Journal of Pharmaceutical and Biomedical Analysis 48 (2008) 356–360

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Compatibility studies between mannitol and omeprazole sodium isomers

S. Agatonovic-Kustrin[a,*], N. Markovic[b], M. Ginic-Markovic[c], M. Mangan[a], B.D. Glass[a]

[a] School of Pharmacy and Molecular Sciences, James Cook University, Townsville, QLD 4811, Australia
[b] Tagara Group, Adelaide, Glynde, SA 5070, Australia
[c] School of Chemistry, Flinders University, Adelaide, SA 5042, Australia

ARTICLE INFO

Article history:
Received 19 September 2007
Received in revised form 5 February 2008
Accepted 8 February 2008
Available online 17 February 2008

Keywords:
Omeprazole sodium isomers
Mannitol
Differential scanning calorimetry
Attenuated total reflectance infrared spectroscopy
Localized thermal analysis
Polymorphism
Tautomerism

ABSTRACT

Omeprazole, commonly used in the treatment of various gastrointestinal disorders degrades rapidly in acidic pHs and results in inter-individual variability due to different rates of metabolism amongst patients. Since *S*-omeprazole shows more predictable bioavailability and excipients have been known to interact with active pharmaceutical ingredients to produce altered bioavailability, it was decided to investigate the compatibility of omeprazole sodium isomers with mannitol, the major excipient in omeprazole formulations using differential scanning calorimetry (DSC) for bulk drug, attenuated total reflectance (ATR) infrared (IR) spectroscopy in a powder mixture and localized thermal analysis (LTA) from a drug disk.

DSC results clearly indicate an interaction between mannitol and *R*-omeprazole sodium due to decreased melting temperatures and broadening peaks. The DSC of *S*-omeprazole sodium does not show melting temperature although the drug was crystalline. Because of the accelerated temperature conditions during DSC experiments applied in this work, ATR-IR was undertaken to determine whether these results occurred at room temperature for the solid dosage form. The ATR-IR results show a difference between *R*- and *S*-omeprazole sodium with mannitol by the appearance of both the amino (N–H) and imino (=N–H) stretching frequencies for *R*-omeprazole and only the N–H for the *S*-omeprazole sodium. It may thus be concluded that different ratios for the tautomeric forms for *S*- and *R*-omeprazole sodium result in changes in the degree of crystallinity and are responsible for the interaction with mannitol, common excipient in formulation. These interactions may be directly related to the difference in terms of bioavailability.

1. Introduction

Omeprazole is a potent non-reversible inhibitor of the enzyme gastric proton pump H^+/K^+-ATPase, which is responsible for gastric acid secretion. It is used to treat and prevent two common disorders: gastrointestinal ulcers and gastroesophageal reflux disease (GERD). Poor aqueous solubility and physicochemical instability however present a formulation challenge to the pharmaceutical industry.

Chemically, omeprazole consists of a substituted benzimidazole ring and pyridine ring connected via a sulfoxide-containing chain. Due to the presence of the pyridine ring, omeprazole is a weak base with a pK_a of 3.97, while the N–H proton in the benzimidazole ring is responsible for the acidity of the molecule (pK_a of 8.70). This acidity is advantageous for the preparation of the more stable alkali salts of omeprazole, such as omeprazole sodium, which is currently used for pharmaceutical applications. Alkaline salts of the isomers were found to be crystalline, which was also advantageous for future clinical studies.

Preformulation studies [1] have shown that moisture, temperature, salts and metal ions [2,3] and an acidic environment [4,5] decrease the stability of omeprazole and should be avoided in pharmaceutical formulations. In aqueous media, the degradation rate proceeds with a half-life of less than 10 min at pH values lower than 4.3 [6]. Omeprazole is thus formulated as enteric-coated granules encapsulated in a gelatin shell (e.g. omeprazole capsules) or as enteric-coated tablets (e.g. omeprazole multiple unit pellet system) [7,8].

