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Heavy metal contamination of nanosuspensions produced by high-pressure homogenisation

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Abstract

High pressure homogenisation is a method for the production of nanosuspensions. In this process crystalline drug particles are pressed with high pressure through a narrow homogenisation gap. Due to the conditions in the gap it seems possible that metal erosion can occur. In this study the heavy metal (Fe) contamination of nanosuspensions produced by high pressure homogenisation was determined. Therefore nanosuspensions were analysed by atom absorption spectroscopy concerning their load of iron which is chosen as reference metal. The results show that the erosion of metal is below 1 ppm and will not cause any toxicological problems. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Nanosuspensions; Heavy metal contamination; High pressure homogenisation; Metal erosion

An increasing number of newly developed high potential drugs show very poor solubility in aqueous and also in organic media. This leads in most cases to a poor bioavailability of these drugs (Müller et al., 1996). An approach to solve this problem is the transfer of crude drug particles into nanoparticles, i.e. by high pressure homogenisation (DissoCubes™) leading to nanosuspensions (Müller et al., 1999a). In this process, the dispersion is pressed with high pressure through a narrow homogenisation gap, which is only a few micrometers in width. High-pressure homogenisation is an established method for the industrial production of fat emulsions for parenteral nutrition for several years. The large number of licensed products on the market shows that the heavy metal contamination during the production of emulsions is not a problem. But compared with emulsions, the production of nanosuspensions requires higher pressure (up to 1500 bar) and the suspension contains hard crystalline drug particles (Müller et al., 1999b). Therefore the friction in the gap between particles and wall is higher. Due to this effect it seems possible that the crystals may erase metal (ions or particles) out of the homogenisation valve which then would lead to a heavy metal contamination in the product. The observance of heavy metal contamination has become more and more important. Although there are no defined limits for most heavy metals in

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parenterals, it would be reasonable to ensure within the scope of quality management that the rate of contamination stays in acceptable ranges.

This paper describes the analysis of nanosuspensions concerning their contamination with metal, i.e. iron, by atomic absorption spectroscopy and shows that the erosion of metal is extremely low thus not causing any toxicological problems. The production unit was challenged by applying extreme production conditions (i.e. maximum possible pressure of 1500 bar and up to 50 cycles).

The drug RMKK98 was obtained from dds-drug delivery services GmbH (Kronshagen, Germany), barium sulfate (as hard model drug), Poloxamer 188, Tween 80, and sodium chloride were purchased from Sigma Aldrich (Deisenhofen, Germany). Fuming hydrochloric acid (37%), fuming nitric acid (100%) and the iron standard Titrisol® 1‰ were purchased from Merck KGaA (Darmstadt, Germany). Acetylene was purchased from Air liquid GmbH (Düsseldorf, Germany)

The following samples were prepared

Sample 1: barium sulfate in a concentration of 5% (w/w) dispersed in a solution of Poloxamer 188 (1% w/w).

Sample 2: RMKK98 in a concentration of 5% (w/w) dispersed in a solution of Poloxamer 188 (1% w/w), Tween 80 (0.5% w/w) and sodium cholate (0.1% w/w)

Sample 3: pure Milli Q water (homogenised)

Sample 4: iron chloride standard solution taken from a 1‰ stock solution leading to a concentration of 25 ppm iron

Sample 5: pure Milli Q water (not homogenised)

Sample 6: hydrochloric acid 7%

Samples 1 and 2: the drug suspensions were first premixed with the aqueous surfactant solution for 1 min using an Ultra-Turrax T25 at 9500 rpm (Janke and Kunkel GmbH, Staufen, Germany). This premix was homogenised using a Gaulin Micron Lab 40 high pressure homogeniser (APV Deutschland GmbH, Lübeck, Germany) applying the following pressures: 150 bar for 2 cycles, 500 bar for 2 cycles and 1500 bar for 20 cycles (sample 1) or 10 cycles (sample 2). Sample 3: the water was homogenised using 1500 bar for 50 cycles.

Iron concentration analysis was performed with flame atomic absorption spectroscopy (AAS) using a PU 9100X atomic absorption spectrometer (Philips GmbH, Hamburg, Germany) with an iron hollow cathode lamp (15 mA, Philips GmbH, Hamburg, Germany) powered with 12 mA. The absorption was measured at a wavelength of 248.3 nm with a detection gap of 0.2 nm. The fuel gas of the burner was a mixture of air and acetylene (1:1).

