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Short communication

Identification of hematite particles in sealed glass containers for pharmaceutical uses by Raman microspectroscopy

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ABSTRACT

Raman microspectroscopy has been shown to enable the identification of micro-particles inside sealed glass containers for pharmaceutical use without any sample preparation. Raman spectra were collected from unknown particles with a maximum size of 1 mm, adsorbed on the inner surface of ampoules. The particles were clearly identified as primarily hematite with traces of magnetite by their characteristic Raman spectral bands. The presence of this deposit was attributed to the projection of iron oxides during the manufacturing process. These oxide particles were not detected by the quality control process of the glass manufacturer, showing that in-process quality controls failed to detect this problem. Particle identification by Raman microspectroscopy appears to be a selective, rapid and reliable analytical procedure for quality control and assurance in the pharmaceutical industry. Identification of the particles was also helpful for evaluating the nature of the contaminant and enables consequences for the toxicological aspects of final product quality to be managed.

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1. Introduction

The feasibility of *in situ* identification of particles inside glass containers for intravenous infusion for safety and quality control is an important analytical procedure in the pharmaceutical industry [1]. Foreign particles inside glass containers in in-use admixed intravenous drug products are contaminant particles, whose presence should be considered as abnormal. These particles may arise from the active drug substances, excipients, container or closure components. The particles may be created by the manufacturing process of the drug substances or from the life cycle of the drug substance and also depending of storage conditions. The formation of particles during the life cycle could be due to specific interactions between drug substances and container or closure components. Finally, the particles may be present in container or closure components before the manufacturing process or are introduced in this step.

The presence of inert particles such as glass from ampoules in in-use admixed intravenous infusions may result in adverse effects such as phlebitis and cause damage to the lungs, brain kidneys, liver and spleen [2]. Particles may have specific toxicity if they are not inert.

Testing for foreign particles is mandatory in the pharmaceutical industry and should be conducted during development studies in order to work out strategies for the control of product formulation. This characterization may include identification, understanding of source, the number of particles and determination of batch-to-batch consistency, shape, and size. The identification of particles is an essential step for determining the root cause in order to manage post-non-compliance and prevent future reoccurrence.

Different microspectroscopic techniques have been used to identify particles with different origins such as scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS), Fourier transform infrared (FT-IR) as well as Raman microspectroscopy [1].

The Raman *in situ* technique provided a complementary and sometimes unique method in particle identification in a pharmaceutical context, in particularly inside a glass container. Raman spectroscopy has the advantages of allowing a real-time molecular analysis without the need for sample preparation, reducing the time of analysis, limiting the risks of cross-contamination and of loss of particle. It is a powerful laser spectroscopic technique that detects the characteristic vibrational energy levels of a molecule. When light irradiates a molecule, most photons are scattered elastically. This elastically scattered light has the same frequency as the incident illumination and is termed Rayleigh scat-

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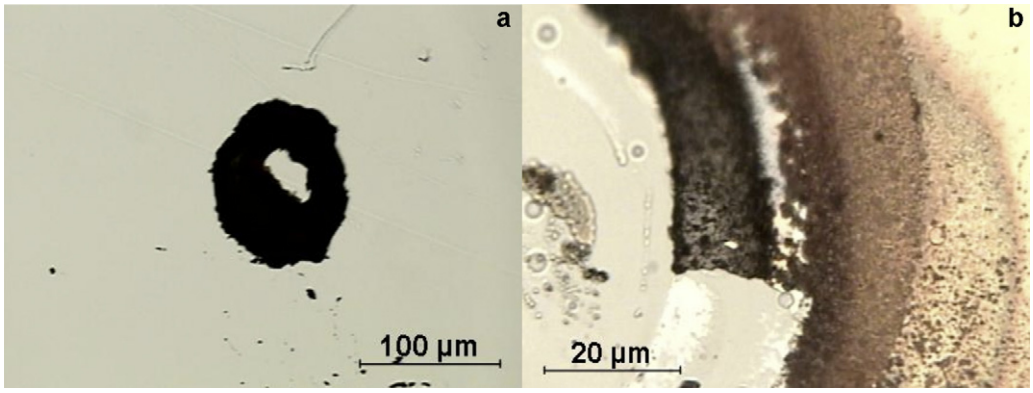

Fig. 1. Example of a microparticle inside a glass ampoule. Photo taken by an Olympus microscope with 10× (a) and 50× (b) MPlan objectives.

tering. A small fraction of light, however, is inelastically scattered. This inelastically scattered light, termed Raman (Stokes and anti-Stokes) scattering, exhibits frequency shifts with respect to the incident light. These shifts precisely correspond to the vibrational energy transitions of the molecule. They can be analyzed by an optical dispersive system to be represented as spectra. Consequently, the Raman spectrum can be considered as a spectral fingerprint of the molecule [3].

