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Research paper

Ageing phenomena of chitosan and chitosan–diclofenac sodium system detected by low-frequency dielectric spectroscopy

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Abstract

The use of natural polymers for design of dosage form has received considerable attention recently, especially from the safety point of view. Among these polymers, chitosan shows very interesting biological, chemical and physical properties which makes it possible to use chitosan for various pharmaceutical applications. Microcrystalline chitosan (MCCh) is a special multifunctional polymeric material existing in the form of either of gelatinous water dispersion or a powder.

Thermal ageing of chitosan and chitosan–diclofenac sodium mixture have been studied using low-frequency dielectric measurements. The ageing was carried out by annealing in ambient atmosphere in the temperature range between 25°C and 100°C. The dielectric losses in the aged samples proved to decrease by about one order of magnitude. The additional measurements of molecular weight distribution and infrared absorption were also carried out for better understanding of nature of the ageing phenomena. Partial evacuation of water, cross-linking and improvement of structural order may be suggested to be a result of thermal ageing of the investigated materials. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Microcrystalline chitosan; Diclofenac sodium; Low-frequency dielectric spectroscopy; IR absorption spectra; GPC molecular weigh

1. Introduction

Chitin is the second, after cellulose, most abundant natural polymer. It is widespread in the animal world, in particular in crustacea, molluscs and insects where chitin is an important constituent of the exoskeleton and appears also in the cells of fungi and moulds [1,2]. Chitosan is produced from chitin by chemical or enzymatic deacetylation of chitin, though it also occurs naturally in some fungi. Owing to its properties, chitosan can be used in many areas, for instance in medicine, pharmacy, biotechnology, agriculture, and the food industry [3,4]. Chitosan is attracted to lipids and has the ability, acting like a ‘sponge’, to prevent fat in the digestive tract from being absorbed. Chitosan is indigestible and can reduce the transit time food pass through the digestive system. The result is that less fat, including cholesterol and fatty acids are available to be absorbed [5]. Chitosan is regarded as a good carrier for other pharmaceutical compounds to release the additional compound gradually. Recently, the use of natural polymers as drug carriers has received much attention in the pharmaceutical field especially from the viewpoint of safety and toxicity. Natural polysaccharides, such as chitosan, have been used to sustain the action of drugs. Chitosan as a drug carrier has been chosen because of its biocompatibility, biodegradability and healing characteristics so as to achieve a modulation in drug release which in turn would lead to reduced irritation effects of drugs [6,7]. Augmentation of the practical applications of this natural polymer is related to modification of its properties by suitable changes of molecular, supermolecular and chemical structure. New useful forms of chitosan with high practical usability are produced by its physical and chemical modification. Microcrystalline chitosan (MCCh) [8,9] is a special multifunctional polymeric material suitable for use in several applications. It exists in the two main forms: gelatinous water dispersion and powder characterised by several specific properties such as: controlled bioactivity, controlled biodegradability, controlled biocompatibility, direct film forming behaviour, developed intrinsic surface, high chelating ability.

Diclofenac sodium (DS) is a non-steroidal anti-inflammatory drug with analgesic and antirheumatic properties. DS is often used as a model drug for experimental testing of pharmaceutical techniques [10–12]. In this paper the dielectric

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properties of thermally aged system of microcrystalline chitosan and diclofenac sodium are reported. The basic purpose of the investigations was to test the applicability of low-frequency dielectric spectroscopy as a tool of characterisation of ageing phenomena in chitosan systems.

Low-frequency dielectric spectroscopy (LFDS) has been developed recently as a new analytical method in order to gain a better insight into the structure and properties of drugs [13]. In this paper LFDS is used for detection of thermal ageing phenomena of chitosan and its system with diclofenac sodium.

The dielectric properties of materials may be described by their complex capacitance $C^*(\omega)$ or permittivity $\varepsilon^*(\omega)$

$$C(\omega) = \mathrm{C}(\omega) - \mathrm{i}\mathrm{G}(\omega)/\omega$$

$$= (A/d)[\varepsilon'(\omega) - \mathrm{i}\varepsilon''(\omega)] = (\varepsilon_0 \mathrm{A/d})[\varepsilon_\mathrm{r}'(\omega) - \mathrm{i}\varepsilon_\mathrm{r}''(\omega)] \quad (1)$$

where C is the ordinary electrical capacitance, G is the conductance, A is the surface area, d is the thickness, $\varepsilon'(\omega)$ is the real part of permittivity and $\varepsilon''(\omega)$ is the imaginary part of permittivity, ε_0 is the permittivity of free space, i is the imaginary unit. Both the real and imaginary parts of capacitance may be also expressed by the relative permittivity ε^*_r being the ratio of capacitance of filled and empty capacitor. ω is the circular frequency of the sinusoidal external electric field.

