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Synthesis and Biological Activity of Mechanocomposites of Piroxicam with Different Molecular Weight Chitosan

T. P. SHAKHTSHNEIDER[1,2], S. A. MYZ[1], A. S. MEDVEDEVA[3], L. P. SAFRONOVA[3], T. M. KONSHINA[4], A. A. BURLUTSKAYA[4], and G. V. GOLDOBINA[4]

[1]*Institute of Solid State Chemistry and Mechanochemistry, Siberian Branch, Russian Academy of Sciences, Novosibirsk, Russia*

E-mail: shah@solid.nsc.ru

[2]*Novosibirsk State University, Novosibirsk, Russia*

[3]*A. E. Favorsky Institute of Chemistry, Siberian Branch, Russian Academy of Sciences, Irkutsk, Russia*

[4]*Perm State Medical University named after Academician E.A. Wagner, Perm, Russia*

Abstract

Mechanocomposites of piroxicam with different molecular weight chitosan were produced. They possessed elevated rates of release of piroxicam into a solution. Anti-inflammatory and analgesic activities of the resulting composites were explored. Piroxicam-chitosan mechanocomposites were superior analgesics compared to piroxicam.

Key words: piroxicam, chitosan, mechanocomposites, mechanochemical activation, analgesic activity

INTRODUCTION

Piroxicam is a representative of a series of N-heterocyclic carboxamides and refers to the oxicam group. The species is widely used in medical science as a nonsteroidal analgesic, antipyretic, and anti-inflammatory drug [1]. Currently, the area of use of piroxicam is being broadened to replace opioids during the post-surgery period [2], and also in cancer chemotherapy [3–6]. It has recently been demonstrated that piroxicam also shows anti-tumour activity [7].

Piroxicam has good characteristics of permeability through membranes, but due to the low solubility in water (0.003 % at pH 5, 37 °C), it slowly dissolves in the gastrointestinal tract, which may cause stomach irritation. We have recently obtained a water-soluble bioconjugate of piroxicam and arabinogalactan sulphate having anti-inflammatory activity [8]. In order to increase the rate of dissolution and solubility of piroxicam, solid dispersed systems are also obtained using various methods and auxiliary substances. Both water-soluble polymers and inorganic carriers are among the latter [9–16].

Chitosan is a linear polysaccharide, macromolecules of which are comprised of randomly bonded β-(1-4)-*D*-glucosoamine linkages and N-acetyl-*D*-glucosamine, is a strong complexing agent due to amino and hydroxyl groups. Lately, chitosan has been extensively investigated as a drug carrier. An example of that is a series of recent research papers describing the synthesis of complexes of anti-cancer agents, such as doxorubicin, tamoxifen [17], and norcantharidin [18], with chitosan. Mixtures of piroxicam with chitin and chitosan (1 : 2 by weight) were produced by the mechanochemical method (grinding in a ceramic

ball mill for 24 h). They had an increased rate of drug release into a solution (pH 1.2) compared to physical mixtures of initial components [9]. It was demonstrated that co-grinding led to piroxicam amorphization, which was a cause for an increase in dissolution rate, whereas changing IR spectra testified the interaction of the components. The authors associate a rise in dissolution rate for physical mixtures of components in relation to the initial piroxicam with the improved wettability of the drug.

Our earlier research works used chitosan produced from the chitin of crustacean amphipod *Gammarus* Altai with a mass average molecular weight of $1.6 \cdot 10^6$ [13]. It was shown that mechanical activation (MA) of chitosan resulting from the mechanocracking of macromolecular chains of biopolymer resulted in a reduction of its molecular weight to a value of $5 \cdot 10^3$ kDa. Mechanoactivated chitosan exhibits increased, albeit slightly, solubility in water ($0.6 \cdot 10^{-2}$ mg/mL). Alterations in IR spectra testify of the interaction of piroxicam with chitosan to form hydrogen bonds between hydroxyl and amino groups of biopolymer macromolecules and functional moieties of the drug. The rate of piroxicam dissolution from mechanoactivated mixtures and its solution concentration are higher than those for both initial and mechanoactivated drug. The maximum solubility of piroxicam is observed for physical mixtures of mechanoactivated components. This may be related to generating water-soluble complexes of piroxicam and chitosan. They may be produced both when mechanochemical processing and dissolving the mechanoactivated components in aqueous solutions.

