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Research paper

Radiolabelling of parenteral O/W emulsions by means of neutron activation

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Abstract

Parenteral O/W emulsions containing lanthanide fatty acid derivatives were prepared. With regard to enhancing the incorporation efficiency of the neutron activatable excipients, the addition of the non-ionic co-emulsifier Solutol HS 15® proved to be most suitable. Comparing the different chain lengths of the fatty acids, the long chain fatty acid derivative lanthanide(tri)stearate seemed to be superior in strengthening the interfacial layer. After neutron activation, the physical and chemical stability of the irradiated formulations was evaluated. The chemical stability, indicated by the concentration of lyso phosphatidylcholine as the degradation product of the main emulsifier, was shown to be dependent on the irradiation time. By applying a neutron flux of 2.1×10^{13} neutrons/cm^2 per s, the maximum should not rise above 60 s. The physical stability indicated by the particle size distribution was affected by the presence of the non-ionic co-emulsifier. Concerning the amount of radiation necessary for in vivo biodistribution studies the maximum load of Samarium fatty acid derivatives did not yield sufficient radioactivity levels. However, Europium derivatives could be shown to be suitable for in vivo studies.

Keywords: O/W Emulsion; Neutron activation; Radiolabelling; Lanthanide

1. Introduction

The application of radionuclides in pharmaceutical dosage form investigations has been established for some time [1]. However, there are certain limitations, which have an impact on the possibilities of radiolabelling. Due to the very short half-lives of radioactive isotopes the manufacturing procedure chosen for the production of the dosage is time-limited. Other restrictions on the manufacturing process as, for instance, the materials and methods used and the production scale are due to the risk of contamination.

Neutron activation has been proved to be a very efficient and safer alternative [2,3]. Low amounts of stable isotopes with suitable nuclide data such as favourable half-lives and neutron capture cross-sections can be incorporated during normal manufacturing procedures. Only the subsequent irradiation of the final dosage form with an appropriate neutron flux leads to radioactive gamma-emitting nuclides. The approximate amounts of isotope derivatives necessary to yield the desired radioactivity levels for biodistribution studies by means of scintigraphic imaging, can be calculated according to the neutron activation equation [4]. In assessing the feasibility of neutron activatable markers, the maximum load and the irradiation time were found to be of crucial importance.

When designing parenteral O/W emulsion formulations as drug delivery systems with targeted functions, there is a need to monitor the in vivo biodistribution in a non-invasive manner. For this radiolabelling by neutron activation appeared to be quite promising. Lipophilic derivatives of lanthanides could be considered as possible neutron activatable excipients in emulsion formulations. However, the incorporation of stable isotope derivatives

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requires the determination of the maximum load that does not affect the physical and chemical stability of the colloidal systems.

Previous investigations concerning the long-term stability of parenteral O/W emulsions showed that the use of mixed emulsifying agents was superior in strengthening the interfacial layer. The emulsion stability could be considerably improved by the addition of a non-ionic co-emulsifier providing a steric repulsion effect [5].

Exposing emulsion formulations to an intense thermal and epithermal neutron flux implies that there will be an impact on the physical and chemical stability of the colloidal systems. The decomposition of radiolabelled compounds by radiation emitted from the radioactive nuclides, which is called radiolysis, is dependent on the photon energies.

The objective of the present work was to evaluate the physical and chemical stability of parenteral O/W emulsions modified with lanthanide fatty acids prior to and after neutron activation. The non-ionic co-emulsifier Solutol HS 15® was added to improve the incorporation efficiency and the long-term stability of the emulsion formulations.

2. Materials and methods

2.1. Materials

The soya bean lecithin, Phospholipon 80®, was kindly provided by Nattermann GmbH (Köln, Germany). The non-ionic surfactant Solutol HS 15® was supplied by BASF (Ludwigshafen, Germany). The lanthanide fatty acids were synthesized and characterized in our laboratory. For the synthesis, Samarium(tri)chloride (>99%) and Europium(tri)chloride (>99.9%) from Aldrich (Germany) and Sodium laurate (>99%) and Sodium stearate (>98%) from Fluka (Germany) were used. The arachis oil was furnished by Lamotte (Bremen, Germany). The used chemicals were of pharmaceutical grade.