The imaginary part of permittivity ε'' is related to the energy loss in the material being under the influence of external electric field. The quantity often used for description of dielectric losses is tangent of the loss angle

$$\tan\delta = \varepsilon''/\varepsilon' \quad (2)$$

Dielectric relaxations in real condensed systems are often well described by the well-known Havriliak–Negami expression:

$$\varepsilon^*(\omega) = \varepsilon' - i\varepsilon''$$

$$= i\left(\frac{\sigma_0}{\varepsilon_0\omega}\right)^n + \sum_{k=1}^{m}\left\{\frac{\Delta\varepsilon_k}{[1 + (i\omega\tau_k)^{\alpha_k}]^{\beta_k}} + \varepsilon_{\infty k}\right\} \quad (3)$$

where σ_0 is the direct current (*dc*) conductivity, $0 < n <= 1$, $\Delta\varepsilon$ is the dielectric strength, τ is the relaxation time, α and β are parameters describing the shape of a loss peak, ε_∞ is the high-frequency value of permittivity, m is the number of relaxation processes.

The shape of the loss peak depends on the distribution of relaxation times. The broader the distribution of relaxation times is, the broader the loss peak becomes. In general the distribution of relaxation times broadens with increasing disorder of structure.

2. Experimental Details

Microcrystalline chitosan (MCCh) obtained according to the original method [8] in a form of gelatinous water dispersion was the precursor material for further preparation of samples. MCCh was characterised by 3.4% of the polymer content, the average molecular weight Mw = 110 kDa and the degree of deacetylation DD equal to 85.7%. Diclofenac sodium was purchased from Sigma Chemical Co. The therapeutic substance, i.e. diclofenac sodium was dissolved in MCCh hydrogel by precise grinding of DS powder with small amount of the base gel. Such a material was then mixed with the rest of base at room temperature. The pure MCCh hydrogel and the MCCh hydrogel containing a drug (the weight ratio MCCh : DS = 3 : 1) were spread on a teflon plate and the water was evaporated at room temperature. After the evaporation of water the xerogel films were obtained and then crumbled. Next the material was mechanically blended and compressed. The final samples were prepared in form of compressed pellets obtained from powdered material. The weight of pellets was 0.1 g and their thickness was about 0.5 mm. The pellets were supplied with metal (gold or aluminium) electrodes of the diameter of 9 mm. The electrodes were evaporated in vacuum of the order of 10^{-5} Torr in order to obtain as good contact with compressed pellets as possible. In particular evaporation of electrodes should enable to avoid air gaps between electrode and sample. However, beam of gold or aluminium atoms falling onto the pellets may give rise to some initial ageing of substance at the very surface. This ageing process has not been detected since the first dielectric measurements were carried out after the evaporation.

The samples were aged in the ambient atmosphere at a few temperatures between 293 K (20°C) and 353 K (80°C) for about 5 h. The dielectric measurements were carried out using Solartron 1260 Impedance Analyser with Chelsea Dielectric Interface. The measurements were carried out in the air.

The distribution of molecular weight of chitosan was obtained by gel permeation chromatography using GPC Hewlett–Packard 1050 instrument. Acetate buffer pH 4.3 was used as a solvent, the measurements were carried out with a flow rate 0.8 ml/min at room temperature.

The IR spectra were recorded with Mattson FT-IR spectrometer at room temperature. Specimens for spectroscopy were prepared in the form of compressed pellets 0.5 mm thick consisting of KBr and the investigated material (1 mg of the investigated material + 300 mg KBr) or in the form of thin films 5 μm thick. The samples of chitosan and chitosan–diclofenac sodium system were annealed at 313, 333, 353 and 373 K for 5 h.