The improvement of the bioavailability and clinical efficiency of poorly water-soluble lipophilic drugs upon oral administration is of great importance. A series of research papers [19–21] noted that the use of low-molecular-weight chitosan (LMWC) yielded better results to increase the solubility and bioavailability of drugs.

The purpose of the work was to produce mechanocomposites of piroxicam with different molecular weight chitosan and research on their properties including pharmacological activity *versus* synthesis conditions and mechanocomposite composition.

EXPERIMENTAL

Chemical

Piroxicam was synthesized at the A. E. Favorsky Institute of Chemistry of the Siberian Branch of the Russian Academy of Sciences according to original technology [22, 23]. High-molecular-weight chitosan (HMWC) (TS 9289-058-04689375) of Invest-Pharm OOO (Moscow, Russia) with molecular weight M_w = 460 kDa, degree of deacetylation of 98 % and LMWC of Bioprogress CJSC (Moscow region, Russia) with molecular weight M_w = 39 kDa and degree of deacetylation of 79 % were used for research.

The mechanical processing of mixtures of piroxicam and chitosan in mass ratios of 1 : 3 and 1 : 10 was carried out using two different mills, SPEX 8000 (CertiPrepInc, USA) mixer mill and a planetary AGO-2 (ISSCM, Russia) ball mill with the water cooling of jars. Processing conditions in SPEX-8000 and AGO-2 mills were as follows: a steel jar with a capacity of 60 mL, a diameter of balls of 6 mm, the ratio of the mass of the sample to the mass of the balls of 1 : 15, the load on the ball of 8–10*g* and steel jars with a capacity of 40 mL, diameter of balls 6 mm, the ratio of the mass of the sample to the mass of the balls of 1 : 30, the load on the ball of 20*g*, respectively. Processing time was 15 min. In order to avoid sample contamination resulting from iron rubbing, the lining of jars and balls was carried out. A small amount of the drug was loaded into the jars and processing was carried out for 2 min immediately before the experiment for these purposes.

X-ray phase analysis was carried out using D8 Discover with GADDS XRD with a Hi-Star two-coordinate detector (Bruker, Germany), CuK_α radiation and STADI MP (STOE & Cie GmbH, Germany), Cu$K_{\alpha 1}$ radiation, 2θ = 5–65°.

Attenuated total reflectance (ATR) IR spectra in the 4000–580 cm^{-1} frequency range were recorded with a resolution of 4 cm^{-1} with a Digilab Excalibur 3100 FT-IR spectrometer (USA) using a Pike Miracle ATR attachment with ZnSe crystal without special sample preparation.

Measuring dissolution rate and drug solubility were carried out using the Varian 705-DS Dissolution Apparatus designed for testing drug substances including tablets and capsules. The drug dissolution rate was explored using two methods.

1) A sample in the 125–315 μm particle size range (grading was carried out using sieves) containing a lack of piroxicam in relation to its equilibrium concentration (solubility) was placed into a glass thermostated at (37±0.5) °C, equipped with a mechanical stirrer in a rotation speed of 100 rpm. Dissolution dynamics was investigated in a buffer solution (pH 6.86), the solvent volume of 130 mL. Samples of the analysed solution of 3.5 mL were taken at certain time intervals, moreover, the same volume of the corresponding

solution was added to the dissolution medium for its preservation. The resulting samples were filtered through filtration paper "Blue tape". Quantitative analysis was carried out by UV-spectrophotometry. The optical density of samples was measured using a Cary 50 Scan spectrophotometer (Varian, USA) with a wavelength of 358–365 nm in a cuvette with a layer thickness of 10 mm. A dilute solution of chitosan ($7 \cdot 10^{-2}$ mg/mL) was used as a reference solution.

2) A sample (particle size ≤125 μm) containing an excess of piroxicam was placed into a thermostated glass beaker with 250 mL of water at a temperature of (37±0.5) °C. The beaker was equipped with a mechanical stirrer with a rotation speed of 250 rpm. Sampling (3.5 mL) was carried out at the end of 1 day. The optical density of the samples was measured as mentioned above. Spectrophotometer calibration according to piroxicam was performed by measuring solutions with different substance concentration and plotting the calibration curve. All experiments were carried out three times.

