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Influence of the sterilization process on alginate dispersions

GEERT M. R. VANDENBOSSCHE, JEAN-PAUL REMON, *Laboratory of Pharmaceutical Technology, University of Ghent, Harelbekestraat 72, B-9000 Ghent, Belgium*

Abstract—The influence of different sterilization procedures on alginate dispersions was studied by measuring viscosity and molecular weight changes. Autoclaving caused a 64% decrease in viscosity. Heating at a low temperature over several cycles was less efficient in sterilizing alginates and there was a progressive breakdown of the alginate chain over the succeeding cycles. Heating during ethylene oxide sterilization also resulted in reduced viscosity and breakdown. Membrane filtration yielded a sterile product with no significant reduction in viscosity or mol. wt.

Because of their natural origin, alginates have to be cleared of impurities and microorganisms before their use in pharmaceutical and biotechnological applications. Applications in implantable biomaterials (Klein et al 1987) or in the immobilization of living cells require a sterile product. Calcium alginate beads with immobilized microorganisms (Burgess & Kwok 1991) or plant cells (Brodelius et al 1979) and alginate–polylysine microcapsules containing insect cells (King et al 1989) or mammalian cells (Duff 1985) are used in bioreactors. Alginate–polylysine microcapsules with a specific mol. wt cut-off are used to prevent immune rejection of implanted cells (Lim & Sun 1980; Sun 1988). The modification of the mol. wt cut-off of the microcapsules requires raw materials with constant physicochemical characteristics.

Although it has been shown that autoclaving alginate has a deleterious effect on the viscosity (Coates & Richardson 1974; Hartman et al 1975; Leo et al 1990), alginate dispersions have been sterilized for 20 min at 121°C (Kupchik et al 1983; Edmunds et al 1989), for 15 min at 100°C (King et al 1989) and for 20 min at 80°C (Roscoe & Owsianka 1982). Sun (1988), Kwok et al (1989) and Burgess & Kwok (1991) used membrane filtration.

This communication describes the influence of different sterilization techniques on the viscosity, the mol. wt distribution and the sterility of the alginate. Sterilization with ionizing irradiation was not performed as both Coates & Richardson (1974) and Hartman et al (1975) showed that after irradiation (2·5 mrad) only 40% of the initial viscosity was preserved.

Materials and methods

A 1% (w/v) dispersion of alginate (Manucol DH, Kelco, London, UK) in Moscona's solution (950 mL distilled water, 8·0 g NaCl, 0·3 g KCl, 0·05 g NaH_2PO_4, 0·025 g $KH_2PO_4 \cdot H_2O$, 1·0 g $NaHCO_3$, 2·0 g dextrose, HCl 1 M to pH 7·2) was divided into several 100 mL fractions. The first fraction was autoclaved (Speedclave, Ritter, Rochester, NY, USA) for 20 min at 120°C. Three other 100 mL fractions were exposed to 2, 3 or 4 heating cycles (30 min at 80°C) and subsequent incubation (24 h at 25°C) (Wallhäusser 1984). Four other fractions were sterilized using two types of membrane filters. Several cellulose acetate membrane filters (0·22 μm; SM16534, Sartorius, Göttingen, Germany) were required to filter the alginate dispersion. The viscosity was determined for the collected filtrates from 0 to 5 mL, 5 to 10 mL and 10 to 150 mL, respectively. Only one cellulose nitrate membrane filter (0·45 μm; SM11306, Sartorius) was required for the filtration of 100 mL alginate dispersion.

Correspondence: J. P. Remon, Laboratory of Pharmaceutical Technology, University of Ghent, Harelbekestraat 72, B-9000 Ghent, Belgium.

The alginate powder was sterilized by ethylene oxide (KGS-2590, Münchener Medizin Mechanik, München, Germany). An under-pressure of 93 kPa was maintained for 60 min at 50°C. The ethylene oxide concentration was 1·4 g L^{-1} and the relative humidity was 50%. The sample was aerated for two weeks at 22°C and at a relative humidity of 45%.

