International Journal of Pharmaceutics, 103 (1994) 125–129

IJP 03345

Use of proton-induced X-ray emission (PIXE) analysis in the evaluation of large volume parenteral rubber stoppers

Jukka Pekka Mannermaa [a], Jyrki Räisänen [b], Mervi Hyvönen-Dabek [b], Erik Spring [b] and Jouko Yliruusi [c]

[a] *Orion Corporation, Orion-Farmos Pharmaceuticals, Oulu (Finland),* [b] *Department of Physics, University of Helsinki, Helsinki (Finland) and* [c] *School of Pharmacy, Division of Pharmaceutical Technology, University of Helsinki, Helsinki (Finland)*

(Received 27 April 1993) (Accepted 15 June 1993)

Key words: Particulate matter contamination; Rubber stopper; External beam proton-induced X-ray emission (PIXE) analysis

Summary

The use of external beam proton-induced X-ray emission (PIXE) analysis for determining the elemental composition of three different types of rubber stoppers used to seal parenteral solutions is described. The stoppers were immersed in 0.9% sodium chloride solution in conical flasks. The flasks were then autoclaved in a pilot autoclave using an F_0 value of 15 min at 121°C. The PIXE measurements were performed at the 2.5 MeV Van de Graaff accelerator in the University of Helsinki. The results show that the PIXE method clearly revealed the differences between different types of rubber stoppers. The elements found were, e.g., Fe, Ti, Zn, Br. The limits of detection were 2 ppm for Ti, 6 ppm for Fe, 2 ppm for Cu, 1 ppm for Zn, 3 ppm for Br and 0.5 ppm for Pb. The concentrations of a certain element vary greatly from sample to sample, for example, that of Zn by a factor of 4000. The elemental compositions of 4482 PH 4001/45 and 4782 PH 4106/50 are nearly identical, whereas the elemental composition of 4443 PH 21/50 is more complicated. Heavy elements were either non-existent or present in very low quantities. PIXE analysis is useful for detecting and quantifying elements with atomic numbers greater than 12. A particular feature of the method is that many elements can be analyzed simultaneously if a detection system with sufficient resolution is available. The sensitivity of the method at the ppm level and the fact that analyses may be performed on samples of small size make it a promising technique for analyzing the chemical composition of rubber stoppers. This method could be used in various pharmaceutical applications, since analysis can be performed under a normal atmosphere without requiring the use of a vacuum.

Introduction

Particulate matter contamination in parenterals is a major problem that has been studied from many points of view during the last few years (Groves and De Malka, 1976; Green et al., 1979; Borchert et al., 1986). In practice, the parenterals are usually filtered through 0.2 μm filters prior to filling. The main contamination risks after filtering are the air-borne particles or the particles released from primary packaging components. In particular, rubber stoppers are considered to be a prominent contamination source for parenteral products. Rubber stoppers contain many different ingredients that form a physical mixture (Technical Methods Bulletin, 1980).

Correspondence to: J.-P. Mannermaa, Orion Corp., Orion-Farmos Pharmaceuticals, Product Development, Orionintie 16, P.O. Box 65, 02101 Espoo, Finland.

SSDI 0378-5173(93)E0197-X

Previously, Mannermaa et al. (1992) studied the elemental composition of the surface of seven different rubber stoppers and the effect of sterilization on the stoppers using an energy dispersive X-ray analyzer. They reported that the elemental composition varied significantly between different types of stoppers.

Proton-induced X-ray emission (PIXE) analysis is useful for detecting and quantifying elements with atomic numbers greater than 12. A particular feature of the method is that many elements can be analyzed simultaneously. The sensitivity of the method at the ppm level and the fact that analyses may be performed on samples of small size without the use of a vacuum make it particularly attractive in environmental, biological and medical work as well as in complex pharmaceutical systems (Johansson and Cambell, 1988).

In this paper, we report the use of external beam PIXE at the Accelerator Laboratory of the University of Helsinki for analyzing the elemental composition of three different types of rubber stoppers used to seal parenteral solutions.