3. Results and discussion

3.1. Dielectric measurements

The dielectric properties of chitosan compressed pellets were carried out at the room temperature (293 K). The samples were annealed at 313, 323, 333 and 353 K for

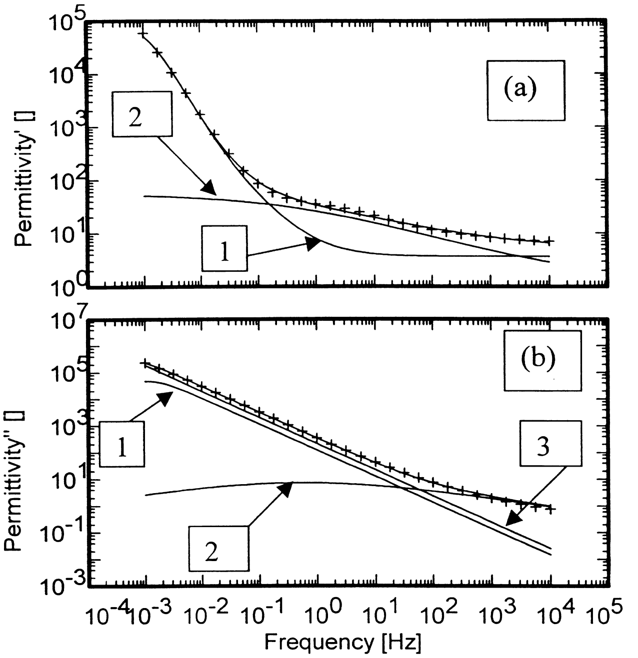

Fig. 1. Dielectric response of virgin compressed chitosan pellet at 293 K. (a) Real part of relative permittivity, (b) imaginary part of relative permittivity. 1, curves corresponding to the low-frequency relaxation; 2, curves corresponding to the high-frequency relaxation; 3, contribution of conductivity $(\sigma_0/\varepsilon_0\omega)^n$. The experimental points are marked with (+). The full curves show the results of fitting carried out with Eq. (3).

about 5 h. Fig.1 shows the dielectric response of virgin compressed chitosan pellet at 293 K. The experimental data were fitted with Eq. (3) assuming that two relaxation processes in the system occur. This assumption results directly from the shape of real part of permittivity. The first low-frequency relaxation process is defined by the relaxation time exceeding 100 s, the second high-frequency one is defined by the relaxation time of the order of 1 s (see Table 1).

The low-frequency relaxation phenomena give rise to a remarkable increase in real part of permittivity exceeding three orders of magnitude. Such a low-frequency dielectric dispersion may be either due to influence of electrode barrier or due to so-called Maxwell–Wagner–Sillars (MWS) relaxations typical of many inhomogeneous polycrystalline structures. Model describing dielectric effects resulting from any material inhomogeneity has been developed by Hill and Pickup [14]. The model allows to study these properties of inhomogeneous structures which result from their inhomogeneity. However, the paper is not focused on this problem so we do not consider the details resulting from the model. In case of electrode potential barrier the dielectric response should be dependent on the external dc voltage which has not been detected in the voltage range 0 ÷ 10 V. In this situation it may be suggested that the low-frequency dispersion results from MWS phenomena. Though the low-frequency values of permittivity are probably dependent on the polycrystalline structure of samples, they may be used for characterisation of the system. In particular it will be shown that they may be used for detection of ageing phenomena in the compressed chitosan pellets.

As far as the high-frequency relaxation is concerned it may be supposed that the relaxation is related to molecular movements. This high-frequency relaxation will be a subject of further investigations. However, it should be noted that the high-frequency loss peak is very broad (parameters $\alpha_{\mathrm{highfr}} = 0.35$ and $\beta_{\mathrm{highfr}} = 1$ describes this) which must correspond to a very broad distribution of relaxation times describing the molecular movements in the chitosan pellets.

Comparison of dielectric properties of the virgin and annealed samples is shown in Fig. 2. As a result of annealing at 313 and 323 K the loss peak shifts towards the lower frequencies by about half an order of magnitude. After annealing at 353 K the loss peak shifts further left. The result of the shift is that the dielectric losses decrease significantly at the frequencies over 0.1 Hz. The decrease in tan δ results from decrease in both the real and imaginary part of electrical permittivity. The influence of annealing on the real part of relative permittivity of chitosan compressed pellets at various frequencies is shown in Fig. 3. The decrease in relative permittivity resulting from annealing is more pronounced at the lower frequencies. The data shown in Figs. 2 and 3 mean that the thermal ageing of chitosan polycrystalline compressed pellets leads to significant change of their dielectric properties.

