

Scientific paper

Preformulation Investigation of Some Clopidogrel Addition Salts

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Abstract

Physico-chemical properties of active substances such as solubility, dissolution rate, chemical stability, pharmaceutical processibility, etc. can be improved by salt formation of active substances. Characterization of physical properties of such salts is important for selection of an optimal salt having required biopharmaceutical properties, stability and manufacturability.

The present study deals with the preformulation study of selected clopidogrel acid addition salts, i.e. hydrogen sulfate, hydrochloride (HCl), hydrobromide (HBr), besylate and (–)-camphor-10-sulfonate salt (CSA) and two commercially available polymorphic forms of hydrogen sulphate salt, i.e. form 1 (HS F1) and form 2 (HS F2).

Clopidogrel salts were characterized by means of thermal analysis (TG, DSC), X-ray powder diffraction (XRPD), infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), dynamic vapor sorption (DVS), true density, scanning electron microscopy (SEM) and solubility. Distinct differences in tested parameters were found among acid addition salts and crystalline forms of clopidogrel. Higher melting point of both hydrogen sulphate salt was attributed to presence of hydrogen bonds among HS anions, connecting them into a chain.

All salts included in the present study were anhydrous, except HBr which was in the form of monohydrate. The two tested polymorphic forms of clopidogrel HS salt are enantiotropically related to each other and showed the highest hygroscopicity among the tested salts. This is important for development of solid dosage form containing both polymorphic forms and for selection of primary packaging.

Solubility studies in different aqueous media showed comparable solubility for clopidogrel hydrogen sulfate (polymorphic forms 1 and 2), hydrochloride (form 1) and hydrobromide hydrate (form 1) whereas clopidogrel camphorsulfonate (CSA) and besylate salt showed slightly lower solubility.

Keywords: clopidogrel acid addition salts, characterization, solubility.

1. Introduction

Salt formation at active pharmaceutical ingredients (API) is usually used to manipulate their solubility (increase or decrease), to improve stability and decrease toxicity and to reduce hygroscopicity of APIs alone and also of finished drug product. It was found that chloride salts have the highest percentage of occurrence among all known pharmaceutical salts; they are followed by bromide, nitrate (V), ammonium, sulfate (VI), etc. Most of these salts can be obtained in different crystalline forms, such as polymorphs and hydrates, which are due to their pro-

erties more and more important in pharmaceutical industry.¹

Polymorphic forms of a compound are identical in chemical composition and have similar properties in liquid state, but poses different crystal structures, what result in differences in physical properties on molecular, particulate and bulk level among different forms.² Among these properties most important are those connected to bioavailability, stability and pharmaceutical processibility of API in bulk or incorporated into solid dosage forms such as solubility, dissolution rate, wetability, true density, hygroscopicity, melting point, particle size, filterability, flowability, compressibility, etc.³

Clopidogrel, (S)-(+)-methyl (2-chlorophenyl)(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate (Figure 1), is a potent platelet anti-aggregation drug. It was launched in 1997 by Sanofi Synthelabo under the brand name Plavix. The corresponding R-enantiomer at carbon 8 does not show anti-aggregating activity and is poorly tolerated.⁴ Its carboxylic acid derivative (S)-(+)-(2-chlorophenyl)-6,7-dihydro-4H-thieno[3,2-c]pyridine-5-yl acetic acid (clopidogrel acid), which can be obtained by the hydrolysis of the ester group, either in vitro, catalyzed by the increased humidity and temperature, or in vivo, as a result of the action of enzyme carboxylesterase, is the main degradation product having no pharmacological activity.⁵

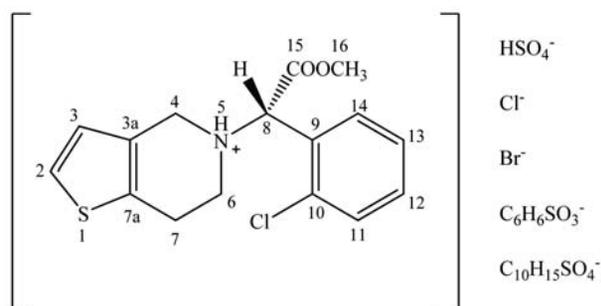


Figure 1. Chemical structure of clopidogrel salts: clopidogrel hydrogensulfate, hydrochloride, hydrobromide, besylate and (-)-camphor-10-sulfonate salt

In a free base form, clopidogrel ($pK_a = 4.5$) is an oily substance with high viscosity, which is relatively unstable under increased moisture and temperature. Usually APIs are purified by crystallization and as clopidogrel does not form solid crystalline phase it is difficult to purify. Accordingly, acid addition salts of clopidogrel, which are more stable solids and simpler for purification by crystallization, were developed.^{6,7}

Physico-chemical properties of clopidogrel salts are very scarcely described in scientific literature. Some limited data on the characteristics and behavior of hydrogen sulphate salt was published.⁸ On the other hand some data on physical characteristics of clopidogrel salts and crystalline forms are known only from patent literature.^{6,7,9} For example, WO patent No. 2007/108604, 2007 discloses a number of acid addition salts of clopidogrel, including hydrochloride, hydrobromide, hydrogen sulfate and taurocholate. Beside to these characterized salts, many other salts are known, i.e. acetate, benzoate, besylate, fumarate, maleate, citrate, mesylate, camphor sulfonate, isopropylsulfate, etc.⁷

Among the above listed acid addition salts of clopidogrel, the most preferred one has been crystalline clopidogrel hydrogen sulfate salt. This salt can crystallize in various polymorphic and pseudopolymorphic forms, but only forms 1 and 2 are used in pharmaceutical products.⁸

Detailed knowledge of physico-chemical characteristics of salts is important for pharmaceutical development to select proper formulation, manufacturing process and packaging which will attribute to acceptable quality of final product.