Materials and Methods

Handling and sterilization of stopper samples

The rubber stoppers used were all manufactured by Pharma-Gummi Wimmer West GmbH, Germany. Two of the stoppers were siliconized brombutyl stoppers, 4443 PH 21/50 representing an older formulation and 4482 PH 4001/45 a modern one. The third stopper was a modern non-siliconized chlorbutyl stopper 4782 PH 4106/50.

10 unwashed rubber stoppers were taken from each batch and were placed into 500 ml conical flasks with 200 ml of filtered (0.2 μm) 0.9% sodium chloride solution in a laminar flow cabinet.

The stoppers were sterilized in a microprocessor controlled pilot autoclave, GEV 612 AR-2 (Getinge Ab, Sweden). The flasks were placed into the autoclave chamber and sterilized to an F_0 value of 15 ± 0.5 min using a sterilization temperature of 121.0 ± 0.3°C.

Proton-induced X-ray emission (PIXE) analysis

PIXE is an analytical method based upon the measurement of characteristic X-rays. A beam of protons of 1–4 MeV energy is used to eject inner-shell electrons from atoms in a target whose composition is to be analyzed. When the thus resulting vacancies are filled by outer-shell electrons, characteristic X-rays whose energies identify the particular atom are emitted. In addition to the excitation of characteristic X-rays, a continuous background X-ray spectrum, mainly caused by secondary electron bremsstrahlung, is produced. This background radiation is very intense at low photon energies. The incident charged particles themselves also cause bremsstrahlung when they are decelerated in the target material. This process makes only a small contribution to the high-energy part of the spectra. A more dominant background at the high proton energy region is due to Compton scattering of gamma-rays originating from nuclear reactions induced by the bombarding protons with the sample nuclei. Taking into account both the variation in the cross-section for characteristic X-rays and the various background processes, 2.4 MeV protons are routinely used for PIXE in the Accelerator Laboratory, Department of Physics, University of Helsinki. The probing depth for 2.4 MeV protons is about 100 μm in typical biological material. The principles of PIXE have been discussed in detail, for example, by Johansson and Cambell (1988).

The measurements were performed in the open air at the 2.5 MeV Van de Graaff accelerator of the University of Helsinki. The X-rays were detected with a 50 mm$^2 \times 6$ mm intrinsic Ge detector having an energy resolution of 170 eV for the FeKα peak during data collection. Also a Si(Li) detector (80 mm$^2 \times 5$ mm) was used for comparison. An absorber of 625 μm thick Kapton (by DuPont, U.S.A.) was used to reduce the proportion of low-energy X-rays. The proton beam ($E_p = 2.4$ MeV) was brought into the air by use of a system described in detail earlier (Anttila et al., 1985). This beam exit set-up is specially designed for multi-element analysis of, in particular, biological samples, as the samples are cooled by cold nitrogen gas at atmospheric pressure. By using an

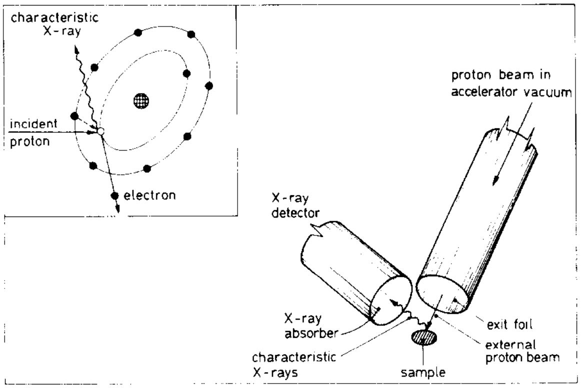

Fig. 1. The experimental arrangement used showing the exit foil, sample and detection geometry. The production process of characteristic X-rays is shown in the inset. Not to scale.

external beam sample preparation is easy and risks of sample contamination are avoided. Moreover, volatile elements may be detected, e.g., bromine.

The experimental arrangement is shown in Fig. 1 together with the schematics of the production procedure of characteristic X-rays.

To study the homogeneity of the samples the spectra were measured both from the intact surface and after cutting with a titanium knife.