2.2. Methods

2.2.1. Emulsion preparation

All emulsion formulations studied consisted of 20% (w/w) arachis oil, 1.5% (w/w) soya bean lecithin and bidistilled water. The phospholipid Phospholipon 80® as well as the lanthanide fatty acid derivatives (0.05–0.25% w/w) were dispersed in the arachis oil. The non-ionic emulsifier Solutol HS 15® (1% w/w) was dissolved in the bidistilled water. Both phases were heated separately at 40°C and dispersed by an ultra-turrax (T25, Jahnke und Kunkel, Staufen, Germany) at 8000 rpm for 3 min. This coarse emulsion was passed seven times at 30 MPa through a high pressure homogenizer (Micron Laboratory 40, APV Gaulin, Lübeck, Germany). The emulsions were sterilized by autoclaving for 20 min at 121°C.

2.2.2. Particle characterization

The volume distribution of the particle size (D 50%-value, D 99%-value) was analyzed using laser diffractometry (Helos, Sympatec, Clausthal-Zellerfeld, Germany) after emulsion preparation, autoclaving and neutron activation, respectively. Particle charge was determined by electrophoresis measurements using a Malvern Zetasizer 3 (Malvern Instruments, UK) and expressed as zeta potential. Samples were diluted in 0.5 mmol/l NaCl prior to measurement.

2.2.3. Neutron activation

For neutron activation the research reactor FRG-1 at the GKSS Nuclear Research Center Geesthacht, Germany was used. This is a swimming pool reactor with a thermal power of 5 MW. The labelled emulsions were irradiated in a neutron flux of 2.1×10^{13} N /cm^2 per s. The irradiation time was varied between 30 s and 10 min. The radioactive purity of the derivatives was determined using a high resolution Ge(Li) detector interfaced with a multichannel analyzer.

2.2.4. Neutron activation equation

As indicated by the neutron activation equation (Eq. (1)), there are a number of parameters that can be varied permitting a maximum flexibility of the technique.

$$A = \sigma \cdot \phi \cdot N \cdot \left(1 - e^{-\lambda \cdot t_{irr}}\right) \quad (1)$$

with:

A: activity (Bq) = (disintegrations/s)
σ: neutron capture cross-section (barn = 10^{-28} m^2)
ϕ: neutron flux (neutrons/s per cm^2)
N: number of target atoms present
λ: decay constant (time^{-1}) = ln $2/T_{1/2}$
with: $T_{1/2:\ \text{isotope half-life}}$
t_{irr}: irradiation time (time)

For in vivo biodistribution studies, the radioactivity level necessary is set to be minimum 30–40 MBq for 1–2 days observations and 50–100 MBq for 3–4 days observations. Eq. (1) shows that the resulting radioactivity is linearly related to the neutron capture cross-section, which is defined by the isotope chosen and to the neutron flux which can be regarded as a constant of the nuclear reactor used. So the only two parameters that can be varied are the number of target atoms which is dependent on the incorporated amount of stable isotope and the irradiation time. If the irradiation time is very small in comparison to the isotope half-life, the resulting radioactivity can be considered to be proportional to it.

2.2.5. Choice of isotope

The radioactive isotopes were carefully chosen so that they can be followed by external scintigraphy. To label O/W emulsion formulations, it was necessary to incorporate lipophilic derivatives of neutron activatable markers.

Table 1

Nuclide parameters of isotopes of interest [11]

Stable isotope (natural abundance)	Thermal neutron capture cross-section (barns)	Radioactive isotope	Half-life (h)	Photon energies (MeV)
Eu-151 (47.8%)	3150	Eu-152m	9.27	0.841 0.963
Sm-152 (26.7%)	206	Sm-153	46.75	0.097 0.103
Na-23 (100%)	0.53	Na-24	14.96	1.369 2.754
K-41 (100%)	1.46	K-42	12.36	1.525

Lanthanides are considered to have very suitable physical properties, such as favourable neutron capture cross-sections and half-lives for neutron activation (Table 1). To increase their solubility in an oil-emulsifier phase fatty acid derivatives were synthesized by a precipitation method [6].

