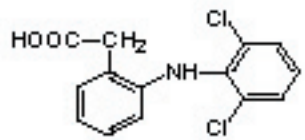
Chitosan is a polysaccharide derived from the chitin of crustaceans, with crab- and shrimp-shell wastes as its principal sources. Its properties as cationic polysaccharide, and its low toxicity and good biocompatibility [1, 2], make it interesting for study as an excipient. Chitosan which contains a number of amino groups in the polymer backbone is polycationic in acidic environments and forms gel. Important chemical properties of chitosan include the extent of deacetylation, and the average molecular weight of the polymer [3].

Both the literature data [4, 5] and the author's results indicate that the chitosan may be considered as pharmaceutical preparation. Applied as an auxiliary substance, it makes it possible to obtain many different dosage forms. An introduction of the polymer to a dosage form may control its properties which may be modified by choosing an appropriate type of the polymer. Because of its gel-forming ability, chitosan has received attention as a possible release-rate modifying excipient in hydrogel-based controlled-release systems.

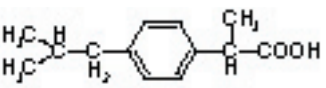
Microcrystalline chitosan (MCCh) is a special multifunctional polymeric material existing in the form of either of hydrogel at neutral pH or a powder. In the available literature there are few reports on the application of MCCh in medicine, pharmacy, and other areas [6-8]. The results of study [9] indicate 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.

2. Microcrystalline chitosan and its use as a pharmaceutical excipient in hydrogel - based controlled – release systems

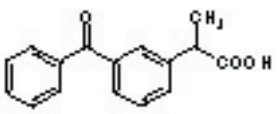
Microcrystalline chitosan was prepared using the previously described method [10] at the Institute of Chemical Fibres, Łódź (Poland). It was obtained from non-modified chitosan (Chemopol Co., India) and had an average molecular weight $\bar{M}_w \approx 100$ kDa. The deacetylation degree (DD), equal to 92%, was determined potentiometrically [11]. Microcrystalline chitosan in the form of hydrogel at neutral pH has not been used in the studies on NSAIDs so far. Our studies [12-16] were the first to evaluate the use of MCCh as NSAIDs carrier. MCCh 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 non-steroidal, anti-inflammatory drugs (NSAIDs). Conducted dermatological studies have confirmed biocompatibility of hydrogels of MCCh with human skin.



Diclofenac (DA)



Ibuprofen (IBA)



Ketoprofen (KTA)

Non-steroidal, anti-inflammatory drugs (NSAIDs)

The apparent viscosity of the MCCh hydrogel containing a therapeutical substance was found to increase in a year's long storage time. Changes in the apparent viscosity of MCCh hydrogel, induced by the presence of therapeutic substance and by storage time, are suggestive of physicochemical interactions between these substances. The more stable hydrogel systems containing a therapeutic substance were produced by using MCCh hydrogels of higher polymer content [12].

The effect of storage time on the yield stress and on the apparent viscosity of the hydrogel systems is presented in Table 1.

The microcrystalline chitosan as hydrogel with glycerol, 1,2-propylene glycol and methylcellulose hydrogel added, appears to be the useful vehicle. The high yield stress of this hydrogel system is an advantageous property. This property ensures good spreading and adhesion to the skin surface, which in turn determines uniform distribution of the therapeutic substance. The results of rheological studies of hydrogel systems suggest a complex nature of the system, which can be pseudoplastic or plastic depending

Table 1. *Changes in yield stress (τ_0) and apparent viscosity of hydrogel systems with storage time at 20 °C*

