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Increase of solubility and transmembrane permeability of niclosamide from its mechanochemically synthesized solid dispersions

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Abstract

More than 4.5 billion people worldwide are affected by parasitic diseases, with helminth infections accounting for 99% of the total number. Niclosamide (NS) is a weakly acidic active pharmaceutical ingredient (API) used to treat helminth infections. However, the pharmaceutical use of pure niclosamide is limited by its low bioavailability due to its poor aqueous solubility. The aim of this work is a mechanochemical preparation of niclosamide solid dispersions with increased solubility. Due to the pH dependence of NS water solubility and possible complexes formation, NS solid dispersions (SD) with 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) and alkalizing agents, such as calcium carbonate ($CaCO_3$) and N-methyl-D-glucamine (MG) are mechanochemically prepared in this study. The physical properties of NS SD in solid state are characterized by differential scanning calorimetry, X-ray diffraction, FT-IR spectroscopy, and scanning electron microscopy studies. The characteristics of the water solutions formed from the obtained SDs are analyzed by HPLC. It is shown that the solubility increases for all studied compositions. These phenomena are obliged by complexation with HP-β-CD, which was shown by 1H-NMR methods, and enhanced ionization in the cases of using calcium carbonate and MG. Results of the parallel artificial membrane permeability assay (PAMPA) have shown that mechanochemically obtained NS/MG SD (1/1 mass ratio, 8 h milling) significantly enhances permeation of NS across an artificial membrane. Thus, the obtained compositions are a promising basis for the development of NS-based preparations for oral administration, with reduced dose and high pharmacological effect.

Keywords

niclosamide
solubility enhancement
solid dispersions
mechanochemical synthesis
hydroxypropyl-β-cyclodextrin
calcium carbonate
N-methyl-D-glucamine

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Key findings

- Niclosamide solid dispersions with increased solubility were prepared using mechanochemical solid state method.
- The enhanced niclosamide solubility is caused by inclusion complex formation with HP-β-CD and by increased ionization when using alkalizing agents.
- The obtained niclosamide/N-methyl-D-glucamine solid dispersion (1/1 mass ratio, 8 h milling) has shown enhanced permeation of niclosamide across an artificial membrane.

1. Introduction

According to the statistics of the World Health Organization, about 5 million deaths occur annually in the world as a result of infectious and parasitic diseases. More than 4.5 billion people wordwide are affected by parasitic diseases, with helminth infections accounting for 99% of the total number of such diseases [1].

Niclosamide (5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide, NS, Figure 1a) is an active pharmaceuti-

cal ingredient (API) used to treat helminth infections [2]. Various formulations containing NS have been shown to be highly effective against sheep and mice cestode infections [3, 4], Moniezia benedeni [5], and Anoplocephalidae [6].

NS has shown preclinical efficacy in the treatment of cancer [7], bacterial infections, type II diabetes, NAFLD and NASH, endometriosis, systemic sclerosis, rheumatoid arthritis and various viral infections [8]. NS can be potentially used to treat COVID-19 [9]. The side effects of NS have been shown to be infrequent, mild and temporary [10].

However, the pharmaceutical use of pure niclosamide is limited by its low bioavailability [11] due to its poor aqueous solubility (5–8 mg/l, 20 °C) [12]. Since NS is characterized by low solubility and good permeability, it is classified as a class II drug in the BCS system [13].

Many different approaches have been proposed to improve the solubility of niclosamide. An incomplete list includes: wet grinding to reduce the size of NS crystalline particles to nanoscale [14], emulsification of NS in an oil-water system with a number of copolymers [15], co-crystallization [16], NS nanosuspensions and incorporation of NS into dendrimer-like biopolymers [17], solid lipid nanoparticles production using stearic acid, polysorbate-80 and polyethylene glycol [18], complexation with O-phosphorylated calixarene or cyclodextrin [19]. Unfortunately, these processes are often associated with high solvent consumption, partial API decomposition, and are quite complicated. Thus, many aspects of niclosamide formulation dosage need to be further improved.

In contrast to the traditional liquid-phase preparation methods of APIs for dosage formulations, rather simple operations and equipment are used in mechanochemical approach. The preparation time is significantly reduced, and no solvent is required [20].

As a result of mechanochemical processing, SDs can be formed. This process is accompanied by a partial or complete transition to an amorphous state with an increase in the entropy of the system, which contributes to the acceleration of the dissolution process.

In addition, mechanical activation allows the combination of substances, regardless of their solubility and, to avoid undesirable side chemical reactions, the reduction of the contribution of rapid aggregation of drug molecules during dissolution [21]. More stable intermolecular complexes can be obtained by mechanical processing, which is indicated by an increase in solubility in comparison with the unprocessed mixtures. Therefore, the application of mechanochemical processing to niclosamide in order to increase its solubility and bioavailability is of considerable interest.

