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Research Article

Theme: Sterile Products: Advances and Challenges in Formulation, Manufacturing, Devices and Regulatory Aspects
Guest Editors: Lavinia Lewis, Jim Agalloco, Bill Lambert, Russell Madsen, and Mark Staples

Complexities of Particulate Matter Measurement in Parenteral Formulations of Small-Molecule Amphiphilic Drugs

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Abstract. Reconstituted parenteral solutions of three surface-active anti-infective small-molecule drugs and solutions of sodium dodecyl sulfate (SDS, a model surfactant) were studied to quantify the impact of sample preparation and handling on particle counts. Turbidimetry and light obscuration profiles were recorded as a function of agitation and shearing with and without the introduction of foam into the solutions. SDS solutions at concentrations above the critical micelle concentration (CMC) show significantly greater sensitivity to shear and foam presence than SDS solution below the CMC: Values of >10 μm particles increased 8 fold over control (an unsheared sample) in the micellar solution *vs*. 4 fold particle count increase over control at a sub-micellar concentration. An even more significant increase in the ratio of particle count in sheared/unsheared solution is seen for >25 μm unit counts, due to the increased interference of foam with the measurement. Two commercial products, injection formulations of teicoplanin and cefotaxime sodium, as well as an investigational compound **1**, showed an increase in scattering as a function of foam production. The impact of foaming was significant, resulting in an increase of turbidity and light obscuration measurements in all solutions. The results illustrate some of the challenges that are inherent to optically clear, homogeneous pharmaceutical injections containing compounds which have a tendency toward self-association and surfactant-like behavior.

KEY WORDS: foam; formulations; HIAC; light obscuration; parenteral; particulate matter; shear; surface-active pharmaceutical ingredients.

INTRODUCTION

Development of parenteral formulations of drugs is a complex endeavor involving aspects of physical chemistry, microbiology, and engineering to generate a sterile, particle-free product (1,2). The process of selecting a composition necessitates an intimate understanding of both the physicochemical properties of the active pharmaceutical ingredient, such as stability towards degradation and detailed thermodynamic solubility data relative to a crystalline drug form (if available), as well as the solution properties of the final injection. The capacity of a drug solution to exhibit surfactancy (for example, through micellization or other self-association mechanisms) along with the effect of excipients on such properties impacts product design (3–5). Ultimately, compatibility with injection components and general handling of the materials is needed to ensure a smooth process of formulation development to support the use of an injectable product in humans.

In addition to an understanding of the properties of the drug substance and its solutions, knowledge and proper interpretation of regulatory requirements, including ICH guidelines, for the final product are required as they pertain to the quality and release of the formulation. Testing for particulate matter requires thorough consideration, specifically as one must decide which tests to use and when. According to the United States Pharmacopeia (USP), "*particulate matter in injections and parenteral infusions consists of mobile undissolved particles, other than gas bubbles, unintentionally present in the solutions*".[1] Quantitative analysis of particulate matter employs two procedures, method 1, a light obscuration particle count test, and method 2, a microscopic particle count test. Both are specified in USP [788]. Method 1 is preferred when examining injections

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[1] Excerpt taken from the USP: http://www.usp.org/USPNF/notices/erratum788.html

and parenteral infusions for sub-visible particles. However, it may be necessary to test some preparations by the light obscuration particle count test followed by the microscopic particle count test, method 2, to reach a final conclusion on conformance to requirements. Unfortunately, not all parenteral preparations can be examined for sub-visible particles by one or both of these methods. For example, emulsions, colloids, and liposomal preparations are intrinsically opaque due to light scattering by droplets and/or particle assemblies present in the injection (6,7). For this reason, filtration and subsequent microscopic analysis of the filter is often used for the particulate matter analysis of these kinds of opaque samples. Similarly, products that produce air or gas bubbles when introduced into a light obscuration sensor may also require microscopic particle count testing. Finally, if the viscosity of the preparation to be tested is sufficiently high so as to preclude its examination by either test method, it may be acceptable to perform a quantitative dilution with an appropriate diluent to decrease viscosity to facilitate the test.