The influence of thermal ageing on the dielectric losses in chitosan–diclofenac sodium compressed pellets is shown in Fig. 4. As a result of annealing at 313, 323, 333 and 353 K tanδ decreases by about half an order of magnitude. No peak on the tan δ curve has been detected in the frequency range used. The real part of relative permittivity also decreases with increasing temperature of annealing (see Fig. 5). Like in the case of pure chitosan pellets, the influence of annealing is more pronounced for the lower frequencies of the measuring sinusoidal signal.

It is shown in Figs. 2 and 4 that the decrease in dielectric losses in chitosan–diclofenac sodium system is greater than in pure chitosan. It will be supported by measurements of

Table 1
Some of parameters defining the dielectric response of chitosan compressed pellets. The subscripts denote the low frequency and high frequency peak

Temperature of annealing (K)	$\tau_{\mathrm{low\ fr}}$	$\alpha_{\mathrm{low\ fr}}$	$\beta_{\mathrm{low\ fr}}$	$\tau_{\mathrm{high\ fr}}$	$\alpha_{\mathrm{high\ fr}}$	$\beta_{\mathrm{high\ fr}}$
293	152	0.98	1	1.19	0.35	1
333	127	0.91	1	3.84	0.87	0.75

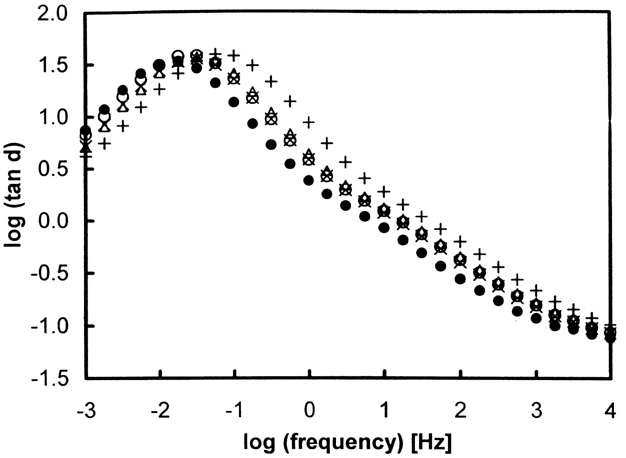

Fig. 2. Dielectric losses in virgin (+) and annealed for 5 h chitosan pellets at the room temperature (293 K). The temperatures of annealing: O313 K, Δ323 K, ×333 K, ●353 K.

molecular weight distribution that this is due to cross-linking in chitosan–diclofenac sodium system leading to increased contribution of bigger molecules and restraint of molecular motions in these samples.

In case of molecular solids annealing often leads to improvement of structure perfection due to recrystallization. The increasing perfection of structure should give rise to narrowing of the distribution of relaxation times related to molecular movements. Fig. 6 shows the results of fitting of dielectric response of chitosan compressed pellet annealed at 333 K for 5 h. The arms of the high-frequency loss peak are much steeper than in case of virgin chitosan pellets (compare with Fig. 1) which is confirmed by the values of parameters $\alpha_{\mathrm{high\ fr}}$ and $\beta_{\mathrm{high\ fr}}$ (see Table 1). As it was mentioned before this may result from improvement of structural order and confirms the suggestion that the structural order increases as a result of annealing.

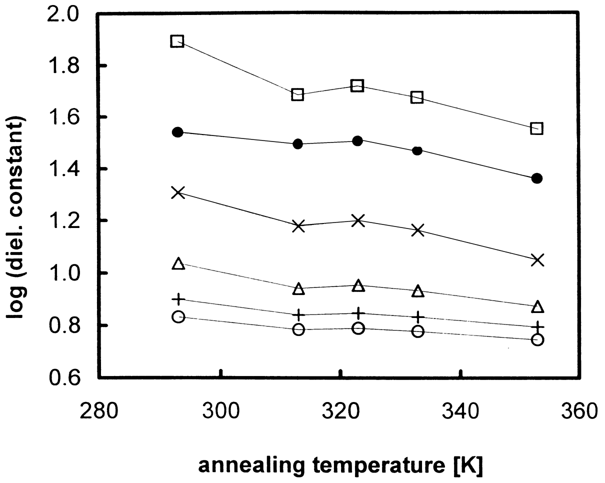

Fig. 3. Dielectric constant of chitosan compressed pellets measured at 293 K as a function of the annealing temperature for various frequencies. □0.1 Hz, ●1 Hz, ×10 Hz, Δ100 Hz, +1 kHz, O10 kHz.

