Journal of Pharmaceutical and Biomedical Analysis 49 (2009) 655–659

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Evaluation of surface and microstructure of differently plasticized chitosan films

János Bajdik[a], Marzia Marciello[b], Carla Caramella[b], Attila Domján[c], Károly Süvegh[d,e], Tamás Marek[e], Klára Pintye-Hódi[a,*]

[a] Department of Pharmaceutical Technology, University of Szeged, Eötvös u. 6, H-6720 Szeged, Hungary
[b] Department of Pharmaceutical Chemistry, University of Pavia, Viale Taramelli 12, 27100 Pavia, Italy
[c] Institute of Structural Chemistry, Chemical Research Center, Hungarian Academy of Sciences, P.O. Box 17, Budapest H-1525, Hungary
[d] Laboratory of Nuclear Chemistry, Eötvös Loránd University, H-1518 Budapest 112, P.O. Box 32, Hungary
[e] Laboratory of Nuclear Chemistry, Chemical Research Center, Hungarian Academy of Sciences, H-1117 Budapest, Pázmány P. s. 1/A, Hungary

ARTICLE INFO

Article history:
Received 21 August 2008
Received in revised form
14 November 2008
Accepted 12 December 2008
Available online 24 December 2008

Keywords:
Chitosan
Plasticizer
Positronium lifetime measurement
Solid-state NMR
Surface free energy

ABSTRACT

Surface and structural investigations of natural biopolymer (chitosan) films containing various conventionally applied hydrophilic plasticizers (glycerol and poly(ethylene glycol) 400) were performed and the results were compared, with the aim of acquiring new information concerning the formation of these plasticized films. The surface tests revealed that the water uptake, the water-binding properties (moisture content) and the polarity were higher for the film containing glycerol as plasticizer. Positronium lifetime measurements and NMR studies performed to evaluate the effects of the plasticizer on the polymer structure demonstrated relevant differences in the effects of the plasticizers. The influence of glycerol on the structure of the film formed was more intensive than that of PEG 400. It can be concluded that the surface properties of the films, which are very important for their storage and application, cannot be established exactly by means of structural tests. Both surface and structural tests must be performed before the formulation of this type of plasticized mucoadhesive films.

1. Introduction

Efforts are currently being made to acquire a more detailed understanding of all technological processes in pharmaceutical technology and hence in industry. Knowledge of film formation from aqueous solution of polymers is very important during the coating of solid dosage forms. This process and the structure of the film can be influenced by different additives [1,2]. An understanding of the formation of macromolecular films is very important, because the field of application of these films is constantly becoming wider. They are used not only as coatings and binders for granulation, but also as separate dosage forms (films for topical application), e.g. the buccal administration of different sensitive agents, such as proteins [3,4].

An exact knowledge of film-formation properties can be informative in an evaluation of the surfaces of films. These are very relevant because the surface can determine the adhesion to the core or to biological membranes (bioadhesion for drug-loaded films) [5]. In spite of the importance of these surface tests, however, they are not frequently performed during optimization of fluids containing film-former agents. In studies conducted to evaluate the properties of the surfaces of films, mainly films prepared from different solutions have been used [6,7]. It this case, the aqueous and organic solution of different types of cellulose ethers was evaluated. This can furnish a better understanding of the effects of different additives. The changes in the surface properties of films due to the additives must not decrease the adhesion of the complex film to the surface. The adhesion between the particles can be calculated from the surface free energies of the components [8]. The levels of adhesion between solid materials and a single binder polymer for granulation have been evaluated [9,10]. In these studies, the work of adhesion and the work of cohesion were calculated and correlated with the pellet properties (friability, bulk and tapped density, and porosity). However, those publications did not deal with the films, but only with the solid components. In the event of the coating of films containing a large number of additives, it is not enough to determine the adhesion between the polymer and the core. Determination of the surface free energy of the complex film is therefore very important. It is well known from our previous results that the surface properties of film can not be predicted from the parameters of the separate components [11]. In that study, taste-masking films were prepared from aqueous citric acid solutions of a cationic polymer with various hydrophilic plasticizers. The wetting properties and surface free energy of the free films were studied. The direction of the change in polarity (a hydrophilic component caused a decrease in the polarity) was

* Corresponding author. Tel.: +36 62 545 576; fax: +36 62 545 571.
E-mail address: klara.hodi@pharm.u-szeged.hu (K. Pintye-Hódi).