The objective of present study was to perform a detailed characterization of the selected acid addition salts of S(+) clopidogrel and two polymorphic forms of hydrogen sulfate salt, which are commercially available and used in the development of pharmaceutical formulations.

2. Materials and Methods

2.1. Materials

Clopidogrel hydrogen sulfate form 1 (HS F1) (M.w. = 419.9 g/mol), clopidogrel hydrogen sulfate form 2 (HS F2) (M.w. = 419.9 g/mol), clopidogrel hydrochloride (HCl) form 1 (M.w. = 358.3 g/mol), clopidogrel hydrobromide (HBr) form 1 (M.w. = 420.7 g/mol), clopidogrel besylate (besylate) (M.w. = 479.9 g/mol) and clopidogrel (-)-camphor-10-sulfonate (CSA) (M.w. = 554.12 g/mol) were prepared by Krka, d.d., Novo mesto.

2.2. Methods

2.2.1 Thermal Analysis

Thermal analysis (DSC) was performed by Mettler DSC 822e (dynamic N_2 atmosphere, heating rate $10\text{ }^\circ\text{C}/\text{min}$). Thermal effects were evaluated using Pyris software.

Thermally induced decomposition was performed by Mettler DSC 822e (dynamic N_2 atmosphere, heating rate $10\text{ }^\circ\text{C}/\text{min}$). Samples of clopidogrel HS F1 and HS F2 were heated to 170, 180, 190 and $200\text{ }^\circ\text{C}$, respectively. The concentration of clopidogrel acid and R isomer in the samples was determined by HPLC analysis.

Thermogravimetric analysis (TGA) was performed by Mettler DSC/TGA instrument (dynamic N_2 atmosphere, heating rate $10\text{ }^\circ\text{C}/\text{min}$).

2.2.2. Solubility

2.2.2.1. Solubility in Aqueous Solutions

The solubility of clopidogrel addition salts was determined in 0.1 M HCl solutions (pH 1,2), acetate buffer (pH 4.5), and phosphate buffer (pH 6.8) and purified water at a temperature of $37\text{ }^\circ\text{C}$. The excess of clopidogrel acid addition salts ($100\text{ mg}/\text{ml}$) was added to 20 ml of buffer solutions and purified water, and shaken for 24 hours (incubated at $37\text{ }^\circ\text{C}$). The concentration of clopidogrel in the filtrate ($0.45\text{ }\mu\text{m}$ pore size filter) was determined by HPLC analysis. The solubility values obtained were expressed in mmol/l solution. The pH of the solution at the time of the solubility determination was also measured.

2. 2. 2. Kinetic Solubility in Acetone

500 mg of clopidogrel HS F1 and HS F2 was suspended in 10 ml of acetone and stirred for 50 min (incubated at 263 K, 273 K, 288 K, 303 K and 329 K). The concentration of clopidogrel in the filtrate (0.45 μm pore size filter) was determined by HPLC analysis after 20 and 50 min. Since the solubility values measured at a particular temperature after 20 min and 50 min were similar for both forms and within the expected standard error of the method, it was assumed that solubility equilibrium was reached. After sampling, polymorphic form of a solid residue was checked by IR spectroscopy to confirm that no polymorphic transition occurred during the dissolution process.

2. 2. 3. FTIR Spectroscopy

Infrared spectra in KBr pellets were recorded within the wave number range of 4000–400 cm^{-1} with a Perkin-Elmer FTIR spectrometer 1720X at resolution 4 cm^{-1} .

2. 2. 4. X-ray Powder Diffraction (XRPD)

Diffractionograms were obtained by a Phillips PW 1710 diffractometer (CuK α radiation, $3 \leq 2\theta \leq 31^\circ$).

2. 2. 5. Dynamic Vapor Sorption (DVS) Isotherms

were obtained on an automatic water sorption analyzer DVS-1 (Surface Measurement System LTD) under the

following conditions: controlled room temperature (25 $^\circ\text{C}$), nitrogen flow: 200 ml/min, two complete cycles 0–95% RH and backwards in 11 steps.

2. 2. 6. Scanning Electron Microscopy (SEM)

was performed using a Field emission scanning electron microscope FE-SEM SUPRA 35 VP (Carl Zeiss, Germany) equipped with energy dispersive spectroscopy Inca 400 (Oxford Instruments, UK).

Unspattered samples in original form were analysed at the low beam energy (1 kV).

2. 2. 7. True Density

was determined by an Accupyc 1330 helium pycnometer.

2. 2. 8. ^{13}C and ^1H Nuclear Magnetic Resonance (NMR)

spectra were obtained by a Varian Unity Inova 300 MHz spectrometer using 5 mm “Magic Angle” and by a Varian Inova 600 MHz NMR spectrometer using 3.2 mm “NB Double Resonance HX MAS”. Larmor frequencies of proton and carbon atoms were 302.98 MHz and 76.19 MHz in NMR spectra and 599.77 MHz and 150.83 MHz on the 600 MHz NMR spectrometer. Carbon and proton chemical shifts were presented relatively to HMB (hexamethylbenzene) external standard used as a secondary reference. Rotation frequencies were 5 kHz on the 300 MHz



Figure 2a. SEM micrographs of clopidogrel HBr (magnification 1.000)

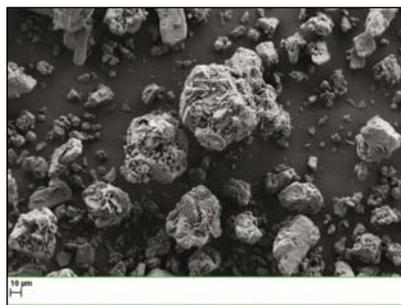


Figure 2b. SEM micrographs of clopidogrel besylate (magnification 1.000)



Figure 2c. SEM micrographs of clopidogrel CSA (magnification 1.000)



Figure 2d. SEM micrographs of clopidogrel HCl (magnification 1.000)