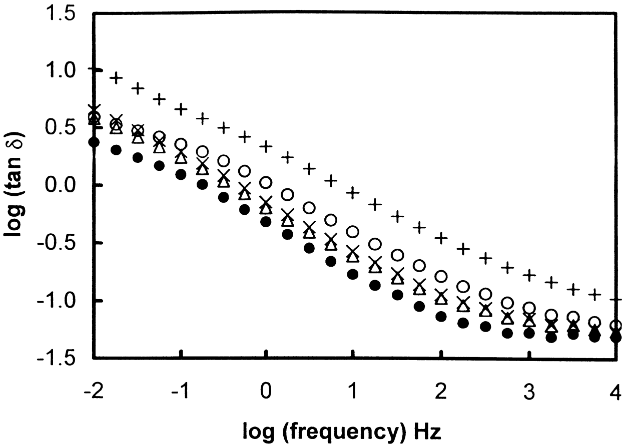

Fig. 4. Dielectric losses in virgin (+) and annealed for 5 h chitosan–diclofenac sodium compressed pellets measured at 293 K. The temperatures of annealing: O313 K, Δ323 K, ×333 K, ●353 K.

The increasing perfection of structure may also give rise to decrease in dielectric losses indirectly. Some localised electronic states in molecular solids may be related to structural disorder [15,16]. Charge carriers localised at the states may jump among them contributing to dielectric losses in this way. The more perfect structure becomes, the smaller the concentration of the electronic states may be expected to be [15]. This also can give rise to decrease in dielectric losses resulting from thermal ageing.

Apart from increased perfection of structure, the following main reasons for the decrease in dielectric losses in the investigated systems should be mentioned:

- the decrease in amount of water as a result of annealing. Polar water molecules may give rise to some additional dielectric losses and polarisation. Besides, water molecules may also give rise to electronic localised states

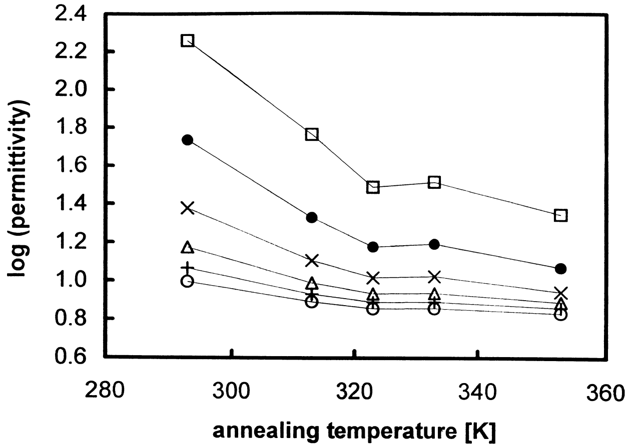

Fig. 5. Dielectric constant of chitosan–diclofenac sodium compressed pellets measured at 293 K as a function of the annealing temperature for various frequencies. □0.1 Hz, ●1 Hz, ×10 Hz, Δ100 Hz, +1 kHz, O10 kHz.