Mechanochemical preparation has already been used to obtain NS dosage formulations. Polyvinylpyrrolidone (PVP) [3, 22], sodium salt of glycyrrhizic acid Na_2GA [4], arabinogalactan (AG), and SiO_2 [5, 6] were used as excipients. As a result, the solubility of NS/PVP SDs was increased from 10 to 25 times compared to pure NS.

The anthelminthic activity of all SDs mentioned above was shown to be improved. Unfortunately, no data have been published on NS the solubility from compositions with Na_2GA, AG and SiO_2. Therefore, it is not known to what extent these excipients affected the solubility of niclosamide.

Another possible excipient is MG (Figure 1b), an alkalizing agent which is able to form supramolecular adducts with lipophilic organic compounds in water, thereby increasing their solubility [23]. In the case of niclosamide MG could play the role of an alkalizing agent and, potentially, a complexing agent.

Mechanochemical processing with alkalizing agents is suggested due to the fact that the NS water solubility has been shown to be pH dependent [24], and can be enhanced by 1–2 orders of magnitude by increasing the pH of the solution up to pH = 9. This phenomenon reflects the fact that NS is a weakly acidic API (pK_a = 7.45 [12]) and is capable of ionization in water solution. This approach has already been used for another APIs: valsartan (VAL) [25] and nimesulide (NIM) [26]. VAL has been mechanically processed with $CaCO_3$ and MG (5/1 mass ratio). NIM has been treated with $MgCO_3$ (5/1 mass ratio). The obtained SDs showed an increased solubility of about 10 times (VAL SDs) and about 100 times (NIM SD) compared to the pure APIs.

HP-β-CD (Figure 1d) is known to form water-soluble complexes due to host-guest interactions. Various lipophilic organic APIs can enter its cylindrical hydrophobic cavity (Figure 1d) [27].

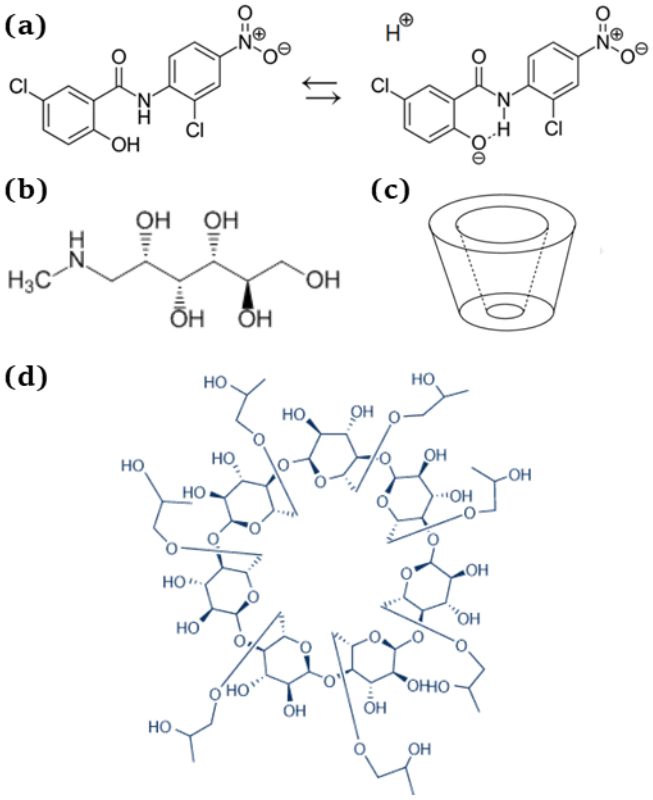

Figure 1 Structure and ionization scheme of the niclosamide molecule (a), structure of the N-methyl-D-glucamine molecule (b), structure of the HP-β-CD molecule (c, d).

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In addition, HP-β-CD has been shown to be more effective than other β-cyclodextrins in increasing the APIs stability, solubility, bioavailability, permeability and duration of action, as well as reducing irritation and toxicity, [28]. Its complexes with NS, obtained by freeze-drying [29] and powder extrusion [30] methods have been described previously and shown to have increased solubility. Unfortunately, these preparation methods are time consuming, require many preparation steps or expensive equipment. Therefore, simpler and less expensive solid-state mechanochemical method might be introduced.

This technology has been used earlier [26] to obtain NIM SDs with a variety of complexing agents, such as HP-β-CD, plant water-soluble polysaccharide arabinogalactan, disodium salt of glycyrrhizic acid (Na_2GA). All SDs were shown to enhance the NIM solubility due to complexes formation in water solutions, which was confirmed by ^{1}H-NMR methods.

