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Heat-induced Formulation Inhomogeneity of a Three-component Suspension

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ABSTRACT

A suspension formulation containing sarafloxacin HCl, triamcinolone acetonide, and clotrimazole was developed for the treatment of otitis externa in dogs. The potency for the three active ingredients in this suspension was monitored at 25°C and 40°C for up to 3 months. The potencies of triamcinolone and clotrimazole were found unchanged, but the potency of sarafloxacin HCl in the samples stored at 40°C for 1 month varied significantly between samples. However, assay inconsistency for sarafloxacin HCl was not seen in samples stored at 25°C. Under an optical microscope, large crystals were found in the 40°C stability samples but not in the 25°C samples. The large crystals in 40°C samples were identified as sarafloxacin by high-performance liquid chromatography (HPLC). This finding suggests that crystal growth of sarafloxacin took place at 40°C during storage, leading to the formation of larger crystals and the consequent sampling nonuniformity and assay inconsistency. The solid-state properties of these crystals were further evaluated using hot-stage microscopy and Fourier transform infrared (FTIR) analysis. The results indicate that the crystal growth of sarafloxacin was most likely attributed to a change in the hydration form of sarafloxacin.

Key Words: Formulation inhomogeneity; Three-component suspension; Otitis externa; High-performance liquid chromatography (HPLC).

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INTRODUCTION

Otic formulations are used in the treatment of one of the most commonly occurring diseases in dogs, otitis externa. Otitis externa is the infection or inflammation of the external and middle ears that can be caused by fungi or bacteria. An otic suspension containing three active ingredients, 1 mg/mL sarafloxacin HCl (antibacterial), 1 mg/mL triamcinolone acetonide (anti-inflammatory), and 10 mg/mL clotrimazole (antifungal), was developed.[1,2] The suspension contains 0.35% (w/w) of sodium carboxymethylcellulose (CMC) as a thickening and stabilizing agent.[3–5] Sodium carboxymethylcellulose has also been commonly used in suspensions for its inhibitory effect on drug crystallization.[6–8] The stability of otic suspension was monitored at 5°C, 25°C, and 40°C. The potency of all three drugs in the samples stored at 5°C and 25°C was found unchanged after 3 months of storage. Triamcinolone acetonide and clotrimazole were stable in 40°C samples, but sarafloxacin HCl was found to be subpotent when an aliquot of the sample was analyzed. However, when the entire container of sample was analyzed, a 100% recovery of sarafloxacin HCl was obtained. The objective of this study was to investigate the cause for the inconsistency in sarafloxacin potency for 40°C stability samples.

MATERIALS AND METHODS

Materials

Micronized sarafloxacin HCl trihydrate (Abbott Laboratories, North Chicago, IL), triamcinolone acetonide (Sicor, Milano, Italy), and clotrimazole (Fabbrica Italiana Sintetici, Vicenza, Italy), with mean particle sizes between 3 and 7 μm and 90% of particles less than 11 μm were used. Carboxymethylcellulose (Sigma Chemical Co., St. Louis, MO) was used as received. Tween 20, ethylenediaminetetraacetic acid (EDTA) disodium, monobasic and dibasic potassium phosphate, and NaCl (Sigma Chemical Co., St. Louis, MO) were all reagent grades.

Preparation of Suspension

The otic suspension formulation consists of sarafloxacin HCl (1 mg/mL), triamcinolone acetonide (1 mg/mL), and clotrimazole (10 mg/mL) in an aqueous medium containing phosphate buffer (0.02 M, pH 7.0), Tween 20 (0.02% w/v), NaCl (1% w/v), EDTA (0.1% w/v), and carboxymethylcellulose (0.35% w/v). A predetermined amount of excipients (EDTA, NaCl, Tween 20) was dissolved in the buffer solution, and carboxymethylcellulose (CMC) was added and stirred until it was completely dissolved. Triamcinolone acetonide, sarafloxacin HCl, and clotrimazole were subsequently dispersed uniformly into the aqueous medium using a Silverson® L4RT high-shear mixer (East Longmeadow, MA) at 7000 rpm. Thirty milliliters of the formulation were filled and sealed in low-density polyethylene bottles and stored in temperature- and humidity-controlled chambers (5°C, 25°C with 60% relative humidity, and 40°C with 75% relative humidity).