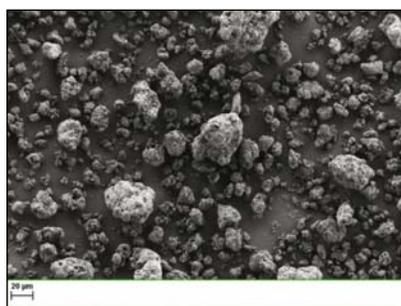


Figure 2e. SEM micrographs of clopidogrel HS F1 (magnification 1.000)

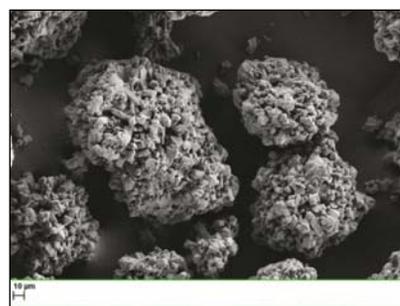


Figure 2f. SEM micrographs of clopidogrel HS F2 (magnification 1.000)

spectrometer, and 20 kHz during ^1H measurements and 10 kHz during ^{13}C measurements on the 600 MHz NMR spectrometer.

3. Results and Discussion

3. 1. Microscopy

Morphology of particles of API is very important for assuring acceptable flow properties and compressibility which are important parameters for compression of API containing powder mixture into tablets. SEM analysis of clopidogrel HBr showed that it occurs as irregular plates or columnar particles where the surface of the majority of particles is cracked (Figure 2a). Clopidogrel besylate (Figure 2b) and clopidogrel CSA (Figure 2c) exist in the form of irregular plates, which are agglomerated in the case of besylate salt. On the other hand, clopidogrel HCl (Figure 2d) exists in the form of long needle-like plates with relatively smooth surface with almost no observable agglomeration.

Irregular columnar aggregated particles of various sizes were found in the case of clopidogrel HS F1 (Figure 2e) whereas big rounded agglomerates, formed from sticky plates, can be seen in powdered clopidogrel HS F2 (Figure 2f). Agglomerated secondary particles observed in form 1 are smaller than those observed in form 2.

3. 2. Thermal Analysis

Thermogravimetric (TG) and differential scanning calorimetry (DSC) curves of clopidogrel salts and polymorphs are shown in Figures 3 and 4. No mass loss can be observed in the TG curves of the samples of clopidogrel HS F2, besylate and CSA salt heated to 170 °C, indicating that no solvent was present in these samples. Only slight weight loss can be observed at clopidogrel HS F1 form indicating surface adsorption of moisture onto the particles. The TG curve of clopidogrel HCl (Figure 3) showed initiation of mass loss at 150 °C corresponding to the melting peak in the DSC curve. The observed weight loss du-

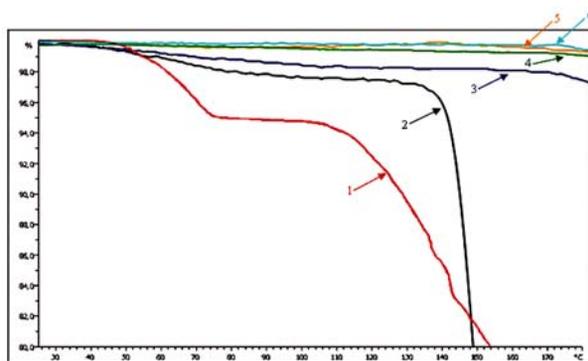


Figure 3: TGA curves of clopidogrel HBr (1), HCl (2), HS F1 (3), HS F2 (4), besylate (5), CSA (6)

ring melting was ascribed to thermal decomposition of the sample.

The TG curve for clopidogrel HBr showed the initiation of mass loss at 50 °C, which ended at 80 °C with a total mass loss of approximately 5%, corresponding to approx. one mole of water per mole of the HBr salt. Evaporation can also be observed in DSC curve (Figure 4) where slow rising of the base line of DSC curves starting at 50 °C can be observed. The data of the mass loss determined by the TGA analysis are comparable with the results of determination of water content by Karl Fisher method. From these results we can conclude that the mass loss in the temperature range 50–80 °C is attributed to the evaporation of the hydrate water in the form of a thermally unstable hydrate. We can also conclude and confirm that clopidogrel HBr F1, which is according to the literature the most stable crystalline form of HBr salt, is a monohydrate form.^{10, 11} From the obtained low energy and temperature of dehydration of monohydrate form of HBr salt it was proposed that water molecules are weakly bonded into the crystal structure of monohydrate form and its thermal stability is low. The formation of anhydrous form of HBr salt at elevated temperatures was confirmed by X-ray analysis. The obtained anhydrous form of clopidogrel HBr salt corresponds to the form described in the literature as 1A.¹² It is stable in the temperature range from 90 to 110 °C, when melting of anhydrous clopidogrel HBr starts.¹²

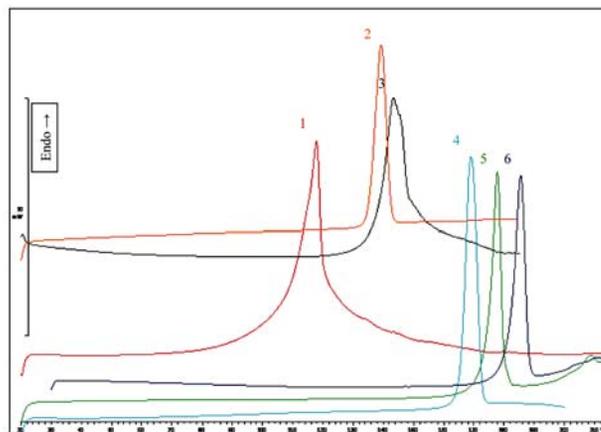


Figure 4: DSC curves of clopidogrel HBr (1), besylate salts (2), HCl (3), CSA (4), HS F2 (5), HS F1 (6)

Anhydrous form 1A is not physically stable. When it is exposed to open air conditions with normal relative humidity (around 60%), anhydrous form transforms spontaneously back to monohydrate form what was confirmed by X-ray analysis.