The second problem is as a result of significant inter-individual variability [9] that is attributed to different rates of metabolism amongst patients. Individual differences were explained by variations in the metabolic enzyme CYP2C19 [10]. For those rapid metabolizers a higher or multiple doses was required, while slow metabolizers are exposed to increased concentrations of the drug. Improving the predictability of the bioavailability of omeprazole was originally attempted by altering the substituent pattern on the heterocyclic rings, but the real breakthrough was achieved when the (*S*)-(−)-enantiomer, later named esomeprazole, was introduced onto the market by Astra Zeneca. Their aim was to achieve a therapeutic benefit due to less inter-individual variation and higher average plasma levels, providing greater dose efficiency in patients. Clinical trials have in fact shown esomeprazole to have

* Corresponding author. Tel.: +61 7 4781 6803; fax: +61 7 4781 5356.
E-mail address: nena.kustrin@jcu.edu.au (S. Agatonovic-Kustrin).

0731-7085/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.02.009

both improved pharmacokinetics and suppression of acid secretion than omeprazole and thus enhanced bioavailability and less patient variation [11]. Both isomers have however identical gastric proton pump H^+/K^+-ATPase, enzyme inhibitor potency in vitro.

The aim of this study was to identify possible incompatibilities between omeprazole sodium and mannitol in formulation that can further contribute to the difference in efficacy. Drug formulation often involves the preparation of multi-component systems based both on drug–drug and drug–excipient combinations. Assessment of the potential incompatibility between active pharmaceutical ingredients (API) and different excipients is an essential part of the preformulation study prior to the final formulation of a solid dosage form [12]. Excipients are known to facilitate administration and release of the API, as well as to protect it from the environment and although essentially pharmaceutically inert, physicochemical interactions with the API may be possible [13]. Mannitol is a commonly used, non-hygroscopic and chemically stable excipient, with good flow properties and high compressibility and as such is suitable for use with APIs sensitive to water. There have however been some reports of the adverse effects of mannitol on stability of drugs, attributed to continued crystallization of mannitol from a system that is initially only partially crystalline. This can result in the increase of water activity in amorphous regions where the drug is located, with subsequent adverse effects on stability [14].

In the case of chiral drugs used as API, such as omeprazole, attention should be paid to interactions of single enantiomer with excipients since these interactions may lead to significant changes in the physicochemical properties of the drug and subsequent bioavailability [15]. This study investigates the compatibility of omeprazole sodium isomers with mannitol, the major excipient in omeprazole formulations, using differential scanning calorimetry (DSC) for bulk drug, attenuated total reflectance (ATR) infrared (IR) spectroscopy in a powder mixture and localized thermal analysis (LTA) from a drug disk.

2. Experimental

2.1. Materials

Racemic *R*,*S*-omeprazole sodium and reference standards for *R*- and *S*-omeprazole sodium (99.4%, w/w drug purity, 99.9% isomeric purity) were kindly donated by Astra Zeneca, Sweden.

2.2. Methods

Physical mixtures of *S*- and *R*-omeprazole sodium and mannitol were prepared in 20:80, 30:70 and 50:50 (w/w) ratios by gently blending in an agate mortar with a spatula at room temperature ($25 \pm 1\,^{\circ}C$).

DSC measurements were conducted using a DSC 822e instrument (Mettler Toledo Instruments, Ohio, USA). Calibrations of base line, cell constant and temperature were performed prior to each measurement using indium (Mettler Toledo) with corresponding heating rates. The samples (~4 mg) were encapsulated in hermetically sealed aluminium pans and all experiments were performed under a nitrogen purge gas rate of 50 ml min^{-1}, in duplicate.

S- and *R*-Omeprazole–mannitol disks of ~1 cm in diameter and approximately 2 mm in thickness were prepared by placing approximately 200 mg of premixed powder sample (85:15, w/w) into a die and applying pressure of ~7 tonnes for 5 min for use in ATR-IR and microthermal analysis experiments.

