ELSEVIER

European Journal of Pharmaceutics and Biopharmaceutics 45 (1998) 157–163

European Journal of Pharmaceutics and Biopharmaceutics

Research paper

NMR relaxometric investigations of solid lipid nanoparticles (SLN) containing gadolinium(III) complexes

Silvia Morel[a], Enzo Terreno[b], Elena Ugazio[a], Silvio Aime[b], Maria Rosa Gasco[a,*]

[a]*Dipartimento di Scienza e Tecnologia del Farmaco, Torino, Italy*
[b]*Dipartimento di Chimica I.F.M., Torino, Italy*

Received 12 July 1996; revised version received 18 March 1997; accepted 29 April 1997

Abstract

This work deals with the preparation and relaxometric investigations of solid lipid nanoparticles (SLN) containing $[Gd–DTPA(H_2O)]^{2-}$ and $[Gd–DOTA(H_2O)]^{-}$. These paramagnetic chelates are commonly used as contrast agents (CA) for magnetic resonance imaging (MRI) owing to their ability to strongly increase the tissue water proton relaxation rate. The amount of gadolinium(III) (Gd(III)) complex included in the SLN has been evaluated and, on this basis, it has been found that the longitudinal relaxivity of these Gd(III) chelates apparently does not vary, at physiological pH, following their inclusion in SLN. We are unable to establish whether this is due to the free exchange of water from the inner compartment containing the Gd(III) chelate to the bulk water or whether the observed relaxation rate is essentially determined by a fraction of the complex which is close to the surface of the SLN in a region easily accessible to the bulk water. At acidic pH values, the relaxivity of the paramagnetic SLN containing the less thermodynamically and kinetically stable $[Gd–DTPA(H_2O)]^{2-}$ markedly increases. This effect may be ascribed to an increased immobilization and/or to an enhanced hydration of the complex on SLN. © 1998 Elsevier Science B.V.

Keywords: Contrast agent; Gadolinium(III) complex; Magnetic resonance imaging; Solid lipid nanoparticles; W/O/W microemulsions

1. Introduction

Specific targeting of tissues and organs is an important goal in the development of contrast agents (CA) for magnetic resonance imaging (MRI).

Basically, there are three ways to attain this purpose: (i) to introduce chemical functionalities on the contrast agent to provide it with the suitable molecular recognition capability [1,2]; (ii) to form covalent or non-covalent conjugates between the contrast agent and the bio-molecules having characteristic tropism [3–6]; and (iii) to include the contrast agent in a suitable delivery system.

Among the latter systems, much attention has been devoted to liposomes because of their ability to be efficiently taken up by the reticuloendothelial system. Liposome-associated CA may consist of both water-soluble agents entrapped within the aqueous inner compartment of liposomes [7] and lipophilic agents which directly participate to form the unilamellar vescicles [8–10].

Another 'carrier' system which has been considered is represented by red blood cells which may be loaded with the CA through a procedure based on change of the osmolarity of the medium [11].

We are interested in exploring the use of solid lipid nanoparticles (SLN) as a targeting system since it is known that nanoparticulates may be taken up by the membranous M cells in the dome epithelium of Peyer's patches [12]. These cells are responsible for the endocytosis of luminal particulates to lymphoid tissue and our aim is to exploit this route for the development of an SLN-based CA suitable for oral administration.

SLN may be prepared by the water dispersion either of

* Corresponding author. Dipartimento di Scienza e Tecnologia del Farmaco, via P. Giuria 9, 10125 Torino, Italy. Tel.: +39 11 6707667; fax: +39 11 6707687; e-mail: m-gasco@silver.ch.unito.it

0939-6411/98/$19.00
PII S0939-6411(97)00107-0

lipids [13–15] or of warm O/W microemulsions [16]. In this work we considered the latter approach and we used, as the oil phase, low melting lipophilic molecules, such as triglycerides or fatty acids. Calorimetric and X-ray diffraction studies [17] as well as transmission electron microscopy (TEM) micrographs [18] support the view that these systems are characterized by a solid-like behaviour.

Whereas lipophilic drugs are easily included in the internal phase of these delivery systems, it has also been shown that inclusion of hydrophilic drugs is possible provided that they are supplied under a suitable ion-paired form [18]. Furthermore, SLN containing small amounts of hydrophilic oligopeptides have been prepared [19,20].

