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Developing a discriminating dissolution test for three mebendazole polymorphs based on solubility differences

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Mebendazole, a broad spectrum anthelmintic drug, is practically insoluble in water and exists in three polymorphic forms, A, B, and C, of which C is pharmaceutically favoured. Since the dissolution of drugs from solid oral dosage forms can depend on the crystal form of the drug an attempt should be made while developing dissolution tests to set test parameters that are sensitive to changes in the crystal form. USP 24 describes 0.1 M hydrochloric acid containing 1.0% sodium lauryl sulphate (SLS) as the dissolution medium for mebendazole tablets. Results showed that the high concentration of sodium lauryl sulphate in the USP dissolution medium does not allow the use of this test to distinguish between the solubility differences of the three mebendazole polymorphs. By decreasing the amount of sodium lauryl sulphate in the dissolution medium clear differences in the dissolution rates of the three forms were observed. The most discriminating medium was 0.1 M HCl, containing no sodium lauryl sulphate.

1. Introduction

Mebendazole is a broad spectrum anthelmintic drug producing high cure rates in infestations by *Ascaris*, threadworms, hookworms and whipworms [1]. Mebendazole is practically insoluble in water and a study of its polymorphism has led to the identification and characterisation of three polymorphic forms (A, B, C) displaying significantly different solubility and therapeutic differences [2, 3]. The polymorphs differ with respect to their IR spectra, x-ray powder diffractograms (XRPD), and differential scanning calorimetry (DSC) thermograms [2, 4]. The solubility of the three polymorphs in 0.03 M hydrochloric acid is in the order B > C > A [4]. Solubility studies and clinical trials have shown that polymorph C is pharmaceutically favoured [5, 6].

Dissolution of drugs from solid oral dosage forms is a necessary criterion for drug bioavailability. Therefore, the dissolution test for solid oral drug products has emerged as the single most important control test for assuring batch-to-batch bioequivalence once its bioavailability has been defined [7]. In developing dissolution tests, attempts are made to design test parameters that are sensitive to manufacturing/process/formulation changes. The determination of dissolution profiles of water-insoluble drug products, like mebendazole, requires dissolution media different from those normally used for water-soluble drug products [8]. Selecting a dissolution medium of acceptable volume and composition as well as having a good discriminating power is difficult for these drugs [9]. Approaches used in the design of dissolution media for poorly soluble drugs include: (a) bringing about drug solubility by increasing the volume of the aqueous sink or removing the dissolved drug [10, 12]; (b) solubilization of the drug by co-solvents, up to 40% [13, 14] and by anionic [8] or non-ionic [8, 15] surfactants added to the dissolution medium in postmicellar concentrations; (c) alteration of pH to enhance the solubility of ionizable drug molecules [8]. The last two approaches seem less cumbersome and have been more widely employed in pharmacopeial dissolution tests. USP 24 [16] prescribes 0.1 M hydrochloric acid containing 1% sodium lauryl sulphate as the dissolution medium for mebendazole tablets.

Surfactants like sodium lauryl sulphate enhance the dissolution rate of poorly water-soluble drug products due to wetting, micellar solubilization, and/or deflocculation [8]. However, the sodium lauryl sulphate present in the dissolution medium may reduce the discriminative power between the three polymorphic forms of mebendazole, in the USP dissolution test. The aim of this study was to investigate the dissolution properties of mebendazole polymorphs. Changes in the USP dissolution medium, involving varying concentrations of sodium lauryl sulphate, were also investigated to determine the medium that would be the most discriminative between the solubility of polymorphs A, B and C. This is important, since products that contain all three polymorphic forms of mebendazole are found on the market in developing countries [17]. For example, in South Africa there is a number of generic mebendazole products from different manufacturers available and these products are widely used because mebendazole forms an integral part of the essential drug list in South Africa.

Table 1: Main absorbencies in the Fourier transform IR spectra of the mebendazole polymorphs

Crystal form	–NH (cm^{-1})	–C=O (cm^{-1})
Form A	3370	1730
Form B	3340	1700
Form C	3410	1720

2. Investigations, results and discussion

2.1. Characterization of mebendazole polymorphs

It is known that mebendazole can exist in three crystal polymorphic forms with different solubilities. The three polymorphic forms of mebendazole, form A, B and C, were identified amongst raw materials available to generic manufacturers or prepared as described by Himmelreich et al. [2] and Costa et al. [4].