μTA was conducted using a 2990 Micro-Thermal Analyser (TA Instruments, New Castle, DE) with a thermal probe. The thermal response of the probe was first calibrated using benzoic acid crystals (TA Instruments) with a melting point of 122 °C after extrapolating to previously saved calibration curve (correlation coefficient of 99.8%). Isothermal imaging was performed on the disks positioned on a metallic sample stage using double-sided non-conductive tape. A constant force equivalent to ~10 nA of sensor deflection was applied and the tip rastered over an area of $100\,\mu m \times 100\,\mu m$ with a scanning rate of $100\,\mu m\,s^{-1}$ (resolution of 300 lines). Scans were performed isothermally at 30 °C, with a scan rate of 1 Hz, in triplicate. Additionally after selecting five points on the surface area, localized thermal analysis, at a heating rate of $10\,^{\circ}C\,s^{-1}$ for up to 300 °C, was applied and reproducibility confirmed.

ATR-IR spectra were collected using a Nicolet "Nexus" FTIR spectrometer with ZnSe prism (4000–650 cm^{-1}) and spectra were recorded with the Nicolet's OMNIC software, by averaging 64 scans for each spectrum with resolutions of 4 cm^{-1} (data point resolution/interval of 1 cm^{-1}).

The ATR-IR powder samples were prepared according to the procedure of the quantitative adsorption experiments. Binary mixture samples were prepared as smear mounts. Approximately 0.5 g of each sample was mixed with water and smeared directly onto the crystal. The ATR-IR spectra of compressed omeprazole–mannitol mixtures were measured directly. *S*- and *R*-Omeprazole–mannitol disks were placed in intimate contact with the ZnSe crystal and spectra were recorded.

3. Results and discussion

Differential scanning calorimetry can be used for a wide range of pharmaceutical applications ranging from the characterisation of raw materials to the evaluation of drug–excipient interactions through the appearance, shift, or disappearance of endo- or exothermal effects and/or variations in the relevant enthalpy values [16–21].

Differential scanning calorimetry has been utilized to study the crystalline nature of pure *S*- and *R*-omeprazole sodium, racemic *R*,*S*-omeprazole sodium and mannitol as an excipient. From the DSC thermogram (Fig. 1), the clear melting peak temperatures at ~170 °C (mannitol) and at ~180 °C (*R*-omeprazole sodium) are observed, indicating the crystalline nature of excipient and drug, respectively. These melting temperatures are in the good agreement with the literature values. However thermal investigations of pure esomeprazole sodium and a racemic omeprazole sodium mixture (Fig. 1, arbitrary units used for *y*-axis) suggest absence in melting event. The thermal profile of racemic *R*,*S*-omeprazole

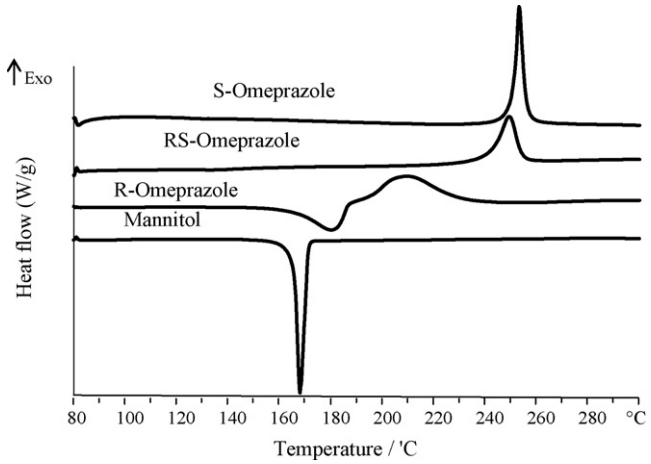

Fig. 1. DSC thermograms of *R*-, *S*-omeprazole and racemic *R*,*S*-omeprazole and mannitol (heating rate of 0.5 °C min^{-1}).