Most of the articles in the literature refer to samarium as the neutron activatable marker of choice, due to its very favourable isotope half-life of 46.7 h and its very suitable photon energies of 103 keV [7]. The radiation detection by means of a gamma camera shows the highest resolution in the range of 70–400 keV. The gamma camera is most sensitive in the range between 100 and 250 keV. However, the relatively low neutron capture cross-section of ^{153}Sm (210 barns) limits its usefulness for several scintigraphic studies due to insufficient radioactivities resulting [8]. So alternatively, an Europium isotope (^{151m}Eu) was used for the production of radiolabelled emulsion systems after neutron activation. ^{151m}Eu has a markedly increased neutron capture cross-section of 3150 barn, qualifying it to lead to suitable radioactivity levels for biodistribution studies of radiolabelled colloidal systems. The major drawback of the Europium isotope is its half-life of 9.27 h. Contamination of emulsion components with traces of sodium and potassium is unavoidable [9]. After irradiation it leads to the formation of the undesired radionuclides ^{24}Na ($t_{1/2}$ = 14.95 h) and ^{42}K ($t_{1/2}$ = 12.36 h) with high photon energies of 1369 and 2754 keV for ^{24}Na and 1525 keV for ^{42}K. To avoid interference with subsequent analyses, the activities due to ^{24}Na and ^{42}K need to decay to background levels prior to measurements to obtain high radionuclide purity. So one has to consider that, for instance, a 15 h decay period is equivalent to 1.67 half-lives of ^{152m}Eu which is equivalent to 1 and 1.33 half-lives of ^{24}Na and ^{42}K, respectively.

2.2.6. Neutron flux

The neutron flux of the nuclear research reactor FRG-I at the Nuclear Research Center at Geesthacht was determined prior to each measurement. It turned out to be very stable and can be stated as follows:

Thermal neutron flux: $2.1 \cdot 10^{13}$ N/cm^2 per s
Epithermal neutron flux: 2.5 ± 0.05%
Fast neutron flux: 16.1 ± 0.08%

2.2.7. Number of target atoms

The number of targeted atoms of the stable isotope chosen is dependent on its natural abundance and can be calculated as follows:

$$N = m \frac{N_L}{M} I \quad (2)$$

with:

m: incorporated amount of stable isotope
N_L: Avogadro constant (Loschmidt number)
M: atom weight
I: isotope abundance

2.2.8. Chromatography analysis

The amount of lyso phosphatidylcholine was determined by high performance thin layer chromatography (HPTLC, Camag, Berlin, Germany). The quantitative analysis was performed by densitometric measurements (CAMAG TLC Scanner II, CAMAG, Berlin, Germany) at 510 nm. The R_f-value of lyso phosphatidylcholine was determined to be 0.30.

3. Results and Discussion

3.1. Incorporation efficiency

The incorporation of lanthanide fatty acids into parenteral O/W emulsion formulations was limited in a dose-dependent manner. The maximum load of the emulsions was found to be 1 μmol/ml independent from the chain length of the lanthanide fatty acid incorporated and from the lanthanide cation used (Fig. 1). However, the addition of polyethylene glycol-660-12-hydroxy stearate (Solutol HS

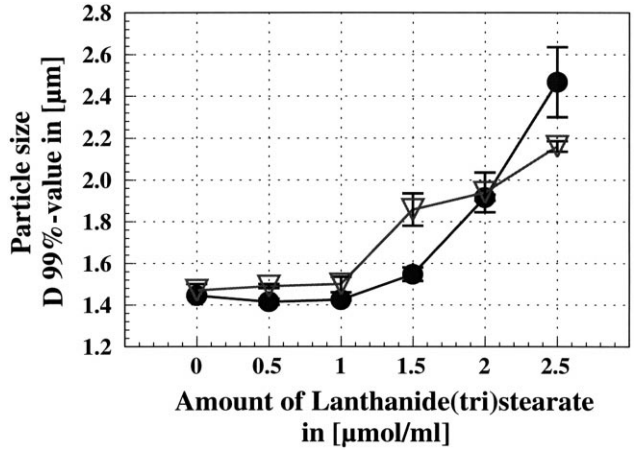

Fig. 1. Particle size distribution (D 99%-value) in dependence on the lanthanide(tri)stearate chosen (•, Sm-Stearate; ▽, Eu-Stearate).