Sample Preparation for Potency Assay

An aliquot sampling method was initially used for the 5°C, 25°C, and 40°C stability samples. However, because of the subpotent and inconsistent results for sarafloxacin HCl in 40°C samples, a whole-bottle sampling method was used for the 40°C stability samples.

When using the aliquot sampling method, a predetermined amount of the suspension sample (1.2 to 2.0 g) was taken from each bottle (containing 30 mL of the formulation) after vigorously shaking the suspension sample for 30 seconds. The suspension sample was subsequently transferred into a 50-mL volumetric flask. Five milliliters of N,N-dimethylformamide (DMF) and 35 mL of mobile phase A [see high-performance liquid chromatography (HPLC) method] were added and the mixture sonicated until the suspension was completely dissolved. The final sample solution was injected onto the HPLC. Duplicate samples were prepared for assay.

For the whole-bottle sampling method, the entire contents of each bottle were transferred into a 1-L volumetric flask followed by a few rinses using mobile phase A. The bottle was finally rinsed with a mixture of 40 mL mobile phase A and 100 mL DMF. All the rinsates were transferred and combined in the flask. Six hundred mL of mobile phase A was added and the mixture sonicated to dissolve the suspension. After complete dissolution of all solid ingredients, mobile phase A was added to the volume and the solution was injected onto the HPLC.

HPLC Method for Potency Assay

The potency of the active ingredients in the sample solution was determined using a Hewlett Packard Series 1100 HPLC system (Kennett Square, PA) equipped with a Waters symmetry C18 column (5 μm, 25 cm × 4.6 mm I.D.). The injection volume was 25 μL and the mobile phase flow rate was set at 1.0 mL/min. The column was kept at 25°C. Ultraviolet (UV)

Table 1. HPLC gradient scheme for potency assay.

Time (min.)	Mobile phase A (%)	Mobile phase B (%)
0	100	0
8.0	100	0
10.0	70	30
17.0	70	30
19.0	0	100
24.0	0	100
26.0	100	0
35.0	100	0

detection was performed at 260 nm. An HPLC run was carried out according to the gradient scheme described in Table 1. The phosphate buffer used for mobile phase preparation was prepared by mixing water (1800 mL) with 5 mL phosphoric acid (85%) and diluting to 2 L with water. The pH was adjusted to 2.5 with 2 N sodium hydroxide. Mobile phase A was prepared by mixing 500 mL acetonitrile with 2000 mL pH 2.5 phosphate buffer. Mobile phase B was prepared by mixing acetonitrile (1200 mL), tetrahydrofuran (600 mL), and water (100 mL). Both mobile phases were filtered through a 0.45-μm membrane filter.

Identification of the Crystals

Stability samples were examined under an optical microscope (Leitz Ortholux, Germany) for the presence of large crystals. The 40°C suspension samples with large crystals present were filtered through a 0.45-μm silver membrane. The crystals were isolated and collected for further analyses. High-performance liquid chromatography, hot-stage microscopy, and Fourier transform infrared (FTIR) were used for crystal identification.

The large crystals in the 40°C samples were collected, washed with purified water, and air-dried prior to use for further analysis. The crystals were dissolved in 2 mL of methanol and analyzed by the previously described HPLC method for peak identification. The chromatograms of the crystal were compared to those of the reference standards.

For the hot-stage microscopic analysis, a single crystal collected from the 40°C sample was heated at a constant rate of 5°C/minute on a hot stage (Mettler Instrument Corporation, Hightstown, NJ) under an optical microscope (Leitz Ortholux, Germany). The melting point of the crystal was determined and compared to that of the bulk sarafloxacin HCl trihydrate.