0731-7085/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.12.020

unexpected. It was explained by the change in orientation of the macromolecules.

Changes in the arrangement of macromolecules can alter the properties of the surface of the film. This can mainly be achieved with different additives (or with the active agent for drug-loaded films). In this case, the network of the polymers is disturbed by these components. An factor affecting the surface chemical properties of the cellulose ether films is the degree to which functional groups and the cellulose backbone orient during film formation [6]. These water-soluble components are surface active and will organize and form structures at the solution–air interface. The properties of the pendant chains will also influence this process. These surface effects during the drying process may contribute significantly to the relative presence of functional groups at the surface in much the same way that solvent composition and annealing can influence surface properties in EC [7]. Methods for a separate evaluation of the internal structure must therefore also be applied for a better understanding of film formation.

In the present work, the surfaces and structures of samples containing the natural polymer chitosan (CS) with various conventionally applied hydrophilic plasticizers (glycerol and poly(ethylene glycol) (PEG) 400) were compared. It is a preformulation study for the preparation of poured drug loaded film. These mucoadhesive films were prepared from the acidic solution of chitosan. The protecting effect of this film is not appropriate for the formulation of coating product. It can be increased with the alkalizing of the film. Our aim was the formulation of free films for individual buccal drug delivery system. In this case, the application of this step is not obviously necessary. The alkalizing of the surface of film was therefore not applied. The moisture contents, wetting properties and surface free energies of the free films were studied in order to evaluate the external surfaces of the films. These studies were supplemented with solid-state nuclear magnetic resonance (NMR) spectroscopy and positronium lifetime measurement so as to evaluate the arrangement of the film-forming molecules and hence the structures of the films, which is very important as concerns an understanding of the formation of plasticized films.

The positron is the anti-particle of the electron. In vacuum, it is stable but it undergoes annihilation when it encounters an electron. In materials, the characteristics of the annihilation are determined by the chemical and spatial structure of the material [12]. When positron spectroscopy is applied for the study of polymers, the most important positron state is the positronium. This is a very light "hydrogen" atom in which the proton is replaced by a positron. This "atom" scans the free volume of materials. The longer its lifetime, the larger is the free volume. In the case of polymers, this free volume is the free space between the polymeric chains.

Solid-state NMR spectroscopy is an effective and powerful tool for the characterization of mixed and amorphous materials that cannot be characterized by means of scattering methods. Besides an analytical characterization of a system, solid-state NMR methods yield structural information on the molecular scale. ^{1}H single pulse and cross-polarization (CP) ^{13}C solid-state NMR spectra were recorded and analyzed in order to investigate the changes in molecular dynamics caused by the plasticizer.

2. Experimental

2.1. Materials

CS is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit). High molecular weight CS (Chitosan 1568, Giusto Faravelli S.p.a., Milano, Italy) was applied (degree of acetylation = 9%). The molecular weight of the chitosan was previously determined by using the viscosimetric method, using the Mark Houwink equation [13,14] The obtained molecular weight was of 1850 kDa. Ascorbic acid (AA), glycerol and PEG 400 were supplied by Sigma Chemicals Co. (Milano, Italy). Ascorbic acid was applied as solubilizing agent and it may improve the permeation enhancement properties of the chitosan [13].