Determining piroxicam quantitative content in mechanocomposites, for which biological activities were analysed, was performed by potentiometric titration (a universal EV-74 ionomer) with a 0.1 M solution of chloric acid in methanol; glass and silver chloride electrodes were used as an indicator and a reference electrode, respectively.

Pharmacological

Anti-inflammatory activity (AIA) of mechanocomposites of piroxicam with chitosan was explored on a classical model of the acute carrageenan edema of the hind leg in outbred white rats of a mass of 190–220 g. Samples under investigation were introduced within 1 h of inducing inflammation through a probe into the stomach at a dose of 5 mg kg per piroxicam alone. Low-molecular-weight chitosan was introduced at a dose of 55 mg/kg considering the introduced dose of piroxicam/LMWH mechanocomposite (1 : 10 by mass). In order to reproduce the edema, 0.1 mL of a 1 % solution of carrageenin was introduced to rats. The initial volume of paws of and the one at the height of inflammation were measured by the oncometric method in 3 and 5 h after administering phlogogen. The severity of AIA was determined according to the ability of the compounds under examination to prevent swelling compared to control.

Analgesic activity was examined with a classical model of acetic cramps in white scrub mice with a mass of 20–35 g. The samples under examination were introduced to the stomach *via* a sonde for 1 h before intraperitoneal injection of 0.25 mL of a 0.75 % acetic acid solution. The number of cramps was computed within 15 min after administering algogene. Analgesic activity was estimated according to a reduction in the number of cramps compared to control.

RESULTS AND DISCUSSION

Resulting from mechanical activation of mixtures of piroxicam with both HMWC and LMWC in SPEX 8000 and AGO-2 mills, there is no complete amorphization of drugs, although the reflexes on X-ray diffraction patterns broaden (Fig. 1), which indicates the partial disordering of the drug crystal structure.

Compared to a mixture of components activated separately, in IR spectra of samples (Fig. 2) obtained in both mills, there are variations within the range of stretching N–H (3340 cm^{-1}), C=O (1631 cm^{-1}), C=N (1531 cm^{-1}), and also bending O–H (1093 cm^{-1}) vibrations. This indicates the interaction of components upon mechanical processing and suggests mechanocomposite formation.

Figure 3 gives curves for release of piroxicam into a solution from mixtures with HMWC. It can be seen that already upon dissolution of a physical mixture of initial components (curve *2*), piroxicam concentrations in the solution are higher

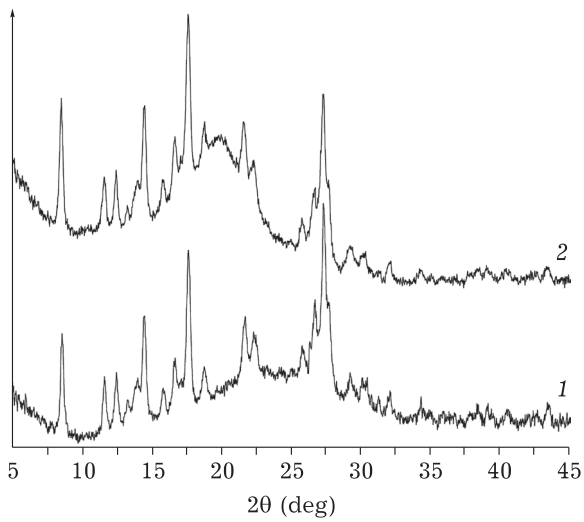

Fig. 1. X-ray diffraction patterns of mixtures of piroxicam with LMWC (*1*) and HMWC (*2*) ball milled in an AGO-2 mill.