USP chapter 788 provides handling instructions for infusion solutions as they will be tested using light obscuration methods and gives clear limits of the acceptable number of particles in a solution. The number of particles which are ≥10 μm cannot exceed 12 in a 1-mL volume, and particles which are ≥25 μm cannot exceed two particles. Light obscuration or extinction methods rely on light blockage caused by a particle, which is correlated to the size of the particle. The advantage of these techniques is that they are rapid, well-established, and require a limited volume of sample for testing. However, a disadvantage is that the methods are sensitive to adventitious effects from sample preparation, including air or gas bubbles. In such cases, the test results do not provide information on the origin or true presence of particles in the test article. While light obscuration remains the "gold standard" quality control assessment for solution-based injectable formulations, the literature is significantly limited in the assessment of amphiphilic small molecules present in parenteral drug products. On the other hand, those groups developing large molecules such as biologics have recognized the limitations of light obscuration and have taken advantage of emerging technologies to address particulates. One emerging technology for this purpose is micro-flow imaging technology, which combines digital microscopy, micro-fluidics, and image processing to fully characterize the physical properties of the solutions (8,9). These tools have allowed the distinction between air entrapment, aggregation, and solid particulates introduced during manufacturing processes. Application of the integrated set of technologies have led to more robust formulations (those which limited self-aggregation) and handling procedures, based on the physico-chemical properties of the drug under development (10).

On account of the potential complexity of using light obscuration methods for particulate analysis, we explore in this contribution the special case of surfactant-like injection formulations of small-molecule drugs. Specifically, we consider the impact of sample handling and foam/gas bubble interference on the particle count in a series of products to illustrate some of the challenges of particulate analysis in aqueous-based injections of low molecular weight (<1,000 Da) molecules with inherent surfactancy.

EXPERIMENTAL SECTION

Surface Tension Measurements. Surface tension measurements were carried out by the pendant drop method using a model FTA-188 contact angle and surface tension analyzer (First Ten Angstroms, Inc.) Concentrations of compound 1 ranging from 0.1–50 mg/mL were prepared by dissolving the drug in a 25-mM citrate buffer and adjusted to a pH range of 4.6–5.2 using sodium hydroxide. Solutions were also prepared in buffers containing 50 mM NaCl to test the effect of ionic strength on surface tension.

Turbidity by Nephelometry. Measurements were done with a plate-reading model BMG NEPHELOstar using a gain setting of 60, a measurement time per well of 1 s and a laser wavelength of 635 nm. Shearing for the nephelometry experiments was done with a small volume (less than 10 mL) syringe and needle by alternately aspirating and dispensing up to ten times. Shearing without foam was achieved by repeated cycles of aspirating and dispensing so that air excluded from the syringe. Shearing with foam was achieved by including air in the syringe during aspirating/dispensing cycles. Ten minutes were allowed to pass before measurement to allow any visible bubbles to dissipate. During these experiments, it was found that even the plates and blank solutions show a certain level of light scattering. Therefore, the turbidity values are considered arbitrary, and the data are shown to indicate relative differences in turbidity as a function of sample handling.

Light Obscuration by HIAC. A Hiac Royco model 9064 counter equipped with a model HRLD-150 sensor and a model 3,000 sampler was used to measure particulate matter by light obscuration for the Stage 1 USP pharmacopoeial test. This instrument determines the size of particles within a range of ca. 1–100 μm and leads to a number distribution. Based on the pharmacopoeial requirements, the particles are typically classified in a limited number of size classes, such as particles smaller than 10 μm (<10 μm), particles between 10 and 25 μm (10–25 μm), and particles larger than 25 μm (>25 μm).

General Procedure for the Preparation of Solutions for HIAC Testing. The following procedure was applied in the investigations: Commercially sourced IV bags containing final infusion solutions were cut open, and the contents were transferred into a beaker that was previously rinsed with particle-free water. In some experiments, solutions were filtered through a 0.5-μm disk filter or a 0.2-μm in-line filter to eliminate foam. Occasionally, the solution was additionally degassed for 30 min under vacuum.

Solution Samples with Shear but Without Foam. Infusion solutions were added to a volumetric flask (100 mL) equipped with a magnetic stir bar and filled to capacity. The flask was then closed using a stopper, and the resulting sample had no air compartment above the liquid. The solution was then stirred at a high speed for 30 min. After stirring, the solutions were left standing up to 30 min.

Solution Samples with Shear and Foam. Infusion solutions were added to a volumetric flask (200 mL) equipped with a magnetic stir bar until half full. The flask was then

closed with a stopper and stirred at a high speed for 30 min. After stirring, the solutions were left standing up to 30 min.