The FTIR spectra of KBr pellets of bulk sarafloxacin HCl trihydrate, clotrimazole, and triamcinolone acetonide and large crystals collected from the 40°C samples were generated using a Nicolet Magna IR 550 spectrometer (Madison, WI) equipped with a KBr beam splitter and an MCT/A detector. All FTIR spectra were acquired at a resolution of 8 cm^{-1} with 164 scans.

RESULTS AND DISCUSSION

Drug Potency Assay

The drug potency of the suspension was monitored for 3 months at 5°C, 25°C, and 40°C (Table 2). Triamcinolone acetonide and clotrimazole were found to be stable within this time period. When using the aliquot sampling method, the sarafloxacin HCl potency results for samples stored at 40°C for 1 month were

Table 2. Stability data of the otic suspension at 25°C and 40°C.

Sampling time (month)	Sampling method	Storage conditions	Sarafloxacin (%)	Triamcinolone (%)	Clotrimazole (%)
0	Aliquot sampling	N/A	100.1±1.8[a]	101.0±1.1[a]	98.0±0.4[a]
1	Aliquot sampling	5°C	104.2±3.7[a]	101.1±0.2[a]	98.2±1.0[a]
		25°C, 60% RH	103.4±3.7[a]	101.2±0.2[a]	97.7±0.6[a]
		40°C, 75% RH	72.7±20.9[a]	99.9±0.7[a]	95.6±1.6[a]
3	Aliquot sampling	5°C	100.0[b]	100.2[b]	96.5[b]
		25°C, 60% RH	100.4[b]	100.0[b]	97.1[b]
		40°C, 75% RH	54.8[b]	98.9[b]	95.0[b]
3	Whole bottle	40°C, 75% RH	95.9[b]	97.3[b]	96.1[b]

[a]The reported value is an average of duplicate samples from the same bottle.
[b]Single measurement.

found to be inconsistent (51.8% and 93.6%). For samples stored at 40°C for 3 months, the potency of sarafloxacin HCl determined using the aliquot sampling method was 54.8%, and 95.9% when determined using the whole-bottle sampling method. Inconsistent assay results for sarafloxacin HCl were not found in the 5°C and 25°C samples.

Microscopic Examination

The samples of the suspension stored at 25°C and 40°C for 1, 2, and 3 months were examined under an optical microscope and compared to a freshly made sample. A photomicrograph of the suspension at initial time point (Fig. 1) showed a mean particle size of approximately 2 to 5 μm, which was comparable to the mean particle sizes of the micronized bulk drugs determined by a dynamic laser light-scattering particle size analysis. The mean particle sizes of the micronized sarafloxacin HCl, triamcinolone, and clotrimazole were 2.1, 3.7, and 4.1 μm, respectively. The drug particles in the 25°C samples remained unchanged after 3 months of storage (Fig. 2). However, large rectangular crystals were detected in the 40°C samples (Figs. 3 and 4). These crystals were found to increase in size as a function of time. The length of the crystals in the 40°C sample was approximately 30 and 400 μm after 1- and 3-month storage, respectively.

Identification of the Crystals

In an attempt to identify the large crystals in the 40°C samples, the crystals were collected, dissolved in methanol, and analyzed using HPLC. Figure 5 shows the HPLC chromatogram of the methanolic solution of the collected large crystals and the chromatogram of a solution containing three bulk drugs. The retention times of sarafloxacin HCl, clotrimazole, and triamcinolone acetonide were 8.5, 15.0, and 16.5 minutes, respectively. The retention time of the large crystals was 8.5 minutes, which was identical to that of the sarafloxacin HCl bulk drug. The small peaks at 15.0 and 16.5 minutes shown in the chromatogram of the large crystals are probably attributed to the presence of trace amounts of clotrimazole and triamcinolone deposited on the surface of the sarafloxacin crystals.