In this regard, we think it is of particular interest to prepare SLN containing hydrophilic paramagnetic chelates currently used as CA for MRI, by following an analogous procedure to that employed in the case of the inclusion of oligopeptides.

The polyaminocarboxylate complexes [Gd–DOTA-$(H_2O)]^-$ and $[Gd–DTPA(H_2O)]^{2-}$ (Fig. 1) have already been used in clinical diagnosis as MRI contrast agents owing to their favourable magnetic and pharmacokinetic properties [21]. In fact, the presence of seven unpaired electrons coupled to a long electronic relaxation time, makes gadolinium(III) (Gd(III)) ion particularly effective in reducing both longitudinal and transverse relaxation times of the tissue water protons. Furthermore, these chelates display a high thermodynamic and kinetic stability which ensures a low toxicity even if it is difficult to foresee whether these favourable properties are maintained when the complexes are included in the SLN.

In addition to their application as CA for MRI, other possible applications may be envisaged for these SLN-based paramagnetic species. For instance, in the case where the SLN contain both a paramagnetic agent and a drug, the contrast enhancement in the MRI images caused by the former would report on the biodistribution and release path of the latter.

2. Experimental

2.1. Materials

Stearic acid, dioctyl sodium sulfosuccinate (AOT) and egg lecithin were purchased from Merck (Darmstadt, Germany), butyric acid and butanol from Fluka (Buchs, Switzerland), taurodeoxycholate sodium salt (TDC) from Sigma (St. Louis, MO, USA). $[Gd–DOTA(H_2O)]^-$ and $[Gd–DTPA(H_2O)]^{2-}$ complexes both as *N*-methyl-glucamine salts were a kind gift from Bracco (Milan, Italy). Egg lecithin was purified as previously described in the literature [22]. Sodium hydroxide was purchased from Sigma (St. Louis, MO, USA). Hydrochloric acid (37% solution) and nitric acid (65% solution) were purchased from Carlo Erba (Milan, Italy).

2.2. Instruments

TCF10A ultrafiltration system Amicon and Diaflo YM100 membrane (cut-off 100 000) (Beverley, MA, USA), Modulyo freeze-dryer (Edwards, Crawley, UK), Zetasizer 2c (Malvern, Worcestershire, UK), Stelar Spinmaster spectrometer (Stelar, Mede (PV), Italy), and HI 9321 pH-meter (Hanna Instruments, Padova, Italy) were used.

2.3. Preparation of the W/O/W microemulsion

The W/O/W multiple microemulsion was prepared in two steps. A warm W/O microemulsion was first prepared by adding a warm aqueous solution containing the Gd(III) complex (0.05–0.2 M) to a mixture of molten stearic acid, lecithin (or AOT) and butanol (or butyric acid), at 70°C.

The W/O/W microemulsion was obtained by adding a mixture of water, lecithin, butanol (or butyric acid) and TDC, warmed at about 70°C, to the warm W/O microemulsion. Both steps resulted in the formation of optically transparent systems. The composition of the different W/O/W microemulsions prepared in this work are reported in Table 1.

2.4. Preparation of SLN

The warm (70°C) W/O/W microemulsion was dispersed in water at a temperature of 2–3°C, under mechanical stirring, obtaining SLN. The ratio between the microemulsion and the dispersion medium was about 1:10.

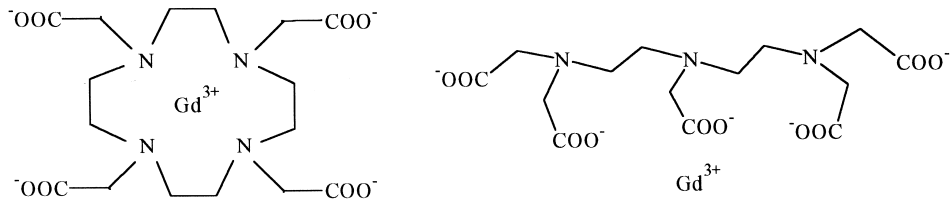
The dispersion of SLN was purified three times by ultradiafiltration, using Diaflo membranes, and both the SLN dispersions and the washing waters were separately freeze-dried.

