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# Analysing the crystal purity of mebendazole raw material and its stability in a suspension formulation

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ABSTRACT

The objective of this study was to develop a simple, direct and non-destructive method to assess crystal purity of mebendazole raw material and to establish its stability in a suspension formulation using diffuse reflectance ultraviolet (DRA-UV) spectroscopy and attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy. Quantitation of mebendazole, found to exhibit polymorphism with three polymorphic forms A, B and C identified, was carried out with ATR-FTIR spectroscopy. Artificial neural network (ANN) was employed as a data-modelling tool. The developed ANN models confirmed that the characteristic absorptions in the infrared (IR) spectral region are directly proportional to the measured amounts of mebendazole crystal forms present in the samples ($r^2 > 0.94$), which was confirmed with X-ray diffraction (XRD) at $r^2 > 0.97$. These models also predicted that the mebendazole raw material contained $7.21 \pm 1.25\%$ (ATR-FTIR data) and $10.38 \pm 0.18\%$ (XRD data) of form A as an impurity. ATR-FTIR data for the suspension formulation showed some dissolution of form C and recrystalisation as the more stable form A. These quantitative results obtained for the binary crystal form mixtures clearly demonstrate the strong potential of ATR-FTIR for use in the determination of the polymorphic content not only in bulk pharmaceuticals but also in liquid formulations.



## 1. Introduction

Human helminthic infestations are common and have significant worldwide health implications. They can be responsible for delayed child growth and development, probably via a mechanism of iron-deficient anaemia. The preferred treatment is anthelmintic therapy such as pyrantel, albendazole or mebendazole, all of which are efficacious and cost effective. Some encapsulated helminthic infestations may however require surgical intervention followed by chemotherapy. Mebendazole (methyl-5-benzoyl benzimidazole-2-carbamate), a broad-spectrum anthelmintic drug of the benzimidazole class, effective against a number of nematodal and cestodal species (Liebenberg et al., 1998), is recommended for the treatment of non-surgical cases and as a supplementary treatment prior to and post-surgery. The World Health Organization (WHO) has identified mebendazole as an essential drug, based upon its clinical efficacy and low cost. However, it has been observed that *in vivo* results are far from being as effective as those demonstrated *in vitro* due to its low absorption at the gastrointestinal level. Mebendazole is practically insoluble in water and only 5–10% of the ingested drug is absorbed from the human gastrointestinal tract. Drug absorption is increased when taken with food, particularly fatty food. Studies of its polymorphism has led to the identification and characterization of three polymorphic forms (A–C) with different solubilities ($0.984 \pm 0.005$; $7.13 \pm 0.05$; $3.54 \pm 0.05\ \mathrm{mg\,mL^{-1}} \times 10^{-2}$) and thermodynamic stabilities (A > C > B) (Himmelreich et al., 1977). The observation that different therapeutic outcomes have been attributed to the different polymorphs supports the idea that low solubility and rate of dissolution are important factors to consider.

Using the above techniques developed by Himmelreich et al. (1977) Liebenberg et al. (1998) undertook a qualitative study to determine the polymorphic purity of four raw materials and generic tablets that are available in the South African market. Although three of their raw material samples were confirmed by contain the preferred polymorph C, dissolution studies on the fourth sample indicated that it was predominantly polymorph A. All tablet samples were confirmed to contain polymorph C, however the outcome was that generic manufacturers were to be aware of the existence of polymorphs other than C and to use more than one technique to determine polymorphic purity. At this stage there was very little evidence to suggest that small amounts of A (<10%) in C could be quantitated. Liebenberg and co-workers (Swanepoel et al., 2003) have continued their work by developing a dissolution

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test capable of discriminating between the three polymorphs using a reduced amount of sodium lauryl sulphate in the dissolution medium. In fact, the best result was achieved using 0.1 M HCl and no sodium lauryl sulphate. This group (De Villiers et al., 2005) in addition have investigated the use of variable-temperature X-ray powder diffraction to study the transformation of form C. Their results indicate polymorph C to be stable at room temperature to 179 °C with transformation to form A happening at between 205 and 220 °C. Since compression of powders into tablets involves an increase in temperature, this was also investigated. Results indicated that compression in fact did not effect a transformation from C to A. Froelich and Gasparotto (2005) using the dissolution method adapted by Swanepoel et al. and IR spectroscopy, further investigated the polymorphism of mebendazole. Their results indicated that all three polymorphic forms were detected in the dosage forms (tablets) and two in bulk raw material locally available. In a more recent study, Souza et al. (2006) compared two locally available generic products and found that they both met pharmacopeial standards.