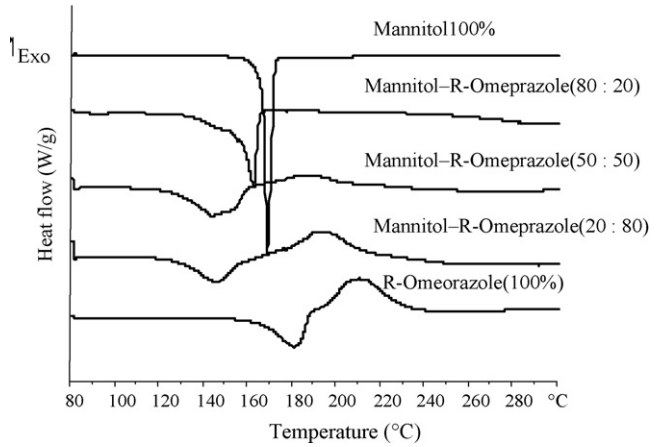

Fig. 2. DSC thermograms of different ratios of *R*-omeprazole–mannitol mixtures, pure *R*-omeprazole and mannitol (heating rate of 0.5 °C min^{-1}).

sodium and *S*-omeprazole sodium do not show endothermic event, suggesting a potential conversion to a metastable or amorphous form. Similar behaviour was recently reported in the literature [22]. It was found that *R*,*S*-omeprazole sodium demonstrates different thermal profile and crystallinity degree compared to omeprazole base, which should be assessed in the development of omeprazole dosage form. The DSC of racemic omeprazole sodium did not exhibit a single sharp endothermic peak, typical for fusion of crystalline substances, although the X-ray powder diffraction (XRPD) confirmed its crystallinity [21].

Furthermore, the onset of degradation temperatures ranged from 195 to 250 °C (absence of mannitol) is indicative of their thermal stability. However, degradation peaks at 229.5 and 227.0 °C for *R*- and *S*-omeprazole sodium salts were reproducible for both forms within experimental limits. These peaks are assigned to degradation of omeprazole and are an indication that these sodium salts are more thermally stable forms in comparison to reported literature values (156 °C) for the neutral form of omeprazole [23].

Overall, mixing an additive mannitol with either omeprazole sodium isomers or racemic omeprazole sodium leads to the decrease in the crystalline nature of the resulting mixtures. In the case of *S*- and *R*,*S*-omeprazole sodium the crystallinity is lost. Mixing of mannitol and *R*-omeprazole sodium leads to the decrease in melting temperatures and broadening the melting peaks that can be attributed to the interaction of two different crystalline structures (Fig. 2).

Localized thermal analysis was used to visualize the spatial distribution in compressed mixtures, display images of the sample's topography and to perform a differential surface thermal analysis on a micron scale within the sample. Specifically, the sample surface was firstly imaged (Fig. 3), and then a specific area was identified for further thermal analysis by localized thermal analysis.

Topographical images have revealed better compression behaviour of the *S*-omeprazole–mannitol mixture resulting in smoother surface compared to that of *R*-omeprazole–mannitol. Significant physical interaction of *R*-omeprazole with mannitol after applied pressure (drug disk) was evident in the LTA, confirming the DSC findings.

Surface roughness was calculated by μTA Lab software (TA Instruments) in terms of the percentage difference for the particle height distribution and is presented in Table 1. For the *S*-omeprazole–mannitol mixture, 13% of the surface height was up to 200 nm, 75% was from 200 to 300 nm and the remaining 12% was between 300 and 400 nm, while for *R*-omeprazole 6% was up to 200 nm, 59% from 200 to 300 nm, 24% from 300 to 400 and remaining 11% was higher than 400 nm. This compared to *S*-omeprazole alone gave a rough height distribution. Since the mannitol surface was mostly homogenous with 93% height from 200 to 300 nm, the *R*-omeprazole particles with the height above 400 nm may be attributed to the presence of different polymorphic form.