Type of hydrogel		τ_0 N m^{-2}	Shear rate, s^{-1}			
			10	100	550	1000
			Apparent viscosity, N s m^{-2}			
MCCh	after preparation	85.0 ± 0.7	15.10	2.63	0.63	0.15
	after 1 year	80.0 ± 0.8	13.58	2.33	0.59	0.36
MCCh-DS	after preparation	115.0 ± 0.5	26.67	3.33	0.88	0.64
	after 1 year	166.8 ± 0.6	30.40	3.91	1.09	0.86
MCCh-DA	after preparation	154.2 ± 1.7	20.87	3.28	0.81	0.52
	after 1 year	158.4 ± 1.9	24.66	3.36	0.83	0.50
MCCh-KTA	after preparation	140.0 ± 1.6	23.87	3.17	0.79	0.46
	after 1 year	150.0 ± 1.5	27.80	3.70	0.99	0.60

on the kind and content of the polymer and also on the presence of other substances and occurrence of physicochemical interactions between the polymer and these substances. The influence of microcrystalline chitosan hydrogel, alone as well as in combination with methylcellulose (MC) or Carbopol (CP), on the release of diclofenac free acid (DA) and its salt (DS) was studied in vitro. The results confirmed that release was dependent on the chemical character of the drug and on the type of vehicle [13]. The least amount of diclofenac acid is released through the membrane from MCCh/DA (F1) after preparation (small amount of diclofenac acid in water after release process). The increase in the release of diclofenac acid after storage time may be explained on the basis of an increase in the solubility of the drug in MCCh hydrogel (Table 2). The obtained results confirm that the introduction of ethanol, triethanolamine (TEA), and hydrophilizing agents (1,2-propylene glycol and glycerol) into MCCh hydrogel leads to a better release rate of diclofenac from MCCh/DTEA hydrogel formulations (F3) and MCCh/MC/DTEA hydrogel formulations (F5). The process of DS release from MCCh hydrogel alone (F2) is quicker than from modified MCCh hydrogel (F6).

From the analysis of the data reported in Table 2, release rate of ketoprofen from MCCh hydrogel formulations was significantly influenced by both, the rheological properties of the basis and drug-base interactions [14]. The release was significantly influenced by the character of the vehicle used as well as by the drug interaction with its components. The change in the modified MCCh hydrogel features (increase the viscosity) following addition of MC hydrogel and hydrophilizing agents (G, PG) is associated with a decrease in drug release in similar as observed for diclofenac [13]. The enhanced pharmaceutical availability of ketoprofen can be attributed to charge transfer complex (CT) formation ($COO^-NH_3^+$) between carboxylic group of drug and free amine group of MCCh [17].

The process of drug release from studied hydrogel formulation was of a two-phase nature in the majority of the analysed systems. The first phase was characterized by rapid release whereas in the second phase the release was much slower.

Table 2. *Values of constants of kinetic equation $C_t = C_1(1\text{-}e^{-k1t}) + C_2(1\text{-}e^{-k2t})$ describing the release of therapeutical substance from hydrogel formulation into water*

Formulation		Phase I			Phase II		
		C_1	$k_1 \times 10^3$	$t_{0.5}$	C_2	$k_2 \times 10^5$	$t_{0.5}$
		%	min^{-1}	min	%	min^{-1}	h
MCCh-DA	F1	19.5 ± 0.7	11.1 ± 0.1	62.4	80.8 ± 0.1	12.6 ±0.1	91.7
MCCh-DS	F2	45.4 ± 2.5	20.0 ± 1.1	34.7	50.3 ± 0.7	213 ± 2	5.4
MCCh-DTEA	F3	98.2 ± 1.3	3.20 ± 0.03	216	-	-	-
MC-DS	F4	38.5 ± 1.1	65.4 ± 0.1	10.6	61.2 ± 0.6	51.7 ± 0.5	22.3
MCCh-MC-DTEA	F5	53.8 ± 1.0	16.4 ± 0.3	42.3	44.4 ± 0.3	31.2 ± 0.9	37
MCCh-MC-DS	F6	50.3 ± 1.5	12.7 ± 0.2	54.6	45.2 ± 0.3	10.0 ± 0.1	115
MCCh-KTA	F7	20.1 ± 0.5	30.0 ± 0.1	23.1	79.5 ± 0.7	185 ± 5	6.3
MCCh-MC-KTA	F8	99.4 ± 0.2	0.508 ± 0.018	1360	-	-	-
MC-KTA	F9	99.2 ± 0.7	1.08 0.05	642	-	-	-