Routinely, the most stable rather than soluble polymorph is selected for drug formulations thus avoiding potential change in form upon processing. In the case of mebendazole, the more soluble, but less stable form C is therapeutically favoured (Rodriguez-Caabeiro et al., 1987; Costa et al., 1991; Charoenlarp et al., 1993). Mebendazole is assayed by a potentiometric titration in the European Pharmacopeia (1997), and by spectrophotometric measurement in the UV region for the oral suspension in the USP 25 (US Pharmacopeia XXV, 2002). Most of these methods deal with the determination of the intact mebendazole molecule. The objectives of this work were thus to develop simple, direct and non-destructive procedures to identify and quantify crystal purity of mebendazole raw material and to establish the stability of mebendazole form C in a suspension formulation.

## 2. Experimental

Due to the opaque nature of samples, spectroscopy measurements were performed using diffuse reflectance techniques, DRA-UV and ATR-IR spectroscopy. Quantitation of polymorphic mixtures, carried out using ANN data modelling were confirmed with results obtained using an XRD method.

### 2.1. DRA-UV spectra

The reflectance UV–vis spectra were collected using a Cary 50 UV–vis spectrophotometer equipped with an external remote diffuse reflectance accessory (DRA) probe. UV–vis spectra of powder samples and wet paste samples were acquired in the region 250–450 nm using appropriate baseline correction (zero/baseline correction). The spectra were acquired at two different locations on each sample to assess surface homogeneity.

### 2.2. ATR-FTIR spectra

IR spectra were examined over the range of 650–4000 $cm^{-1}$ using a Varian 'Scimitar' MIR FTIR spectrometer equipped with a Peltier-cooled DTGS (deuterated triglycine sulphate) detector and a horizontal ATR accessory with a zinc selenide (ZnSe) crystal as the reflection element. All adsorption samples were analysed as wet pastes. Spectra were recorded with the Digilab FTIR resolution Version 4.0 software, by averaging 64 scans for each spectrum with resolutions of 4 $cm^{-1}$ (data point resolution/interval = 1 $cm^{-1}$). Background spectra were obtained for each experimental condition.

### 2.3. XRD spectra

Data for the samples were collected with a Siemens D5000 front-loading X-ray diffractometer fitted with a Copper tube (Cu Kα = 1.54178 Å), operating at 40 kV and 30 mA, and a post-diffraction graphite monochromator.

### 2.4. Methods and materials

Mebendazole (R 17635); reference polymorphic forms A, B, and C (lots V890-167, V890-223, and ZR017635PUA721, respectively) were supplied by Janssen Pharmaceutica Biotech. N.V. The mebendazole raw material was kindly donated by Aspen-Pharmacare Ltd., Port Elizabeth, South Africa.

### 2.5. Sample preparation

#### 2.5.1. Bulk drug assay

For calibration purposes, 14 binary mixtures containing different proportions of form C and A were prepared. The weight fractions of form C in the mixtures with form A were 0, 2, 5, 20, 30, 40, 50, 60, 70, 80, 90, 95, 98 and 100%. All the sample mixtures were mixed geometrically and then stored in a desiccator at room temperature (25 ± 2 °C) and protected from light. The bulk drug was gently ground using a glass mortar and pestle to reduce variation in particle size, as this can have a significant influence on the diffuse reflectance measurements (Fuller and Griffiths, 1978).

#### 2.5.2. Mebendazole suspension

2% mebendazole suspension in a 10% sucrose solution was prepared and stabilized with cellulose esters, sodium lauryl sulphate as a surfactant and preserved with parabens. Simethicon emulsion as wetting and antiflatulent agent and citric acid and Tutti–Fruitti as flavouring agents were added to the formulation. The suspension was stored in a closed container at 25 ± 2 °C (constant temperature), protected from direct light.