It is apparent from the surface topographical images that the "high" parts correspond to a significant extent with a particular type of large 'aggregate' morphology. A key property of a bulk particulate material is a typical image pattern or texture. Texture is related to the distribution of the spatial variation in grey scale levels (or colour levels in colour images) and can be connected to general bulk-particle characteristics. The surface topography may have a profound effect on the thermal conductivity signal resulting in peaks appearing as low thermal conductivity (dark) areas and troughs as high conductivity (light) areas [24]. It is not unreasonable to suggest that this morphology is related to one of the polymorphic forms of omeprazole and that this fact results in the thermal contrast observed. Great variations in the depth of the peaks probably arise from a varying contact areas and packing density of material under the probe but could be also due to varying ratios of amorphous and different crystalline material [25,26]. However, the origin of the differing surface thermal conductivity of the *R*- and *S*-omeprazole with mannitol is not clear, as they display very similar bulk thermal behaviour (DSC).

μTA (LTA) results for the *R*-omeprazole–mannitol mixture was unexpected (Fig. 4). The derivative of power signal was used to aid data analysis since it allows the identification of exothermic and

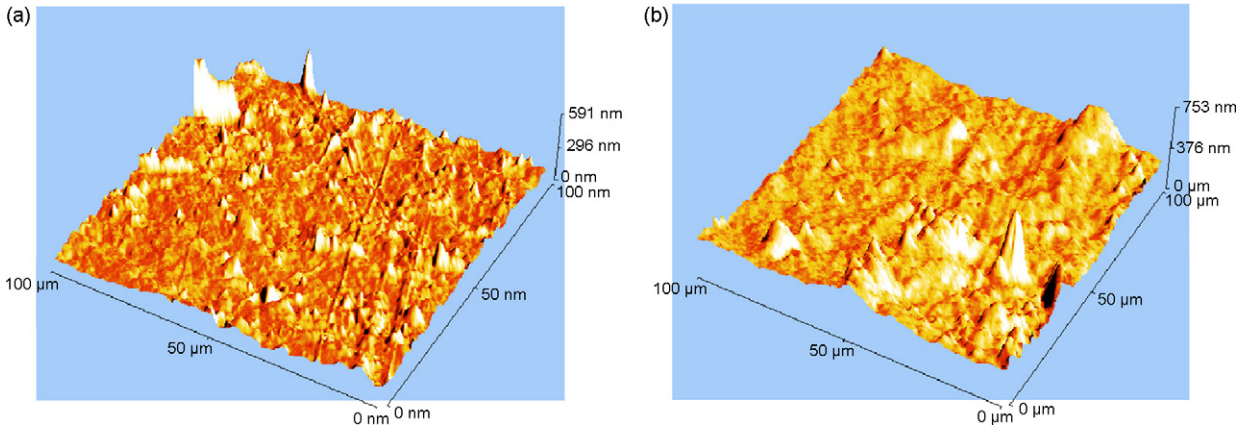

Fig. 3. Surface topographical images of (a) *S*-omeprazole–mannitol and (b) *R*-omeprazole–mannitol compressed disks.

Table 1
Height distribution as a percentage for compressed disks of *R*- and *S*-omeprazole:mannitol mixtures and mannitol

Height (nm)	*R*-Omeprazole:mannitol (%)	*S*-Omeprazole:mannitol (%)	Pure mannitol (%)
<200	6	13	7
200–300	59	75	93
300–400	24	12	0
>400	11	0	0

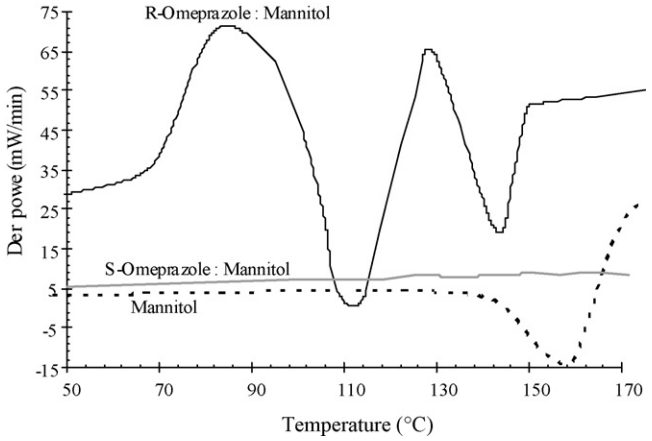

Fig. 4. μTA (LTA) overlaid runs of *S*- and *R*-omeprazole:mannitol mixtures and pure mannitol for changes of derivative of power.