In Fig. 1 the DSC thermograms, Fig. 2 the XRPD patterns and in Table 1 the main FTIR signals of the three forms are given. The thermogram of form A was characterised by two melting endotherms at 264 and 331 °C. Form B displayed three melting endotherms at 243, 254 and 327 °C. The second endotherm at 243 °C was an endo-exothermic recrystallization with the exotherm following at 254 °C. Form C has a small endotherm at 195 °C followed by two endotherms corresponding to that of form A. This suggests that form C might be a non-crystalline form that is converted to the more crystalline form at 255–260 °C. XRPD patterns were similar to those reported by Costa et al. [4]. Characteristic IR-signals, Table 1, were also an easy way to identify the three forms [17].

The solubilities of the polymorphs in 0.1 M HCl were in the order B (0.07 mg/ml) > C (0.04 mg/ml) > A (0.02 mg/ml). However, the solubility in water after 24 h at 30 °C was extremely low, less than 0.2×10^{-4} mg/ml for form C. In some samples no discernible concentration of mebendazole could be detected by UV-analysis.

2.2. Powder properties and particle morphology

The importance of geometrical form (i.e., size and shape) of the particles in the dissolution rate of fine particulate sparingly soluble drugs should be emphasized. It was observed that the dissolution rates of sparingly soluble drugs, like mebendazole, are related to particle shape and size [18]. Form A and B were free-flowing powders while form C was strongly aggregated. The mean volume particle sizes of the powders were: A = 3.56 ± 1.54 μm; B = 6.18 ± 1.72 μm; C = 3.35 ± 1.02 μm. For particles of the same size, the dissolution rate decreased as the level of flakiness and irregularity increased. This phenomenon can be explained by an increase in the average hydrodynamic boundary layer thickness as the particles become more irregular [18]. The differences in aggregation behaviour of the three forms were inspected closer with a SEM. In Fig. 3 these photomicrographs are shown. From these results it was clear that form A, was the least aggregated. Form B contained a large number of agglomerates with a mean size around 20–50 μm. Form C was also agglomerated but these were not as big or tightly packed.

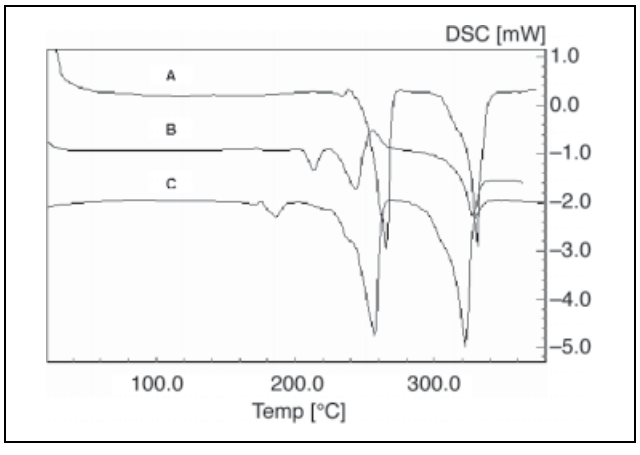


Fig. 1: DSC thermograms of the three mebendazole polymorphs

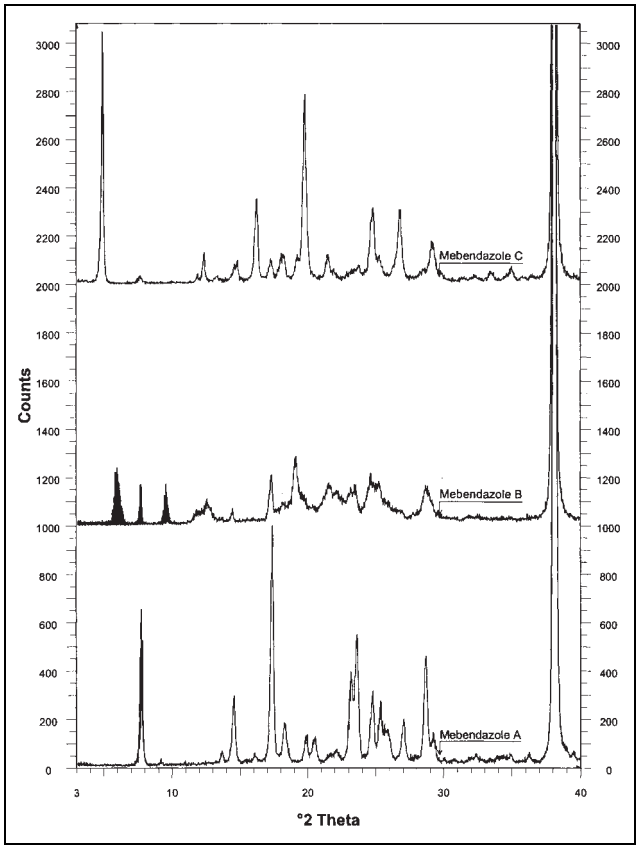
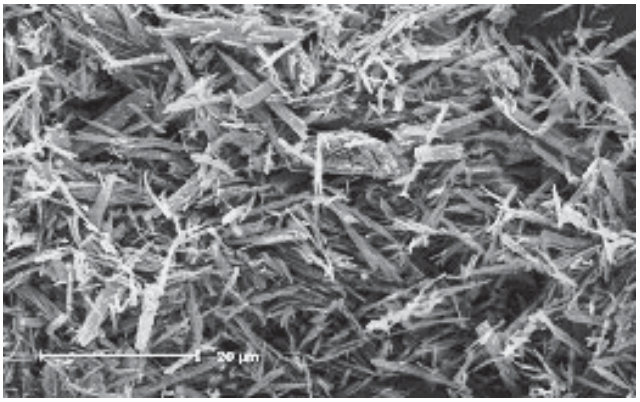