2.5. Characterization of SLN

The size and polydispersity of the SLN were measured by photon correlation spectroscopy (PCS) using a Zetasizer 2c. The wavelength of the laser light (He/Ne) was 632.8 nm. The measurements were carried out at 25°C. The pH of the solutions was brought to the desired value by adding small aliquots of concentrated solutions of NaOH or HCl.

The NMR work was carried out by measuring the longitudinal water proton relaxation rate ($R_1 = 1/T_1$) of both SLN suspension and of the washing waters. In the first case, the samples were prepared by dispersing 80–200 mg of the freeze-dried SLN in 1 ml of bidistilled water. All the NMR measurements were carried out at 25°C and at a magnetic field strength of 0.47 T, corresponding to a proton Larmor frequency of 20 MHz. The measurements were carried out by using the inversion recovery pulse sequence $(180–\tau–90)_n$.

In this sequence, the spin system is first subjected to a



Gd-DOTA Gd-DTPA

Fig. 1. Schematic representation of the Gd(III) chelates used in this work.

180° pulse (pulse length 7 μs), along the +*x*-axis, in order to bring the net magnetization from the +*z* (equilibrium position) to the −*z*-axis. Following this pulse there is a delay period, τ, whose length is varied as the sequence pulse is repeated ($n = 16$). During this interval, the spin system relaxes and returns to equilibrium. After τ, a second 90° pulse (pulse length 3.5 μs) is applied, always along +*x*, in order to detect the magnetization on the *y*-axis. Finally, the longitudinal relaxation rate may be obtained from the exponential τ dependence of the signal intensity $I_{(\tau)}$:

$$I_{(\tau)} = I_{\infty}(1 - 2e^{-R_1\tau}) \quad (1)$$

where I_{∞} represents the equilibrium value, i.e. the net magnetization for the fully relaxed spin system. The signal intensity was obtained by averaging the first 128 data points of the free induction decay after four scans. A phase cycle was applied on the 90° observation pulse in order to cut off the *y*-scale receiver offset. A reproducibility test gave an uncertainty of 0.5% in the measured longitudinal relaxation rate.

3. Results and discussion

We have considered three types of microemulsion (see Section 2), basically differing in the nature and concentration of the surfactant and co-surfactant agents. Their water content is very similar (≈70%) as well as their size, as estimated from their mean diameters (about 180 nm, polydispersion 0.30) measured by a quasi-elastic light scattering technique. The determination of the Gd(III) content in the SLN system was carried out by means of the NMR relaxometric technique.

In fact, the value of the observed longitudinal water proton relaxation rate (R_{1obs}) is dependent upon the millimolar concentration of the paramagnetic species in solution ([GdL]) according to the following relationship:

$$R_{1obs} = r_{1p}[\mathrm{GdL}] + R_{1d} \quad (2)$$

where r_{1p} is the water proton longitudinal relaxivity of the Gd(III) complex and R_{1d} is the diamagnetic contribution measured in the absence of the paramagnetic solute.

Therefore, the longitudinal relaxivity of a paramagnetic species represents the increment of the longitudinal water proton relaxation rate per millimolar unit concentration of the paramagnetic complex.

The evaluation of the amount of Gd(III) complex included in the SLN system was performed by measuring the Gd(III) content both in the washing waters and SLN suspension.

In the first case, we dealt with the evaluation of the concentration of the Gd(III) chelate recovered in the collected washing waters during the SLN preparation. To this aim, we used the r_{1p} values determined in pure water for the two complexes (4.7 mM^{-1} s^{-1} at 25°C, pH 7 and 20 MHz for both Gd(III) complexes). This determination is based on the assumption that the washing waters only contain the intact Gd(III) chelate.

Fig. 2 shows the comparison between the pH dependence of the longitudinal water proton relaxivity for [Gd–DTPA $(H_2O)]^{2-}$ both in washing and pure water.