Preparation of Solutions of Compound 1. A lyophilized powder cake of 1 was reconstituted with 10 mL water for injection or 5% dextrose in water (D5W) for injection. The vial was then shaken vigorously to allow for complete dissolution. Before dilution, to give the final infusion solution, any visible foam was allowed to dissipate. A 10-mL aliquot of the reconstituted solution was then removed from the vial and injected into a suitable container (*e.g.*, polyvinyl chloride (PVC) or polyethylene (PE) infusion bags, glass bottles) containing 250 mL of D5W. The infusion solution was gently inverted five to ten times to form a homogenous solution. Vigorous agitation was avoided to prevent foaming.

Preparation of SDS Solutions. Using a 50-mL syringe (Luer Lock, ref 300865) equipped with a needle (BD Microlance, 0.7×30 mm), 20 mL of the solution of interest was sampled from a beaker of solution into the syringe with a high velocity sufficient to produce foaming. The syringe with 20 mL solution was than emptied with the same velocity into the same beaker, positioning the needle tip just above the liquid surface to create a large amount of foam.

Preparation of Cefotaxime (Claforan®) Solution for Injection. Claforan 1,000 mg IM/IV powder for reconstitution vials were obtained from a pharmacy in the EU. A 20 mg/mL solution was prepared by dissolving 1 g of powder in 10 mL of water for injection and diluting further to 50 mL with D5W.

Preparation of Teicoplanin (Targocid®) Solution for Injection. Targocid 200 mg IM/IV powder for reconstitution vials were obtained from a pharmacy in the EU. A 66-mg/mL solution was prepared by injecting sterile water slowly into a vial containing Targocid powder. The vial was then gently shaken until the dry substance is completely dissolved. Care was taken to avoid the formation of foam. If foam developed during the preparation of the injection solution, the solution was left to stand for approximately 30 min until the foam disappeared.

RESULTS AND DISCUSSION

Light obscuration methods were employed in order to determine the effect of handling on the properties of the infusion formulations in common diluents. A HIAC particle counter was used to obtain the number of 10- and 25-μm particles in solutions as a function of their handling: A given solution that was handled without agitation was compared with samples of the same solution that was subjected to vigorous shaking as well as shaken solution measured after standing for varying periods of time. Additionally, nephelometry testing was performed on formulations that were prepared according to the USP, as well as those which had been sheared both with and without the introduction of foam. The sample preparations, while controlled, were not performed in the exact way to generate data suitable for the comparing release data for the tested drug product. Instead, the work was done to highlight the trends observed based on sample preparation and handling, and to demonstrate the variability in unit count under different handling conditions. More precisely, the hypothesis is that, due to the amphiphilic nature of the drug molecules, preparation methods that introduce high shear rates such as rapidly injecting or mixing the drug before administration can result in increased particle count in solution.

Light obscuration methods readily detect and report the presence of submicron "particles" in solution. However, the method yields no information on the nature of any particles detected. The exact nature of these particles have not been investigated in this study, but based on dissipation by visual observation, it is reasonable to assume that these are microscopic stabilized bubbles resulting from the rapid shearing of the air–water mixture (11–14). The amphiphilic nature of these drugs lowers the surface tension, thus facilitating the formation of microscopic bubbles in solution. Moreover, adsorption of these amphiphilic molecules at the air–water interface can help to stabilize these bubbles (15), allowing them to persist for longer periods of time (16).

INVESTIGATIONAL COMPOUND 1

Compound 1 is an investigational small molecule in development as an injectable formulation. During establishment of the compatibility of the drug product with various infusion solvents, lines, and containers, variable data were obtained for particulate count testing by the stage 1 light obscuration method. The variability was observed within a single lot when tested several times, as well as in testing of different lots. It was suspected that the source of the increased particulate count was entrapped air (due to the ampiphilic nature of the molecule) during the handling procedures. This notion was further corroborated when the solutions were analyzed using stage 2 microscopic methods, highlighting that no solids particles were recovered on 0.2-um filters.