Fig. 2: XRPD patterns of the three mebendazole polymorphs

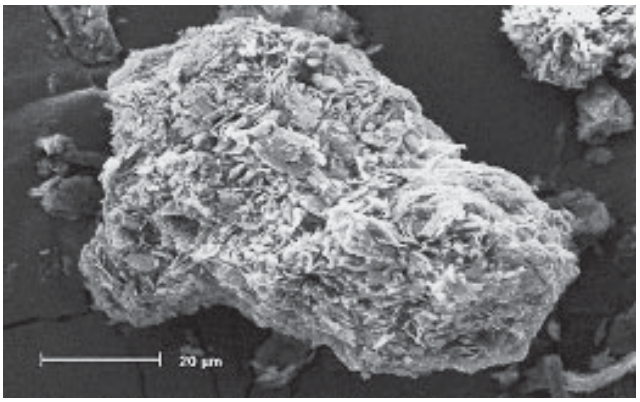
2.3. Powder dissolution behavior

The dissolution rates of the three forms, as dispersed powders with particle sizes below 10 μm, were measured according to the method of the USP. The dissolution medium was 0.1 M HCl containing 1% sodium lauryl sulphate and the dissolution profiles obtained therein are shown in Figs. 4–6. These figures also show the dissolution in 0.1 M HCl without surfactant and the effect of surfactant concentration on dissolution when the concentration of sodium lauryl sulphate was varied from 0.1–1%.

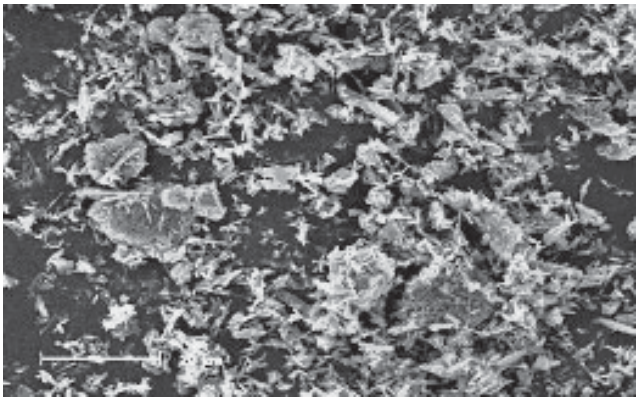
According to the USP not less than 75% (Q) of the drug must be dissolved within 120 min (Fig. 7). In the USP medium all three polymorphs dissolved more than 75% within 120 min. Form C = 102% > Form A = 95% > Form B = 94%. In 0.1 M HCl the dissolution rates were significantly lower but this medium distinguished between the differences in the solubility of the three forms. Form C = 72% > Form B = 45% > Form A = 20%. By increas-