### 2.6. Pre-processing the data

Spectral intensities at all wavenumbers from the ATR-FTIR spectra and at all the degrees of the diffractogram had a weak correlation with weight fraction of forms A and C in the mixtures. Reduction and transformation of the input data was necessary to enhance the ANN performance and to reduce the number of outliers and variance among values.

For the powder XRD analysis samples were prepared as smear mounts. Approximately 0.5 g of each sample was mixed with water and smeared onto a round glass slide (15-mm diameter). This slide was then inserted into a plastic cavity mount suitable for insertion into the X-ray diffractometer. The sample was scanned from 1.3° (2$\theta$) to 65° (2$\theta$) and 3195 steps (2$\theta$) were recorded. These steps were further processed to reduce the data being fed to ANN and to smooth noise in the diffractograms. The 3195 steps were reduced into 319 averaged intensities from 10 consecutive steps.

The ATR-FTIR 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 original spectra were scanned between 650 and 4000 wavenumbers ($cm^{-1}$) and reduced to 1730 spectral intensities during the data recording. These spectral data were further processed to smooth the noise in the spectra. The 1730 absorbances were reduced into 173 averaged spectral values, each of ten consecutive wavenumbers. The spectral intensities were used as the inputs,

together as the weight fraction of forms A and C as outputs to train the ANN.

For the suspension stability analysis the wet paste was uniformly applied directly onto the zinc selenide (ZnSe) crystal and ATR-FTIR spectra collected. For each spectrum, 32 scans were collected at a resolution of 4 $cm^{-1}$. The time from sampling of the suspension to completion of the paste spectrum was 5 min. Preparation and collection of data was undertaken with the samples protected from light due to their inherent photo-instability.

### 2.7. Data modelling

The commercial software used to model the spectral data was Statistica Neural Networks 7.0 (StatSoft Inc., Tulsa, OK, USA).

#### 2.7.1. Designing neural networks and selecting inputs

ANN, a powerful non-linear modelling technique is a type of artificial intelligence that simulates the neurological functions of the brain and imitates the way that human brain works and learns from data. This inherent learning capability and good generalisation behaviour makes them useful in complex system pattern identification and classification (Bishop, 1995). It is composed of a large number of highly interconnected artificial neurons organized in layers. Generalized regression neural networks (GRNNs) have four layers: input, a layer of radial centres, a layer of regression neurons, and output (Speckt, 1991). The radial layer neurons represent the centres of clusters of known training data. This layer must be trained by a clustering algorithm such as K-means, subsampling, or Kohonen training. The radial layer is typically large but not necessarily as large as the number of training cases. The regression layer must have exactly one unit more than the output layer and contains linear neurons of two types. There is one type A neuron for each output unit and one type B neuron. Type A neurons calculate the "desired" regression outputs for the cases; the type B neuron calculates the probability density. The output layer executes a special postsynaptic division. Each unit simply divides the output of the type A unit by the output of the type B unit in the previous layer. The primary advantage of GRNN is the speed at which the network can be trained. There are no training parameters such as learning rate and momentum in back propagation network, but there is a smoothing factor that is applied after the network is trained. The smoothing factor allows the GRNN to interpolate between data in the training set.

The most straightforward approach was used to build the ANN model. Spectral data sets were divided into three data sets: training, testing, and validating. Intelligent problem solver was used for the initial training to select the best ANN type. In contrast to traditional linear techniques in statistics, there is no method known that will automatically locate the optimal neural network to fit a particular data set. The best network, or perhaps a few of the best neural networks is traditionally selected by running training algorithms a number of times. Therefore, a number of experiments with different designs are conducted, and a generalized regression neural network (GRNN) with a 173:8:3:2 structure was selected to train ATR-FTIR data and GRNN with 319:9:3:2 structure was selected to train XRD data, as the networks with the best topology.

## 3. Results and discussion

### 3.1. DRA-UV spectra

It was reported that mebendazole solution should have has three absorption bands at $\lambda_{max}$ values of 210, 250 and 315 nm (Karim et al., 1996). According to Clark, mebendazole base shows absorption maxima at 270 and 355 nm (Moffat, 1986), while the acid form absorbs at 234 and 288 nm. Fig. 1 showing diffuse reflectance UV spectra of the mebendazole bulk drug and polymorphs A and C as powders and in the paste indicates obvious spectral differences between forms A and C. Spectral differences between form C and bulk drug are however minimal.