2.2. Preparation of free films

2.43 g of AA was dissolved in distilled water. After its dissolution, the plasticizer was added at 1% (w/w) followed CS to obtain a concentration of 2.5% (w/w). The pH of the CS + AA solution containing PEG 400 was about 4.62, and that of the solution containing glycerol was about 4.8. 3 g of each liquid was poured onto an even Teflon surface with a diameter of 3.3 cm, and dried under ambient conditions ($25 \pm 2\,^{\circ}C$ and $50 \pm 5\%$ RH). The dried samples (after 24 h drying) were stored for 24 h in a desiccator (<20% RH/room temperature) and were examined. Film thickness was measured with an accuracy of 0.001 mm with a screw micrometer (Mitutoyo Ltd., Kawasaki) at the middle of the specimens.

2.3. Water uptake of films

An Enslin apparatus with a glass filter and a pipette with an accuracy of 0.01 ml were used to determine the water uptakes of the free films. A piece of film took up the maximum quantity of water possible through a filter paper under these conditions. The quantity of fluid was controlled every 10 s. 0.10–0.15 g of film was tested. Three parallel experiments were performed.

2.4. Moisture content

The moisture contents of the free films were determined with a moisture analyzer (HR73 Halogen Moisture Analyzer, Mettler-Toledo GmbH, Greifensee, Switzerland). The standard drying program was used and the drying temperature was 105 °C. The sample was heated to the drying temperature and then held constant at that temperature. The films were dried to constant weight. Three parallel experiments were performed.

2.5. Determination of wetting and surface free energy of films

One major factor to consider in solid–solid contact is the surface free energy. The surface free energy is the free energy change when the surface area of a medium is increased by unit area. For solids, the surface free energy is commonly denoted by γ_s and is given in units of energy per unit area, mJ/m^2 (or mN/m).

The surface free energy of a sample can provide very important information (e.g. adhesion, spreading, etc.) concerning the processibility of the solid product. It is therefore advisable to know this parameter during the formulation of a film-coated product. The solid surface free energy was calculated according to the method of Wu [15]. It is the sum of the polar (γ_s^p) and dispersion (γ_s^d) components of the solid. The solid-surface free energy can be assessed by measurements of the contact angle of two liquids of known polarity and the solution of two equations with two unknowns:

$$(1 + \cos\,\Theta)\gamma 1 = \frac{4(\gamma_s^d \gamma_l^d)}{\gamma_s^d + \gamma_l^d} + \frac{4(\gamma_s^p \gamma_l^p)}{\gamma_s^p + \gamma_l^p}$$

where γ_l is the liquid surface tension and γ_s is the solid-surface free energy.

The polarity, as a percentage, can be derived from the surface free energy. It is the ratio of the polar part and the total surface free energy.

An optical contact angle measuring device (OCA 20, DataPhysics Instruments GmbH) was utilized to determine the wetting proper-

ties of the samples. An automatic syringe was used for the dropping, and circle fitting was applied to determine the contact angle. The test fluids were water and diiodomethane (Merck KGaA, Darmstadt). The dispersion part of the surface tension was 21.8 mN/m for water and 50.8 mN/m for diiodomethane. The polar part of the surface tension was 51 mN/m for water and 0 mN/m for diiodomethane [16]. The dried samples (after 24 h drying) were stored for 24 h in a desiccator (<20% RH/room temperature) and were examined with the two solvents. The contact angles were calculated from results of 20 drops.

2.6. Positronium lifetime measurement

The positron source consisted of carrier-free ^{22}NaCl. It was placed between two very thin (2 mg/cm^2) kapton foils and two identical polymer samples. The activity of the source was around 105 Bq. The lifetime spectra were collected by a conventional fast–fast coincidence unit. The time resolution of the system was around 250 ps.

The spectra were evaluated by the computer program RESOLUTION [17]. Three lifetime components were identified but only the longest one, the positronium lifetime, was used to characterize the samples. The intensity of positronium formation in the samples was around 8–10%.