15®) led to a significant increase in incorporation efficiency prior to autoclaving (Fig. 2). This might be correlated to a steric stabilization of the interfacial layer. The fatty acid chain of the lanthanide derivative is bound up into the interface of the oil droplet. So the non-ionic PEG-derivative provides stability in the surrounding medium by a repulsion effect. After autoclaving the deviation of the particle size of the Solutol HS 15® modified emulsion formulations prepared with up to 1 μmol/ml of lanthanide fatty acid was negligible. However, loads of lanthanide fatty acids higher than 1 μmol/ml lead to an increased instability as well (Fig. 3). The reason for it was determined to be the zeta potential of the modified formulations. With increased amounts of lanthanide fatty acids the negative zeta potential increased leading to a marked instability of O/W emulsions. Regarding long-term stability, the zeta potential of a stable O/W emulsion should not rise above −30 mV. By the addition of lanthanide(tri)laurate derivatives, the zeta potential was shown to be increased to values within the critical range of −30 ± 5 mV. In the presence of Solutol HS 15®, the negative zeta potential increased even further due to a shielding of the negatively charged particle surface by the non-ionic PEG-chains of the co-emulsifier. As the electrostatic stability of the colloidal formulations became insufficient with increased amounts of lanthanide(tri)laurate, the incorporated amount of lanthanide(tri)laurate should not rise above 1 μmol/ml. By using long chain fatty acid derivatives, lanthanide(tri)stearate derivatives, the impact of the incorporated amount was less pronounced. The zeta potential of those formulations was measured to be in the range of −40 ± 5 mV. After autoclaving, there was a decline of the negative zeta potential. However, the absolute values did not rise above −35 mV. By incorporation of Solutol HS 15®, the negative zeta potential was increased by another 5 mV reaching the critical range of −30 mV. The results showed lanthanide(tri)stearate to be most favourable due to sufficient electrostatic stabilization (Fig. 4).

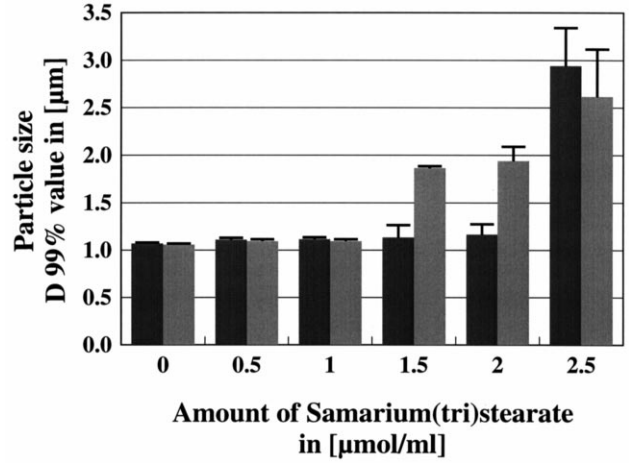

Fig. 3. Particle size distribution of Solutol HS 15® modified O/W emulsions prior to (■) and after autoclaving (shaded box).

Lanthanide fatty acid derivatives are metallo-organic salts of a trivalent cation (Samarium, Europium) and monovalent anions (fatty acids carboxylates) in the ratio 1:3. By incorporating trivalent cations in the emulsifier interface layer, an immense compression of the inner Helmholtz layer occurred resulting in a sharp decrease of the potential curve near the particle surface and causing the zeta potential to be diminished. The lower the zeta potential of colloidal particles the less the energy barrier between adjacent droplets and the more likely coalescence may occur. In the presence of Solutol HS 15®, the electrostatic barrier is lowered but the non-ionic co-emulsifier provides an additional steric stabilization to the emulsion particles. However, if a closer look is taken at the particle size distribution after autoclaving, the maximum possible load of 1 μmol/ml corresponds to the maximum zeta potential values as well.