A



B



C

Fig. 3: SEM photomicrographs of (A) form A, (B) form B and (C) form C

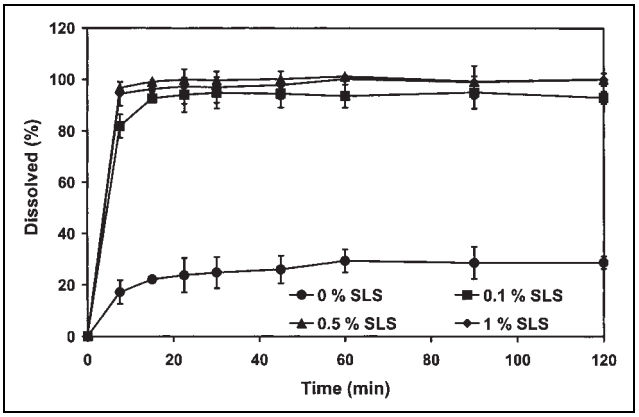


Fig. 4: Powder dissolution profiles of form A

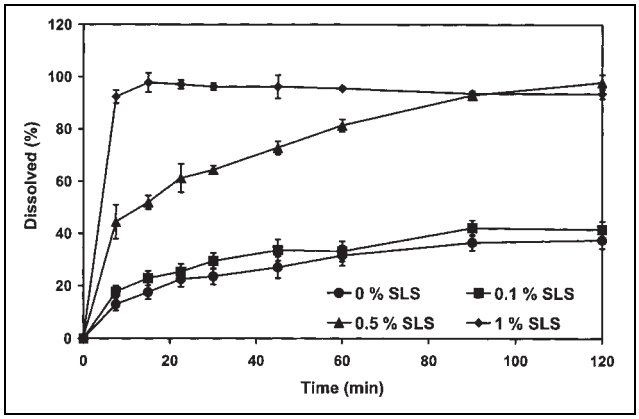


Fig. 5: Powder dissolution profiles of form B

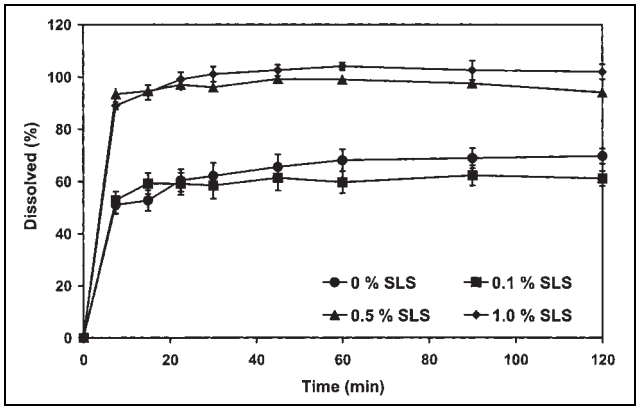


Fig. 6: Powder dissolution profiles of form C

ing the concentration of sodium lauryl sulphate in the dissolution medium the discriminating power of the medium was diminished.

These results suggest that the solubility of the mebendazole polymorphs depended on how the powdered drug is wetted by the medium in which it is dissolving. As shown in Fig. 3, the mebendazole polymorphs spontaneously aggregate to form large poorly wettable aggregates.

2.4. Effect of pH and surfactant concentration on solubility

The effect of pH and SLS concentration on the solubility of three polymorphs of mebendazole was studied individually (Table 2). The results indicate that both SLS concentration and pH significantly influence the solubility of form A and C. At 1% SLS concentration, the solubility of

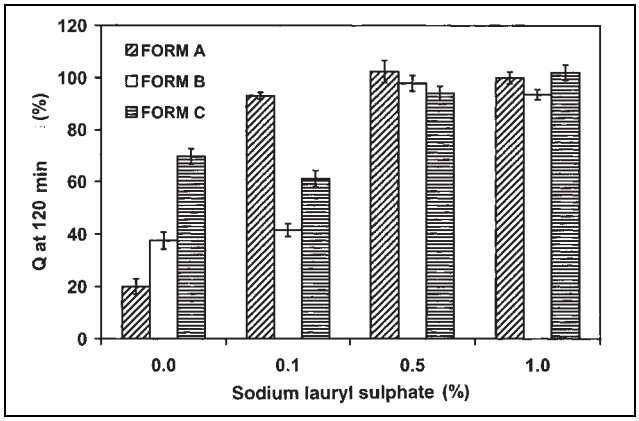


Fig. 7: Effect of sodium lauryl sulphate concentration on Q at 120 min

Table 2: Solubility of mebendazole polymorphs at increasing pH with and without the addition of 1% sodium lauryl sulfate (SLS)