2.7. NMR study

The solid-state magic angle spinning (MAS) ^{1}H and ^{13}C NMR spectra of the samples were recorded on a Varian NMR System operating at a ^{1}H frequency of 600 MHz, with a Chemagnetics 3.2 mm narrow bore triple resonance T3 probe in double resonance mode. The spinning rate of the rotor was in all cases 10 kHz. The ^{13}C NMR spectra were measured with a CP technique under Hartmann–Hahn conditions [18]. The contact time was 2 ms and SPINAL-64 [19] proton decoupling was used. The measuring temperature was 25 °C and adamantane was used as external chemical shift reference.

3. Results and discussion

3.1. Surface properties

First the general surface properties of the samples were tested. The mechanical property of the CS film without plasticizer was so poor that it was not appropriate for the preliminary tests. The film formed from the CS solution was inadequate and the film formed broke during drying ("spider web"-like). The development of the small pieces of this film (width was about 1–2 mm) was detected. These segments partly detached from the surface. Because of the cracked surface, the wettability and the contact angle of this film could not be determined. There was no significant difference in the thickness of the free films formed (Table 1). The enhanced water-binding tendency was higher for the films containing glycerol. This can be explained by the higher amount of uptake water and the increased moisture content.

The contact angles of the apolar diiodomethane were significantly ($p < 0.05$) different for the samples containing the different plasticizers (Table 2). Wetting of the films with the polar agent led to values that differed less. The calculated surface free energy was very similar for both samples. These results demonstrated that the polarity (the ratio of the polar part and the total surface free energy) of the films prepared with glycerol was slightly higher.

Increased moisture content can cause several stability problems, and differences in water uptake and wetting can influence the behaviour of the films in aqueous media (e.g. bioadhesion).

Table 1
General properties of the films.

	PEG 400	Glycerol
Thickness (μm)	96.0 ± 11.9	100.7 ± 6.9
Water uptake (ml/g)	1.91 ± 0.13	2.36 ± 0.09
Moisture content (%)	3.63 ± 0.38	4.60 ± 0.30

Table 2
Surface properties of the films.

	PEG 400	Glycerol
ΘDiiodomethane (°)	42.84 ± 2.69	47.24 ± 1.06
ΘWater (°)	31.03 ± 1.50	32.19 ± 1.94
γ^d (mN/m)	38.84	36.75
γ^p (mN/m)	32.45	32.69
γ^{Total} (mN/m)	71.29	69.43
Polarity (%)	45.52	47.08

3.2. Positronium lifetime measurement

The surface properties of the films (moisture content, water uptake and polarity) were changed, and the texture of these samples should also be evaluated for a better understanding of the film formation. The free volume holes are relatively small in CS, the positronium lifetime data indicating an average hole radius of around 4 Å (Table 3).

The two plasticizers applied to modify the structure of CS behave differently. While both slightly increase the positronium formation intensity, only glycerol modifies the lifetime. The lifetime is decreased very considerably, which indicates a significant modification of the original CS structure. In view of the chemical compositions of the given molecules, there are two plausible explanations for such a large lifetime decrease: the formation of inclusion complexes, or a large-scale destruction of the original system of secondary bonds. In the former case, the holes in the CS become filled with glycerol molecules; in the latter case CS chains move closer to each other.

Positronium lifetimes alone are insufficient to decide between the two possibilities. The NMR measurements performed in parallel with the positron study suggested that structural breakdown is much more probable than the formation of inclusion complexes. Thus, glycerol destroys the rigid structure of CS strong secondary bonds. The average hole radius in CS (glycerol) becomes a little less than 4 Å.

On the other hand, the other plasticizer, PEG 400 does not modify the structure of CS. The positronium lifetime data revealed that the free volume remains unchanged even after plasticization.

3.3. NMR study

Because of the homonuclear couplings, the signals in the solid-state proton NMR spectra of the rigid amorphous CS are very broad. The molecular motions averaging these couplings reduce the line widths. In consequence of the influence of the plasticizer, the segment and chain motions become faster in the polymers, and thus the proton signal narrows. The solid-state MAS proton spectra of the pure and the plasticizer (glycerol and PEG 400)-containing films

Table 3
The measured o-Ps lifetimes and the calculated void radii for the samples.*.