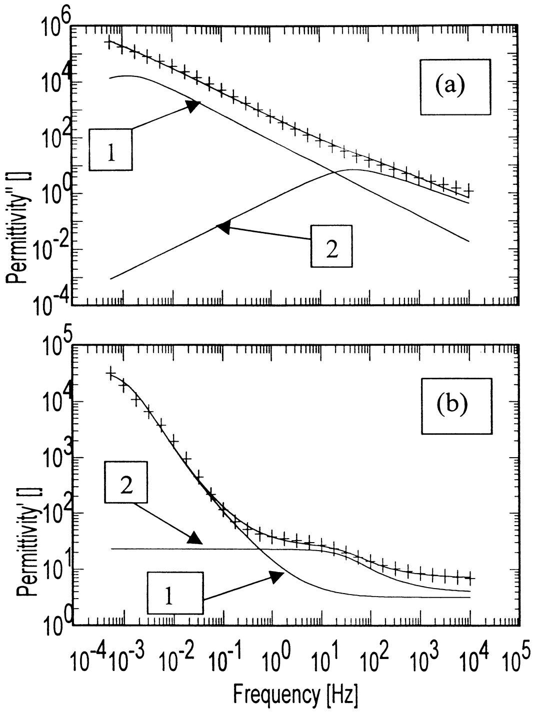

Fig. 6. Dielectric response of an annealed compressed chitosan pellet at 293 K. The annealing temperature was 333 K, the time of annealing was 5 h. (a) Imaginary part of relative permittivity. (b) Real part of relative permittivity. 1, curves corresponding to the low-frequency relaxation, 2, curves corresponding to the high-frequency relaxation. The experimental points are marked with (+). The full curves show the results of fitting carried out with Eq. (3).

[16]. Hopping transport of charge among such localised states also leads to increase in electrical permittivity,

- cross-linking resulting in additional confinement of molecular movements which in turn give rise to decrease in polarisation and dielectric losses.

In order to estimate the contribution of the above phenomena the investigations of infrared absorption and distribution of molecular weight have been carried out. Direct measurements of the water content have also been carried out to test the suggestion that the decreasing amount of water give some contribution to the decrease in dielectric losses as a result of annealing.

Table 2
Peak values of absorbance of chitosan film corresponding to 1090 cm^{-1} band and 3500 cm^{-1} band

Temperature of annealing (°C)	Band 1090 cm^{-1} C—O—C	Band 3500 cm^{-1} OH, NH
20	1.39	1.31
40	1.44	1.27
60	1.3	1.11
80	1.38	1.115
100	1.31	1.115

3.2. Infrared absorption

Three main absorption bands have been detected in chitosan samples (see Fig. 7), namely:

- the band in the range 3500–2500 cm^{-1} corresponding to the vibrations of OH, NH and CH groups,
- the complex band in the range 1690–1300 cm^{-1} corresponding to absorption of carbonyl and amide groups (Amide I band at 1665 cm^{-1} and Amide II band at 1565 cm^{-1}),
- the strong absorption band between 800 and 1200 cm^{-1}, which is characteristic of pyranose rings of chitosan.

The infrared spectra of chitosan–diclofenac sodium system is of similar shape though the relation between absorbencies corresponding to the three main absorption bands are a little different. Besides, some new absorption bands at 1452 cm^{-1} and 1576 cm^{-1} appear in chitosan–diclofenac sodium system.

We focus on the first (O—H) band at 3450 cm^{-1} and on the third one at 1090 cm^{-1} related to absorption of C—O—C bonds, because the first one may depend on the amount of water absorbed and the third one may give evidence of molecular degradation or cross-linking. The peak absorbence values of the first and the third band are shown in Tables 2 and 3. The above data may be summarised as follows:

1. in chitosan film the absorbance corresponding to OH group decreases from about 1.3 to about 1.15, which may be due to decreasing amount of water molecules. In case of chitosan–diclofenac sodium system the decrease is not so well pronounced,
2. the absorbance corresponding to C—O—C band increases above the annealing temperature equal to 313 K in chitosan–diclofenac sodium system. This may be due to cross-linking leading to increase in molecular weight. The above suggestion will be confirmed by the investigations of molecular weight of chitosan–diclofenac sodium system.

3.3. Molecular weight

Cross-linking of chitosan molecular structure may lead to restraint of molecular movement, which in turn should lead

Table 3
Peak absorbency values of chitosan–diclofenac sodium film corresponding to 1090 cm^{-1} band and 3500 cm^{-1} band

Temperature of annealing (°C)	Band 1090 cm^{-1} C—O—C	Band 3500 cm^{-1} OH, NH
20	1.4	1.32
40	1.41	1.35
60	1.5	1.33
80	1.47	1.27
100	1.47	1.31

Table 4
Percentage contribution of macromolecules of various molecular weight in chitosan and chitosan–diclofenac sodium systems

Samples (temperature of ageing)	Percentage contribution of macromolecules of molecular weight						
	>800 000	800 000 to 400 000	400 000 to 200 000	200 000 to 100 000	100 000 to 50 000	50 000 to 5000	<5000
Chitosan virgin	1	2	8	17	24	44	4
Chitosan (333 K)	1	2	8	17	24	45	3
Chitosan (373 K)	1	5	10	15	20	45	4
Chitosan after 1 year	1	3	8	13	17	52	6
Chit/DS virgin	0	0	2	7	15	70	6
Chit/DS (333 K)	1	3	7	12	16	54	7
Chit/DS (373 K)	1	3	8	14	18	51	7

to decrease in dielectric polarisation and losses. In order to estimate the influence of cross-linking on the detected changes of dielectric properties the investigations of molecular weight were carried out.