pH	Form A		Form B		Form C	
	0%	1%	0%	1%	0%	1%
1.2	0.011 ± 0.0010	0.101 ± 0.0080	0.077 ± 0.0050	0.107 ± 0.0080	0.039 ± 0.0040	0.096 ± 0.0020
3.6	0.008 ± 0.0009	0.070 ± 0.0060	0.032 ± 0.0030	0.074 ± 0.0030	0.025 ± 0.0030	0.073 ± 0.0030
4.6	0.005 ± 0.0006	0.058 ± 0.0030	0.015 ± 0.0010	0.061 ± 0.0020	0.010 ± 0.0010	0.058 ± 0.0030
6.0	0.003 ± 0.0004	0.017 ± 0.0008	0.004 ± 0.0003	0.011 ± 0.0001	0.003 ± 0.0001	0.011 ± 0.0005
7.4	0.001 ± 0.0003	0.007 ± 0.0001	0.003 ± 0.0002	0.008 ± 0.0003	0.002 ± 0.0001	0.008 ± 0.0030

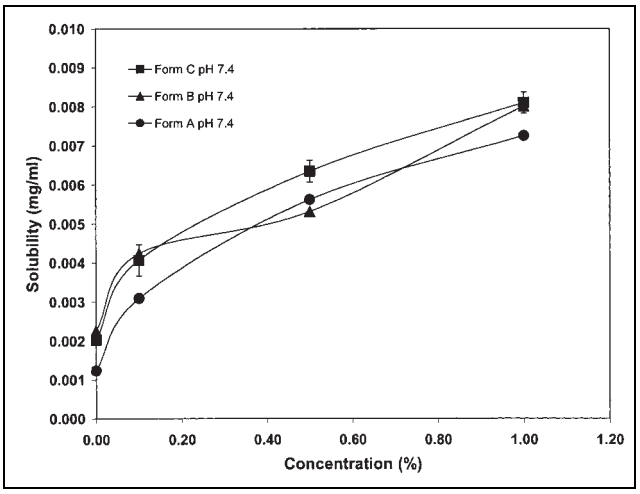


Fig. 8: Change in solubility of mebendazole polymorphs at pH 7.4 with an increase in SLS concentration

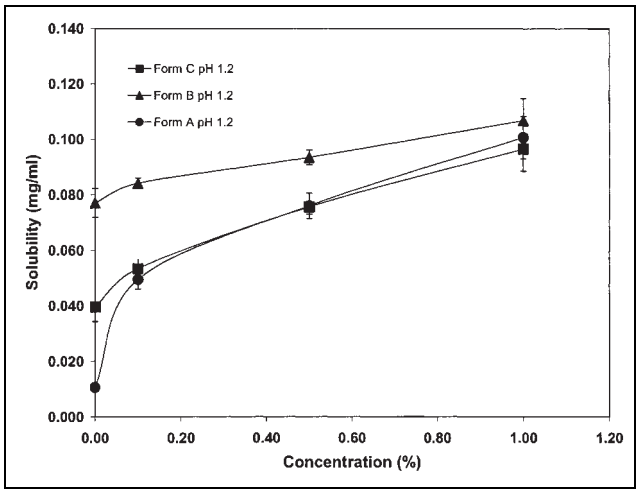


Fig. 9: Change in solubility of mebendazole polymorphs at pH 1.2 with an increase in SLS concentration

all the polymorphs is more or less the same at any given pH (Fig. 8, 9). Significant differences in solubility began to appear when the amount of SLS in the dissolution was reduced. In the absence of SLS and at pH 1.2, pH 3.6, and pH 4.6 all the polymorphs showed significant differences in the solubility with maximum differences at pH 1.2 (Fig. 9). This shows that in the USP dissolution medium, the surface properties of the powders are sufficiently changed by sodium lauryl sulfate so that aggregates are dispersed, improving the dissolution rate and eliminating any differences in dissolution due to changes in the crystal form.

2.5. Conclusion

Drug solubility studies and clinical trials have shown that form C of mebendazole is preferred. However, the high concentration of sodium lauryl sulphate in the USP dissolution medium does not allow the use of this test to determine if Form C is used or not. By decreasing the amount of sodium lauryl sulphate in the dissolution medium clear differences in the dissolution rates of the three forms were observed. The most discriminating medium was 0.1 M HCl, containing no sodium lauryl sulphate.

Manufacturers and regulatory agencies should take note of this effect. When buying or sourcing raw material, tablets or suspensions, dissolution results obtained using the USP conditions would not ensure that the products contain polymorph C. Consideration should therefore be given to changing the dissolution medium to improve its discriminating power. Simply eliminating sodium lauryl sulfate from the USP dissolution medium would give manufacturers and regulatory agencies a simple method to ensure that mebendazole tablets and suspensions contain the right crystal polymorph.