From the shape of the DSC curves of clopidogrel HBr and HCl samples, high values of fusion enthalpies (Table 1) and the rapid mass change in the TG curves, as a result of a rapid weight loss during melting, we can conclude that both halogen salts are thermally unstable at their

melting point temperatures, resulting in their chemical decomposition.

The DSC analysis (Figure 4) of clopidogrel HS F1 showed a melting endotherm with onset temperature at 181.7 °C (peak temperature at 185.5 °C) and enthalpy of fusion of 31.43 kJ/mol, while F2 showed a melting endotherm with onset temperature at 178.7 °C (peak temperature at 181.7 °C) and melting enthalpy of 36.36 kJ/mol. The observed values for melting point and melting enthalpy for both forms are in good agreement with the values published in the literature.^{8,9} Our results confirm enantiotropic relationship between both forms of HS salt, found by Koradia et al.⁸ Slightly broader melting peak for clopidogrel HS F1 compared to HS F2 was attributed to thermally induced decomposition, which starts at temperatures higher than 180 °C (Table 1, Figure 5).

In order to verify the extent of chemical degradation of both polymorphic forms of clopidogrel HS salt, both samples were thermally treated and the impurity profile was checked by HPLC analysis. Samples of both polymorphic forms of clopidogrel HS salts were exposed isot-

3. 3. True Density

True density measurements for clopidogrel HS F1 and HS F2 were performed using a helium pycnometer. Higher true density (1.512 g/cm³) was obtained for clopidogrel HS F1 compared to HS F2 (1.472 g/cm³). Both values are similar to those reported in the literature.⁹ Differences in the true density between both polymorphic forms of HS salt were attributed to different packaging of molecules in the crystalline structure and, therefore, to the different crystalline structure of both polymorphic forms.

3. 4. Spectroscopic Characteristics

The FTIR is one of the most widely reported spectroscopic techniques for solid state characterization of pharmaceutical substances; it can be used for both, qualitative and quantitative analyses of drug substances and excipients.¹² FTIR spectra of clopidogrel addition salts are given in Figure 6.

Table 1: Melting points, enthalpies of fusion and water content for clopidogrel HS F1, HS F2, HCl, HBr, CSA and besylate salts

	HS F1	HS F2	HCl	HBr	besylate	CSA
Onset (°C)	181,7	178,7	137,7	103,7	135,1	165,5
Peak max. (°C)	185,5	181,7	143,4	114,7	139,2	169,0
ΔHf (kJ/mol)	31,43	36,36	51,11	69,35	44,71	51,63
Water content (%), KF analysis	<0,5	<0,5	<0,5	4,35	<0,5	<0,5

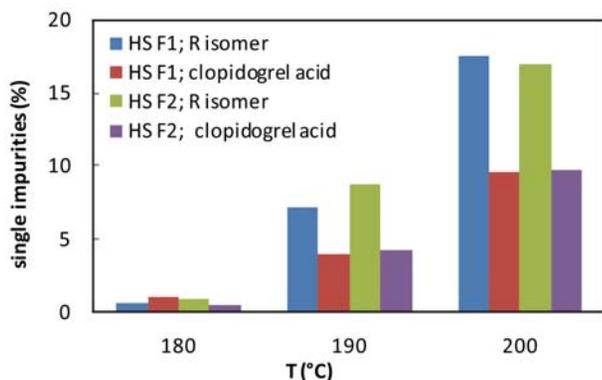


Figure 5: Impurities determined in thermally treated samples of clopidogrel HS F1 and HS F2

hermally for 10 min to increased temperatures, i.e. 180, 190 and 200 °C. The content of two known pharmacologically inactive degradation products, i.e. clopidogrel acid and clopidogrel R isomer, were quantified (Figure 5). From the obtained results we can conclude that both polymorphic forms have similar thermal stability at temperatures close to melting point and that thermal decomposition accelerates in liquid (melted) state.

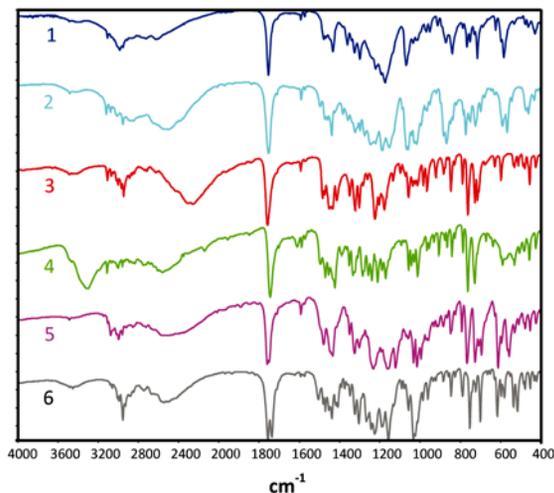


Figure 6: FTIR spectra of clopidogrel HS F1 (1), HS F2 (2), HCl (3), HBr (4), besylate (5), CSA (6)

The absorption bands arising from aromatic CH stretching vibrations were observed at wavelengths from 3050 to 3150 cm⁻¹; this aromatic band for clopidogrel HS F2 is observed at 3121 cm⁻¹ but it is shifted to 3103 cm⁻¹ in

the case of HS F1. This indicates that CH bonds in chlorophenyl ring have different strengths at HS F2 compared to HS F1. Furthermore, ^{13}C solid state NMR spectra of both hydrogen sulfate salts show significant difference in the aromatic region of spectra (region from 124 to 136 ppm) (Figure 7b). This observation can be attributed to different orientation of chlorophenyl ring in HS F2 crystal lattice compared to HS F1, leading consequently to different conformation of clopidogrel molecule in the crystal lattice.