The hot-stage microscope was used to determine the melting points of the large crystals and the bulk

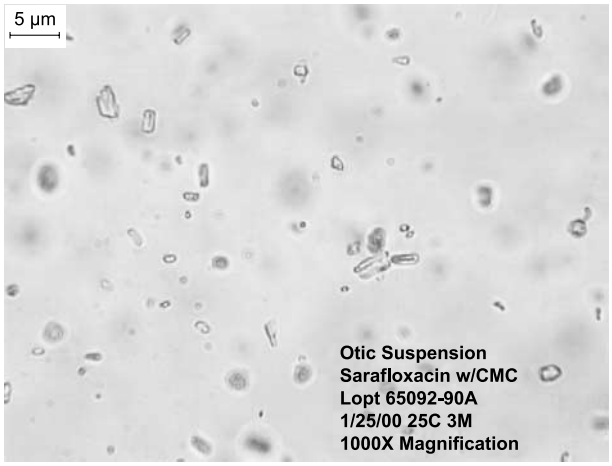

Figure 2. Photomicrograph of suspension sample stored at 25°C for 3 months.

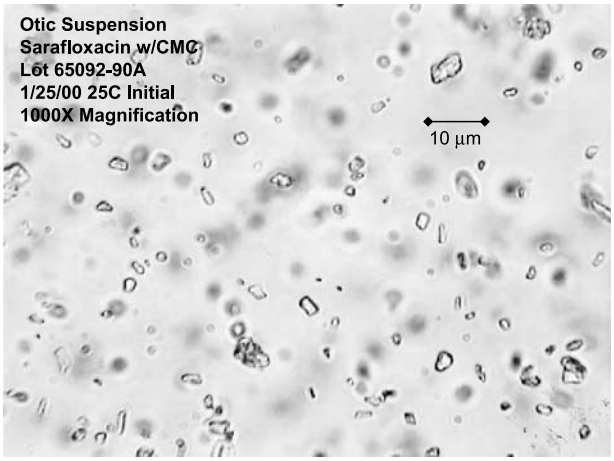

Figure 1. Photomicrograph of suspension sample at time 0.

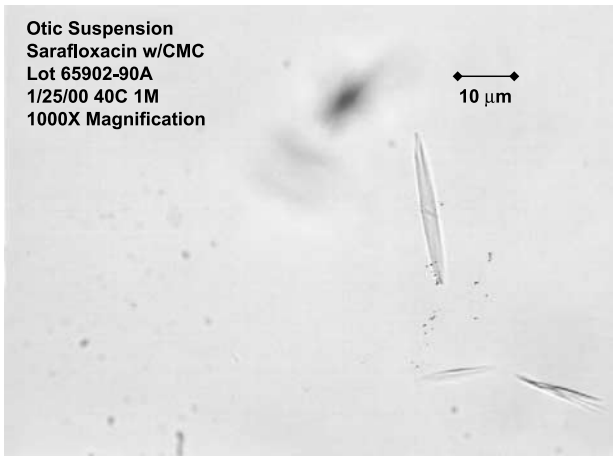

Figure 3. Photomicrograph of suspension sample stored at 40°C for 1 month.

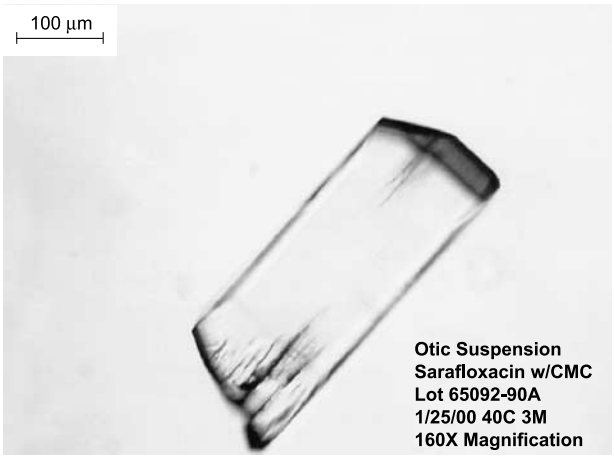

Figure 4. Photomicrograph of suspension sample stored at 40°C for 3 months.

sarafloxacin HCl trihydrate. The melting of the bulk sarafloxacin HCl trihydrate was observed at 259°C. The melting of the large crystals from the 40°C samples was observed at temperature ranging from 262 to 278°C. The FTIR spectra of the three bulk drugs and the large crystals are shown in Fig. 6. It is clear that the spectrum of the large crystals is not identical to that of the bulk drugs. Both hot-stage microscopic and FTIR results indicate that the form of the large crystals found in the 40°C samples differed from that of the bulk sarafloxacin HCl trihydrate.