Several published reports have addressed the application of Raman microscopy to the identification of particles or mapping to provide the special distribution: Raman microscopy has been used in many pharmaceutical applications [4–11], in particular for the identification of foreign particles [1,12,13]. This technique has been adapted for the detection of microparticles in heterogeneous sample matrices. It is in fact very valuable for *in situ* studies and when only a small quantity of particle material is available [6].

We report here the identification of iron oxide particles inside glass ampoules from different batches, observed as dark spots by Raman microspectroscopy.

2. Materials and methods

2.1. Materials

A cardioplegic solution contained potassium (0.80 M), chloride (1.10 M) and magnesium (0.15 M) ions. This cardioplegic solution is used in heart surgery to stop the heart, thereby facilitating the work of surgeons.

2.2. Methods

The microparticles were directly observed on the inner wall of glass ampoules filled with aqueous cardioplegic solution.

2.3. Apparatus

Raman spectral acquisitions were performed with a HR Labram microspectrometer (Horiba Jobin Yvon, Lille, France). The excitation source was a 633 nm single-mode diode laser (TOPTICA PHOTONICS, Germany) generating at 35 mW on the sample. The microspectrometer was equipped with an Olympus microscope and measurements were recorded using 10 and a 50× MPlan objectives (Olympus, Japan). Light scattered by the sample was collected through the same objective. Rayleigh elastic scattering was intercepted by a Notch filter which reduced its intensity by a factor of 10^6. A Peltier cooled (−65 °C) multichannel CCD detector (Coupled Charge Device) (1024 × 256 pixels) detected the Raman Stokes signal dispersed with a 100 µm slit width and an 1800 grooves/mm holographic grating. Spectral resolution calculated from the full width at half maximum of the silica wafer band at 521 cm^{-1} was 1 cm^{-1}. The mean separation power of the dispersion system, *i.e.* the distance between two CCD pixels was 0.2 cm^{-1}. The spectral region studied was 200–1800 cm^{-1}. The acquisition time of each spectrum was 2 × 20 s.

Spectral acquisition and data pre-processing were conducted with Labspec5 software (Horiba Jobin Yvon SAS, Lille, France).

3. Results and discussion

Raman microspectroscopy was selected because this technique can identify organic and inorganic compounds, as well as polymers. Particles could be formed during the manufacturing process from inorganic drug substances or from contamination products (inorganic or organic) present in drug substances or starting materials. In the present work, the dark spots could also have arisen from carbonization of organic compounds during autoclaving.

The particles were dark reddish-brown or black spots, small (<1 mm), located on the inner wall of type I glass ampoules and their distribution appeared to be random. Fig. 1 illustrates the adhering aspect of the particle to the surface of the glass.

This observation was first made on ampoules containing an aqueous cardioplegic solution of potassium, chloride and magnesium ions. This contamination was present on approximately 10% of the test sample of two production batches, with 1–3 spots per ampoule (first batch: 9.6% (n = 240); second batch: 12.1% (n = 240)). The maximal size of the black spots was 1 mm. These dark spots were not formerly observed in this type of production (cardioplegic solution). 480 ampoules from another batch of this production were observed and no ampoule with a black spot was found. It was initially supposed that the spots were formed during the manufacturing process. The heat sterilization step (121 °C during 20 min) could in fact be the origin of carbonization of organic molecules. The inorganic nature of the raw materials (pharmaceutical qualities) used for this manufacturing was inconsistent with this hypothesis. Possible cross-contamination during formulation was also incompatible with the presence of dark spots (because all manufacturing runs were heat-sterilized). The manufacturer never observed dark spots before.