The spectrum of powder form A shows two absorption bands at $\lambda_{max}$ of 299 with a shoulder at 365 and 250 nm. On the other hand, spectra from the powder form C and bulk drug do not have distinct peaks but absorb significantly in the UV range from 200 to 380 nm.

The UV diffuse reflectance spectra of the paste samples of the bulk drug and form A were quite different. The structural distinction between the two polymorphic forms was achieved by comparing the UV absorbance data of form C with that of form A. The UV absorbance spectra of the form C and bulk drug exhibit a maximum absorbance at ~320 nm and shoulder peak at ~280 nm. The UV absorbance spectra of form A, on the other hand, showed two distinct peaks, one broad peak at ~345 and another peak at ~280 nm. This indicates that the principal chromophore responsible for the absorption peak at higher wavelength is changed. Furthermore, form A has a significant absorption at 355, while form C and bulk drug did not absorb in this spectral region.

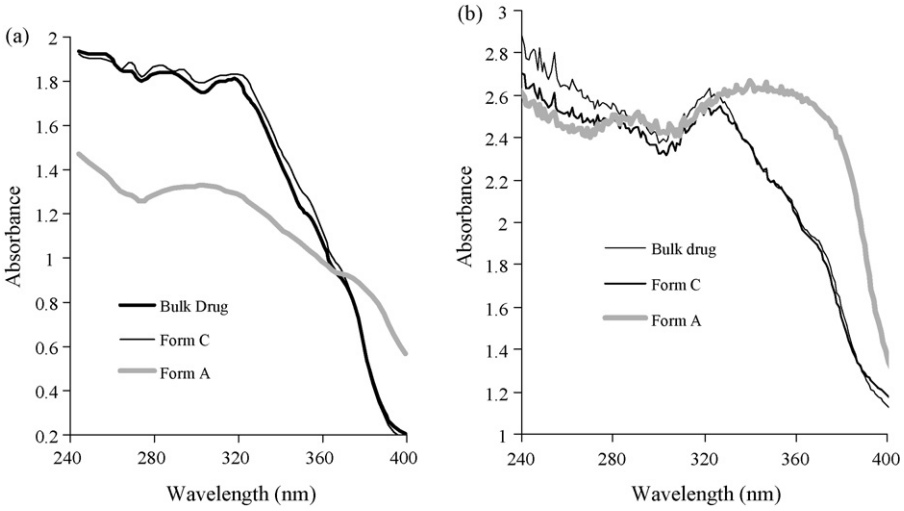


**Fig. 1.** Diffuse reflectance UV–vis spectra of the mebendazole bulk drug and polymorphic forms A and C as powders (a) and in a paste (b).