Crystal growth occurring in a suspension can be attributed to several mechanisms, including Ostwald ripening, temperature fluctuation, or transformation of the drug from a metastable to a more stable crystal form.[9,10] Dziki et al. reported that sarafloxacin can form multiple hydrates.[11] The transformation of sarafloxacin HCl trihydrate to another hydrate form is likely the mechanism for crystal growth observed with the 40°C samples. The identification of this new crystal form and its subsequent use in formulating the suspension appear to be the best approach in solving the content uniformity and assay consistency problem of sarafloxacin in the suspension.

CONCLUSIONS

The potency of sarafloxacin in an otic suspension stored at 40°C was found to be inconsistent. This problem was found to be caused by the formation of large crystals, which were identified to be sarafloxacin. Hot-stage microscopy and FTIR results of the large sarafloxacin crystals found in 40°C samples were different from those of the bulk drug. It is hypothesized

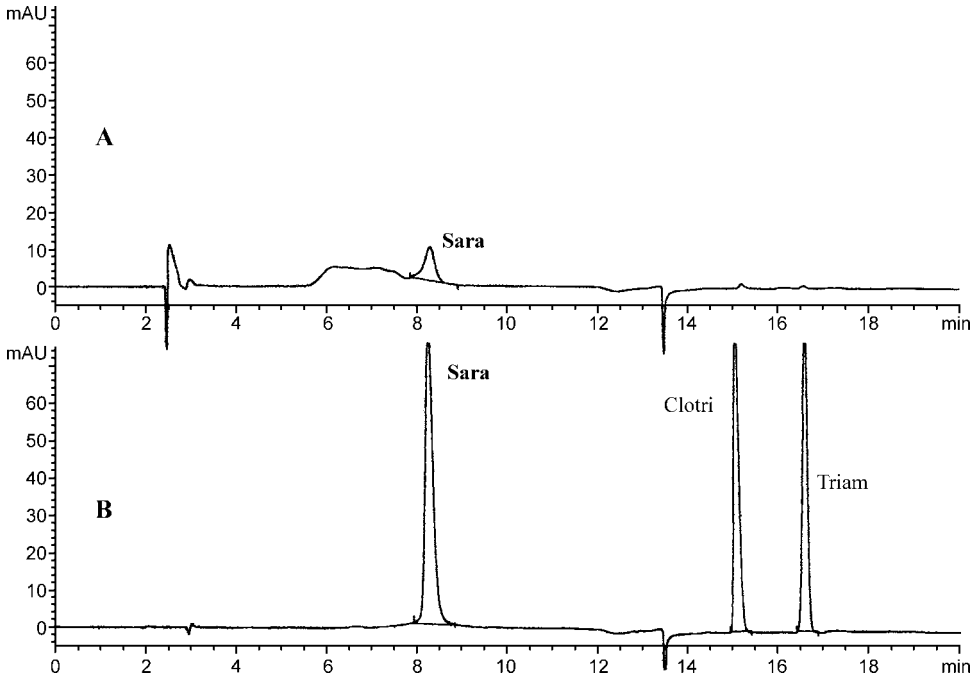

Figure 5. HPLC chromatograms of (A) large crystals collected from 40°C sample and (B) standard solution containing three bulk drugs.