The presence of hydrate water in the case of clopidogrel HBr F1 resulted in the typical broad absorption band in its FTIR spectrum at 3300 cm^{-1} .

The characteristic broad absorption band (for all salts) associated with stretching vibrations of bonded N-H (due to salt formation) is observed from 2500 to 2600 cm^{-1} . The spectra of all tested salts showed strong absorbance band, corresponding to C=O stretching vibrations at approximately 1750 cm^{-1} .

Some differences in spectra of tested salts were observed in the range bands for proton donor functional groups (-NH- and -OH). For HS F1, these bands are located at app. 2750 cm^{-1} whereas in the case of HS F2 the band was shifted to app. 2510 cm^{-1} , indicating stronger H bonds in the case of HS F2 compared to HS F1. Pronounced differences were observed also in the finger print regions for all clopidogrel addition salts.

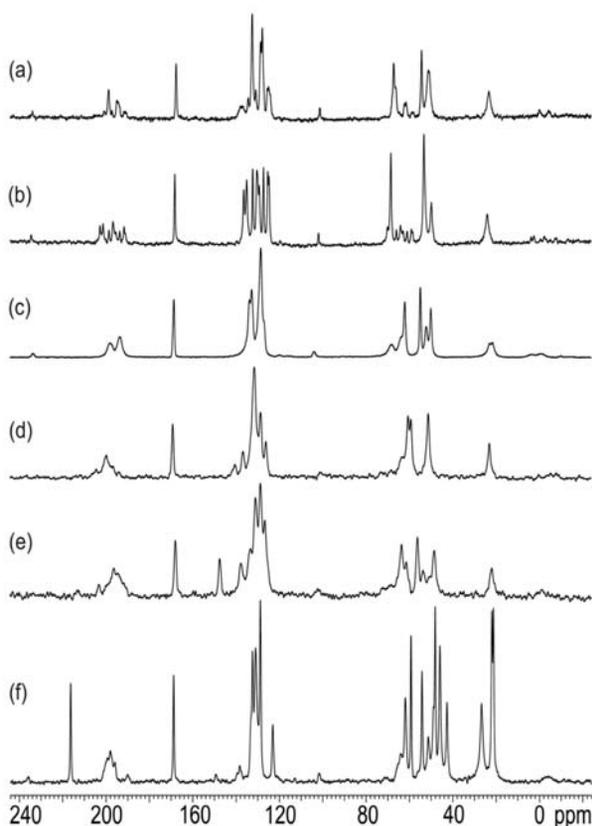


Figure 7: ^{13}C solid state NMR spectra of clopidogrel HS F1 (a), HS F2 (b), HCl F1 (c), HBr F1 (d), besylate (e) and (f) CSA.

Solid state ^{13}C NMR spectra of tested clopidogrel salts are shown in Figure 7 where significant differences among spectra can be observed showing differences in chemical environment of individual carbon atoms in the molecule from which different packaging of clopidogrel molecules in crystals of different salts can be proposed.

No significant difference in the C13 spectrum of HS F1 and HS F2 for carbonyl (C15) and methoxy (C16) carbons can be observed. These two functional groups are important as they could participate in the hydrogen bond formation. As the chemical environment around C atoms in both mentioned functional groups is similar for HS F1 and HS F2, since there is no significant difference in the C13 spectrum of HS F1 and HS F2 for carbonyl and aliphatic carbons (methoxy) we could assume that hydrogen bond between hydrogen sulphate anions is formed also in HS F1. Presence of hydrogen bonds in crystal structure of HS F1 and HS F2 was proposed to attribute to physico-chemical stability and higher melting point of HS salt in comparison to the other studied salts.

^{13}C CPMAS NMR spectrum of HS F2 (Figure 7) clearly shows only one set of well resolved signals. This implies that there is one molecule of clopidogrel hydrogensulphate in unit cell. ^{13}C CPMAS NMR spectrum of HS F1 is very similar to spectrum of HS F2 with the observable differences in chemical shifts of aromatic carbons in the region of cca. 124 ppm to 136 ppm. Small changes can be observed in the aliphatic region of methylene carbons (C4, C6), quaternary carbon (C8) and methoxy C16 carbon.

Comparison of ^{13}C solid state NMR spectra for HCl F1 and HBr F1 shows small difference in the chemical shifts of aromatic carbons with peaks overlap in the region 125–132 ppm. Observable differences are seen for the

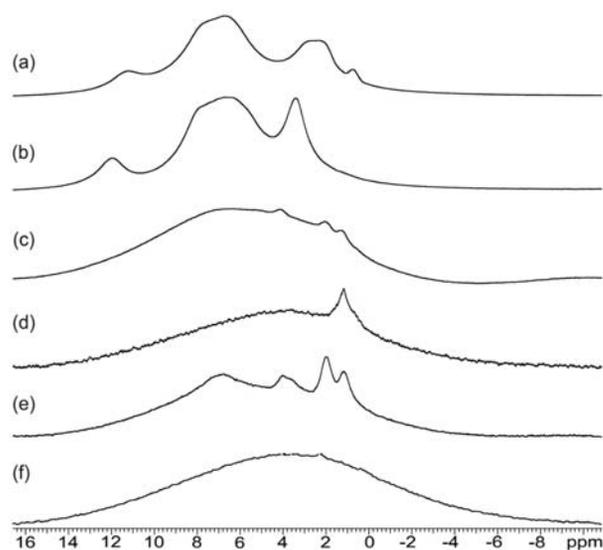


Figure 8: ^1H MAS NMR spectra of clopidogrel salts: a – HS F1, b – HS F2, c – HCl, d – HBr, e – besylate, f – CSA

methylene C4, C6 carbons which are well resolved in HCl F1. Significant change to lower frequency is observed for C16 methoxy carbon in HBr F1.