The profiles are very similar, being characterized by a plateau region between pH 3 and 7.5. At more acidic pH values, there is an increase of the longitudinal water proton relaxivity which appears to be related to an increased num-

Table 1

Composition % of the W/O and W/O/W microemulsions

Components	W/O microemulsions		
	Microemulsion 1	Microemulsion 2	Microemulsion 3
Stearic acid	37.2	44.0	83.2
Water	7.0	7.0	3.4
Lecithin	25.3	27.3	–
AOT	–	–	13.4
Butyric acid	30.5	–	–
Butanol	–	21.7	–
	W/O/W microemulsions		
	Microemulsion 1	Microemulsion 2	Microemulsion 3
W/O	13.0	14.1	12.0
Water	68.6	67.5	67.5
Lecithin	4.2	4.2	8.3
Butyric acid	6.1	–	5.0
Butanol	–	6.0	–
TDC	8.1	8.2	7.2

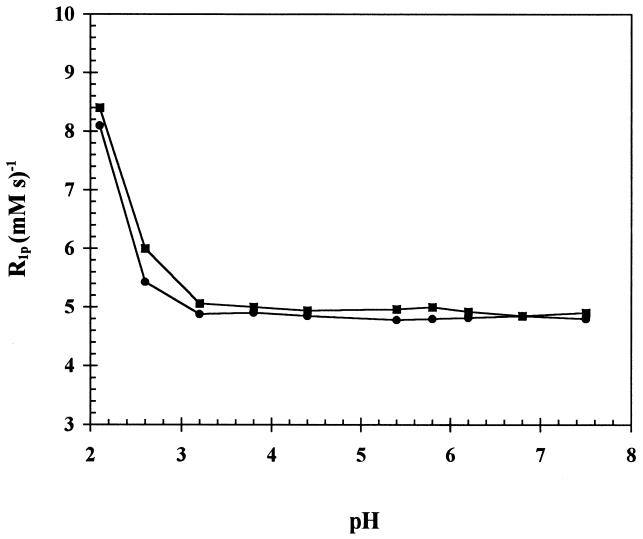


Fig. 2. Comparison between the pH dependence of water proton relaxivity for $[Gd–DTPA(H_2O)]^{2-}$ in pure water (●) and in the washing waters (■) collected during the SLN preparation (microemulsion 1) (25°C, 20 MHz).

ber of water molecules coordinated to the paramagnetic centre as a consequence of a decreased stability of the chelate [23]. The close similarity between the two profiles supports our assumption that the intact $[Gd–DTPA(H_2O)]^{2-}$ is present in the washing waters. Indeed, in the case of $[Gd–DOTA(H_2O)]^{-}$, no change in relaxivity was observed upon changing the pH of the solution down to pH 1, as expected on the basis of the much higher thermodynamic (and kinetic) stability of this complex.

Once the concentration of the Gd(III) complex in the washing waters is known, it is possible to determine its content in the SLN by knowledge of the amount of the chelate initially added during the W/O/W microemulsion preparation.

In the case of the direct determination of Gd(III) content in the SLN suspension, we first transformed all the Gd(III) present in the system into Gd(III) aquoion. This procedure was carried out by treating the suspension with a concentrated (2.6 M) solution of HNO_3.

Then, the quantitation of Gd(III) aquoion was obtained by comparing the measured relaxivity with the relaxivity determined for Gd(III) aquoion at the same pH (r_{1p} = 13.0 mM^{-1} s^{-1} at 25°C, 20 MHz).

A definite agreement about the Gd(III) content in the SLN has been found from the data obtained following the two methods. In Table 2, the amounts of Gd(III) chelate included in the SLN are reported along with the uncertainty related to the differences between the two approaches.

These results show that a good percentage of the supplied $[Gd–DTPA(H_2O)]^{2-}$ has been included in the SLN and, furthermore, no significant difference has been noticed in the extent of the inclusion on varying the composition of the W/O/W microemulsion. The inclusion of $[Gd–DOTA(H_2O)]^{-}$ appears more difficult as only about 18% of the supplied complex is found in the SLN. However, it is important to note that the absolute amounts of the paramagnetic complexes included in the SLN system are rather modest, as the weight ratio between the Gd(III) complexes and the freeze dried SLN is in the range 0.1–0.3%.