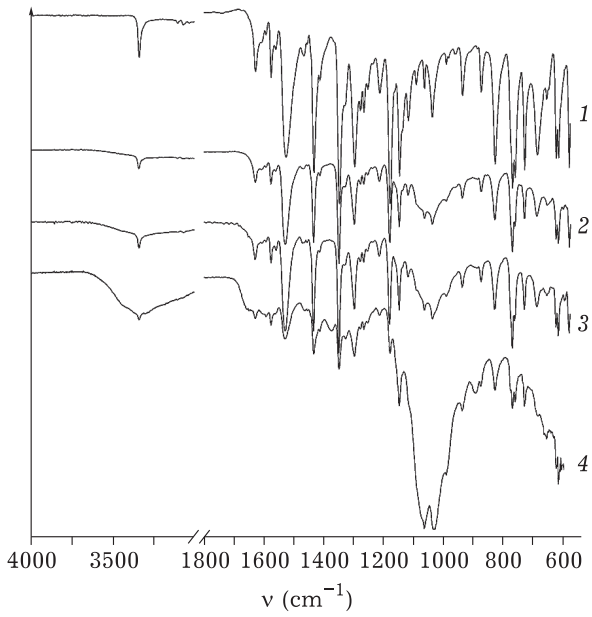

Fig. 2. ATR IR spectra: *1* – initial piroxicam; *2*, *3* – piroxicam mixtures processed in the mill SPEX 8000 with low and high molecular weight chitosan, respectively; *4* – a physical mixture of piroxicam with high molecular chitosan processed separately in therein.

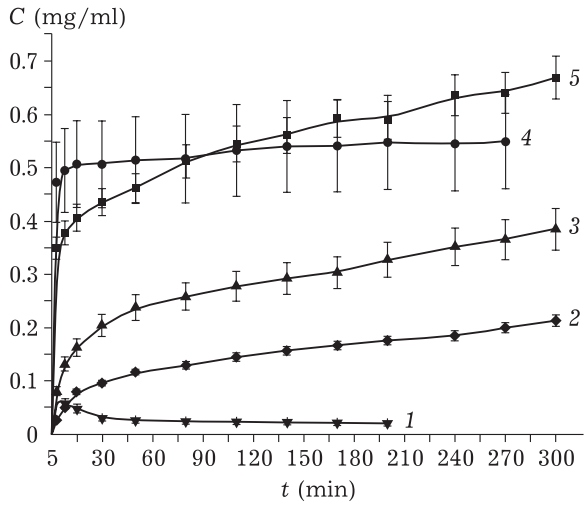

Fig. 3. Curves for release of piroxicam into a solution from mixtures with chitosan (1 : 3 by mass): *1* – mechanically activated piroxicam; *2* – physical mixture of the initial components; *3* – same of the initial piroxicam with mechanically activated chitosan; *4* – mechanically activated mixture; *5* – physical mixture of mechanically activated components.

than the solubility of the drug alone (curve *1*), which is presumably related to generating complexes of piroxicam with chitosan therein. This outcome is different from that acquired in [13] when chitosan with a greater MM (M_w = 1600 kDa) was used and the concentration of the drug in the solution was almost in agreement with solubilities of the initial and mechanically activated piroxicam. A cause may be the aggregation of macromolecules of chitosan with a high molecular weight, as was noted by the authors in [17], which prevents complex formation. On the other side, it is apparent that complex formation rate depends on the dissolution rate of individual components and thus, should be increased with reducing biopolymer molecular weight. Apparently, this is what leads to an elevated concentration of piroxicam in the solution upon dissolution of the physical mixture with chitosan that has a lower molecular weight compared to that used in [13]. The experiment using mechanically activated HMWC proves this. In this case, there is even a higher concentration of piroxicam in a solution (see Fig. 3, curve *3*), which may also be associated with a decrease in the molecular weight of chitosan resulting from the microcracking of biopolymer chains upon MA.

A physical mixture of separately activated components (see Fig. 3, curve *5*) has the maximum solubility. Nevertheless, the rate of piroxicam transition into the solution in the case of mechanocomposites (curve *4*) is higher than that for a physical mixture of mechanoactivated components. This can be seen more visually in Fig. 4 where dissolution curves are given when placed in a solution of samples containing a lack of piroxicam in relation to its solubility (method 1).

Piroxicam is distributed over the surface of chitosan during MA, which leads to an increase in its surface and, consequently, contributes to an increase in the rate of dissolution. Furthermore, in the case of mechanocomposites, the transfer of the ready-made molecular complexes of piroxicam with chitosan generated upon MA to a solution is probable.