Sample	o-Ps lifetime (ns)	Void radius (Å)
Chitosan	1.578	4.09
Chitosan + glycerol	1.203	3.63
Chitosane + PEG	1.556	4.07

* The radii given are those detected by positrons. In the case of glycerol, the real radius might well be around 4.08 Å, but a glycerol molecule occupies in the void.

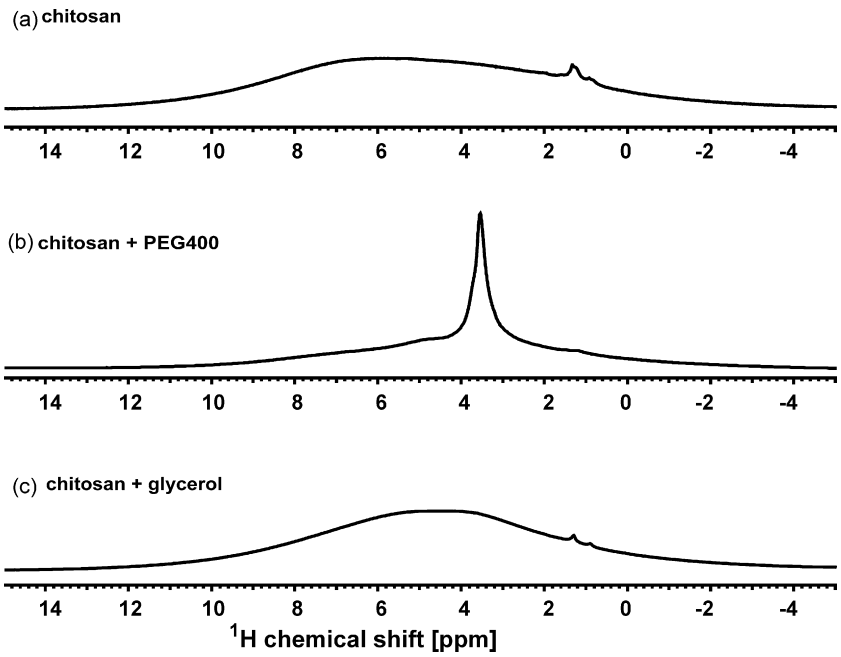

Fig. 1. (a–c) ^{1}H single-pulse solid-state MAS spectra of the untreated and plasticizer-containing films. The spinning speed of the rotors was 10 kHz.

are shown in Fig. 1. The signals relating to CS are broad and do not indicate any significant narrowing caused by the additives. The two proton signals of the glycerol cannot be identified because all the small molecules are strongly H-bonded to the polymer chains. This observation is in good agreement with a previous report [20] that the glycerol signals can be clearly recognized at over 50% glycerol content. The PEG 400 signal is much narrower than the CS signals, pointing fast segment motions, which results in averaging of the homonuclear couplings. The differences to between the proton spectra of the glycerol and the PEG 400-containing films demonstrate that the glycerol molecules are immobilized in the film because of the formation of H-bonds with the polymer matrix, while the PEG 400 molecules are more mobile.

The 13C NMR spectra provide more structural information than the 1H NMR spectra because of the larger chemical shift range. The 13C CP MAS NMR spectra of the samples are shown in Fig. 2. The deacetylation ratio was found by solution-state 13C NMR (spectra not shown) to be 50%. The ratio of acetylated and deacetylated anomeric carbon signal intensities was 1:1. The two C1 carbon signals are not distinguishable from each other. Because of the highly amorphous structure of the prepared films and the partial deacetylation, the carbon signals are relatively broad as compared with those of pure chitin [21] and CS [22].