The aim of the present study is to increase the aqueous solubility of niclosamide, its membrane permeability and thus to improve its bioavailability. With the NS acidity and possible complex formation taken into consideration, NS SD with an alkali–pharmacopeia grade $CaCO_3$, MG and HP-β-CD have been obtained by mechanochemical processing.

2. Materials and methods

2.1. Materials

Niclosamide (Shandong Chenxing New Material Co. Ltd., China) of pharmaceutical grade was used without further purification. N-methyl-D-glucamine (MG, purity of MG ~99.5%) was purchased from Aladdin Industrial Co. Ltd., Shanghai, China. Calcium carbonate ($CaCO_3$, PC-000658, 2013-07-30) was purchased from Shanghai Nuochen Pharmaceutical Co. Ltd., China. Hydroxypropyl-β-cyclodextrin (HP-β-CD, purity of HP-β-CD ~98.0%, substitution degree of HP-β-CD – 5.4, content of β-CD ~0.17%) was purchased from Zhiyuan Bio-Technology Co. Ltd., Binzhou, China.

2.2. Preparation of mechanochemically treated niclosamide solid dispersions

Mechanical treatment of NS compositions with $CaCO_3$, MG and HP-β-CD was carried out in a VM-1 roll mill with a cylindrical vessel which was coated with Teflon and possessed 300 mL volume. Acceleration of grinding bodies is 1 g (free fall). Rotational speed of cylindrical vessel is 156 rpm. Steel balls (diameter 22 mm, 675 g load) were used as grinding bodies. The total load of the treated powders mixture was 18 and 20 g. The duration of mechanical processing was from 2 to 16 h – (2, 4, 8, and 16 h). Mass ratios of 5/1 and 1/1 were used to prepare NS/MG and NS/$CaCO_3$ SDs to provide the desired pH range (up to pH = 10). Molar ratio of 1/1 was used to prepare NS/HP-β-CD SD considering the fact of possible complex formation.

Physical mixtures of the same compositions were prepared by shaking (for about 10 min) the previously noted powdered compounds in closed test tube.

2.3. HPLC Analyses

An Agilent 1200 HPLC system (Agilent Technologies, Palo Alto, CA, USA) was used to determine the concentration of NS. The HPLC system was equipped with a reverse phase column (5 μm, 4.6 × 50 mm, Zorbax Eclipse XDB C18) at 30 °C and diode-array detector. The mobile phase consisted of a 2/3 (v/v) mixture of acetonitrile and acetate buffer (pH = 3.4), the flow rate was 0.8 mL/min, the detection wavelength – 336.8 nm, and the injection volume – 5 μL.

2.4. Content test for niclosamide solid dispersions

To determine the content of NS in compositions, the weighted samples (10 mg) were dissolved in 50 mL of a mixture solution ($CH_3CN/C_2H_5OH/CH_2Cl_2$, 10:9:1, v/v/v). In all cases, all components of complexes were completely dissolved.The samples were then suitably diluted and assayed by HPLC.

2.5. Determination of solubility of niclosamide and its solid dispersions

The mechanically treated products, which were picked up from different milling time, were separately added into a 25 mL flat-bottomed flask with 5 mL of distilled water. Then they were shaken in the orbital shaker for 24 h with 200 rpm at +37 °C. At last, sample solutions were centrifuged at 12,000 rpm for 10 minutes, filtered using a paper filter (pore diameter of 2–3 μm) and determined by HPLC method.

2.6. Fourier transform infrared spectrophotometry

Fourier transform infrared spectrophotometry (FT-IR) spectra of samples were collected from 500 to 4000 cm^{-1} using Fourier spectrophotometer “Infralum FT-801” (“Simeks”, Novosibirsk, Russia). All samples were taken in thin tablets with KBr.

2.7. Powder X-ray diffraction

Powder X-ray diffraction analysis of niclosamide and its solid dispersions was carried out on a DRON-4 equipment (‘‘Byrevestnik’’, St. Petersburg, Russia) using Cu Kα radiation, counter speed 2 deg/min, range of intensity measurement ~1000, angle range – from 4° to 65°.

2.8. Differential scanning calorimetry

Thermal analysis of niclosamide and its solid dispersions was carried out by differential scanning calorimetry (DSC) with the DSC-550 instrument (Instrument Scientific Specialists Inc., Omaha, NE) in Ar atmosphere. Temperature program: 20–250 °C, the heating rate 10 °C deg/min.

2.9. Scanning electron microscopy (SEM)

Electronic images were acquired using a TM-1000 microscope (Hitachi, Tokyo, Japan). Coating of samples with

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gold was performed using a JFC-1600 auto fine coater (JEOL, Tokyo, Japan). The coating parameters were as follows: sputtering time 30 s, amperage 30 mA, and film thickness 15 nm.