The initial degree of contamination was determined on Agar Bacteriological nr 1 (Oxoid, Hampshire, UK) after preparation of serial dilutions in Bacto peptone (Difco, MI, USA). The presence of bacteria and yeasts after sterilization was determined by the addition of 1 mL of the alginate dispersion to 20 mL Clausen (Oxoid, Hampshire, UK) and Sabouraud (Oxoid, Hampshire, UK) liquid media, respectively. Ten tubes of each medium were incubated at 25 and 37°C and evaluated after 3 weeks. Sterility was concluded if none of the tubes showed growth.

The viscosity of the alginate dispersions was measured at 25°C with an RV12 rotoviscositymeter (Haake, MV I, Karlsruhe, Germany). The shear stress was read after 2 min at 256 rev min^{-1} and the viscosity was calculated. The mol. wt distribution of the alginate was determined by high pressure gel permeation chromatography. Samples were injected via a WISP 710A injector (Millipore, Brussels, Belgium) on two serial columns (Ionpack S 805 and Ionpack S 804, 50 cm length, Shodex, Eupen, Belgium) and eluted with 0·9% NaCl at 70°C and 7·6 MPa. An Erma refractive index detector (ERC-7510, Brussels, Belgium) was used at 35°C. The average number ($M\bar{n}$) and average weight ($M\bar{w}$) mol. wt were calculated using pullulan standards (P-82, Shodex) with mol. wts ranging from 850 000 to 5800 Da.

Results

Microbiology. The initial load of the alginate dispersion was about 10^5 mL^{-1}. Ethylene oxide sterilization, followed by aseptic dispersion, autoclaving, heating for 4 cycles and membrane filtration with a 0·22 or a 0·45 μm membrane filter, all yielded sterile products. After 2 or 3 heating cycles a sterile product was not obtained and staining revealed mainly Gram-positive rods.

Viscosity. Autoclaving caused a decrease in viscosity from 31 to 11 mPa s (Fig. 1). Heating resulted in a progressive decrease in viscosity over multiple cycles. After 4 cycles the viscosity was 19 mPa s. The filtrates obtained from membrane filtration through a 0·22 μm membrane filter showed a slight decrease in viscosity in function of the volume filtered. This was inversely related to the increasing pressure needed to continue the filtration.

The viscosity of samples from the untreated dispersion was 31 mPa s with a standard deviation of 0·9 mPa s, whereas membrane filtration of six samples of an alginate dispersion through a 0·45 μm membrane filter showed a viscosity of 30 mPa s and a standard deviation of 1·0 mPa s. According to the Mann-Whitney U test (Siegel 1956) no significant difference was found after membrane filtration compared with the untreated dispersion ($n = 6$, $P > 0·05$).