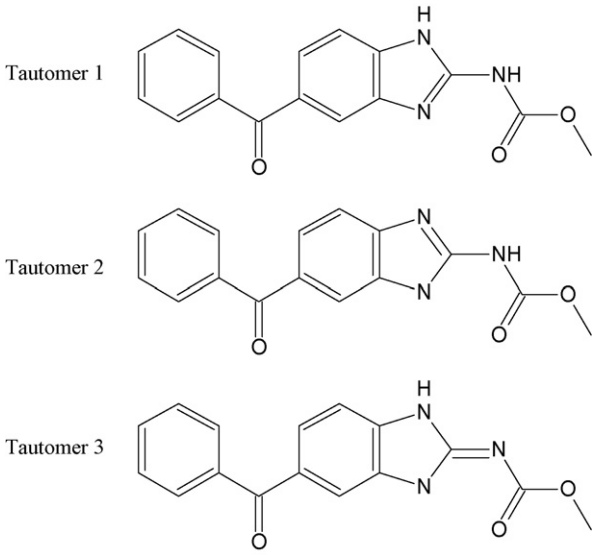


**Fig. 2.** Mebendazole tautomers.

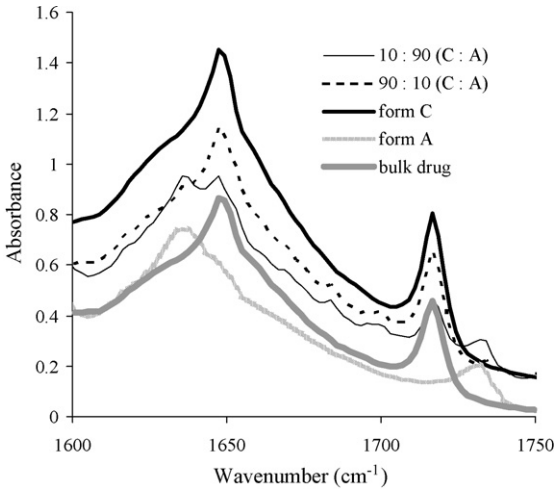


**Fig. 3.** The ATR-FTIR absorption curves of the mebendazole raw material, selected crystal mixtures and pure polymorphic forms C and A.

The two types of polymorphs are packing and conformational polymorphs. Conformational polymorphism is due to molecular ability to change conformation or exist in different tautomeric forms. Tautomers are inherently different molecules and as such cannot be considered different phases of the same molecules. Mebendazole can exist in three tautomeric forms (Fig. 2) leading to a conclusion that imino-enamine tautomerism is responsible for mebendazole polymorphism. Conjugated enamines (C=C–C=N) show an absorption at ~220 nm. Tautomers 1 and 2 have a highly conjugated enamine group and these will absorb at ~220 nm. The shift of the double bond between tautomers is probably responsible for the difference in the peak position between form A and C and therefore the polymorphism. Tautomer 3 does not have conjugated enamine and probably will not absorb in this spectral region. The carbonyl (ketone) group will be responsible for the peak at 340 nm.

As shown in this paper, even at the qualitative level, reflectance spectra can be used to distinguish between different polymorphic forms. Spectra in Fig. 1 suggest that there is little difference in the electronic band structures of the two mebendazole polymorphs. The extensive delocalization of the electrons in the tautomers is reflected in the shift of the longer wavelengths. Thus, the tautomers 1 and 2, are expected to absorb at a lower wave number (higher energy) than the tautomer 3.

### 3.2. ATR-FTIR spectra

The ATR-FTIR absorption curves of the mebendazole raw material, selected crystal mixtures and pure form C and A (Fig. 3), show characteristic >NH– and C=O stretching frequencies at 3404 and 1718 $cm^{-1}$, respectively, that can be used to distinguish between crystal form. In addition to these peaks, a characteristic strong absorption at 1650 $cm^{-1}$ from amide can also be used. These IR frequencies are similar to the measured values for the polymorph C, thereby providing evidence for the presence of polymorph C in the bulk drug.

The ATR-FTIR spectrum of the mebendazole bulk material, shows the –NH, >C=O and amide stretching frequencies at 3402, 1716 and 1647 $cm^{-1}$, respectively. These IR frequencies are identical to the observed values for the pure polymorph C, therby providing further evidence for the presence of polymorph C form in the raw material.

Each crystalline form produced a characteristic IR spectrum containing regions with differences evident in the detailed shape and intensities of the major absorption bands (Fig. 2). The application of IR spectroscopy revealed spectral features in the carbonyl stretching region, from the carbamate component of the drug (carbonyl and amide stretching vibrations) and despite the fact that the raw spectrum of the wet paste was dominated by the strong contributions from water, peaks at 3370 and 3402 $cm^{-1}$ due to the NH stretching vibration can be used to distinguish between polymorphs (Table 1).

However, using single peaks to qualify and quantify crystal purity was not sufficient, and full spectral pattern classification using the ANN is necessary. The ANN was trained with spectral data obtained from different mixtures of the two polymorphs, C and A. Polymorph B was excluded from this study, because although the most soluble, it is also the least stable of all three polymorphs and polymorphic conversion is most likely to occur from C to A. By using mixtures with different mass ratios of polymorphs C and A, a database of spectra was constructed. A close look at the recorded spectra containing a mixture of two polymorphs revealed that the addition of a small amount of form A to form C did not influence the characteristic >NH-stretching frequency of form C at 3402 $cm^{-1}$. Only impurities of 10% and above of form A resulted in two clearly distinguishable characteristic peaks for form A and form C at 3370 and 3402 $cm^{-1}$ for the NH stretching, at 1647 and 1635 $cm^{-1}$ for amide and 1716 and 1730 $cm^{-1}$ for carbonyl stretching vibration, respectively. Full spectral data of mixtures were used to train (80%) and test (20%) the ANN model. Spectral data from the mebendazole raw material (five samples) were used to validate the trained models and estimate crystal purity of bulk drug.