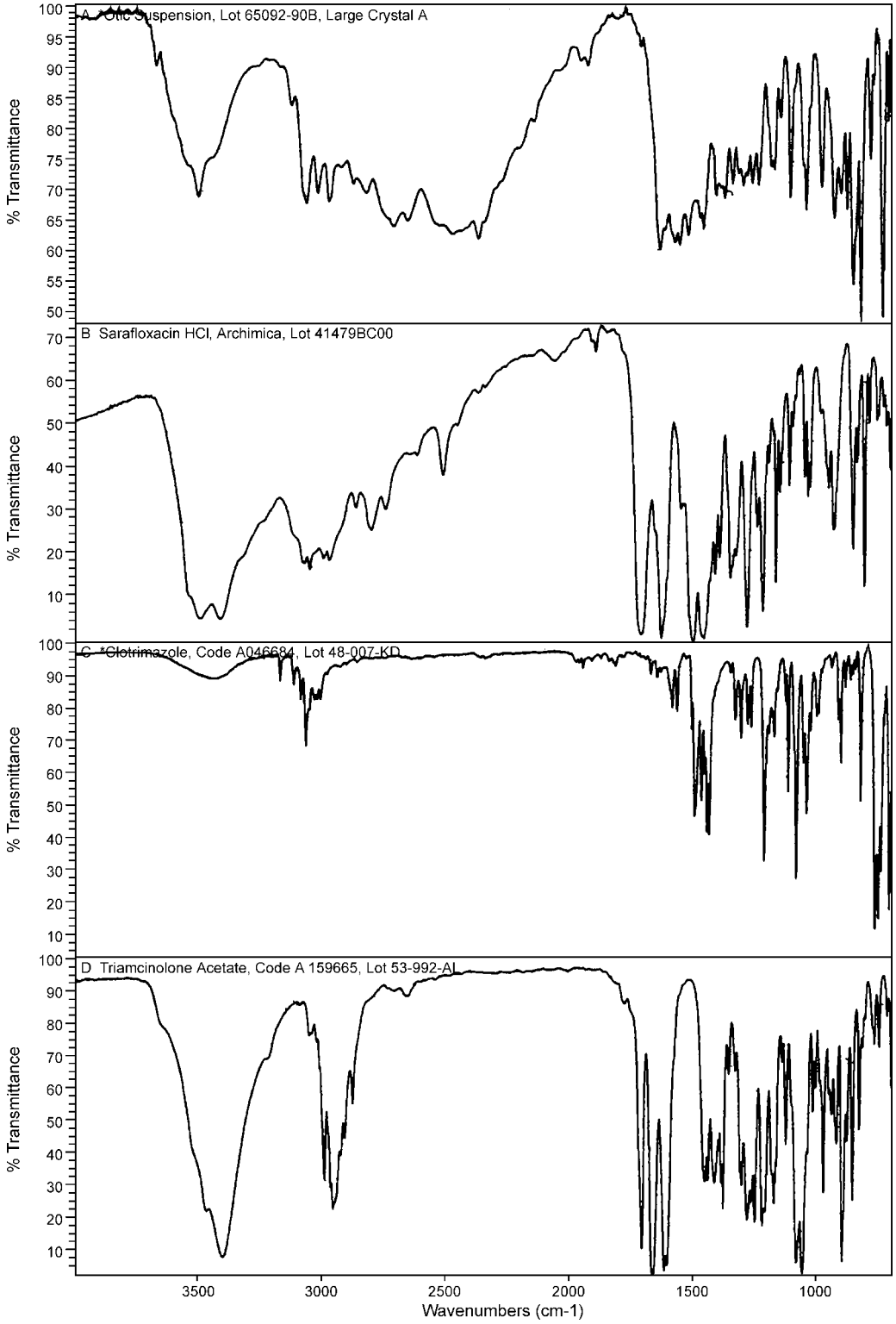

Figure 6. FTIR spectra of (A) large crystals collected from otic suspensions after 3-month storage at 40°C, (B) bulk sarafloxacin, (C) bulk clotrimazole, and (D) bulk triamcinolone.

that the crystal growth of sarafloxacin in the suspension at an elevated storage temperature occurred via a change in the hydration state of the drug.

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