3. The influence of drug-polymer interactions on solubility of selected non-steroidal anti-inflammatory drugs

The interactions between ketoprofen and analysed microcrystalline chitosan as carrier were clearly confirmed by the shift of the endothermic peak, characteristic for pure ketoprofen in the DSC curve of MCCh-KTA lyophilized formulation at lower temperature. The occurrence of interactions between the microcrystalline chitosan and ketoprofen was also confirmed by the results of IR and ^{1}H NMR spectroscopic measurements. In the MCCh-KTA lyophilized system, the crystallinity of ketoprofen was found decreasing to a considerable extent as a result of its interactions with the chitosan of amorphous form [14].

The interaction of IBA with MCCh in the solid state was similar to that observe for ketoprofen. IR spectra of IBA-MCCh system supported the previous results and confirmed the formation of charge transfer complex ($COO\text{-}NH_3^+$) between the carboxyl group of IBA and the amine group of chitosan. The band at 1723 cm^{-1}, characteristic of IBA carbonyl stretching band, was retained in the physical mixture, whereas it had disappeared in case of the IBA-MCCh system [15]. The interaction of ibuprofen with MCCh both, in aqueous dispersion and in the solid state was examined by performing solubility analysis, differential scanning calorimetry (DSC), powder X-ray diffractometry, and IR spectroscopy. Dissolution rate of the pure drug and its solid formulation

(film) or physical mixture with MCCh was examined according to the solid dispersed amount method using the modified paddle apparatus. The dissolution rates of the physical mixture and film, 44.8×10^{-3} and 47.4×10^{-3} min^{-1} respectively, are faster than that of the drug alone (7.49×10^{-3} min^{-1}). The enhanced dissolution rate can be attributed to an increase in solubility and a decrease in crystallinity of the drug. Therefore, the drug solubility was evaluated in the presence of MCCh. The phase-solubility diagram of IBA in aqueous MCCh dispersions at 37 °C is presented in Figure 1.

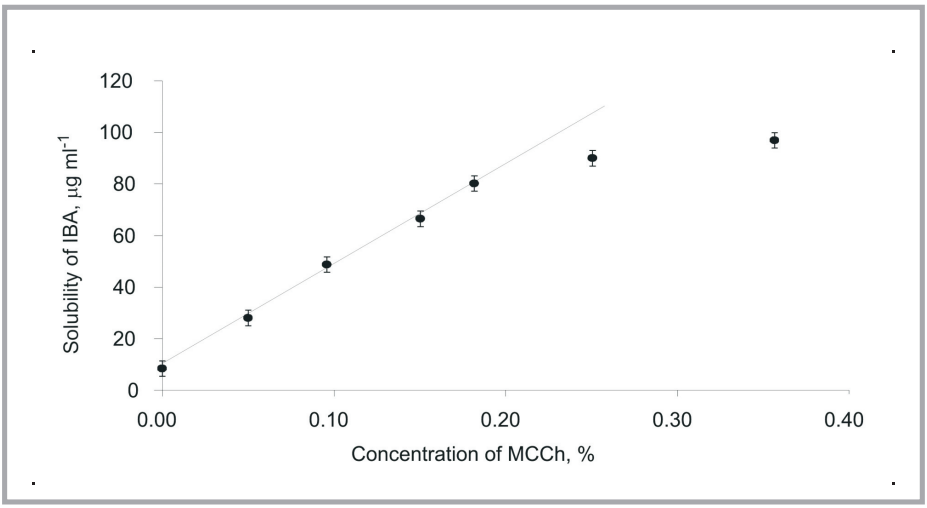


Figure 1. *Phase solubility diagram of ibuprofen – MCCh system in water at 37 °C; data are the mean ±standard deviations, n = 4.*

The graphical illustration of the maximum concentration of the drug as a function of MCCh concentration reveals the existence of nearly linear relationships (mean $r^2 = 0.9979\pm0.0015$) at the concentration of MCCh below 0.20%. At concentrations above 0.20%, a non-linear decrease in solubility of IBA was observed. The improvement of IBA solubility was attributed to salt formation between the carboxylic group of anionic drug and the free amine group of MCCh, which was similar to that, observes for ketoprofen [14].