**Table 1**
Regions of difference in ATR-FTIR spectra obtained for mebendazole polymorphs A and C, bulk drug and selected crystal mixtures

| | Principal absorption peaks (cm−1) | | | | | |
|---|---|---|---|---|---|---|
| | –CO–NH– | | >C=O | | >NH | |
| Form C | 1647 | – | 1716 | | | 3402 |
| Row material | 1647 | – | 1716 | | | 3402 |
| 90:10 | 1647 | – | 1716 | | | 3402 |
| 50:50 | 1647 | 1635 | 1716 | 1730 | 3370 | 3402 |
| 10:90 | 1647 | 1635 | 1716 | 1730 | 3370 | 3402 |
| Form A | – | 1635 | – | 1730 | 3370 | |

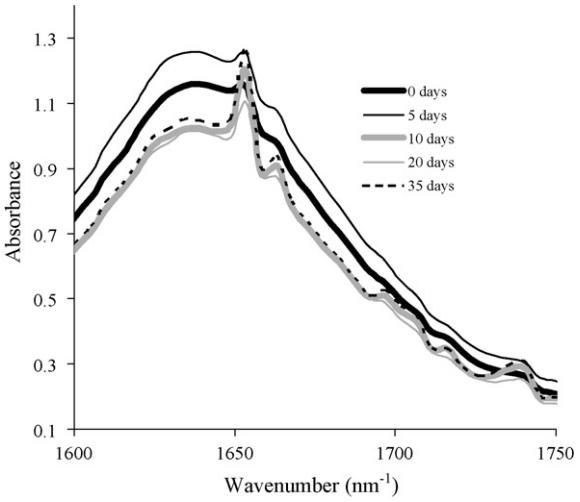


**Fig. 4.** Stability of mebendazole in suspension; peak position as a function of time.

The developed ANN model confirmed that the characteristic absorptions in the IR spectral region and XRD are directly proportional to the measured amounts of different mebendazole crystal forms presented in the samples. Good linearity ($r^2 = 0.95$) of the ATR-FTIR method indicates that this technique can be used to measure mebendazole crystal purity accurately and specifically in the presence of other impurities. The results were confirmed using XRD analysis ($r^2 = 0.97$). The developed ANN models predicted that the mebendazole raw material contained $7.21 \pm 1.25\%$ (ATR-FTIR data) and $10.38 \pm 0.18\%$ (XRD data) of polymorph A present as an impurity in the supplied raw material, which is claimed to contain polymorph C.

### 3.3. Suspension

Suspension formulation generally causes manufacturing, storage and stability problems due to sedimentation, caking, the re-dispersibility of a sediment and crystal growth (Pearson and Varney, 1969; Zatz, 1985; Idkaidek and Najib, 2000). Aggregation of particles, settling rate and the density of sediment will depend upon the choice of the excipients, their concentration, as well as concentration and particle size distribution of active ingredients (Deicke and Süverkrüp, 2000). Mebendazole suspension contains the metastable form C. Since a metastable form is pharmaceutically desired, some precautions have to be taken as conversion is most likely to occur between form C and A. The reduced solubility and dissolution has therapeutic consequences, with the effect of altered bioavailability. Often the metastable polymorphic form will dissolve, and then be followed by crystallization of more stable, less soluble form. Furthermore, in order to obtain sufficient bioavailability, micronization of the mebendazole is generally required in the formulation process of a suspension or paste. Thus, we also wanted to assess the crystallinity of mebendazole in suspension. Mebendazole plane type structure can result in high crystallinity, demonstrating low solubility.