Figs. 8 and 9 show the differential distribution of molecular weight in chitosan and chitosan–diclofenac sodium system. Both in case of pure chitosan and chitosan–diclofenac sodium system the influence of thermal ageing on the molecular weight distribution has been observed. In case of pure chitosan the curves of differential molecular distribution shifts left. This suggests that some degradation of chitosan both due to the annealing and ageing for 1 year at room temperature takes place. However, in the case of the chitosan–diclofenac sodium system the influence of ageing is

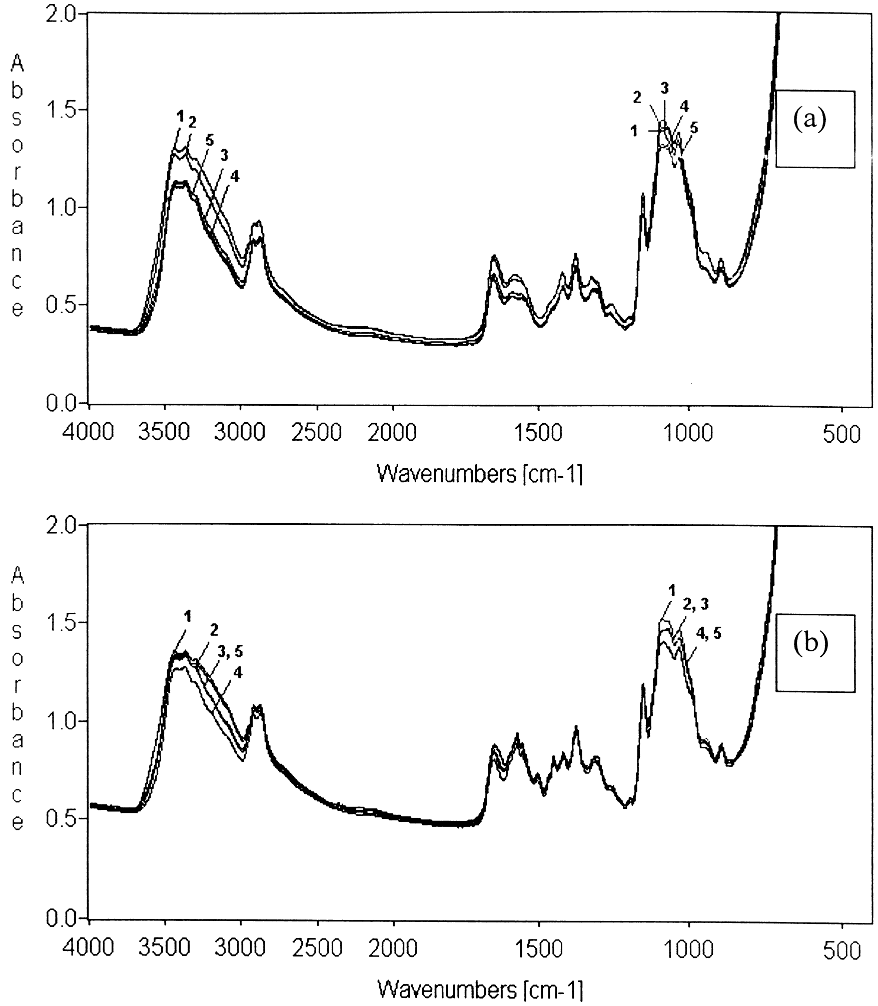

Fig. 7. IR spectra of chitosan film (a) and chitosan–diclofenac sodium system (b).The numbers of curves are: 1, virgin sample; 2, sample annealed at 313 K; 3, sample annealed at 333 K; 4, sample annealed at 353 K; 5, sample annealed at 373 K.