The different behaviour of the lanthanide(tri)laurate and the lanthanide(tri)stearate derivatives was only due to the different chain length of the fatty acid. The kind of lanthanide cation chosen turned out not to have a significant effect

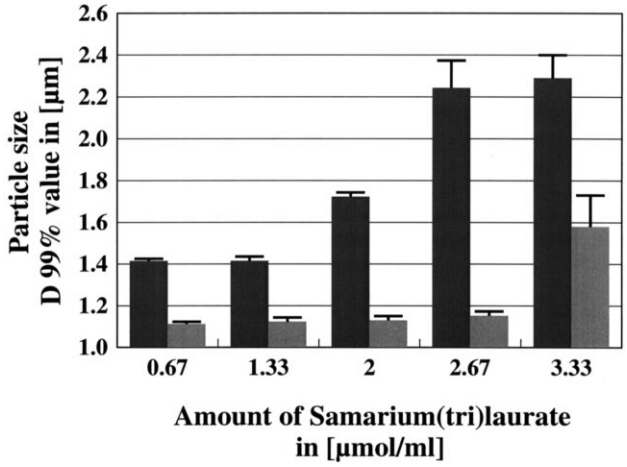

Fig. 2. Particle size distribution (D 99%-value) depending on the addition of Samarium(tri)laurate after manufacturing. (■) without Solutol HS 15®, (shaded box) with Solutol HS 15®.

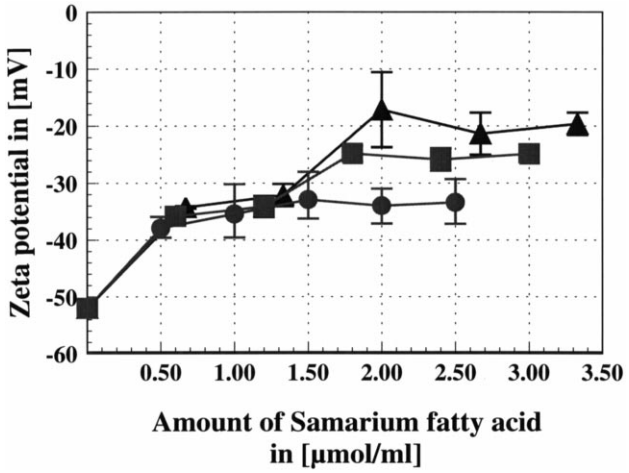

Fig. 4. Zeta potential of Solutol HS 15® modified O/W emulsions depending on the amount of samarium fatty acid added (▲ Sm-Laurate, ■ Sm-Myristate, • Sm-Stearate).

Table 2

Calculated irradiation times for lanthanide fatty acid derivatives according to the neutron activation equation: thermal neutron flux, 2.1×10^{13} N/cm^2 per s; desired radioactivity, 30–40 MBq (1–2 days observation), 50–100 MBq (3–4 days observation)

Desired radioactivity	Amount of activatable isotope (in μg/0.5 ml emulsion)	Irradiation time (min)	
		30–40 MBq	50–100 MBq
Samarium	45.2	608–833	1071–2538
	135.7	192–259	326–681
	226.1	114–153	192–395
Europium	50.7	3.80–8.89	6.34–12.74
	152.2	1.26–2.95	2.11–4.22
	253.5	0.76–1.70	1.26–2.53

on the zeta potential. This was probably due to the very similar physico-chemical properties of those lanthanide cations based on the comparable ionic radii. Since the chain length of the fatty acid derivative used, had such a pronounced impact on the absolute values of the zeta potential, a difference in the charge density at the particle surface can be assumed. It was most likely that the medium chain fatty acid derivatives such as Samarium(tri)laurate and Europium(tri)laurate lead to an increased packing ratio within the interfacial layer, thus resulting into a higher charge density of the cations which is expressed as less negative zeta potentials. On the contrary, lanthanide derivatives of the long chain fatty acid stearic acid showed a more negative zeta potential as a result of a diminished presence of trivalent cations at the particle surface.