2.10. ^{1}H-NMR spectra in solution

^{1}H-NMR spectra were recorded on an Avance III 500 MHz spectrometer (Bruker, Rheinstetten, Germany) in D_2O (99.8%, Aldrich, Moscow, Russia) solutions. Measurement of the spin–spin relaxation time T_2 was carried out using the standard Carr– Purcell–Meiboom–Gill (CPMG) sequence: $P_1(90°)$ – (s – $P_2(180°)$ – s) n – registration, where s = 0.5 ms-fixed time delay, and varied from 0 to 2000 ms.

Selective NOESY (selective nuclear overhauser effect correlation spectroscopy) spectra were recorded using a special pulse sequence from the Bruker library. Measurements were carried out at pH 7.0 and 10.0.

2.11. *In vitro* parallel artificial membrane permeability assay (PAMPA)

PAMPA experiments were carried out in 12-well filter plates (polycarbonate membrane, 12 mm diameter inserts, 0.4 μm pore size, 1.12 cm² area, Corning Inc., Corning, NY, USA). The ability of compounds to diffuse from a donor compartment into an acceptor compartment is evaluated. The artificial membrane was first impregnated by pipetting 60 μL of the 5% (v/v) hexadecane in hexane solution to each of the donor plate wells. The wells were then placed into a fume hood for 1 h to ensure complete evaporation of the hexane. After the hexane had evaporated, 1.5 mL of water was added to each of the wells of the acceptor plate. The hexadecane treated donor plate was then placed on top of the 12-well acceptor plate. Then, 0.5 mL of NS or its compositions in water was added to each well of the donor plate, and the resulting PAMPA device was incubated at 37° and shaken for 3 h with 200 rpm. Samples (1 mL) were collected from the acceptor plate at appropriate time points (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 h) and were analyzed with HPLC method. The acceptor compartment was refilled with the same volume of distilled water.

3. Results and Discussion

3.1. Content test

The drug contents of NS SDs with $CaCO_3$, (1/1 mass ratio) MG (1/1 mass ratio), MG (5/1 mass ratio) and HP-β-CD (1/1 molar ratio) were determined to be 100.0%, 100.0%, 96.7% and 100.0%, respectively. It suggests the preparation of NS SDs by mechanochemichal processing was with high content uniformity.

3.2. Physical characterization studies of NS SDs

3.2.1. Analysis on DSC thermograms

The DSC thermograms of free NS, physical mixtures (PMs), and NS SDs are shown in Figure 2a, 2b.

Significant changes are observed after processing the mixtures NS/MG (1/1 mass ratio) and NS/HP-β-CD (1/1 molar ratio) in the VM-1 mill. The DSC curve of the NS/HP-β-CD PM (1/1 molar ratio) contains an NS endothermic peak (EP, 231 °C, 107.8 J/g) corresponding to the NS intrinsic melting points which is significantly lower in intensity (by 49%) compared to the original substance (235 °C, 212.6 J/g), and disappears completely after the mixture is mechanically processed.

The same NS endothermic peak is absent in the DSC curve of the NS/MG PM (1/1). A decrease (by 9%) of MGendothermic peak in the PM (126°, 272.7 J/g) in comparison with the original substance (131°, 302.1 J/g) and in theDSC curve of themechanochemically treated mixture (by 52%, 105°, 144.2 J/g) is observed. Another change is the MG melting point shift in comparison with the original MG substance.

The disappearance of NS EP indicated the existence of intermolecular interaction or the formation of a new solid phase after the treatment of mechanochemical activation.

3.2.2. Analysis on powder X-ray diffraction

In this experiment, the powder X-ray pattern of NS in the formation of free molecule and compositions were investigated. Powder X-ray diffraction data of some obtained compositions are displayed in Figure 3. The intensity of NS diffraction peaks decreased significantly in mechanically treated compositions.

The decrease of the NS characteristic peaks demonstrated that the main crystalline particles of NS were destroyed by the mechanical activation treatment . It means that the mechanically treated compositions are in amorphous form, which is in accordance with the results of DSC.

3.2.3. Scanning electron microscopy analysis

The electron micrographs of the obtained SDs are shown in Figure 4.