The signal intensity of a CP spectrum depends strongly on the molecular and segment motions. The efficiency of the CP is enhanced from the more rigid region of the sample. The spectrum

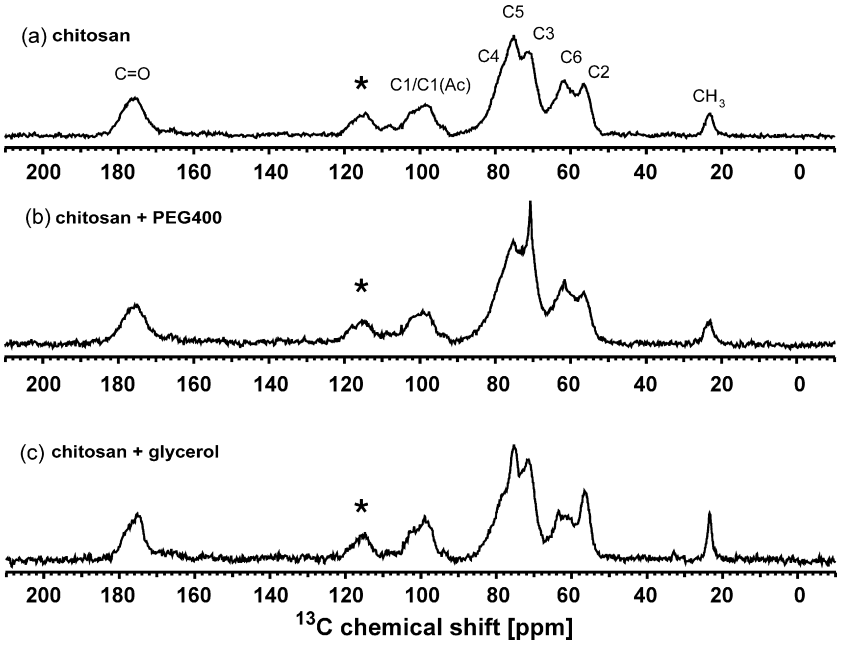

Fig. 2. (a–c) ^{13}C CP MAS solid-state spectra of the untreated and plasticizer-containing films. The spinning speed of the rotors was 10 kHz. The asterisks denote the spinning side band of the carbonyl atom.

(Fig. 2b) of the film containing PEG 400 differs only slightly from that of the untreated CS (Fig. 2a). The signal of the PEG 400 is clearly observable at 70.7 ppm and there are small changes in the signals of C6 and C2. The PEG 400 content of the sample is 30%, but its signal intensity does not reflect this because of the rapid segment motions which decrease the efficiency of the CP. This agrees very well with findings based on the ^{1}H NMR spectrum of the sample. The small changes in the signal of the C6 and C2 signals, however, show that there are some interactions between the CS and the PEG 400. The ^{13}C NMR signals of glycerol are not clearly identifiable, similarly as in the ^{1}H spectrum (Fig. 2c). Strong intermolecular interactions occur between the glycerol and CS molecules, which leads to broadening of the glycerol signals. Despite this broadening, they are definitely narrower than the signals of the other two samples. The signal narrowing indicates changes in the molecular and segment motions. The glycerol molecules form H-bonds with the CS and in parallel destroy the inter- and intramolecular H-bonds between the polymer chains. The partial decomposition of the H-bonded network structure leads to plasticized films. It is important, that all the spectra were acquired with the same experimental setup, in order to minimize the errors caused by different measuring settings. Further solid-state NMR investigations are in progress to investigate the changes in the H-bonds so as to obtain a better understanding of the role of the plasticizer.

4. Conclusions

It can be concluded, that the water uptake, the water-binding properties, and the polarity were higher for the CS film containing glycerol as a plasticizer. The application of glycerol therefore led to a more polar surface than that obtained with PEG 400. This surface can induce better wetting in aqueous media. The explanation of this phenomenon may be the enrichment of the plasticizer on the surface of the film and the special directed orientation of the molecules, and thus the performance of structural studies was advisable.

The positronium lifetime measurements and the NMR studies revealed that the effects of the plasticizer components differed. The connections between CS and glycerol were stronger than those between CS and PEG 400. The H-bond-forming action of glycerol was more intensive. The effects of further components in breaking down the H-bonds must therefore be considered during the design of CS films containing glycerol. The effect of PEG 400 on the structure of the film was less relevant.