3. Experimental

3.1. Materials

Three mebendazole polymorphs were identified among raw material samples obtained from a number of manufacturers or prepared by recrystallization [11, 12]. The purity of the powders was between 99–101%.

3.2. Identification and characterization of crystal forms

IR spectra were recorded on a Nicolet Nexus 470-FT-IR spectrometer (Nicolet Instrument Corporation, Madison WI 53711) over a range of 600–4000 cm^{-1}. The diffuse reflectance method was used. Powders of the samples were mixed with KBr prior to measurement. Results are shown in Fig. 2 and main differences in IR-peaks are listed in Table 1. The aggregation properties of the three polymorphic powders were studied with a scanning electron microscope (Fig. 3). A Philips XL 30 scanning electron microscope (Philips, Netherlands) was used to obtain photomicrographs of the different mebendazole crystal forms. Samples were adhered to a small piece of carbon tape mounted on a metal stub and coated under a vacuum with carbon (Emscope TB500 sputter-coater) before being coated with a thin gold-palladium film (Eiko Engineering ion Coater IB-2). The thermal properties of the crystal forms were measured by differential scanning calorimetry. DSC thermograms were recorded with a Shimadzu DSC-50 instrument (Shimadzu, Kyoto, Japan). The measurement conditions were sample weight, approximately 2 mg; sample holder, aluminium crimp cell; gas flow, nitrogen at 20 ml/min.; heating rate, 10 °C/min. All ambient X-ray powder diffraction patterns (XRPD) of the crystal forms were obtained at room temperature (unless otherwise indicated) using a Bruker D8 Advance diffractometer (Bruker, Germany). The measurement conditions were: target, Cu; voltage, 40 kV; current, 30 mA; divergence slit, 2 mm; anti-scatter slit, 0.6 mm; receiving slit, 0.2 mm; monochromator; detector slit, 0.1 mm; scanning speed, 2°/min (step size 0.025°, step time, 1.0 s). Approximately 300 mg samples were weighed into aluminium sample holders, taking care not to introduce a preferential orientation of crystals.

3.3. Powder dissolution studies

Powder dissolution studies of forms A, B and C were performed using Method 2, paddle, of the USP 24 [16]. The paddles were rotated as 75 rpm and samples were withdrawn from the dissolution medium at 7.5, 15, 22.5, 30, 45, 60, 90 and 120 min. The powder sample, 50 mg, was rinsed from the glass weighing boat into a 10 ml test tube with exactly 2 ml of dissolution medium. Glass beads, 50 mg, with a mean size of 0.1 mm,

were added to the suspension and the mixture was agitated for 120 s using a vortex mixer. The contents of the test tube were then transferred into the dissolution medium. The dissolution media used were 0.1 M hydrochloric acid and 0.1 M hydrochloric acid containing sodium lauryl sulphate in concentrations of 0.1%, 0.5% and 1%, which is the medium prescribed by the USP 24. The concentration of dissolved powder was calculated from the UV absorbancy values obtained at 254 nm. Results are the mean of three determinations.

3.4. Solubility determination

To determine the solubility in water, 12 ml vials containing distilled, deionised water with increasing concentrations of sodium lauryl sulfate, and excess amounts of each crystal form were rotated (100 rpm) in a water bath kept at 30 ± 0.1 °C. After 48 h, equilibrium was reached and aliquots of the solution were withdrawn from the vials and filtered through a 0.25 µm filter. The solutions were suitably diluted with methanol and assayed with a spectrophotometer at 254 nm. Results are the mean of five determinations.

3.5. Statistical evaluation of data

An experiment was set up to compare the effect of different pHs and SLS additives on the increase in solubility for 3 mebendazole polymorphs. Experiments were conducted at pH value levels of 1.2, 3.6, 4.6, 6.0, and 7.4. Four levels of SLS (0.00%, 0.10% 0.50%, and 1.00%) were also applied. All factor-level combinations of these two variables were used in the experiment. At the end of 48 h, five solubility readings were taken at each factor-level combination.

Statistical model: $Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$

Where i = 1, 2, 3, 4, and 5; j = 1, 2, 3, and 4; k = 1, 2, 3, 4, and 5.
Since pH and % SLS interaction is significant (P is 0.0001) for all the 3 polymorphs, we could not test for the main effects. So the effect of pH on the solubility was analyzed at a fixed level of SLS and vice versa.

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