To obtain a rough estimate of the absolute concentrations of the rubber samples, PIXE spectra of a standard (NBS Orchard leaves 1571, U.S.A.) were measured under conditions similar to those of the samples themselves. This standard was chosen because rubber standards were not available.

Results and Discussion

The elemental composition of the rubber stoppers studied are presented in Table 1. Fe was present in large quantities in all stoppers, as was expected due to the coloring pigment used in the stoppers. Differences were detected between the stoppers in the amount of Zn, which decreased from 4782 PH 4106/50 to 4443 PH 21/50 to PH 4001/45 and Br decreased from 4482 PH 4001/45 to 4443 PH 21/50 to 4782 PH 4106/50.

Heavier elements are non-existent in the stopper formulations 4482 PH 4001/45 and 4782 PH 4106/50, and present in very low concentrations in the 4443 PH 21/50.

Typical X-ray spectra from samples are shown in Figs 2–4. The peaks are denoted with the chemical symbols of the elements. Two peaks (Kα and Kβ) from each element are generally seen in the spectra. The measuring times were typically about 10 min.

The concentrations of elements in the three different samples are given in Table 1 in ppm dry weight. As can be seen, the concentrations of Zn vary from sample to sample even by a factor of 4000 (from 50 to 20000 ppm) followed by Br ranging from 50 to 7500. The amount of Fe used as a coloring agent is lower in the more modern stopper formulations, down from 7500 to 4500 ppm. Pb was present only in 4443 PH 21/50 at a concentration of 2 ppm.

The detection limits were evaluated on the basis that a peak can be observed if its area is $> 3\sqrt{N_b}$ where N_b is the integrated counts under the peak. The limits of detection were 2 ppm for Ti, 6 ppm for Fe, 2 ppm for Cu, 1 ppm for Zn, 3 ppm for Br and 0.5 ppm for Pb.

The results of the PIXE method separate the stoppers better than the scanning electron microscope/energy dispersive X-ray analyzer (SEM/EDXRA) data (Mannermaa et al., 1992). Average concentration values were obtained, since local variations in elemental concentrations were avoided by using a larger analysing beam spot

TABLE 1

Elemental concentrations of rubber stoppers in ppm (according to weight)

Element	Sample		
	4443 PH 21/50	4482 PH 4001/45	4782 PH 4106/50
Ti	500[a]	1600[a]	200[a]
Fe	7500[a]	8000[a]	4500[a]
Cu	10	10[a]	–
Zn	50	5[a]	20000
Br	100[a]	7500[a]	50[a]
Pb	2	–	–

[a] Elements detected by SEM/EDXRA (Mannermaa et al., 1992).

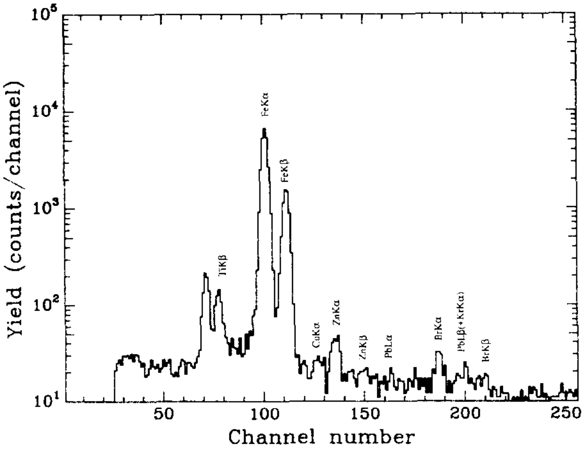

Fig. 2. A PIXE spectrum of stopper 4443 PH 21/50 obtained with 2.4 MeV protons showing clear peaks from titanium and iron. Peaks arising from copper, zinc, lead and bromine are also distinguished. The charge collected was 3.5 μC. The small KrKα peak is due to krypton in the air.

(the diameter of the beam spot was about 7 mm). Together with a greater probing depth, this explains the differences found between the results obtained with the SEM/EDXRA and PIXE analyses, since the rubber stoppers are physical mixtures of material particles in the millimeter range.