Finally, it can be stated that the surface properties must be known for the application of films. The connections between the film-former macromolecule and the plasticizer must also be known for understanding of film-forming. Both surface and structural studies must therefore be performed before the formulation of this type of plasticized mucoadhesive films.

Acknowledgements

This work was supported by Hungarian Scientific Research Fund (OTKA) grant T-49815. We gratefully acknowledge financial support from the Hungarian project GVOP-3.2.1.-2004-04-0210/3.0.

References

[1] G. Cole, J. Hogan, M. Aulton, Pharmaceutical Coating Technology, Taylor & Francis Ltd., London, 1995.
[2] K.H. Bauer, K. Lehmann, H.P. Osterwald, G. Rothang, Coated Pharmaceutical Dosage Forms, Medpharm GmbH Scientific Publishers, Stuttgart, 1988.
[3] I. Diaz del Consuelo, F. Falson, R.H. Guy, Y. Jacques, J. Control. Rel. 122 (2007) 135–140.
[4] C.D. Brown, L. Kreilgaard, M. Nakakura, N. Caram-Lelham, D.K. Pettit, W.R. Gombotz, A.S. Hoffman, J. Control. Rel. 72 (2007) 35–46.
[5] G. Buckton, Interfacial Phenomena in Drug Delivery and Targeting, Harwood Academic Publishers, Chur, 1995.
[6] P.E. Luner, E. Oh, Colloid Surf. A 181 (2001) 31–48.
[7] E. Oh, P.E. Luner, Int. J. Pharm. 188 (1999) 203–219.
[8] F. Podczeck, Particle-particle Adhesion in Pharmaceutical Powder Handling, Imperial College Press, London, 1998.
[9] Zs. Tüske, G. Regdon Jr., I. Erős, S. Srčič, K. Pintye-Hódi, Powder Technol. 155 (2005) 139–144.
[10] O. Planinšek, R. Pišek, A. Trojak, S. Srčič, Int. J. Pharm. 207 (2000) 77–88.
[11] J. Bajdik, M. Fehér, K. Pintye-Hódi, Appl. Surf. Sci. 253 (2007) 7303–7308.
[12] K. Süvegh, A. Vértes, T. Hyodo, Adv. Mol. Struct. Res. 5 (1999) 313–357.
[13] S. Rossi, M. Marciello, G. Sandri, M.C. Bonferoni, F. Ferrari, C. Caramella, Pharm. Dev. Technol. 13 (2008) 513–521.
[14] Z. Jia, D. Shen, Carbohydr. Polym. 49 (2002) 393–396.
[15] S. Wu, J. Polym. Sci. 34 (1971) 19–30.
[16] R. Dreu, J. Širca, K. Pintye-Hódi, T. Burjan, O. Planinšek, S. Srčič, Int. J. Pharm. 291 (2005) 99–111.
[17] P. Kirkegaard, M. Eldrup, O.E. Mogensen, N.J. Pedersen, Comput. Phys. Commun. 23 (1981) 307–343.
[18] S.R. Hartmann, E.L. Hahn, Phys. Rev. 128 (1961) 2042–2053.
[19] B.M. Fung, A.K. Khitrin, K. Ermolaev, J. Magn. Reson. 142 (2000) 97–101.
[20] I. Quijada-Garrido, V. Iglesias-Gonzalez, J.M. Mazon-Arechederra, J.M. Barrales-Rienda, Carbohydr. Polym. 68 (2005) 173–186.
[21] M.F. Cervera, J. Heinämäki, M. Räsänen, S.L. Maunu, M. Karjalainen, O.M.N. Acosta, A.I. Colarte, J. Yliruusi, Carbohydr. Polym. 58 (2004) 401–408.
[22] A. Webster, P.O. Osifo, H.W.J.P. Neomagus, D.M. Grant, Solid State Nucl. Magn. Reson. 30 (2006) 150–161.