Surface tension measurements were carried out on compound 1 in aqueous solutions in order to characterize its amphiphilic behavior**.** Since the compound is hydrolytically labile in solution over a period of days, aqueous solutions of 1 at varying concentrations were made and used immediately to minimize chemical degradation effects. All measurements were conducted at room temperature in a 25-mM citrate buffer, with and without the addition of 50 mM NaCl at pH 4.8. It should be noted that prepared solutions of compound 1 showed a tendency to foam and were therefore allowed to stand for up to 15 min prior to testing in order to allow the foam to dissipate. The surface tension plots of 1 are shown in Fig. 1. From the data in 25-mM citrate buffer

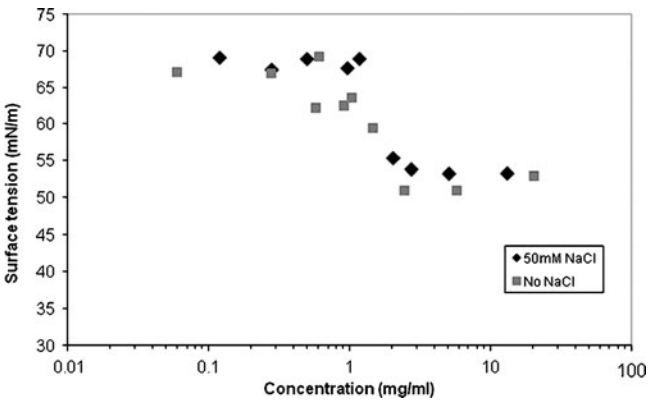

Fig. 1. Surface tension *vs.* concentration for investigational compound 1 in 25 mM citrate buffer, with and without 50 mM NaCl, pH 4.8

(pH 4.8), the main observation is that up to about a 0.8 mg/mL concentration, the surface tension of the solutions remain unchanged. The surface tension drops steeply in the concentration range of 0.9 to 2 mg/mL and plateaus above 2 mg/mL at approximately 51–53 mN/m. For reference, the surface tension of pure buffer in the absence of compound 1 was recorded at 66–68 mN/m, which is close to the surface tension of pure water at room temperature (72 mN/m; (17)). The transition at ~2 mg/mL of 1 has the appearance similar to CMC transitions of surface tension values in surfactant systems. Similarly, the surface tension of the solutions prepared with 50 mM NaCl at low concentrations of the drug candidate (<1 mg/mL) appears unaffected by the presence of compound 1. A sharp transition was once more observed starting at approximately 1 mg/mL, indicating that the presence of NaCl did not significantly affect the surfactancy behavior of the solutions.

Further testing using light obscuration techniques were conducted to further illustrate the impact of sample handling on particle count. This was done by exaggerating sample preparation procedures to maximize the impact of shear and introduction of foam. Samples containing 2.67 mg/mL solutions of 1 which had been sheared with foam, showed an initial increase in turbidity, which gradually decreased to the level of measured in the samples which had not been sheared. Inspection of the data in Fig. 2 reveals that, from the beginning of the measurement, samples sheared without foam had similar turbidity values to the samples which were not sheared. These observations indicate that the presence of foam increases the measured turbidity in solutions of 1.

In addition to the studies described to this point, the effect of filtration of solutions of 1 has also been evaluated. Presented in Fig. 3 are turbidity data for samples which were filtered after they were sheared with foam, along with control samples that were not filtered after being sheared with foam. The filtered samples showed lower turbidity, as measured by nephelometry, compared with samples which were not filtered. Over time, however, the turbidity of the samples that were unfiltered decreased to values similar to those that were filtered. These results confirm that the presence of foam is responsible for the increase in nephelometry readings. Removal of the foam, either by allowing solutions to stand or by filtering the solutions, results in lower turbidity readings by nephelometry.

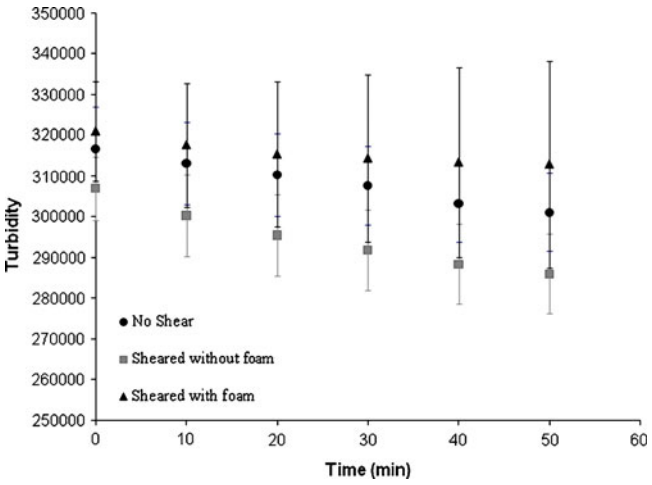

Fig. 2. Time-dependent turbidity in an infusion solution (2.67 mg/mL) of compound 1 in D5W as a function of shear with and without foam