Once the Gd(III) content in the SLN is known, it is possible to evaluate the longitudinal relaxivity of these systems from the observed longitudinal water proton relaxation rate. In regard to this, it has been necessary to assess the diamagnetic contribution to the water relaxation rate in the presence of the SLN. For this reason, we first prepared SLN without adding the paramagnetic chelates and then we measured the dependence of the water proton relaxation rate (R_{1d}) versus the SLN concentration. The interaction between solvent water molecules and the solid nanoparticles, and the consequent decrease of their molecular tumbling, results in an increase of the observed relaxation rate. The correlation between R_{1d} and the SLN content was linear in the SLN concentration range used in this work (0–200 mg/ml). The diamagnetic contribution is, thus, computable, at a given SLN concentration, from the slope of the resulting straight line. Interestingly, the diamagnetic contribution is not affected by the type of the microemulsion used in the SLN preparation.

Table 3 shows that the longitudinal water proton relaxivity values obtained for the Gd(III) chelates included in the SLN are very similar to those determined for these complexes in pure water.

Two possible explanations may be forwarded to account for this result. (i) The whole amount of the Gd(III) complex is included in the inner compartments of the SLN and the water molecules of these compartments are in fast exchange with the outer bulk water. Support for this view comes from the observation that the free induction decay of the proton magnetization in each relaxation rate measurement shows a mono-exponential behaviour. In the case where the water

Table 2

Percentage of Gd(III) complexes recovered in SLN system with respect to the initial value

SLN from microemulsion	% Gd–DTPA	% Gd–DOTA
1	57.6 ± 3	18 ± 2
2	47.6 ± 2	–
3	49.7 ± 4	–

Table 3

Comparison between the longitudinal relaxivity for Gd(III) complex in SLN system and in pure water (25°C, pH 7, 20 MHz)

SLN from microemulsion	r_{1p} Gd–DTPA (mM^{-1} s^{-1})		r_{1p} Gd–DOTA (mM^{-1} s^{-1})	
	SLN	Water	SLN	Water
1	4.8	4.7	4.9	4.7
2	5.1	4.7	–	4.7
3	5.0	4.7	–	4.7

molecules were in slow exchange between the two compartments, a bi-exponential decay would have been observed. The fast component, i.e. the one characterized by a short transverse relaxation time, would refer to the inner compartment containing the paramagnetic species, whereas the slow component, characterized by a long transverse relaxation time, would represent the almost pure bulk water contribution. (ii) There is no exchange (on the NMR time scale) between the inner and outer compartments and, therefore, the Gd(III) chelate in the inner compartment does not contribute at all to the observed relaxivity. Only the portion of the Gd(III) complex close to the surface of SLN (i.e. in sites accessible to the bulk water) is responsible for the observed relaxation enhancement. In this case, the relaxation enhancement caused by this fraction of the complex is much higher with respect to that of the free chelate, as it is likely that it represents only a minor part of the whole amount of the chelate included in SLN. High relaxation rates may be expected as a consequence of a binding interaction of the Gd(III) complexes with the components of the SLN.

In fact, according to the well established theory [24,25], the contribution to the longitudinal proton relaxivity arising from water molecules in the inner coordination sphere of a Gd(III) complex present in molar concentration [GdL], is determined by:

$$R_{1p}^{is} = \frac{q[\mathrm{GdL}]}{55.56(T_{1M} + \tau_M)} \quad (3)$$

where q is the number of water molecules involved in this interaction, T_{1M} is their longitudinal proton relaxation time and τ_M is their mean residence time in the first coordination sphere of the metal. In the presence of a fast exchange between the inner-sphere water molecules and the bulk ones, the inner-sphere relaxivity is dominated by T_{1M} and the value of this parameter is given by the following equation:

$$\frac{1}{T_{1M}} \propto Df(\tau_c,\ \omega_I,\ \omega_s) \quad (4)$$

where D represents the dipolar constant relative to the interaction between the water protons and the paramagnetic centre, τ_c is the correlation time of this process and ω_s and ω_I are the electron and proton Larmor frequencies, respectively. Finally, at the observation frequency of 20 MHz and for small-sized Gd(III) chelates like those considered in this work, τ_c usually corresponds to the molecular reorientational time τ_R. Thus, the interaction of a Gd(III) chelate with the components of the SLN might cause a lengthening of τ_R which would result in a shortening of T_{1M} and, consequently, in an increase of the inner-sphere relaxivity term.