Dissolution rate for HMWC composites is lower than that when using a high-molecular-weight biopolymer (see Fig. 4). Nevertheless, the former is significantly higher than the dissolution rate of the initial substance. It would be expected that an AGO-2 high-energy mill that is characterised by a high grinding and activation rate of solids [24] would be more efficient to prepare composites. On the other side, processing conditions in a SPEX 8000 mill facilitate the interaction of components due to their vigorous mixing and shear stress implemented for contact particles. As a result, both mills turned out to be efficient to prepare composites in the piroxicam–chitosan system.

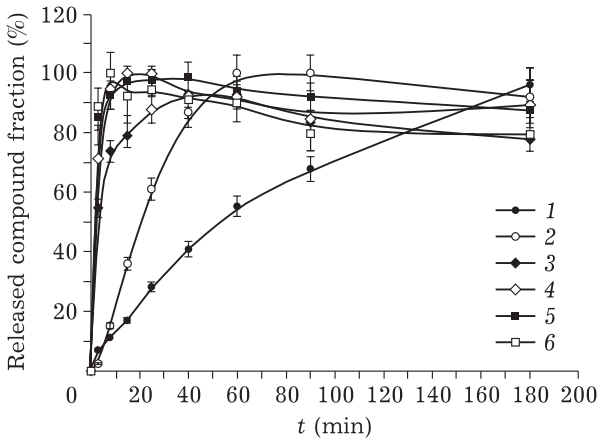

Fig. 4. Dissolution curves: *1* – initial piroxicam; *2* – a physical mixture of mechanically activated piroxicam and mechanically activated HMWC; *3*, *4* – piroxicam–LMWC mixture mechanically activated in AGO-2 and SPEX 8000, correspondingly; *5*, *6* – piroxicam–HMWC mixture mechanically activated in AGO-2 and SPEX 8000, respectively.

Table 1 gives data for piroxicam solubility (method 2) in mechanically activated mixtures with LMWC and HMWC. The water solubility of piroxicam used in the research is 0.03 mg/mL (pH 7) at 37 °C. It can be seen that piroxicam solubility for composites with HMWC is increased by 3 times, whereas when using a high-molecular-weight biopolymer – in less than 2 times. As may be assumed, this may be related to the structure and properties of the resulting complexes. For example, it was determined in [17] that chitosan with a greater molecular mass forms stronger complexes in a solution compared to a low-molecular-weight biopolymer.

Furthermore, it is possible that complexes of different structures may also be generated in case of high and low molecular weight biopolymers. Indeed, differences in the range of stretching N–H, O–H, C–O, and also bending CH_3 (1435 cm^{-1}) and OH (1093 cm^{-1}) vibrations can be seen in IR spectra of mechanically activated mixtures of piroxicam with LMWC and HMWC (see Fig. 2). Similarly to the structure of complexes in a solution [17], one may assume that piroxicam molecule, forming a complex with chitosan, turns out to be encapsulated by a polymer. It has earlier been demonstrated in [25] that mechanical activation of chitosan with piroxicam results in a decrease of the geometrical free volume of the biopolymer, which indirectly supports this hypothesis.

It has been found by potentiometric titration that piroxicam content in mechanocomposites of piroxicam-chitosan (1 : 3 by mass) almost does not differ from that for HMWC and LMWC and is 245 and 242 mg/g, correspondingly, whereas for a composite of piroxicam–HMWC (1 : 10), it is 92 mg/g. The resulting values correspond to piroxicam content in the initial mechanically activated mixtures.

As demonstrated by research on biological activities of all investigated piroxicam mechanocomposites upon oral administration in a dose of 5 mg/kg per piroxicam alone, there is an apparent anti-inflammatory and analgesic activity (Table 2).

TABLE 1

Solubility of mechanically activated piroxicam–chitosan mixtures (1 : 3 by weight)

Mills	Piroxicam concentration in aqueous solution, mg/mL	
	Piroxicam–LMWC	Piroxicam–HMWC
SPEX 8000	0.04 ± 0.01	0.10 ± 0.01
AGO-2	0.06 ± 0.01	0.09 ± 0.01

TABLE 2

Anti-inflammatory and analgesic activity of piroxicam, chitosan, and mechanocomposites of piroxicam with chitosan

Piroxicam and its mechanocomposites	Anti-inflammatory activity, percentage of edema suppression at MA time, h		Analgesic activity, percentage of reduction in number of cramps
	3	5	
Piroxicam–substance	72.8	75.7	47.9
LMWC	22.8*	10.2*	–
Piroxicam–LMWC (1 : 10)	60.0	47.6	65.2
Piroxicam–LMWC (1 : 3)	58.9	50.9	69.1
Piroxicam–HMWC (1 : 3)	69.8	68.9	57.6

* $p > 0.05$; other data are reliable ($p < 0.05$).