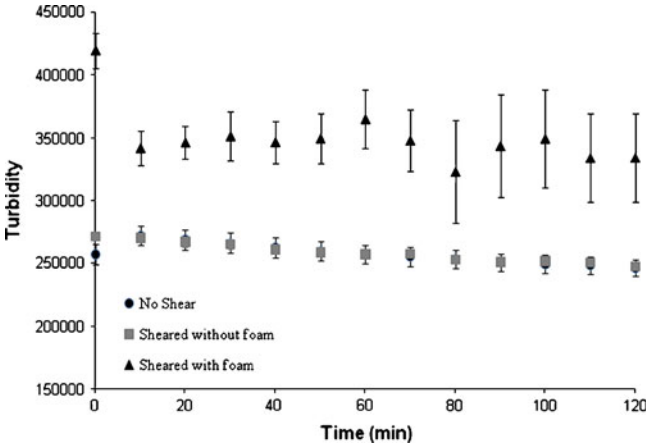

Fig. 3. Time-dependent turbidity in a filtered and unfiltered infusion solution (2.7 mg/ml) of compound 1 in D5W as a function of shear with and without foam

The effect of filtration on particulate counts was also evaluated using light obscuration. Once again, filtered solutions which were sheared without the introduction of foam did not exhibit a meaningful increase in the number of counts, as shown in Table 1. In line with previous observations, filtered solutions which were sheared with the introduction of foam resulted in an approximately eightfold increase in the number of counted particles.

In the remaining sections of this article, the approaches described hereto were used to evaluate sodium dodecyl sulfate (SDS), a model surfactant, and two marketed injectable products, in an effort to highlight the significance of sample preparation for formulations containing amphiphilic small molecules.

SODIUM DODECYL SULFATE A MODEL SURFACTANT SOLUTION

Solutions of SDS at 2.9 mg/mL were prepared at a concentration above the critical micelle concentration of ~2.3 mg/mL at room temperature (17,18). Over a period of 50 min, samples which were sheared with and without presence of foam did not show a statistically significant increase in turbidity relative to the samples which were not sheared, as shown in Fig. 4. This lack of differentiation between handling techniques can be attributed to the fact that the foam generated while shearing solutions of SDS subsides rapidly (within the timeframe of the first measurement),

Table 1. Light Obscuration by Solutions of Compound 1 Filtered Over a 0.2-μm Filter in Water for Injection with and without the Introduction of Foam

Shearing	Foaming	> 10 μm Particles (counts/mL)	>25 μm Particles (counts/mL)
No	No	20	3
Yes	No	28	2
Yes	Yes	166	5

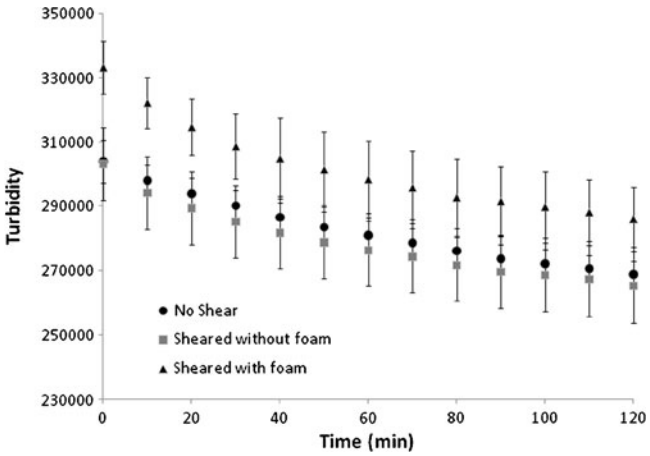

Fig. 4. Turbidity *vs.* time in a solution of approx. 2.3 mg/mL sodium dodecyl sulfate (SDS) in deionized water subjected to shearing with and without foam production