A mebendazole suspension is a supersaturated solution of metastable form C. Due to its supersaturation, the small amount of metastable form C will dissolve while the small amount of stable form A will crystallise after 5 days. The presence of two crystal forms will contribute to the difference in IR peaks over time using in situ ATR-FTIR spectroscopy. Imine and carbamate NH bending modes for form C and form A occurred at 1653 and 1639 $cm^{-1}$ (Fig. 4). Characteristic peak at 1639 $cm^{-1}$ previously seen in the powder forms could not be used to distinguish between polymorphs due to peak interference with the excipients used in the formulation. The peak at 1663 $cm^{-1}$ is characteristic for the C=N stretching vibration. Furthermore, the band centred at 1653 $cm^{-1}$ presents two components associated to NH bending and C=O stretching mode.

The peak at 1653 $cm^{-1}$, characteristic for the form C increased in intensity after 5 days, as well as the peak at 1663 $cm^{-1}$, characteristic for the form A, and peak at 1740 $cm^{-1}$, due to aryl carbonyl stretching vibrations (1735–1750 $cm^{-1}$). For conjugated systems with an aromatic ring attached to the imine nitrogen atom, the C=N absorption is usually observed at lower frequencies (1630 $cm^{-1}$) in comparison with other systems, where this absorption was observed in the 1674–1650 $cm^{-1}$ frequency range (Bellamy, 1975). After 10 days the crystals of both forms started to grow which is evidenced by an increase of the peak intensities at 1653 $cm^{-1}$ and peak at 1740 $cm^{-1}$ characteristic for the forms C and stable form A, respectively. This remained stable after 14 days, with the ratio of these two peaks remaining constant. The total crystallinity of mebendazole in the suspension did not change substantially over the investigated time.

To use any spectroscopic technique for reliably determining crystallinity, the spectra of the crystalline and amorphous materials must be appreciably different in some way. The ATR-FTIR spectra of mebendazole suspension in time demonstrated marked differences. The crystalline spectrum shows stronger and more well-defined absorbances across the entire wavelength range. Also, there are peaks at 1653 and 1663 $cm^{-1}$ that are better defined over time due to the change in crystallization.

The IR spectrum is sensitive to the level of order/disorder in the crystal structure of the sample. The index Ic can be calculated as a ratio of intensities of absorption bands at 1372 $cm^{-1}$ for the deformational oscillations of CH groups (disorder) and covalent vibrations of $CH_2$ and CH groups at ~2900 $cm^{-1}$ (order) (Nelson and O'Connor, 1964). This ratio was constant at approximately Ic = 1.7 during the investigated time frame. Therefore, we can conclude that there is no polymorphic transition in the suspension, however there is a significant hydrogen bonding. In addition upon storage, the shoulder peak at 1740 $cm^{-1}$ appears due to C=O stretching.

Stability of the metastable crystal form in suspension was investigated using the ATR-FTIR method. The good stability of the metastable form in suspension allowed for the observation of the simultaneous growth of both polymorphs with different growth rates after 10 days. The ratio of two forms was maintained constant during the observed time.

## 4. Conclusion

ATR-IR spectroscopy coupled to ANN data-processing and modelling proved to be a useful and practical approach to identify and also to quantify mebendazole polymorphic forms in supplied raw material, as the results achieved correlated with the XRD findings, thus confirming the value of this technique. The presented results have shown that ATR-FTIR spectroscopy can be used to resolve the individual spectroscopic features of mebendazole polymorphs. Using single peaks to qualify and quantify crystal purity was not sufficient and full spectral pattern classification using ANN was necessary. The developed ANN model confirmed that the characteristic absorptions in the IR spectral region are directly proportional to the ratio of different mebendazole crystal forms presented in the samples. In addition, the methodology was useful to confirm the polymorphic stability of mebendazole (polymorphic form C) in a suspension. Overall, it is clear from the obtained results that quantitative assessment of the solid-state polymorphic composition of mebendazole drug substances in complex formulations by ATR-FTIR spectroscopy is possible.

This study thus presents a distinct advantage over the previous work conducted only on solid-state formulations, in that the quantitation of polymorph A in the raw material at <10% is possible as well as the detection of the presence of polymorph A in a suspension formulation.

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