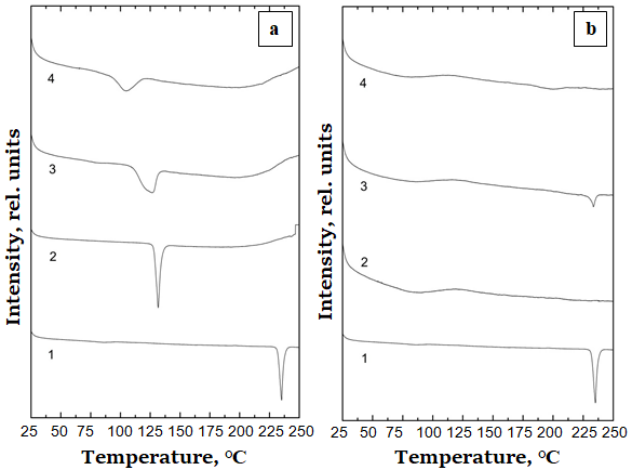

Figure 2 DSC thermograms of NS (a, 1), MG (a, 2), NS/MG (1/1 mass relation) physical mixture (a, 3) and solid dispersion of NS/MG (1/1 mass ratio) treated in, in roll mill for 8 h (a, 4); NS (b, 1); HP-β-CD (b, 2), NS/HP-β-CD (1/1 molar ratio), physical mixture (b, 3) and solid dispersion of NS/HP-β-CD (1/1 molar ratio) treated in roll mill for 8 h (b, 4).

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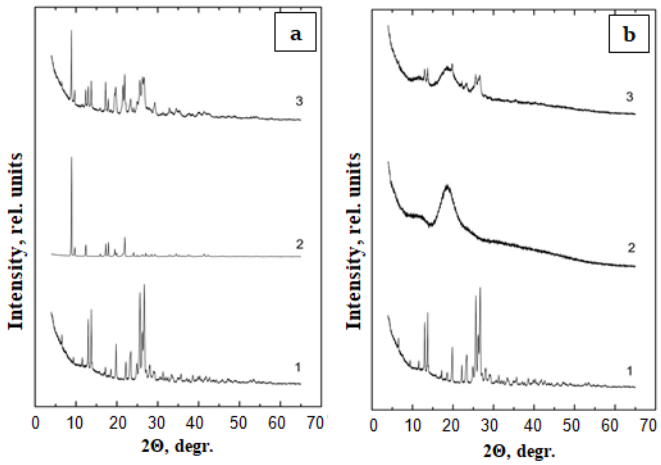

Figure 3 X-ray diffractograms of NS (a, 1); MG (a, 2) and solid dispersion of NS/MG (1/1 mass relation) treated in roll mill for 8 h (a, 3); NS (b, 1); HP-β-CD (b, 2) and solid dispersion of NS/HP-β-CD (1/1 molar relation) treated in roll mill for 8 h (b, 3).

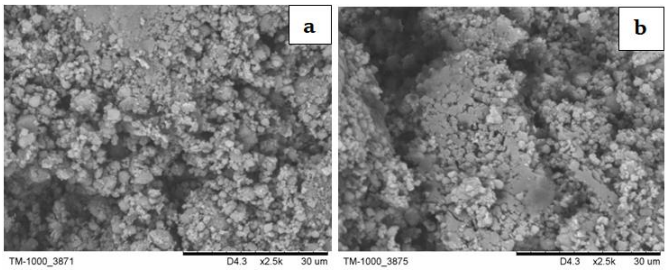

Figure 4 Electron micrographs of NS/MG (1/1 mass relation) solid dispersion, treated in roll mill for 8 h (a); NS/HP-β-CD (1/1 molar relation) solid dispersion, treated in roll mill for 8 h (b).

The initial substances are seen to have characteristic crystalline and intact shape. However, during the mechanochemical milling process, the destruction of the particles occurred followed by the formation of polydisperse particles with irregular shape. The average particle size of the obtained SDs was about 0.6–1 μm.

3.2.4. Analysis on FT-IR spectroscopy

FT-IR spectroscopy was used to investigate possible chemical reactions. The pure NS characteristic peaks did not take any shifts, indicating NS was still in a free acid form after the mechanical treatment. Moreover, the spectra of the milled complexes were found to be a simple overlap of their initial components, demonstrating no chemical reaction took place between NS and the auxiliary components during the milling process.

3.3. Characteristics of NS SDs in solutions

3.3.1. Solubility test

The solubility data of pure NS, its PMs and mechanically treated compositions are shown in Table 1. By comparison with the pure API, the solubility of NS was improved to different extents in the formation of PMs and SDs. For NS/$CaCO_3$ with mass ratio 1/1 (4 h milling) mechanically treated composition, the maximum solubility is 13.8 mg/L, which is larger than that of PM with the same components.

For the NS/MG mechanically treated composition, the largest solubilities are 397.0 mg/L with mass ratio 1/1 (8 h milling) and 145.2 mg/L with mass ratio 5/1 (8 h milling), both of which are larger than those of the same PM components.

For NS/HP-β-CD mechanically treated composition, the maximum solubility of complex is 78.1mg/L (molar ratio 1/1, 8 h milling), which is larger than same components physical mixture.

From the above results, the solubility of NS was sufficiently improved for all of the compositions, but the most significant results were obtained for NS/HP-β-CD and NS/MG mechanically treated compositions. The observed solubility values for the NS compositions with $CaCO_3$ and MG are in line with the curve of the solubility of NS vs pH presented in [24]. For NS/HP-β-CD the mechanism of improved solubility refers to the formation of inclusion complex.