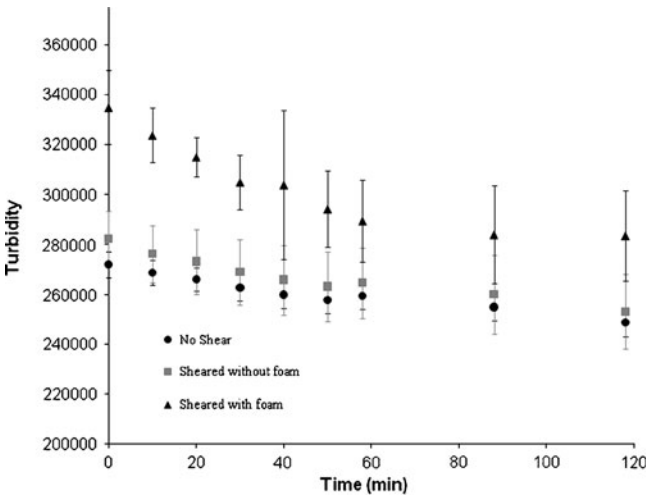

Fig. 6. Time-dependent turbidity in 20 mg/mL solutions of Claforan® (cefotaxime sodium) in water for injection

Table 2. Light Obscuration Results from SDS Solutions in Water for Injection with and without the Introduction of Foam

Concentration of SDS relative to the CMC	Shear	Foam	>10 μm Particles (counts/mL)	>25 μm Particles (counts/mL)
0.1×CMC	Yes	No	39	1.2
0.1×CMC	Yes	Yes	152	2.0
10×CMC	Yes	No	131	8.3
10×CMC	Yes	Yes	1,069	299

SDS sodium dodecyl sulfate, *CMC* critical micelle concentration

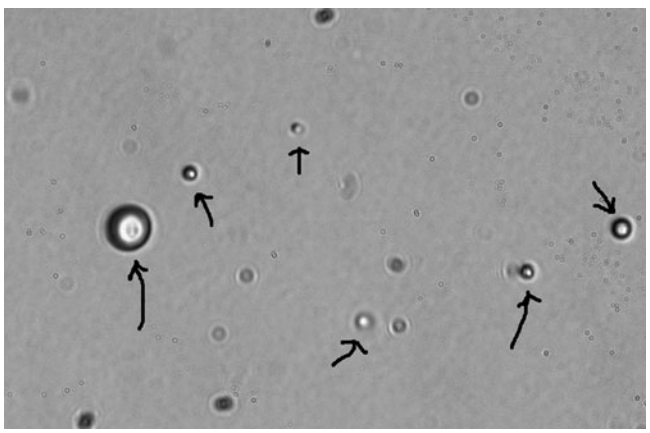

Fig. 5. Microscopic image of a micellar SDS solution (2.3 mg/mL in water; approximately tenfold above the CMC) sheared with the introduction of foam, indicating the presence of air bubbles

making it essentially impossible (with the techniques used) to measure any change in light scattering which would arise from interference of foam in the solution.

The impact of shearing in combination with foam on the light obscuration measurement for SDS was studied using the syringe as the shear-creating device. Contrary to the turbidimetric measurements, the data in Table 2 indicate that when foam was introduced into the solution, an increase in counts can be measured by the HIAC. At a concentration of one tenth the CMC value of SDS, a SDS solution subjected to shear shows up to a fourfold higher unit count of >10 um, compared with the same solution left unsheared. At a concentration of tenfold above the CMC of SDS, the same ratio increases to about eightfold and 36-fold for >10 and >25 um unit counts, respectively, demonstrating vastly increased sensitivity to handling of the micellar system relative to non-micellar SDS solution. The effect of foam on the properties of the SDS solutions was further confirmed by capturing a microscopic image of the solutions immediately following foaming. The micrograph image in Fig. 5 indicates the presence of air bubbles in micellar SDS solution.