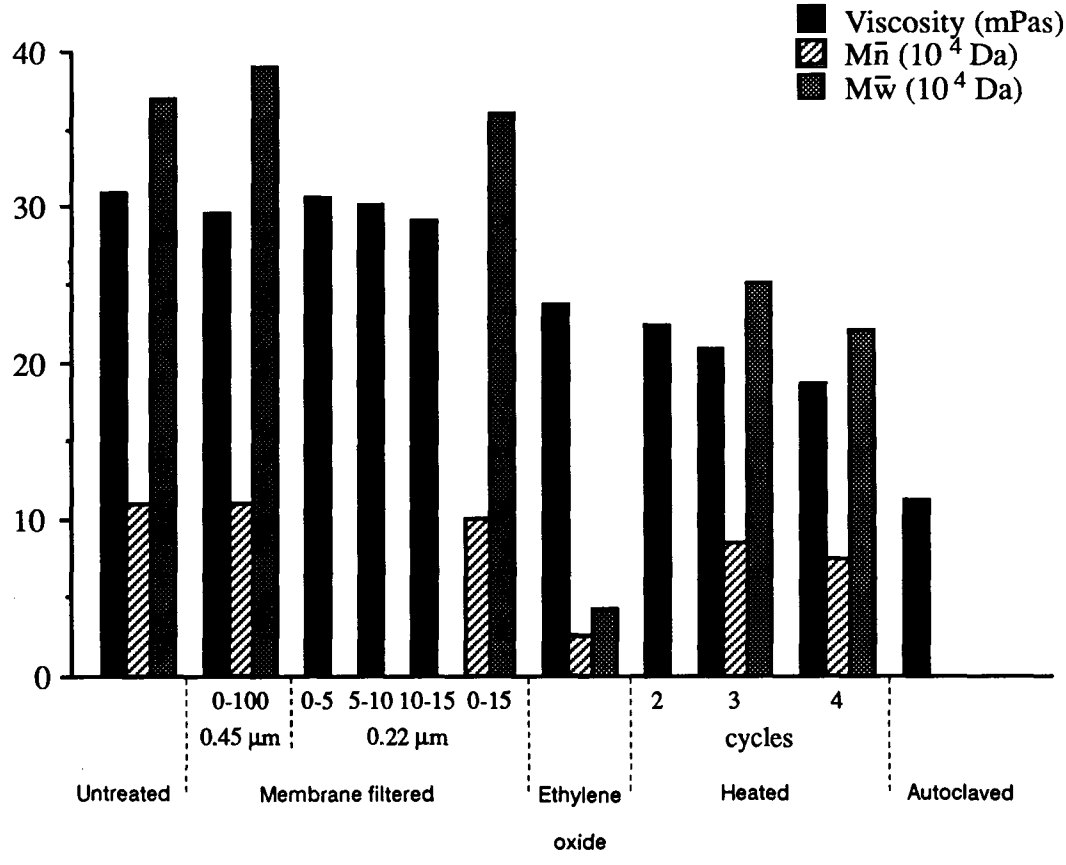

FIG. 1. Viscosity (mPa s) and (if available) average number (Mn̄) and weight (Mw̄) mol. wt of alginate dispersions: untreated, membrane filtered through a 0·45 µm filter, collected (0–5, 5–10, 10–15 mL) after membrane filtration through a 0·22 µm membrane filter, ethylene oxide sterilized, heated over 2, 3 or 4 cycles or autoclaving.

Gel permeation chromatography. Sterilization by 4 heating cycles resulted in a decrease of the Mn̄ and Mw̄ from $1{\cdot}1 \times 10^5$ to $7{\cdot}4 \times 10^4$ and from $3{\cdot}7 \times 10^5$ to $2{\cdot}2 \times 10^5$, respectively. Only a slight decrease in Mn̄ and Mw̄ to $1{\cdot}5 \times 10^5$ and $3{\cdot}6 \times 10^5$, respectively, was observed after membrane filtration through a 0·22 µm membrane filter, and no decrease was found after filtration through a 0·45 µm membrane filter with a standard deviation of $5{\cdot}2 \times 10^4$ (Mn̄) and of $2{\cdot}7 \times 10^4$ (Mw̄).

Discussion

Autoclaving an alginate dispersion caused a decrease in viscosity of about 64%. This is in agreement with data reported by Coates & Richardson (1974) and Leo et al (1990). The degree of breakdown was related to the temperature (Leo et al 1990) and to the time of exposure. During heating cycles used here the sample is submitted to lower temperatures than during autoclaving and therefore the strongly sporulating bacilli are not eliminated. This might explain the necessity to include three incubation periods at 25°C during which the spores germinate. Leo et al (1990) did not find a decrease in viscosity after 15 min at 80°C, whereas in our study a progressive breakdown of the alginate chains was found after multiple exposures during heating cycles. This was confirmed by the decrease in viscosity and average mol. wt (Mn̄ and Mw̄).

The longer exposure time and higher temperature during ethylene oxide sterilization of 7 h at 57°C (Leo et al 1990) as compared with 1·5 h at 45°C (Coates & Richardson 1974) might partially explain the difference in decrease of viscosity (60% vs < 1%). In our experimental conditions an exposure time of 1 h at 50°C induced a decrease in viscosity of 24%.

The decrease in viscosity and average mol. wt, observed as a function of the volume filtered through a 0·22 µm membrane filter, was probably caused by clogging of the filter. Large molecules or impurities might block the pores and therefore reduce the pore size. This is also indicated by the progressively increasing counter-pressure as a function of the volume filtered. The filtrate is more transparent than the turbid, brown-tinted, untreated alginate dispersion. Filtration through a 0·45 µm membrane filter showed a minimal influence on the viscosity and the mol. wt distribution of the alginate dispersion, but yielded nevertheless a sterile product. Although the filtrate from the 0·45 µm filter obtained in this study was sterile, it is advisable to perform filtration through a 0·22 µm membrane filter. Filtration of large quantities of alginate dispersions through a 0·22 µm membrane filter is facilitated by a prefiltration step through a 1·0 µm filter or can be directly applied on a Pronova MVG (Protan, Drammen, Norway) alginate dispersion which is already filtered through a 0·22 µm membrane filter by the supplier (data not shown).