^{13}C NMR CPMAS NMR spectrum for clopidogrel besylate shows peak overlap in the aromatic region and differences in the aliphatic region compared to other spectra.

Additional signals for well crystalline clopidogrel CSA salt are observed in ^{13}C solid state NMR spectrum (carbonyl group at cca. 216 ppm, signals in aliphatic region)

^1H solid state NMR spectra of HS F1 and HS F2 (Figure 8) showed resolved signal at 10.9 ppm and 11.7 ppm, respectively. We can conclude that acidic proton is involved in the hydrogen bonding. The differences in the chemical shift suggest the difference in the strength of hydrogen bonding in clopidogrel HS F1 and HS F2 forms.

From the comparison of X-ray powder diffraction data of different clopidogrel salts and polymorphic forms (Figure 9) we can conclude that all samples were crystalline and showed different diffraction patterns as a consequence of different arrangement of the molecules in the crystal lattice.

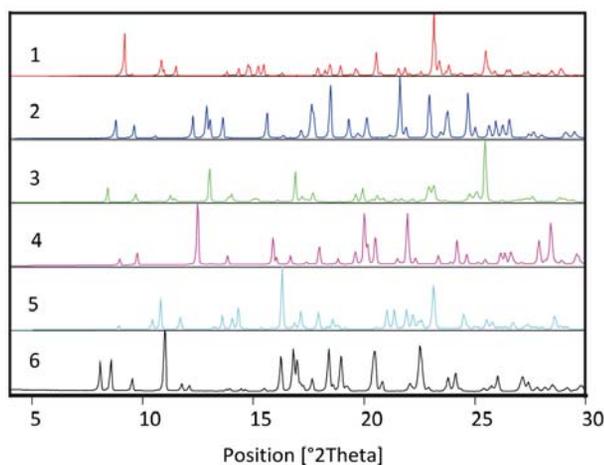


Figure 9: X-ray powder diffractograms of clopidogrel HS F1 (1), HS F2 (2), HCl (3), HBr (4), CSA (5) and besylate salts (6)

According to the single crystal structure of HS F2, a possible hydrogen bond exists between OH protons of one hydrogen sulphate anion and oxygen atoms of next anion.⁹ From the computer model developed from data published by Bouquet et al.⁹ for crystal structure of clopidogrel HS F2 we proposed that hydrogensulphate (HS) anions are connected via hydrogen bonds into a chain (Figure 10). The calculated distance $-\text{O}-\text{H}\cdots\text{O}=\text{}$ between two neighboring HS anions is 0.265 nm.

The solubility determination of clopidogrel acid addition salts in aqueous media was performed in aqueous solutions having different pH: 0.1M HCl solution (pH 1.2), acetate buffer solution (pH 4.5), phosphate buffer (pH 6.8), and purified water (pH 5.5) at a temperature of 37 °C. It was recognized that solubility of all clopidogrel acid

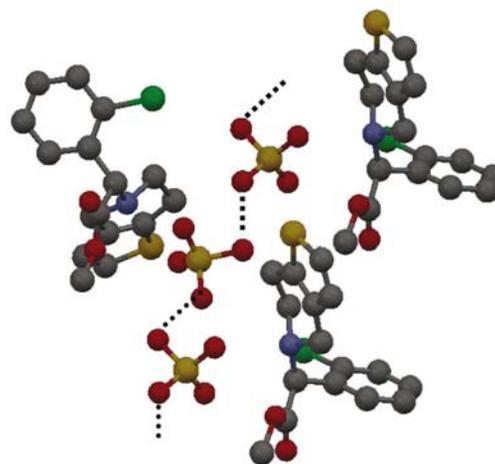


Figure 10: Computer derived model of hydrogen bonds (....) in clopidogrel HS F2 crystal structure

addition salts is strongly affected by the pH of the solution (Figure 11). The solubility of all clopidogrel salts is very high in strong acidic media (pH 1.2) and is significantly lower in phosphate buffer pH 6.8 due to formation of unionized free base form having lower solubility.

The solubilities of both polymorphic forms of clopidogrel HS salt are very similar in all tested media and are slightly higher when compared to the other salts. This observation is in contradiction with the literature data where it was reported that HS F2 exhibits lower solubility than HS F1 as a result of a greater thermodynamic stability.⁹

The solubility of clopidogrel besylate and especially CSA salt were significantly lower, with CSA salt exhibiting the lowest solubility among the tested salts. This observation was attributed to the properties of the counter

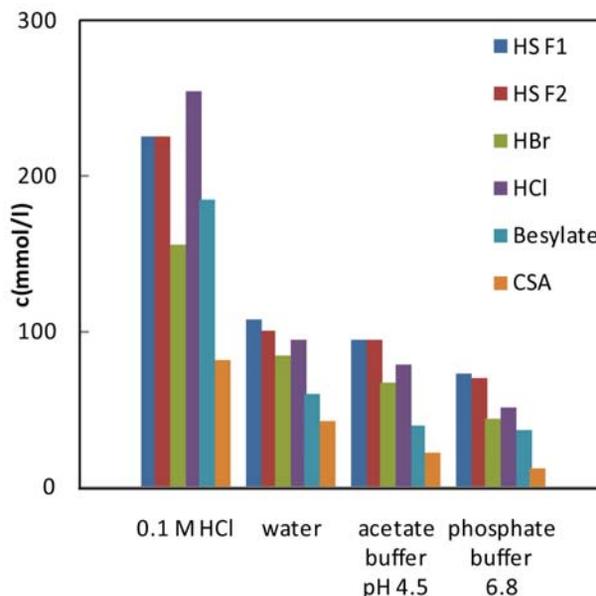


Figure 11: Comparison of solubility of clopidogrel addition salts in different aqueous solutions at 37 °C

ions; it was noted that the camphorsulfonate (CSA) ion is the largest and the most hydrophobic one among the tested salts whereas the camphorsulfonic acid is the weakest acid among the acids forming counter ions in studied clopidogrel salts.