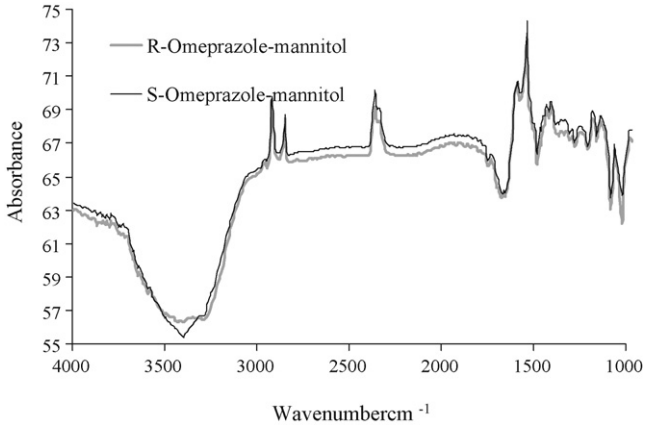

Fig. 5. ATR-IR spectra of *S*- and *R*-omeprazole with mannitol (70:30, w/w) from compressed disks.

endothermic events. Exothermic events are typically seen for crystallization or water loss from the sample and endothermic events are typically seen for the melting. We propose that the observed exothermic peak at 80 °C is due to a water loss from a hygroscopic polymorph (not seen by DSC) accompanied by a dominant endotherm from the subsequent re-crystallization process at 115 °C and melting at 145 °C. DSC was done with samples sealed in aluminium pans. DSC could be performed with open pan so that water loss can be determined.

Thus, localized thermal analysis on the omeprazole–mannitol mixtures has identified the darker and the lighter regions as different polymorphs. Generally, such behaviour was not observed with bulk thermal analysis (DSC), where a peak corresponding to a statistical average of the varying crystalline and amorphous content of the material was observed. However, it should be noted that the area melted by the thermal probe is in the order of several micrometers in all directions. The volume melted can be approximated through scanning probe analysis of the sample after localized thermal analysis, where pits corresponding to the melted points are seen. Hence, regions of a particular polymorph in a mix smaller than or similar to this size could not be conclusively identified.

For *S*-omeprazole the lack of any significant peaks was recorded confirms its amorphous nature. Though there are certain advantages to this technique, such as small sample sizes and fast results, limitations include exposure of drug–excipient mixtures to high temperatures (up to 300 °C or more), which, is not experienced by the dosage form in real situations. Moreover, the presence of a solid–solid interaction does not necessarily indicate an incompatibility. Therefore, the use of other analytical techniques, such as infrared spectroscopy, is advisable [27].

The ATR-IR spectra of *S*- and *R*-omeprazole–mannitol powder mixtures did not show significant differences. However ATR-IR spectra obtained from the disk samples showed characteristic changes in the infrared absorbance spectra in the N–H vibration region (Fig. 5). The *R*-omeprazole–mannitol mixture has two distinct peaks due to amino (N–H) and imino (=N–H) stretching frequencies, at 3425 and 3318 cm^{-1}, respectively, while the *S*-omeprazole–mannitol mixture has only peak due to amino N–H stretching vibration and a small shoulder peak for the imino stretching vibration. It was recently confirmed that crystalline racemic omeprazole exists as a solid solution of two tautomers in a continuous composition range and this raises questions pertaining to the definition of the term polymorph. Tautomeric compounds can present polymorphism but in addition they can present desmotropy, crystallization of a compound in two different tautomers. The term polymorphism is a very well-known descriptor for tautomeric heterocycles [28]. Therefore, *S*-omeprazole sodium consists of predominantly only one tautomeric isomer, while *R*-omeprazole contains both tautomeric forms. Tautomeric proton is highly effective in making strong hydrogen bonds. The interactions between the omeprazole and mannitol can also thus be explained in terms of different hydrogen bonding between the amine groups of the drug and the hydroxyl groups of mannitol. The tautomers of omeprazole, 5- and 6-methoxy derivatives (Fig. 6) have been detected in a solution [29]. On the other hand three polymorphic forms, A–C, were identified and patented for the solid forms of