CEFOTAXIME (CLAFORAN®): A CEPHALOSPORIN ANTIBIOTIC

Solutions of cefotaxime sodium at 20 mg/ml were prepared in water for injection and measured for turbidity using a nephelometer. As shown by the turbidity data over time in Fig. 6, samples which were sheared without foam do not show an increase in turbidity relative to the samples

Table 3. Light Obscuration Data for 20 mg/mL Cefotaxime Sodium Solutions in Water for Injection with and without Foam

Shearing	Foaming	>10 μm Particles (counts/mL)	>25 μm Particles (counts/mL)
No	No	9	0
Yes	No	15	0
Yes	Yes	45	0

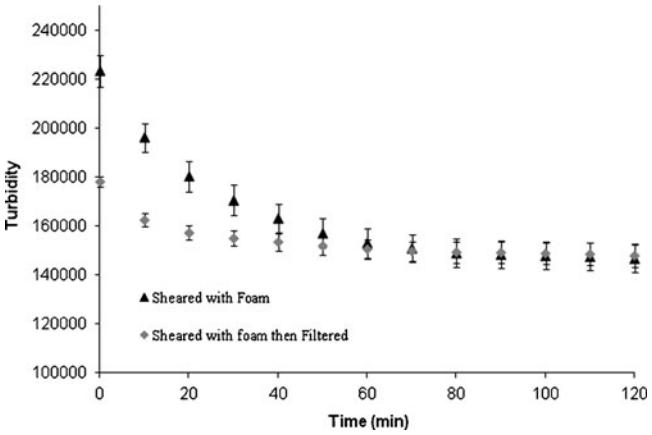

Fig. 7. Time-dependent turbidity in 66.7 mg/ml solutions of Targocid® (teicoplanin) in water for injection

which were not sheared. Samples sheared with foam initially show an increase in turbidity and then exhibit a decrease to approach the turbidity value of the samples which were not sheared.

An analogous experiment with the drug solution was carried out and analyzed using light obscuration. Data from this experiment are shown in Table 3. Solutions which were sheared without the introduction of foam did not result in a significant increase in the number of counts. However, solutions which were sheared with the addition of foam resulted in a fivefold increase in the number of counts >10 μm. These data for cefotaxime sodium are akin to the observations made for SDS. An initial, significant increase in counts may have been difficult to measure using this technique given the data obtained from the nephelometry experiment, which indicated a rapid drop in turbidity following the introduction of foam. The data are also consistent with the visual observation that foam dissipates rapidly once agitation is stopped.

TEICOPLANIN (TARGOCID®): A GLYCOPEPTIDE ANTIBIOTIC

Reconstituted solutions of Targocid at 66.7 mg/mL which were sheared without foam do not show an increase in turbidity relative to the samples which were not sheared, as shown by the data in Fig. 7. However, samples sheared with foam show a large increase in turbidity relative to other samples. The elevated turbidity readings persist for more than 2 h. Turbidity increases are consistent with those observed for solutions of SDS and cefotaxime sodium, though more pronounced for teicoplanin due to the strong surfactancy of the solutions (19,20).

Light obscuration testing of Targocid solutions at 66.7 mg/mL resulted in large particle counts, as shown in Table 4. High counts were observed even without the introduction of excess foam. These solutions were particularly sensitive to handling. This sensitivity is appropriately reflected in the instructions in the product label, which states that if foam is generated during reconstitution, sufficient time be allowed for the dissipation of the foam.[2]

[2] For product label see: http://www.sanofiaventis.com.au/products/aus_pi_targocid.pdf

Table 4. Light Obscuration Data for 66.7 mg/mL Targocid Solutions in Water for Injection with and without the Introduction of Foam

Shearing	Foaming	>10 μm Particles (counts/mL)	>25 μm Particles (counts/mL)
No	No	1,579	112
Yes	No	1,824	30
Yes	Yes	2,579	165

CONCLUSIONS

Injectable solutions of compounds having surface-active properties show a time-dependent impact of mixing with air entrapment and resulting foam on both turbidity values and particulate count by light obscuration. The findings point to the need for highly precise instructions for handling of solutions during particulate count measurements in order to avoid confounding artifacts and "false positives" for particulate matter. These conclusions are made based on work with four small molecule compounds in aqueous solutions: A model surfactant, SDS, two approved products at concentrations relevant to use in patients, as well as for an investigational compound 1. It should be stressed that the present study in no way calls into question either the monographs for particle measurements or the formulation design of the approved compounds tested herein. The study merely highlights sensitivities that are inherent to optically clear, homogeneous pharmaceutical injection formulations containing compounds that have a tendency for self-association and surfactant behavior.

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