As anticipated in the introduction, one of the potential applications of these SLN-based CA may be in the targeting of the lymphatic system by oral administration. Therefore, it was thought useful to evaluate their relaxivity over an extended range of pH values.

Fig. 3 reports the relaxivity profiles obtained for SLN (prepared from microemulsion 1) containing [Gd–DOTA$(H_2O)]^-$ or [Gd–DTPA$(H_2O)]^{2-}$ on varying the pH of the suspensions from 2.5 to 8.0.

The results obtained for microemulsion 2 and 3 are very similar to that reported for microemulsion 1.

Whereas the longitudinal water proton relaxivity of the solution containing [Gd–DOTA$(H_2O)]^-$ is almost constant over all the pH range, the behaviour of [Gd–DTPA$(H_2O)]^{2-}$ is remarkably different, being characterized by a strong relaxivity increase at pH < 5.5. The achieved relaxivity value (≈ 28.0 mM^{-1} s^{-1}) is maintained down to pH 2.5. As the pH is lowered beyond pH 2.5, the relaxivity is further increased to a small extent. The overall behaviour is fully reversible.

The relaxivity shown by the [Gd–DTPA$(H_2O)]^{2-}$ SLN system at pH < 5.5 is significantly higher than the one measured for free Gd(III) aquoion in the presence of SLN, thus ruling out the possibility that the observed relaxation enhancement has to be related to the release of free Gd(III) ions.

The observed relaxation enhancement at acidic pH values might be the consequence of an enhanced immobilization of the paramagnetic species included in the SLN system and/or an increase of the overall hydration shell around the paramagnetic centre.

One may envisage that a strengthening of the binding interaction in the ternary adduct [Gd–DTPA$(H_2O)]^{2-}$ SLN might occur at acidic pH values through the stabilization of hydrogen bond interactions between protonated components present on the SLN and the carboxylic oxygens of DTPA ligand. The reasons why this happens with [Gd–DTPA$(H_2O)]^{2-}$ and not with [Gd–DOTA$(H_2O)]^-$ might lie on the higher residual negative charge of the former complex. Another possible explanation involves the displacement of one or more coordinating acetate arms of the chelate, promoted by the high H_3O^+ concentration, eventually replaced

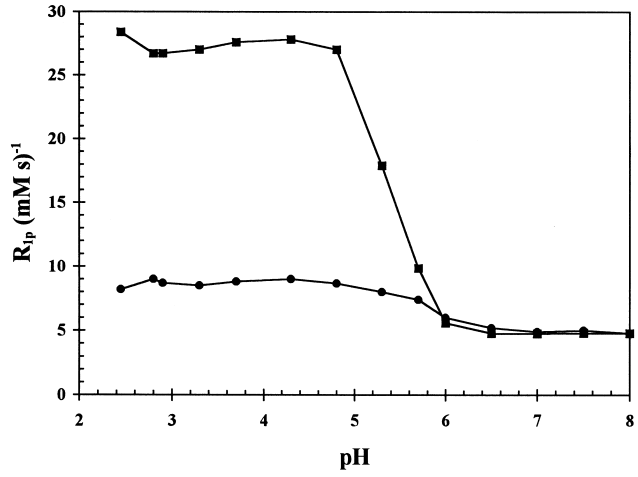


Fig. 3. pH Dependence of the longitudinal proton relaxivity rate for [Gd–DOTA$(H_2O)]^-$ (●) and [Gd–DTPA$(H_2O)]^{2-}$ (■) included in SLN system prepared from microemulsion 1 (25°C, 20 MHz).

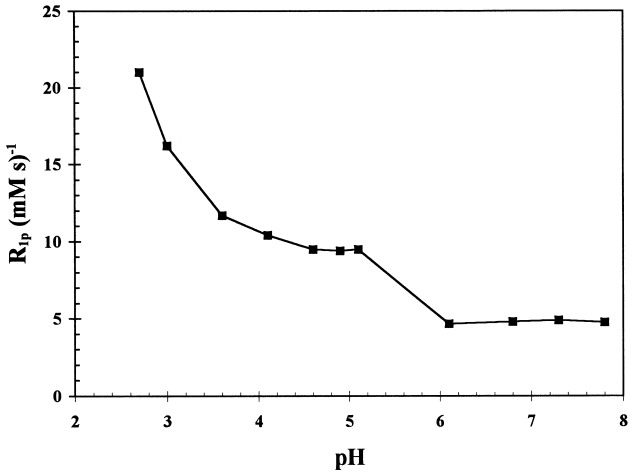


Fig. 4. pH Dependence of the longitudinal water proton relaxivity for $[Gd–DTPA(H_2O)]^{2-}$ externally added to an SLN suspension prepared from microemulsion 3 (25°C, 20 MHz).