Microscopic observations revealed the presence of particles of various colors, ranging from black to red and brown. Spectra of a darker part inside the inclusion did not provide interpretable Raman spectra. Fig. 2 shows a typical Raman spectrum collected from one of the red/brown particles and that was identified as hematite (α-Fe_2O_3). Hematite belongs to the D^6_{3d} crystal space group and seven phonon lines are expected in the Raman spectrum [14]. Six of the seven Raman active bands of hematite that are predicted by group theory were observed with the same intensities, namely two A_{1g} modes (225.5 and 495.9 cm^{-1}) and four E_g

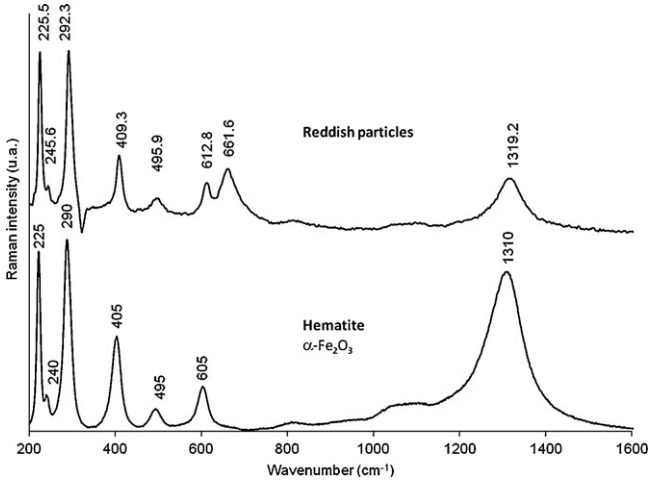

Fig. 2. Comparison of the Raman signatures of the reddish particles and hematite (adapted from Ref. [15]).

modes (245.6, 292.3, 409.3 and 612.8 cm^{-1}). The two magnon scattering bands of hematite at 1319.2 cm^{-1} were observed and closely matched those in the hematite spectrum of Froment et al. [15]. The results make it possible to distinguish hematite from magnetite (Fe_3O_4) characterized by a specific band at 661.6 cm^{-1} [15].

Spectra were acquired using a 35 mW exciting beam that caused partial degradation of the oxide samples after two irradiations with lower band intensity and a dark spot on the irradiated zone was observed. The investigation of the effect of the laser power on the Raman spectra of some of the iron oxides revealed that sample degradation frequently occurs under intense sample illumination and may lead to the misinterpretation of spectra [14]. Even if the attribution of the highly intense band around 660 cm^{-1} is not clear yet in the literature [16], spectrum reveals the presence of an iron oxide.

Hematite and magnetite are metal corrosion products [17]. Magnetite is known to undergo a phase transition to hematite at a temperature higher than 300 °C [14]. The raw materials in this solution are controlled according to the current version of the European Pharmacopoeia (6.8). The drug substances are free of iron (Iron <10 ppm and <20 ppm for magnesium chloride hexahydrate and potassium chloride, respectively) and contents were from 98% to 101%, demonstrating correct purity.

The formation of iron oxides during autoclaving was excluded because the drug substances did not contain iron and no step of the manufacturing process could explain the presence of the dark spot of hematite. Furthermore, the microscopic aspect of the spot showed that the iron oxide spots adhered to the inner wall of the ampoule and were slightly included. The appearance of spots on the glass was reminiscent of a projection of the contaminating agent, probably on the glass that was still warm, which could explain this slight degree of inclusion. Following this observation, a larger number of ampoules from the same batch (empty or filled with a different formulation from that of the cardioplegic solution) were tested. No contamination was found in empty ampoules ($n = 400$) but two other formulations containing organic compounds were contaminated by the same dark spots with a low incidence (1/360 and 2/160 for the two batches).

It was shown that the contamination was a spot flaw in the manufacturing process of glass ampoules because the presence of iron oxide spots was not uniform in the batch process, resulting in a non-uniform contamination from one batch to another.

It was concluded that iron oxides formed during the manufacturing process of ampoules and were probably introduced into the containers during the step of glass blowing, possibly from materials containing iron (or steel).

Iron oxide inside glass ampoules cannot cause adverse effects if the particles remain adsorbed to the ampoule wall as a result of the insolubility of iron oxides in most infusion solvents and their electrostatic interactions with silanols of the glass. If particles are resuspended, however, they could cause considerable problems if infusion solutions are not filtered before use.

4. Conclusion

This case study shows that Raman microspectroscopy can be very helpful for the identification of microparticles inside pharmaceutical glass ampoules. In this short communication, the technique can detect an artifact, demonstrating the value of direct Raman microspectroscopy on the manufacturing site.

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