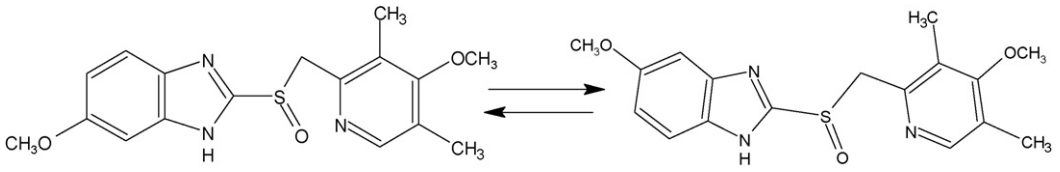

Fig. 6. Tautomers of omeprazole, 5- and 6-methoxy derivatives.

omeprazole using PXRD, single-crystal XRD and Raman analysis, but there is little correlation between them. Patented forms (A–C) are defined in terms of properties (stability, ease of preparation) rather than in terms of structure. US patent 6,780,880 claims that omeprazole crystals contain mixtures of omeprazole tautomers and states that single-crystal X-ray methods may be used to estimate the relative amounts of the two tautomers, without providing any further information [30]. However the literature is confusing. Bhatt and Desiraju have obtained single crystals of five different forms of omeprazole, with the 1:2 ratio varying from 0:100 to 15:85 [31]. There is adequate evidence that tautomer 2 is more stable than 1 and correspondingly that crystals containing greater proportions of 2 are more stable than those containing less. According to the literature, 2 is photostable while 1 is photosensitive. Crystals of 1 are white and do not change colour. As the proportion of 1 increase the crystals darken with increasing ease upon standing. It is also possible that, at the crystal level, 2 pack slightly better than 1. These interactions and changes in the crystal structure are probably directly related to the increase in the dissolution rate observed.

4. Conclusions

The omeprazole sodium isomers demonstrated different thermal profile and crystallinity degree, which should to be assessed in the development of omeprazole dosage forms.

LTA results also illustrate the presence of different polymorphic forms for the *R*-omeprazole and a smoother surface for the *S*-omeprazole–mannitol mixture. These results were consistent with the ATR-IR findings, where for the *R*-omeprazole–mannitol mixture two distinct peaks for the N–H and =N–H stretching frequencies were observed and only one (N–H) distinct peak in the case of the *S*-isomer.

It may thus be concluded that the findings from this paper explain the unpredictable bioavailability of *R*-omeprazole as compared to *S*-omeprazole.