3.3.2. ^{1}H-NMR study of NS/MG and NS/HP-β-CD complexes in solution

In the present study, selective NOESY experiment and NMR relaxation method were applied to prove the intermolecular interactions and confirm NS complexes formation [31]. The spin–spin T_2 relaxation time is known to be very sensitive to the intermolecular interaction and diffusion mobility of molecules [32, 33]. When the ‘‘guest’’ molecule moves into a ‘‘host’’ molecule and bounds in complexation, its diffusion and rotational mobility reduce and the proton relaxation times decrease significantly. The relaxation times (T_2) of NS protons were measured in the aqueous solution for free NS as well as for its mechanically processed SD, namely NS/MG (1/1 mass ratio, 8 h milling) and NS/HP-β-CD (1/1 molar ratio, 8 h milling).

The NMR spectrum of the NS/MG aqueous solution at pH = 7.0 contains only MG signals. The precipitate was analyzed at pH = 10 and its spectrum contains only NS signals. This suggests that when the dry NS/MG dispersion is dissolved at pH = 7.0, the rate of NS self-association is much higher than the rate of complexation. Preparation of a 1% aqueous NS/MG solution at pH 10.0 allowed recording a spectrum containing both NS and MG protons. Under these experimental conditions, NOESY spectra show the presence of cross-peaks between NS and MG protons (Figure 5). The observed cross-peaks with MG protons (2–4 ppm) indicate the presence of the NS/MG complex at pH = 10. However, no difference in chemical shifts is observed for this complex compared to pure NS solution.

Additional evidence for the formation of the NS/MG complex in aqueous solution at pH = 10 was obtained by NMR relaxation for 1% and 0.1% solutions. The NS protons relaxation times at 8.65, 8.4, and 7.8 ppm are summarized in Table 2. For comparison, the table also shows the proton relaxation times of pure NS and its complex with HP-β-CD (1% solution).

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Table 1 Solubility of NS and its mechanical treated products in water (+37 °C).

No.	Samples, mass or molar ratios	Mechanical processing duration, h	Solubility, mg/L	Increase insolubility, times	pH
1	NS	Without processing	0.29	–	4.01
2	NS/$CaCO_3$ (1/1 mass ratio)	Without processing	9.9	34	8.0
3	NS/$CaCO_3$ (1/1 mass ratio)	4	13.8	48	
4	NS/MG (1/1 mass ratio)	Without processing	253.7	875	9.9
5	NS/MG (1/1 mass ratio)	8	397.0	1369	
6	NS/MG (5/1 mass ratio)	Without processing	46.7	161	9.2
7	NS/MG (5/1 mass ratio)	8	145.2	501	
8	NS/HP-β-CD (1/1 molar ratio)	Without processing	8.4	29	7.2
9	NS/HP-β-CD (1/1 molar ratio)	8	78.1	269	

Table 2 Relaxation times T_2 (ms) for NS protons (8.65, 8.4 и 7.8 ppm) for pure NS, NS/MG (1/1 mass ratio) and NS/HP-β-CD (1/1 molar ratio) compositions. The error in the calculation of T_2 does not exceed 5%.

Chem. shift, ppm	Relaxation times T_2, ms			
	NS	NS/MG, 0.1%	NS/MG, 1%	NS/HP-β-CD, 1%
8.65	1900	1100	60	140
8.4	4900	3450	190	190
7.8	4000	2160	145	70

The significant decrease in the T_2 relaxation time in the presence of MG is direct evidence for the formation of the complex. This confirms the conclusions drawn from the NOESY experiments. However, the absence of changes in the chemical shifts of the NS protons in the presence of MG, as well as a significant increase in the relaxation times when the solution is diluted to 0.1% indicates the low stability of this complex.

Excitation of all NS aromatic protons (8–9 ppm) was performed to record the selective NOESY spectrum for NS/HP-b-CD complex (1/1) (Figure 6); the cross peaks at 1.1 ppm correspond to methyl protons of HP-β-CD and the cross peaks at 3.8 ppm correspond to intrinsic protons of HP-β-CD. This indicates the formation of the inclusion complex and the localization of NS near the narrow end of HP-β-CD (Figure 1c).

When the pH of this solution was increased to 10.0 by adding KOH, the solution turned yellow, indicating the formation of the deprotonated form of NS. At the same time, intense NS NMR signals appeared in the NMR spectrum (Figure 7). Selective NOESY spectra of NS/HP-β-CD aqueous solution recorded at pH = 7.0 also show the presence of cross-peaks between NS and CD protons. Considering that only the cross-peaks of closely spaced protons (less than 5 angstroms) are observed in the NOESY experiments, the cross-peaks mentioned above indicate existence of a complex with HP-β-CD.