by basic groups of the SLN components (for instance, stearic carboxylate or lecithin phosphate) and/or by other water molecules. In this case, the selectivity shown by the two complexes may be related to the much higher stereochemical rigidity and kinetic inertia of the macrocyclic $[Gd–DOTA(H_2O)]^{-}$ with respect to the linear $[Gd–DTPA(H_2O)]^{2-}$.

In order to get some more insight into the observed behaviour, we carried out analogous relaxivity measurements on a suspension obtained by adding $[Gd–DTPA(H_2O)]^{2-}$ to an SLN suspension prepared without the inclusion of any paramagnetic complex. Again (Fig. 4), we noted that as the pH is decreased there is an increase of the observed relaxation rate which parallels the behaviour reported in Fig. 3. However, the lower relaxation enhancement observed in this experiment supports the view that the procedure followed to include the paramagnetic complex in the SLN has not resulted in the simple interaction with the polar groups on the outer SLN surface. Indeed, it has dealt with a more extensive involvement of the SLN system. Unfortunately, these data alone do not allow us to establish the exact nature of the occurring interactions.

In conclusion, these systems are potentially useful as oral CA for MRI. The observation of a lack of any pH-dependence in the case of SLN containing $[Gd–DOTA(H_2O)]^{-}$ suggests that for this purpose, at this stage, this system seems to better suited than $[Gd–DTPA(H_2O)]^{2-}$.

Acknowledgements

This work was supported by a 40% MURST grant.

References

[1] S. Aime, M. Botta, G. Ermondi, F. Fedeli, F. Uggeri, Synthesis and NMRD studies of Gd^{3+} complexes of macrocyclic polyamino polycarboxylic ligands bearing β-benzyloxy-α-propionic residues, Inorg. Chem. 31 (1992) 1100–1103.

[2] S. Aime, M. Fasano, S. Paoletti, F. Viola, C. Tarricone, P. Ascenzi, ^{1}H-NMR relaxometric study of pancreatic serine (pro)enzyme inhibition by a Gd(III) chelate bearing boronic functionalities, Biochem. Mol. Biol. Int. 39 (1996) 741–746.

[3] S. Aime, M. Botta, M. Fasano, S. Geninatti Crich, E. Terreno, Gd(III) complexes as contrast agents for magnetic resonance imaging: a proton relaxation enhancement study of the interaction with human serum albumin, J. Biol. Inorg. Chem. 1 (1996) 312–319.

[4] S. Aime, P. Ascenzi, E. Comoglio, M. Fasano, S. Paoletti, Molecular recognition of R- and T-states of human adult hemoglobin by a paramagnetic Gd(III) complex by means of the measurement of solvent water proton relaxation rate, J. Am. Chem. Soc. 117 (1995) 9365–9366.

[5] R.C. Brasch, Rationale and applications for macromolecular GD-based contrast agents, Magn. Res. Med. 22 (1991) 282–287.

[6] E.C. Wiener, M.W. Brechbiel, H. Brothers, R.L. Magin, O.A. Gansow, D.A. Tomalia, P.C. Lauterbur, Dendrimer-based metal chelates: a new class of magnetic resonance imaging contrast agents, Magn. Res. Med. 31 (1994) 1–8.

[7] S.H. Koenig, Q.F. Akhong, R.D. Brown III, M. Lafleur, M. Spiller, E.C. Unger, C.P.S. Tilcock, Permeability of liposomal membranes to water: results from the magnetic field dependence of T_1 of solvent protons in suspensions of vesicles with entrapped paramagnetic ions, Magn. Res. Med. 23 (1992) 275–286.