References

[1] A.A. Riedel, C.S. Leopold, Drug Dev. Ind. Pharm. 31 (2005) 151–160.
[2] I.I. Hamdan, Pharmazie 56 (2001) 877–881.
[3] A. Pilbrant, C. Cederberg, Scand. J. Gastroenterol. Suppl. 108 (1985) 113–120.
[4] M. Mathew, V. Das Gupta, R.E. Bailey, Drug Dev. Ind. Pharm. 21 (1995) 965–971.
[5] A. Ekpe, T. Jacobsen, Drug Dev. Ind. Pharm. 25 (1999) 1057–1065.
[6] S. Agatonovic-Kustrin, D. Williams, N. Ibrahim, B.D. Glass, Curr. Drug Discov. Technol. 4 (2007) 192–197.
[7] M. Erickson, L. Josefsson, Pharmaceutical Formulation for Omeprazole, AstraZeneca. US Patent Number 6,090,827 (2000), Astra Zeneca AB.
[8] C. Scarpignato, I. Pelosini, Aliment. Pharmacol. Ther. 23 (2006) 23–34.
[9] T. Andersson, K. Rohss, E. Bredberg, M. Hassan-Alin, Aliment. Pharmacol. Ther. l5 (2001) 1563–1569.
[10] Highlights from the symposium of the Society for Medicines Research, held on December 6, 2001, in London. Case Histories in Drug Discovery and Design 2001, Drug News Perspect. 15 (January–February 2002).
[11] T. Lind, L. Rydberg, A. Kyleback, et al., Aliment. Pharmacol. Ther. 14 (2000) 861–867.
[12] A. Marini, V. Berbenni, M. Pegoretti, G. Bruni, P. Cofrancesco, C. Sinistri, M. Villa, J. Therm. Anal. Calorim. 73 (2003) 547–561.
[13] R. Fassihi, P.H.R. Persicaner, Int. J. Pharm. 37 (1987) 167–170.
[14] C. Ahlneck, G. Zografi, Int. J. Pharm. 62 (1990) 87–95.
[15] L. Yu, S.M. Reutzel, G.A. Stephenson, Pharm. Sci. Technol. Today 1 (1998) 118–127.
[16] D. Giron, J. Pharm. Biomed. Anal. 4 (1986) 755–770.
[17] S.A. Botha, A.P. Lotter, Drug Dev. Ind. Pharm. 16 (1990) 673–683.
[18] S.Y. Lin, R.Y. Han, Pharmazie 47 (1992) 266–268.
[19] F. Giordanoa, G.P. Bettinetti, J. Pharm. Biomed. Anal. 6 (1988) 951–955.
[20] K.R. Verma1, S. Garg, J. Pharm. Biomed. Anal. 35 (2004) 449–458.
[21] M. Tomassetti, A. Catalani, V. Rossi, S. Vecchio, J. Pharm. Biomed. Anal. 37 (2005) 949–955.
[22] F.S. Murakami, R.N. Pereira, A.P. Cruz, P.O. Rodrigues, M.A.S. Silva, Thermoanalysis and Crystallinity Study of Omeprazole Sodium. In: Latin-American Symposium on Polymorphism and Crystallization in Drugs, 2007, Fortaleza. Anais do Latin-American Symposium on Polymorphism and Crystallization in Drugs, 2007.
[23] S. Budavari (Ed.), The Merck Index, 12th ed., Merck Res. Lab., New Jersey, 1996.
[24] N. Markovic, S. Agotonovic-Kustrin, B. Glass, C.A. Prestidge, J. Pharm. Biomed. Anal. 42 (2006) 25–31.
[25] A. Bauer-Brandl, Int. J. Pharm. 140 (1996) 195–206.
[26] L. Bond, S. Allen, M.C. Davies, C.J. Roberts, A.P. Shivji, S.J.B. Tendler, P.M. Williams, J. Zhang, Int. J. Pharm. 243 (2002) 71–82.
[27] P. Mura, M.T. Faucci, A. Manderioli, S. Furlanetto, S. Pinzauti, Drug Dev. Ind. Pharm. 24 (1998) 747–756.
[28] J. Bernstein, in: G.R. Desiraju (Ed.), Organic Solid State Chemistry, Elsevier, Amsterdam, 1987 (Chapter 13).
[29] R.M. Claramunt, C. López, I. Alkorta, J. Elguero, R. Yang, S. Schulman, Magn. Reson. Chem. 42 (2004) 712–714.
[30] R.R. Whittle, F.D. Sancilio, G.W. Stowell, US Patent 6,780,880 B1 (2004).
[31] P.M. Bhatt, G.R. Desiraju, Chem. Commun. (Camb.) 28 (2007) 2057–2059.