Figure 8 shows the NMR relaxation experiment for NS/HP-β-CD complex (molar ratio 1/1), as well as for the free form of NS. These results showed a decrease in the relaxation time of NS protons in the presence of HP-β-CD. This is an independent direct evidence of the intermolecular complex formation in this system. Simultaneously, some NS protons showed a significant change of chemical shifts relatively to the free molecule in the ^{1}H-NMR spectrum.

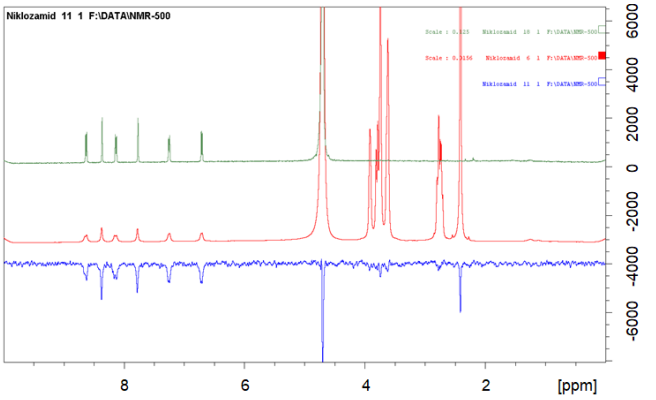

Figure 5 ^{1}H NMR spectra (middle) and selective NOESY (bottom) of a 1% aqueous NS/MG solution recorded at 303 K and pH = 10.0. Selective excitation of all NS signal was performed. The spectrum of pure NS is shown above for comparison.

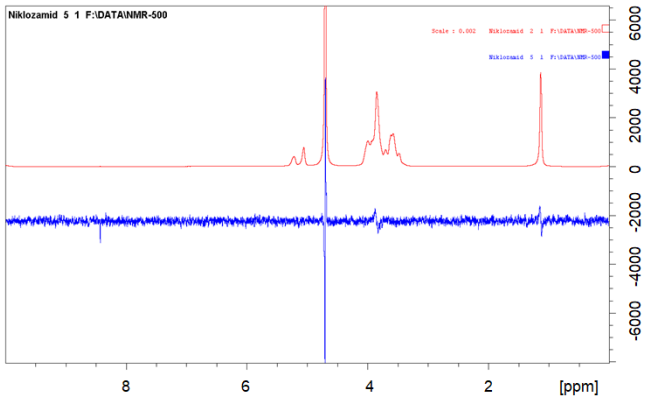

Figure 6 ^{1}H NMR (top) and selective NOESY (bottom) spectra of NS/HP-β-CD aqueous solution recorded at 303 K and pH = 7.0.

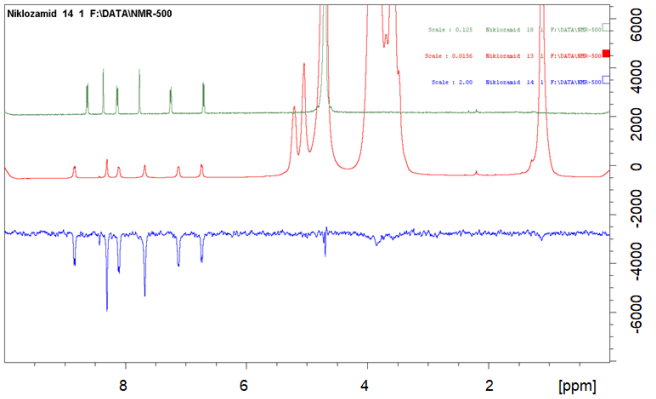

Figure 7 ^{1}H NMR spectra (middle) and selective NOESY (bottom) of a 1% aqueous solution of NS/HP-β-CD registered at 303 K and pH = 10.0. Selective excitation of all NS signal was performed. The pure NS spectrum is shown at the top for comparison.

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3.4. *In vitro* permeation study

A PAMPA assay enabled rapid determination of the trends in the ability of the compounds to permeate the membrane by passive diffusion [34–36]. In the plots (Figure 9), it can be seen that the amount of NS from mechanochemically treated NS/MG composition permeated is higher than from the pure NS, indicating that the co-grinding compositions have enhanced mass transport of NS across an artificial membrane compared to that of the pure drug.

The low NS permeation throw artificial membrane of SD with HP-β-CD seen surprising, considering the facts that it had a high solubility, as well as the known HP-β-CD ability to enhance the drug transport through membrane. The possible explanation is that in this case an increase in solubility is achieved by a strong complexation with HP-β-CD, which prevents theNS release into the free molecule form in solution, whereas the NS/HP-β-CD complex cannot be accepted by the hydrophobic membrane.