This indicates a high bioavailability of the gastrointestinal tract for not only piroxicam but also its mechanocomposites with both HMWC and LMWC.

When using piroxicam–chitosan mechanocomposites, the number of cramps is reduced (by 57.6–69.1 %) compared to control, whereas the analgesic activity becomes higher (by 47.9 %).

Piroxicam–HMWC mechanocomposites show the strongest analgesic effect. For example, with a ratio of 1 : 3, the number of cramps is decreased by 69.1 %, whereas compared to piroxicam alone, it is higher by 1.4 times. The analgesic effect of a composite of piroxicam–LMWC (1 : 10) reducing cramps by 65.2 % compared to control and exceeding piroxicam alone by 1.3 times is somewhat lower.

Chitosan alone without piroxicam does not exhibit anti-inflammatory and analgesic activity. When using LMWC, there is unreliable ($p > 0.05$) suppression of edema by 22.8 and 10.2 % in 3 and 5 h, respectively, after phlogogen administration, which cannot be recognized as sufficient for a therapeutic effect.

The anti-inflammatory effect of mechanocomposite of piroxicam–HMWC (1 : 3) turned out to be strong and became apparent almost at a level of the effect of piroxicam alone. For example, piroxicam–HMWC (1 : 3) suppressed exudation after inflammation induction in 3 h by 69.8 %, and in 5 h by 68.9 %, whereas piroxicam alone reduced the swelling by 72.8 and 75.7 % in 3 and 5 h respectively. Mechanocomposites of piroxicam with LMWC showed anti-inflammatory activity that was somewhat weaker in relation to piroxicam alone. In spite of this, the anti-inflammatory activity of both mechanocomposites of piroxicam–LMWC may be assessed as high, because they inhibited the development of edema by at least a half compared to control. For example, piroxicam-LMWC (1 : 10) reduced the inflammatory response by 60.0 and 47.6 %, in 3 and 5 h, respectively, whereas piroxicam–LMWC (1 : 3) – by 58.9 and 50.9 %, correspondingly.

CONCLUSION

Thus, the resulting mechanocomposites of piroxicam with LMWC and HMWC have high anti-inflammatory activity and are superior to piroxicam alone according to the strength of the analgesic effect.

As demonstrated by the findings, mechanical processing allows using both LMWC and HMWC to improve drug properties. Moreover, unlike literature data [19–21], in order to produce piroxicam-chitosan mechanocomposites with elevated rates of piroxicam release into a solution and drug concentration therein, the use of HMWC yields the best results. The mechanocracking of chains and a reduction in the molecular weight of the biopolymer upon mechanical processing is a likely cause. On the other side, mechanical activation results in generating molecular complexes of piroxicam with chitosan due to the formation of hydrogen bonds between functional groups of the components. Mixtures of separately activated components, dissolution of which is accompanied by complexation in a solution, have the best solubility. Nevertheless, piroxicam release into the solution from the mechanoactivated mixture proceeds with a higher rate due to the formation of molecular complexes upon mechanoactivation and a more developed surface of the drug resulting from polymer distribution in the matrix. The use of LMWC as a carrier material results in enhanced piroxicam solubility to a lesser extent that may be due to the structure of intermolecular piroxicam–chitosan complexes. The research on pharmacological properties of piroxicam–chitosan mechanocomposites has demonstrated significant anti-inflammatory and analgesic activity, which indicates high bioavailability of the gastrointestinal tract of not only piroxicam, but also its complexes with both LMWC and HMWC.

In order to compare bioavailability of different forms of piroxicam, it is advisable to determine their pharmacokinetics. Furthermore, it is interesting to make a comparison for their ulcerogenicity under animal experiment conditions, as this is high gastrotoxicity that constraints the application of piroxicam in the clinic. In order to select the optimum piroxicam type according to the efficiency/safety criterion, further research is required.

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