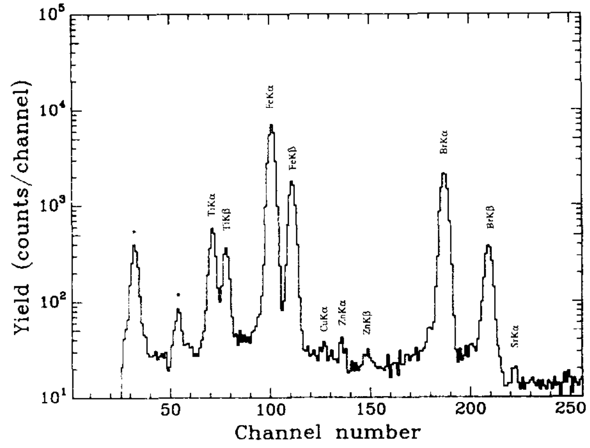

Fig. 3. A PIXE spectrum of stopper 4482 PH 4001/45 shows clear peaks from titanium, iron and bromine and smaller peaks from Zn and Sr. The peaks denoted by an asterisk are escape peaks. The charge collected was 3.5 μC.

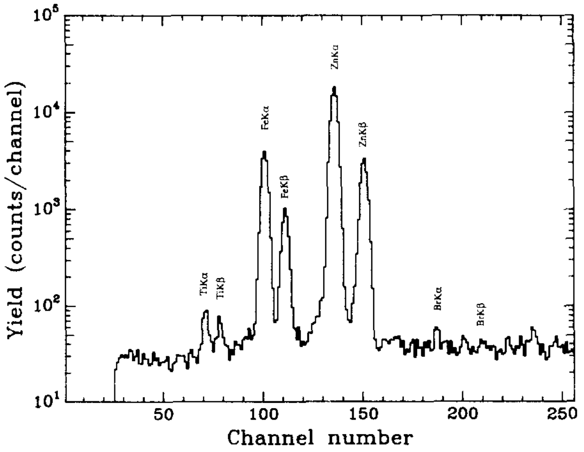

Fig. 4. A PIXE spectrum of stopper 4782 PH 4106/50 shows clear peaks from titanium, iron and zinc and smaller peaks from Br. The charge collected was 3.5 μC.

Because of the sensitivity and the versatility of the method, PIXE analysis is a powerful elemental analysis technique in pharmaceutical applications. For example, the quantitative elemental composition of tablets could be effectively determined with PIXE analysis. It could also be used in identifying particulate contamination in parenterals.

The leaching of the heavy metals to the solutions was not determined in this study. However, when they are found even in minute quantities, a certain probability of contamination is always present, especially when the stopper is a physical mixture of different components.

References

Anttila, A., Räisänen, J. and Lappalainen, R., On the optimization of an external PIXE arrangement. *Nucl. Instrum. Methods Phys. Res. B*, 12 (1985) 245–247.

Borchert, S.J., Abe, A., Aldrich, D.S., Fox, L.E., Freeman, J.E. and White, R.D., Particulate matter in parenteral products: A review. *J. Parenter. Sci. Technol.*, 40 (1986) 212–224.

Green, H., McNellis, J. and Steinman, S., The container size/volume relationship in particulate contamination. *J. Parenter. Drug Assoc.*, 33 (1979) 319–321.

Groves, M.J. and DeMalka, S.R., The relevance of pharmacopeial particulate matter limit tests. *Drug. Dev. Commun.*, 2 (1976) 285–288.

Johansson, S.A.E and Cambell, J.L., *PIXE: A Novel Technique for Elemental Analysis*, Wiley, Chicester, 1988.

Mannermaa, J.-P, Muttonen, E., Yliruusi, J. and Juppo, A., Comparison of different rubber stoppers; the effect of sterilization on the number of particles released. *J. Parenter. Sci. Technol.*, 46 (1992) 73–77.

Technical Methods Bulletin No. 1, Extractables from elastomeric closures: Analytical procedures for functional group characterization/identification, Parenter. Drug Assoc., 1980.