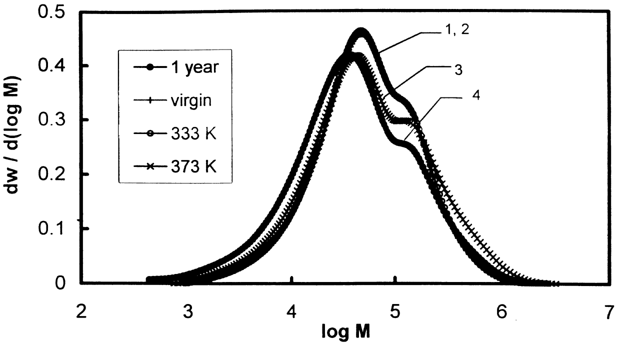

Fig. 8. Differential molecular weight distribution of chitosan samples aged at various temperatures. Curve 1 (+), virgin sample; curve 2 (○), sample aged at 333 K for 5 h; curve 3 (×), sample aged at 373 K for 5 h; curve 4 (●), sample aged for 1 year at room temperature.

different. The increase in contribution of bigger molecules in chitosan–diclofenac sodium samples aged at 333 K (60°C) and 373 K (100°C) should be noted. This is confirmed by the percentage contribution of molecules of various molecular weights shown in Table 4.

3.4. Water content

Additional measurements of water loss resulting from the annealing were carried out. The weight m_1 of initial substance used for preparation of compressed pellets was measured at the beginning. Then the substance was annealed for 5 h at 60°C and its weight after the annealing m_2 was determined. Finally, the substance was annealed at 100°C until its weight became constant (achieved at 5 h). The water content was found from

$$w = \frac{m_1 - m_2}{m_1} 100\% \tag{4}$$

The total loss of weight of pure chitosan was found to be 7% for annealing at 60% and 9% for annealing at 100%. In the case of the MCCh/DS mixture the loss of weight was 6% and 8%, respectively. These results confirm that the decrease in dielectric losses at least in part results from the decrease in water content in the investigated materials.

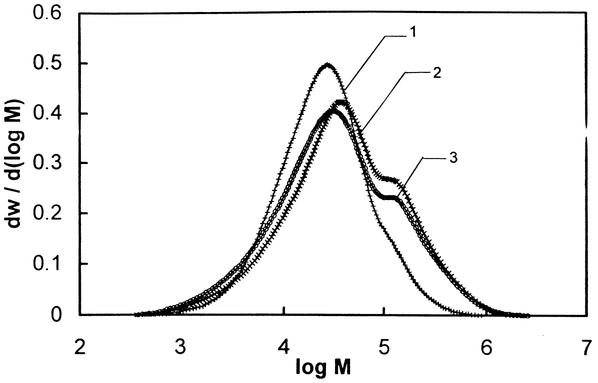

Fig. 9. Differential molecular weight distribution of chitosan–diclofenac sodium samples aged at various temperatures. Curve 1 (+), virgin sample; curve 2 (○), sample aged at 333 K for 5 h; curve 3 (×), sample aged at 373 K for 5 h.

3.5. Summary

Summarising the above discussion it may be suggested that the annealing of the compressed chitosan and chitosan–diclofenac sodium pellet leads to:

- a decrease in the amount of water absorbed which is confirmed by the infrared spectra,
- improvement of structural order confirmed by spectral shape of dielectric response,
- cross-linking confirmed by measurements of distribution of molecular weight in the chitosan–diclofenac sodium system. In pure chitosan pellets the effect of cross-linking has not been detected.

All the above phenomena lead to a decrease in dielectric losses and polarisation, which can be easily detected by low-frequency dielectric measurements. How the thermal ageing influences the release of drug from the chitosan/drug system is going to be a subject of other studies.

4. Conclusions

The following conclusions resulting from the investigation may be put forward:

1. thermal ageing of chitosan and chitosan–diclofenac sodium compressed pellets leads to substantial change of dielectric properties of the systems in the frequency range between $10^{-3} \div 10^{4}$ Hz,
2. two relaxation process in the frequency range are detected. The first one is due to so-called Maxwell–Wagner–Sillars polarisation phenomena related to inhomogeneous polycrystalline structure of the samples. The second one is probably due to molecular motions in the structures investigated,
3. The IR spectra suggest that the amount of water decreases as a result of thermal ageing. This may give rise to the observed decrease in the dielectric losses,
4. The distribution of molecular weights also changes as a result of thermal ageing. The changes of distribution of molecular weight are particularly well pronounced in chitosan–diclofenac sodium system where cross-linking may be suggested to occur,
5. Low-frequency dielectric spectroscopy may be a sensitive analytical method for detection of ageing phenomena in chitosan-related pharmaceutical systems.

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