3.1.1. Neutron activation

As the maximum incorporation efficiency was known, it was possible to calculate the irradiation time necessary according to the neutron activation equation (Table 2). As can be seen in Table 2, the irradiation times necessary for Samarium and Europium fatty acid derivatives to yield sufficient radioactivity levels for in vivo biodistribution studies vary by a factor of about 150. For Samarium derivatives, irradiation times of an hour and above must be considered to cause radiolysis within the colloidal systems due to the high photon energies. In the case of Europium derivatives, irradiation times of less than 15 min have been calculated. They have been assumed to be suitable for neutron activation.

The Europium-labelled emulsion formulations were irradiated in a neutron flux of 190 MGy/h for 60 s. An almost linear relationship between the amount of Europium fatty acid derivative incorporated and the resultant radioactivity could be determined (Fig. 5). The presence of Solutol HS 15® had no significant impact on the total radioactivity level. The nuclide purity was found to be >99% as indicated by Table 3. The gamma spectrum revealed minor quantities of impurities ^{24}Na, ^{42}K and ^{56}Mn. These impurities arose from minor contaminants in the other emulsion formulation components such as bleaching residues from the emulsifier.

3.1.2. Physico-chemical stability after irradiation

For stability, the activation time turned out to be the most important factor. An increase of the irradiation time led to a significant instability of the emulsion formulations (Fig. 5).

After neutron activation the increase in lyso phosphatidylcholine concentration has been taken as a measure for the chemical stability of parenteral O/W emulsions towards radiation, in terms of an indicator of hydrolysis/radiolysis. Lyso phosphatidylcholine is the degradation product of the main emulsifier. Degradation may occur due to temperature and/or pH-induced hydrolysis and to radiolysis as well. Depending on the exposure time, the reference emulsion formulations showed a radiation dose-dependent stability. The lyso phosphatidylcholine concentration was measured to be increased by 50% after 60 s of irradiation. An exposure time of 5 and 10 min led to marked instabilities of the emulsion formulations. The high performance thin layer chromatograms could not be differentiated. Instead of receiving single bands due to the separated components in relation to their R_f-values a broad zone was detected overlaying the areas of interest.

The chemical stability of Europium-labelled emulsions after 60 s of neutron activation was found to be critical. An increase of about 30% in lyso phosphatidylcholine con-

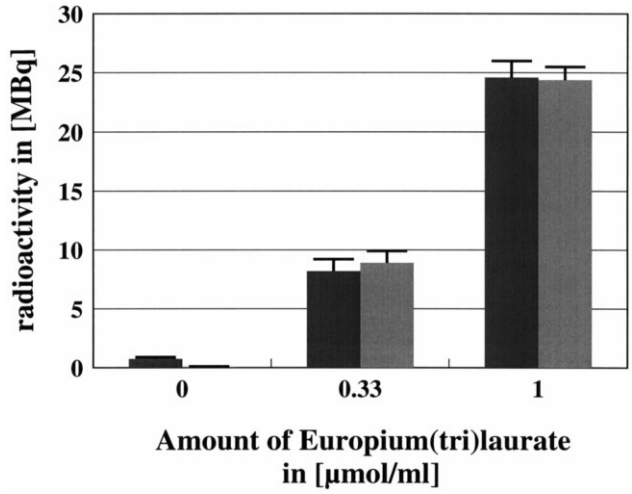

Fig. 5. Radioactivity of Europium(tri)laurate labelled O/W emulsions as confirmed by gamma spectroscopy (60 s irradiation time). (■) without Solutol HS 15®, ( ) with Solutol HS 15®.

Table 3

Nuclide purities of the isotopes of interest

Isotope	Average (%)	Standard deviation (%)
Samarium - 153 (Incorporated as Samarium fatty acid derivative)	83.5	±4.10
Europium - 152m (Incorporated as Europium fatty acid derivative)	100.5	±4.46

tent was measured indicating that radiolysis occurred (Fig. 6). However, the absolute values were below 10% (related to the total phosphatidylcholine content of the main emulsifier Phospholipon 80®). The literature differs about the maximum tolerable amount of lyso phosphatidylcholine in parenteral formulations. Some authors even allow up to 20% [10]. Sauders reported about a complex formation between phosphatidylcholine and lyso phosphatidylcholine which reduces the toxic potential of the degradation product and improves the emulsion stability. However, since lyso phosphatidylcholine was proved to show hemolytic activity, toxic side effects need to be taken into account. However, radiolabelled emulsion formulations by means of neutron activation are supposed to be used for single applications, which allows higher amounts of lyso phosphatidylcholine to be tolerable.