[8] G.W. Kabalka, M.A. Davis, T.H. Moss, E. Buonocore, K. Hubner, E. Holmberg, K. Maruyama, L. Huang, Gadolinium-labeled liposomes containing various amphiphilic Gd–DTPA derivatives: targeted MRI contrast enhancement agents for the liver, Magn. Res. Med. 19 (1991) 406–415.

[9] R.W. Storrs, F.D. Tropper, Y.H. Li, C.K. Song, J.K. Kuniyoshi, D.A. Sipkins, K.C.P Li, M.D. Bednarski, Paramagnetic polymerized liposomes: synthesis, characterization, and applications for magnetic resonance imaging, J. Am. Chem. Soc. 117 (1995) 7301–7306.

[10] W.P. Cacheris, R.C. Grabiak, A.C. Lee, T.J. Richard, T.H. Goodin, R.J. Kaufman, Emulsions of lipophilic paramagnetic complexes as MRI contrast media, Proc. 4th Special Topic Seminar of the European Magnetic Resonance Forum, Santiago de Compostela, Spain, 1994, pp. 37–51.

[11] R. Kravtzoff, E. Urvoase, C. Chambon, C. Ropars, Gd–DOTA loaded into red blood cells, a new magnetic resonance imaging contrast agents for vascular system, in: M. Magnani, J.R. De Loach (Eds.), The Use of Resealed Erythrocytes as Carriers and Bioreactors, Plenum Press, New York, 1992, pp. 347–354.

[12] J. Kreuter, Peroral administration of nanoparticles, Adv. Drug Del. Rev. 7 (1991) 71–86.

[13] A.J. Domb, M. Maniar, Lipospheres for controlled delivery of pharmaceuticals, pesticides and fertilizers, US Patent No. 435,546 (1989).

[14] C. Schwarz, W. Mehnert, J.S. Lucks, R.H. Muller, Solid lipid nanoparticles (SLN) for controlled drug delivery. I. Production, characterization and sterilization, J. Control. Rel. 30 (1994) 83–96.

[15] B. Siekmann, K. Westesen, Submicron-sized parenteral carrier systems based on solid lipids, Pharm. Pharmacol. Lett. 1 (1992) 123–126.

[16] M.R. Gasco, S. Morel, R. Carpignano, Optimization of the incorporation of deoxycorticosterone acetate in lipospheres, Eur. J. Pharm. Biopharm. 38 (1992) 7–10.

[17] D. Aquilano, R. Cavalli, M.R. Gasco, Solid lipospheres obtained from hot microemulsions in the presence of different concentrations of cosurfactant: the crystallization of stearic acid polymorphs, Therm. Acta 230 (1994) 29–37.

[18] R. Cavalli, M.R. Gasco, S. Morel, Behaviour of timolol englobed in lipospheres in presence of a series of phosphate esters, STP Pharm. Sci. 2 (1992) 514–518.

[19] S. Morel, M.R. Gasco, R. Cavalli, Incorporation in lipospheres of [D-Trp-6]LHRH, Int. J. Pharm. 105 (1994) R1–R3.
[20] S. Morel, E. Ugazio, R. Cavalli, M.R. Gasco, Thymopentin in solid lipid nanoparticles, Int. J. Pharm. 132 (1996) 259–261.
[21] B.L. Engelstad, G.L. Wolf, Contrast agents, in: D.D. Stark, W.G. Bradley (Eds.), Magnetic Resonance Imaging, C.V. Mosby, Toronto, 1988, pp. 161–181.
[22] D.J. Hanahan, N.B. Turner, M.E. Deyko, Isolation of egg phosphatidylcholine, J. Biol. Chem. 191 (1951) 623–629.
[23] C.A. Chang, H.G. Brittain, J. Telser, M.F. Tweedle, pH Dependence of relaxivities and hydration numbers of gadolinium(III) complexes of linear amino carboxylates, Inorg. Chem. 29 (1990) 4468–4473.
[24] L. Banci, I. Bertini, C. Luchinat, Nuclear and Electron Relaxation, VCH, Weinheim, Germany, 1991, pp. 91–142.
[25] S.H. Koenig, R.D. Brown III, Field-cycling relaxometry of protein solutions and tissue: implications for MRI, Progr. NMR Spectr. 22 (1990) 487–567.