Based on the findings, it was concluded that the MCCh is a suitable carrier for enhancing the availability of poorly water-soluble drugs, like ibuprofen. The enhanced dissolution of IBA from the IBA-MCCh system (film) could be mainly attributed to amorphization of the drug (as was demonstrated by X-ray diffractometry) [15]. As reported for non-modified chitosan, its microcrystalline form is a suitable carrier for water soluble and insoluble drugs and for enhancing the availability of poorly water – soluble drugs.

4. Stability of microcrystalline chitosan systems with selected non-steroidal anti-inflammatory drugs

The stabilities of microcrystalline chitosan systems with selected non-steroidal anti-inflammatory drugs 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 [16]. 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 interaction 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 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) [18]. The release of DA from MCCh-hydrogel system increases during the storage because of the interactions between the drug and the MCCh-hydrogel, probably occurring after a long storage time. Increased solubility of DA in MCCh-hydrogel had been confirmed by earlier studies [13].

For ketoprofen, which is better soluble in water than diclofenac, storage time does not affect the amount of released drug. The release rate of KTA from MCCh film was not greatly influenced by storage at ambient temperature for 12 months. This 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 [16]. The amount of a drug released from the film heated 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.

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. 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. This fact may be used for pharmaceutical purposes to improve the solubility, stability and biological availability of drugs poorly soluble in water.
The obtained results [13-16] show that microcrystalline chitosan is useful for the preparation of gels and films.

5. Physicochemical features of membranes made of microcrystalline chitosan

Controlled biodegradability and biocompatibility of MCCh makes it possible to use this polymer as resorbed membranes for guided tissue regeneration. These membranes are an alternative for those currently used for guided bone regeneration (GBR). The physicochemical features of new type of GBR membrane have presented in the study [19]. At present the above barrier membranes seem to be a suitable material for applications in oral surgery.

The main purpose of the paper [20] is to present the use of the membranes made of microcrystalline chitosan as a drug (NSAIDs) carrier. A method of the modified membranes production is presented. The membrane was made from microcrystalline chitosan plasticized with glicerol or 1,2-propylene glycol and another neutral polymer – methylcellulose (MC). The modified membrane structure had three layers: MCCh-MC-MCCh or MC-MCCh-MC. Methylcellulose introduce for correcting mechanical properties of membrane, thermal stability and abilities to swell of waters.

Research was led in such directions as thermal stability of the membranes, as well as estimate of susceptibility on biodegradation of employed material. The results of analytical studies are presented [20]. It is shown, that the membranes can be totally destroyed in the biodegrading environment.

The pharmaceutical availability of a drug (ketoprofen) from so received membranes and their mechanical properties, as well as ability to swell has been presented earlier [21]. The process of drug release from a majority of membranes can be described with a first order equation with two exponential functions. The release of ketoprofen into buffer was much quicker and one-phase for chitosan non-modified membrane and with glycerol [14] as compared with chitosan membrane with 1,2-propylene glycol, for which it was two-phase [21]. Thus, 1,2-propylene glycol seems to be effective as both plastificator and substance delaying the release of a drug by decreasing the permeability of a membrane. Pharmaceutical availability of ketoprofen depends on the type and amount of polymer used for the formation of a membrane and on its construction. We have observed slower release of ketoprofen from composite membranes. Two-layer distribution of ketoprofen allows for controlled release of the drug.