The pH values of all solutions decreased during the dissolution of clopidogrel addition salts. As expected, the most significant pH drop was observed for water solutions having pH at the beginning of the experiment 5.5, which does not have any buffer capacity. The measured pH of the saturated aqueous solutions of salts was 1.2 (for HS F1 and HS F2), 1.36 (for HCl), 1.43 (for HBr), 1.7 (for besylate) and 2.5 (for CSA). The pH decrease of the clopidogrel solutions was attributed to the hydrolysis of the clopidogrel addition salts, which are salts of a weak base and of acids of different strengths. The smallest drop in the pH of the solution was observed for besylate and CSA salts. The solubility of clopidogrel free base in 0.1 M HCl was similar to solubility of its salts. High solubility of base in highly acid medium was attributed to formation of protonated clopidogrel as is present in salt form. Clopidogrel base is practically insoluble (0.0034 mmol/l) in water and dissolution of base has no impact on pH of the medium (measured pH of the clopidogrel base suspension in purified water was 5.5).

As acetone is the most frequently used solvent for purification and crystallization of clopidogrel HS F1 and HS F2, it was used as a non-aqueous solvent to perform comparative solubility study of two polymorphic forms of clopidogrel HS, where no influence of pH on the solubility of tested salts can be observed.⁹ From our results we can conclude that the solubility of both polymorphs in

acetone is lower in comparison to their solubility obtained in aqueous solutions.

Furthermore, the experimental results of solubility study confirm our expectation, which was postulated from finding that HS F1 and HS F2 are enantiotropically related, that the solubility of clopidogrel HS F1 is higher than the solubility of clopidogrel HS F2 at room temperature. A plot of $\ln(x)$ versus $1/T$ (Figure 12), where x is mole fraction of the solute in the solution, exhibits a linear relation for both forms of clopidogrel HS salts at lower temperatures, however deviation from proposed behavior of the solution is observed at higher temperatures where the observed solubility was higher than proposed by extrapolation of linear part of the curve obtained at lower temperatures. As shown in Figure 12, the solubility measured at higher temperatures is higher than predicted from the ideal solution theory ('negative deviation'), which means that the solute strongly interacts with the molecules of the solvent. From the extrapolated linear part of solubility curves for both forms of HS salt we can obtain interception point, i.e. the temperature where the solubilities of both forms are equal. With extrapolation of the lines in Figure 12 we can obtain two different interception points (909 and 388 K). On the other hand the interception point calculated using van't Hoff equation for ideal solutions from the melting points (T_f) and melting enthalpies (H_f) for HS F1 and HS F2, is approximately 430 K.¹³ The differences in values of the interception points were attributed to non ideal behavior of solubility of clopidogrel HS polymorphic forms.

$$\ln x = H_f/R \cdot (1/T_f - 1/T) \quad (1)$$

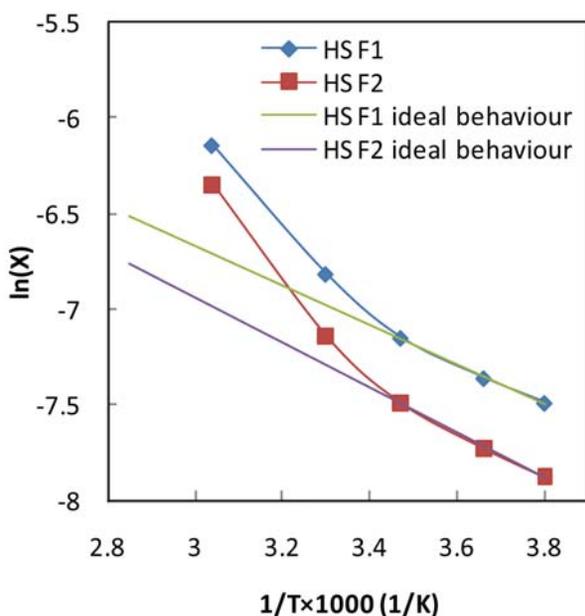


Figure 12: Solubility (in mol fraction) of clopidogrel HS F1 and F2 in acetone at different temperatures (T is expressed as $1/T$, multiplied by 1000)

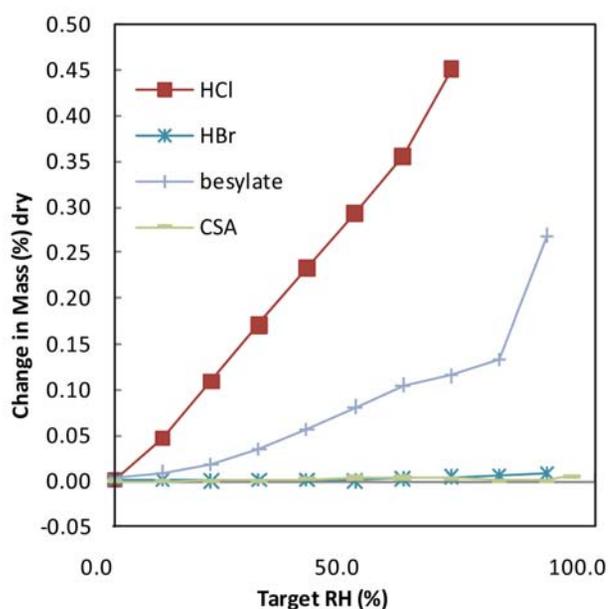


Figure 13: DVS sorption isotherms of clopidogrel HCl, HBr, besylate and CSA

Where x is the molar fraction of solute, H_f is the melting enthalpy, T_f is melting point, T is the temperature of the experiment, R is the gas constant.