In conclusion, sterilization by heat causes a breakdown of the alginate molecules and should therefore not be used to sterilize alginate dispersions. No significant changes in viscosity and average mol. wt were observed after membrane filtration. For applications in which the mol. wt distribution of the alginate is critical, membrane filtration is the method of choice.

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Light stability of molsidomine in infusion fluids

G. M. R. VANDENBOSSCHE, C. DE MUYNCK, F. COLARDYN*, J. P. REMON, *Laboratory of Pharmaceutical Technology, University of Ghent, Harelbekestraat 72, B-9000 Ghent, Belgium, and *Intensive Care Department, University Hospital, De Pintelaan 185, B-9000 Ghent, Belgium*

Abstract—The influence of artificial light, daylight or stimulated sunlight on the stability of molsidomine was investigated. In static experiments an 80 $\mu g\ mL^{-1}$ solution of molsidomine in saline was stored in an unprotected infusion bag. During dynamic experiments the molsidomine solution (80 $\mu g\ mL^{-1}$) was dropped at 12·5 $mL\ h^{-1}$ from an unprotected infusion bag, from an infusion bag covered with aluminium-foil, or from an infusion bag protected with a UV-cover. Either unprotected infusion tubing or infusion tubing with a UV-filter were connected to the infusion bags. Static as well as dynamic experiments showed a half-life of about 20 min for the unprotected molsidomine solutions, when placed behind a window during a sunny day. Protection from light of the infusion bag but not of the infusion tubing had only a minor influence on the drug half-life. Protection of the infusion bag and the infusion tubing with a UV-filter increased the half-life to several days. These results confirm that both the infusion bag and the infusion tubing need adequate light protection during molsidomine administration.

In current clinical practice, antianginal drugs such as nitroglycerin and isosorbide dinitrate during the acute phase of myocardial infarct are often replaced by molsidomine. In addition to its vasodilating effect, molsidomine has anti-platelet aggregation properties and shows a lower tolerance than other anti-anginal drugs (Mukharljamov et al 1986). Apart from these pharmacological differences, some important physicochemical characteristics need to be considered. Nitroglycerin and isosorbide dinitrate are subject to adsorption and absorption to administration sets. This results in a decrease (up to 35%) of the amount of the drug available during the first 5 h of administration, depending on the polymer materials of the medical devices used during therapy.

Correspondence: J. P. Remon, Laboratory of Pharmaceutical Technology, University of Ghent, Harelbekestraat 72, B-9000 Ghent, Belgium.

In this study the stability of molsidomine in adminstration sets was investigated. The leaflet of molsidomine i.v. ampoules (Corvaton, Therabel Pharma, Brussels, Belgium) recommends protection from light. As this advice is not always followed in daily clinical practice, the stability of molsidomine was compared in several experimental arrangements. The stability in unprotected, partially protected and UV-protected administration sets, exposed to different light sources, was compared. Preliminary data have already been presented (De Muynck et al 1992).

Materials and methods

Dilution and administration sets. For both static and dynamic experiments, molsidomine ampoules were diluted to a concentration of 80 $\mu g\ mL^{-1}$ in 50 mL saline (0·9% NaCl w/v) in polyvinylchloride (PVC) infusion bags (Viaflex, Lessines, Belgium). Static experiments were performed with unprotected infusion bags. Samples were taken every 10 min for 3 h, every hour over 10 h and after 24 h. In the dynamic experiments an unprotected infusion bag and an infusion bag covered with aluminium foil were connected to unprotected PVC infusion tubing (Shore A hardness 65, plasticizer: DEHP, Rehau, Rehau, Germany), whereas an infusion bag protected with a UV-cover (Sureset A261, Avon Medicals, Reddich, UK) was connected to