We have compared the mechanical properties of membranes. Microcrystalline chitosan membranes with glycerol or propylene glycol were much more elastic and less fragile than membrane without additional substances. Methylcellulose membrane showed high values of mechanical strength. However, the mixture membrane obtained from microcrystalline chitosan and methylcellulose showed decreased breaking stress. Combining layers of microcrystalline chitosan with methylcellulose improved mechanical strength parameters of obtained membranes.

We have also compared the ability of membranes to swell. Profiles of swelling for the studied membranes are presented in Figure 2.

Methylcellulose membrane completely dissolved after 1 hour, and mixture membrane after 3 hours fell into pieces. For the remaining membranes an increase of membrane mass was observed in comparison with initial dry membrane mass. As described in [14] the film, prepared from MCCh hydrogel by evaporation, was swelling slowly in releasing environment and was water resistance in contrary to hydrophilic film of methylcellulose that swelled in the some environment into a hydrogel of high viscosity. The highest ability to swell was noted for microcrystalline chitosan membrane without any additional substances. After 3 hours its mass increased three-fold and continued to increase. Also, chitosan membrane with glicerol showed high ability to swell. For layer chitosan-methylcellulose membranes, varying ability to swell was noted. Composite membrane with methylcellulose inner layer showed higher ability to swell. This membrane presented a totally different profile of swelling (Figure 2). The mass of the membrane after 1 hour increased four-fold and after 3 hours reached maximum of swelling (300%). The membrane of a reversed construction showed decreased ability to swell. After 3 hours the mass increased two-fold and after 6 hours a hydrogel formed from methylcellulose coating. Decreased ability to swell was also observed for membrane with propylene glycol. This may be explained by lower porosity of membrane.

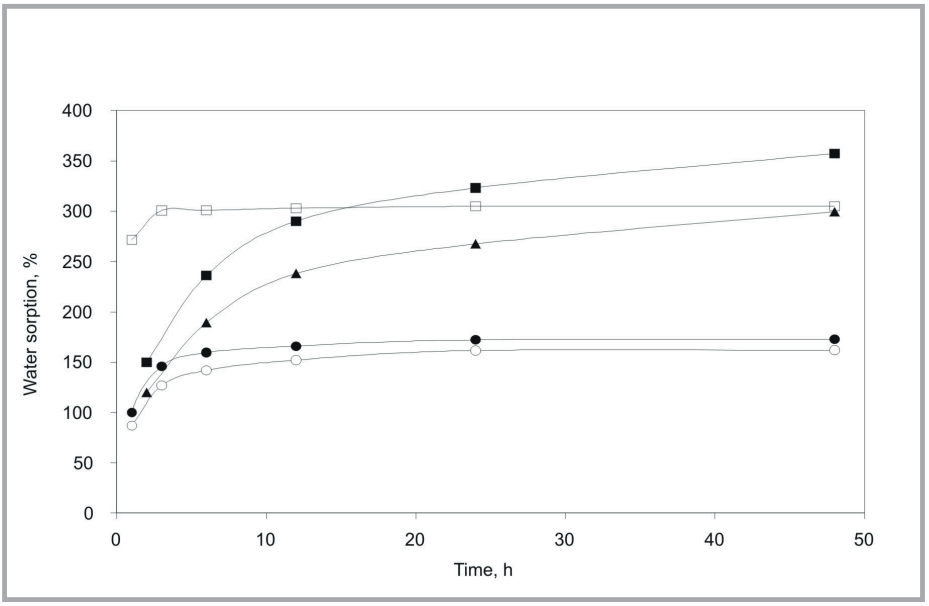


Figure 2. *Sorption water profiles of membranes at temperature 25 °C; MCCh (■), MCCh-G (▲), MCCh-GP (●), MCCh-MC-MCCh (□), MC-MCCh-MC (○)*

Consideration of the MCCh as an excipient is based on the following aspects:
- definition of the applicability of the MCCh in the production of gels with ointment consistency as a base for therapeutic substances,
- definition of the usefulness of MCCh in the production of films and membranes.

These membranes may at the same time serve as a carrier NSAIDs.

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Acknowledgments

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