For the analysis it is necessary that the samples do not contain any particles which could block the nozzle of the liquid dispenser. In order to analyze the iron, it has to be transformed into ionic state since only ions can be detected by this method.

Therefore all samples were treated the same way: they were first mixed with 60 ml Aqua regia, which consists of fuming hydrochloric acid (37%) and fuming nitric acid (100%) in the ratio $3+1$ (v/v), and then boiled down to dryness. The residues were again dissolved in 30 ml Aqua regia and boiled down to dryness two more times. The residues of these processes were dissolved in hydrochloric acid 7% and subsequently analyzed using AAS.

A calibration curve was established using the iron standard solution Titrisol® 1‰ as a stock solution. Sample No. 4 is the 25 ppm standard solution. Several dilutions were made to check the analytical limits. The linear range of the atomic absorption spectrometer was found between 1 and 25 μg/ml iron. The samples were diluted to show absorptions in the linear range and then four consecutive measurements were performed (Table 1).

The homogenisers are made of stainless steel (Standard No. 1.4401 and 1.4542) accepted for production of parenterals and also food products. That means it is generally accepted material with no human hazards. Nevertheless when using high pressure metal erosion can occur. For the analytical procedure iron was chosen as reference metal because it represents the largest metal fraction in steel and will therefore be most likely to be detected in the product. It is obvious that the treat-

Table 1
Concentration of iron in samples measured by atomic absorption spectroscopy (AAS) (1 μg/ml = 1 ppm)

Sample	A	B	C	D	Average	S.D.
	(μg/ml)	(μg/ml)	(μg/ml)	(μg/ml)	(μg/ml)	
Suspension 1	0.59	0.60	0.60	0.62	0.60	0.020
Suspension 2	0.25	0.26	0.25	0.26	0.26	0.011
Water (50 cycles)	0.65	0.63	0.61	0.65	0.64	0.007
Iron-standard	24.32	23.76	24.60	24.04	24.18	0.364
Milli Q water	0.00	0.00	0.00	0.00	0.00	0.000
Hydrochloric acid	0.14	0.09	0.14	0.09	0.12	0.027

Table 2
Steel composition of the homogeniser

Standard no.	C (<%)	Si (<%)	Mn (<%)	Fe (%)	Cr (%)	Mo (%)	Ni (%)	Cu (%)
1.4401	0.07	1.00	2.00	62.5–68.0	16.5–18.5	2.00–2.50	10.5–13.5	–
1.4542	0.07	1.00	1.00	70.0–77.0	15.0–17.0	–	3.00–5.00	3.00–5.00

ment of the samples with highly concentrated acids and boiling to dryness does not lead to a great loss of iron in the solution (the deviation to the expected value was below 4%). The iron concentration in Milli Q water (sample 5) is under the detectable limit but hydrochloric acid shows a contamination of 0.12 ppm iron. This concentration can be taken as the background, because hydrochloric acid was used as solvent for all samples during the sample preparation.

As mentioned before there are no generally defined limits for most heavy metals in parenterals, neither in the German Pharmacopoeia (DAB) nor in any other pharmacopoeia. For some i.v. injectable solutions the guidelines are based on the contamination limits of the bulk material, which has own monographies in the pharmacopoeia. The obtained data show that in spite of the hard production conditions (crystalline particles, maximum pressure, many cycles) the metal erosion is very low i.e. < 1 ppm (Table 1). Compared to the monography of water for injection which sets the limit for heavy metals at 0.1 ppm, it exceeds the critical concentration. However, the volume of nanosuspensions which is injected at once is much smaller than the volume of parenteral nutrition. Nanosuspensions are only a few milliliters in contrast to 0.5 l of fat emulsion. If the suspensions would be diluted to the same volume, the contamination would be even within these limits. Nevertheless in this analysis the load of the bulk material was not determined.

To sum up, the heavy metal contamination of nanosuspensions which are produced by high pressure homogenisation is higher than in other parenterals (e.g. water for injection) because of the metal erosion during the process. However, a foreign contamination below 10 ppm can be considered acceptable in most cases from the industrial point of view. The nanosuspensions were found even below 1 ppm. In addition the small volumes of nanosuspension typically administered lead to a total metal load that is mostly lower than an administration of an infusion based on water for injection. In our continued studies the contamination with other metals, which are ingredients of steel (Table 2) like chrome, nickel or copper, will be determined.

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