Concerning the physical stability, the particle size distribution prior to and after neutron activation was taken as an indicator. As is shown in Fig. 7, the maximum particle sizes of irradiated Europium-labelled emulsions were detected to be well above 35 μm, which excludes them from parenteral applications. However, in the presence of a non-ionic co-emulsifier it was possible to expose emulsion formulations modified with Europium derivatives to irradiation without affecting the physical stability. For parenteral applications, maximum particle sizes of 5 μm are tolerable.

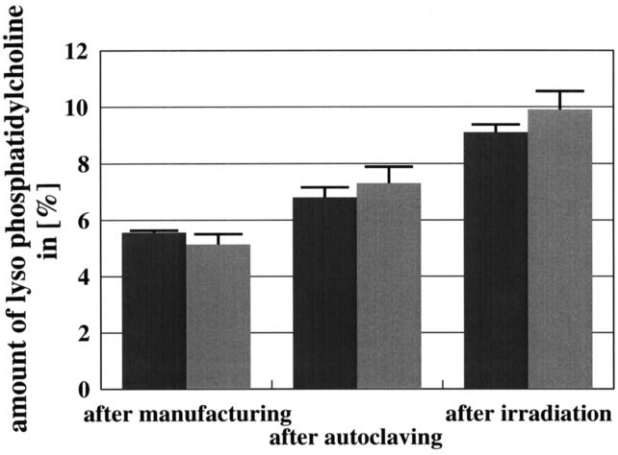

Fig. 6. Chemical stability of Europium(tri)laurate labelled emulsions. (■) without Solutol HS 15®, ( ) with Solutol HS 15®.

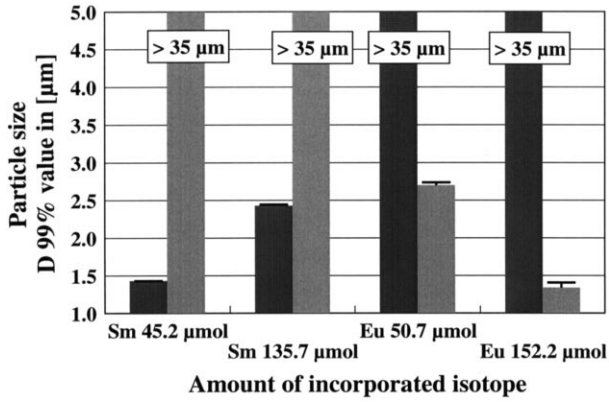

Fig. 7. Physical stability of Europium(tri)laurate labelled emulsions as determined by laser diffractometry. (■) without Solutol HS 15®, ( ) with Solutol HS 15®.

4. Conclusions

It was possible to prepare stable parenteral O/W emulsions with incorporated neutron activatable lanthanide fatty acid derivatives. Concerning the incorporation efficiency of lanthanide fatty acids and long-term stability, a load of 1 μmol/ml turned out to be maximum. When comparing different chain lengths of fatty acids, the long chain fatty acid derivative lanthanide(tri)stearate seemed to be superior in strengthening the interfacial layer due to a more effective electrostatic stabilization. In the presence of the non-ionic co-emulsifier Solutol HS 15®, the incorporation efficiency was enhanced up to 2 μmol/ml after manufacturing. However, this effect was compensated by a reduced electrostatic stability after autoclaving, due to a shielding of the negative surface charge of the particles by the neutral PEG-chains.

For in vivo biodistribution studies, the maximum loads of Samarium fatty acid derivatives were shown not to yield sufficient radioactivity levels. The alternative use of the Europium isotope could be proved to result in appropriate radioactivities.

Careful selection of the neutron activation parameters, such as short irradiation times compatible with the stability of the investigated formulations could be proved to yield the desired radioactivity level for in vivo biodistribution studies.

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