It is known from the literature,^{14, 15} that both polymorphic forms of HS salt exhibit difficulties in formulating of chemically and physically stable solid dosage forms. It is also known that clopidogrel salts tend to chemically degrade even in the presence of low moisture, generating significant amount of impurities, such as hydrolyzed products (acid). In order to study the moisture uptake of different clopidogrel salts the dynamic vapor sorption (DVS) analysis (Figures 13 and 14) was performed. From the results it can be concluded, that clopidogrel besylate and CSA salts are not hygroscopic at normal RH and both halogenide salts showed only negligible moisture sorption.

On the contrary both polymorphic forms of clopidogrel HS F1 and HS F2 became very hygroscopic at RH above 70%. This information is crucial for development of solid dosage form and selection of manufacturing processes, climate and primary packaging of tablets containing clopidogrel HS salt.

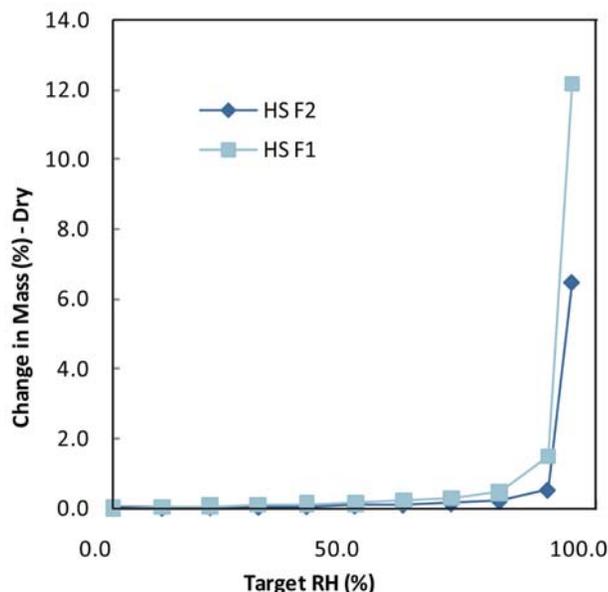


Figure 14: DVS sorption isotherm of clopidogrel HS F1 and HS F2

4. Conclusion

Clopidogrel HS F1, clopidogrel HS F2, clopidogrel HCl, clopidogrel HBr, clopidogrel besylate and clopidogrel CSA salts were characterized by thermal, spectroscopic and crystallographic methods.

Our results of the thermal analysis confirmed the literature proposed an enantiotropic relationship between both clopidogrel HS salt polymorphic forms F1 and F2. The results also showed that melting point of tested salts

depends on counter ion type, where HS salt having the highest melting point and HBr the lowest. High melting point of HS salt was attributed to stabilization of crystal structure of both polymorphic forms of clopidogrel HS salt by hydrogen bonds among HS anions.

Higher values for the enthalpy of fusion observed for clopidogrel HCl and HBr, compared to other tested salts, were partially attributed to the rapid thermal decomposition of both halogen salts occurring during melting.

The comparative solubility study of clopidogrel HS F1 and F2 in acetone showed nonlinearity in van't Hoff solubility plot for both forms, with nonlinearity being more pronounced for F2.

From the results shown and discussed above we can conclude that all tested clopidogrel acid addition salts have good solubility in acidic media and can be used in the pharmaceutical production by taking into account specific limitations for some of them. Lower solubility of clopidogrel CSA and besylate salts and lower thermal stability of clopidogrel HBr salt should be overcome by formulation and manufacturing process adjustments.

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Povzetek

Fizikalno-kemijske lastnosti zdravilnih učinkovin, kot so topnost, hitrost (profil) raztapljanja, kemijska stabilnost, farmacevtsko-tehnološka procesibilnost itd., lahko izboljšamo s tvorbo različnih soli. Karakterizacija fizikalnih lastnosti tovrstnih soli je pomembna za izbiro optimalne soli, ki ima ustrezne biofarmacevtske lastnosti, ustrezno stabilnost in procesibilnost v industrijskem merilu. V nadaljevanju v prispevku predstavljamo predformulacijske študije nekaterih soli klopidogrela z nekaterimi organskimi in anorganskimi kisljinami, tj. klorid (HCl), bromid (HBr), besilat, (-)-kafra-10-sulfonat(CSA) ter hidrogen sulfat, pri slednji smo karakterizirali dve komercialno dostopni polimorfni obliki I (HS F1) in II (HS F2).

Soli klopidogrela smo okarakterizirali z uporabo termične analize (TG, DSC), praškovne rentgenske difrakcije (XRPD), infrardeče spektroskopije (FTIR), jedrske magnetne resonance (NMR), dinamične sorpcije par (DVS), prave gostote, elektronske mikroskopije (SEM) in topnosti. Ugotovljene so bile nekatere bistvene razlike v testiranih parametrih med različnimi solmi in kristaliničnimi oblikami klopidogrela. Višje tališče, ugotovljeno za obe polimorfni obliki klopidogreljeve HS soli, je posledica prisotnih vodikovih vezi med hidrogen sulfatnimi anioni, ki slednje povezujejo v verigo. Klopidogreljev bromid, uporabljen v naši študiji, je bil v obliki monohidrata, vse preostale preiskovane soli pa so bile anhidrične. Preiskovani kristalni obliki soli klopidogreljevega hidrogen sulfata HS F1 in HS F2 sta enantiotropni in kažeta najvišjo higroskopnost med vsemi preiskovanimi solmi. To je zlasti potrebno upoštevati pri razvoju trdnih farmacevtskih oblik, ki vsebujejo hidrogensulfatno sol in pri izbiri ustrezne kakovosti in tipa ovojnine.

Pri preučevanju topnosti soli klopidogrela v različnih vodnih medijih smo ugotovili primerljivo topnost za soli z anorganskimi kisljinami (hidrogensulfat, klorid in bromid), medtem ko smo za obe soli z organskima kisljinama, tj. klopidogrel CSA ter klopidogrel besilat določili nekoliko nižjo topnost.