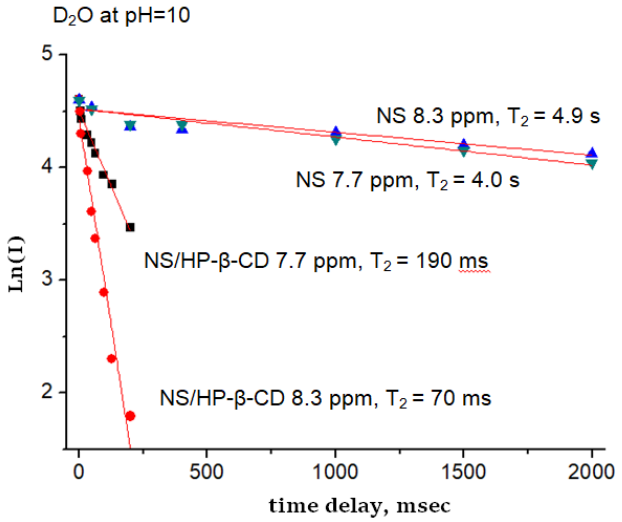

Figure 8 Kinetics of the echo signal decay of NS protons (on a logarithmic scale, protons at 7.7 and 8.3 ppm) for pure NS and its HP-β-CD complex measured for 1% water solution at 30 °C and pH = 10.0.

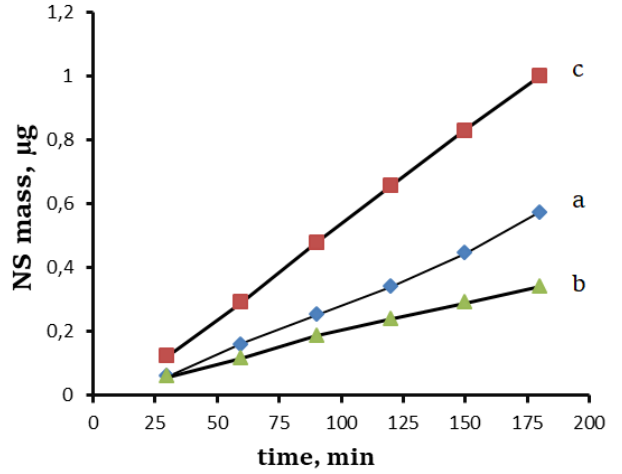

Figure 9 Permeation profile of niclosamide (a) and compositions: NS/MG (1/1 mass ratio) treated in roll mill for 8 h (b), NS/HP-β-CD (1/1 molar ratio) treated in roll mill for 8 h (c).

4. Limitations

A limitation of the mechanochemical method is the difficulty in adapting the process to industrial scale. Depending on the required capacity, the parameters of the equipment used and the mechanochemical treatment process itself must be determined.

5. Conclusions

In this study, we investigated the possibility of improving the solubility of NS by preparing solid dispersions with $CaCO_3$, N-methyl-D-glucamine, and HP-β-CD using mechanochemical technology. The physical properties of NS SD in the solid state were characterized by differential scanning calorimetry, X-ray diffraction, FT-IR spectroscopy and SEM studies. The properties of the water solutions formed from the obtained solid dispersions were analyzed by HPLC for intrinsic solubility and ^{1}H-NMR spectroscopy.

An increase in solubility was observed for all compositions studied. In the case of the NS/HP-β-CD composition, this phenomenon is caused by the formation of inclusion complexes, which was confirmed by ^{1}H-NMR relaxation and selective NOESY methods. In the cases of $CaCO_3$ and N-methyl-D-glucamine ionization was proposed. Although, the formation of the NS and N-methyl-D-glucamine complex was also confirmed by ^{1}H NMR spectroscopy, this complex did not appear to be stable enough to significantly affect the NS solubility. On the contrary, the solubility of niclosamide from the NS/MG SD is consistent with the previously mentioned NS solubility pH dependence.

The PAMPA assay was used to predict passive intestinal absorption. It was found that the mechanochemically obtained SD with N-methyl-D-glucamine (1/1 mass ratio, 8 h milling) shows enhanced permeation of NS across an artificial membrane compared to that of the pure NS and NS/HP-β-CD SD. We suggest that a strong NS complexation with HP-β-CD prevents the NS release into the free form in solution, whereas the NS/HP-β-CD complex cannot be accepted by the hydrophobic membrane.

Therefore, the compositions of NS with $CaCO_3$, N-methyl-D-glucamine, and HP-β-CD obtained by using the mechanochemical manufacturing method are a promising basis for the development of NS-based preparations for oral administration, with reduced dose and high pharmacological effect.

• Supplementary materials

No supplementary materials are available.

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• Conflict of interest

The authors declare no conflict of interest.

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