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Tuning the MR properties of blood-stable pH-responsive paramagnetic liposomes

Knut-Egil Løkling [a,*], Roald Skurtveit [b], Knut Dyrstad [b], Jo Klaveness [a], Sigrid L. Fossheim [b]

[a] *Department of Medicinal Chemistry, School of Pharmacy, University of Oslo, P.O. Box 1155 Blindern, N-0318 Oslo, Norway*
[b] *Amersham Health AS, P.O. Box 4220 Nydalen, N-0401 Oslo, Norway*

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Abstract

Paramagnetic pH-responsive liposomes have recently been suggested as a promising approach for monitoring by magnetic resonance imaging (MRI) pH changes in tumours. In the present study, the effects of variations in bilayer composition on the relaxometric properties of diacylphosphatidylethanolamine (PE)/dipalmitoylglycerosuccinate (DPSG) liposomal GdDTPA-BMA were investigated both in buffer and blood. A factorial experimental design was used with the variables PE chain length and mol% DPSG. All the relaxometric profiles displayed a semi-sigmoidal shape with a minimum plateau at high pH (r_1^{min}) and a maximum at low pH ($r_1^{max,E}$). Relevant sigmoidal curve fit parameters were evaluated by partial least squares regression. Systematic variations in the relaxometric response ($r_1^{max,E} - r_1^{min}$) were shown for the liposomal systems both in buffer and blood. The pH value at which the r_1 was 20% of $r_1^{max,E}$ relative to r_1^{min}, i.e. pH_{20}, decreased significantly both in buffer and blood as a function of the mol% DPSG. This phenomenon could be understood by the increased surface charge density with increasing mol% DPSG and, hence, higher barrier against liposome aggregation with consequent leakage of contrast agent. Furthermore, the pH relaxometric profiles in blood were shifted laterally to higher, and likely more clinically relevant pH values than the corresponding profiles in buffer. The liposome formulations displayed minimal leakage of contrast agent after prolonged incubation in blood at physiological pH and retained their pH sensitivity after pre-incubation in blood.

Keywords: Paramagnetic liposomes; pH-responsive; Bilayer composition; Stability in blood; Tumour pH; Relaxivity

1. Introduction

Magnetic resonance imaging (MRI) contrast agents can enhance the differentiation between tissues and represent today a significant part of this diagnostic modality (Caravan et al., 1999). The majority of gadolinium (Gd)-based contrast agents used in the clinic is low molecular weight Gd-chelates, most of them with extracellular distribution. However, responsive agents that can be used to "visualise" pathology based on alterations in physiological parameters are presently investigated. The interstitial pH in human tumours, for instance, is known to be lower than in healthy tissue (Stubbs et al., 2000; Wike-Hooley et al., 1984). Quantification of this acidity could

* Corresponding author. Tel.: +47-23-18-5605; fax: +47-23-18-6014.
E-mail address: knut-egil.lokling@amersham.com (K.-E. Løkling).

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be important for the assessment of disease severity and for therapy selection (Holahan et al., 1982; Raghunand et al., 2001; Russo et al., 1995). Consequently, paramagnetic contrast agents have recently been reported to display a contrast efficacy modulated by pH both in vitro (Aime et al., 1999; Mikawa et al., 2000) and in vivo (Raghunand et al., 2003). A common feature for most of these novel contrast agents is their ability to function as "off-on" switches, i.e. the MR efficacy is markedly enhanced at a given pH value. The pH value at which the contrast agents switch "on" is an important feature, and should be in a clinically relevant pH range for tumours.

One approach to monitor pH in tumours by MRI, employing pH-sensitive paramagnetic liposomes, has recently been suggested (Lokling et al., 2001). If properly designed, such liposomes can accumulate in the tumour interstitium due to the enhanced permeability and retention effect (Drummond et al., 1999), and switch "on" depending on the interstitial pH. Leakage of contrast agent and a consequent diminished pH-sensitivity in blood has been a major challenge for this liposomal concept (Lokling et al., 2003b). However, a pH-sensitive liposomal contrast agent with improved properties has recently been presented (Lokling et al., 2003a). The liposomal formulation comprises GdDTPA-BMA encapsulated within dipalmitoylphosphatidylethanolamine (DPPE)/dipalmitoylglycerosuccinate (DPSG) liposomes (20 mol% DPSG). At physiological pH, the in vitro T_1-relaxivity (r_1) of this system in blood was significantly lowered compared to that of non-liposomal Gd-chelate, due to an exchange limited relaxation process (Fossheim et al., 1999). Lowering the pH, however, gave a marked increase in r_1, owing to liposome aggregation and subsequent leakage of GdDTPA-BMA.

In the present study, alteration in the bilayer composition was investigated as a mean to modify the relaxometric properties of diacylphosphatidylethanolamine (PE)/DPSG liposomal GdDTPA-BMA. A reduced three-level factorial design with the variables PE chain length and mol% DPSG was therefore used. PE chain length refers to the number of carbon atoms in the PE acyl chains. Seven different liposomal formulations, with duplicates of the centre point, were prepared and characterised by relaxometry in both buffer and blood.

2. Materials and methods

2.1. Materials

Gadolinium diethylenetriamine pentaacetic acid bismethylamide (gadodiamide, GdDTPA-BMA) was obtained from Amersham Health AS, Lindesnes, Norway. 1,2-Dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE) and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE) were purchased from Sygena Ltd., Liestal, Switzerland. 1,2-Dipalmitoyl-*sn*-glycero-3-succinate (DPSG) was supplied from Avanti Polar Lipids Inc., Alabaster, AL, USA. Glucose monohydrate was obtained from Fluka Chemie AG, Buchs, Switzerland. Sodium hydroxide (NaOH), fuming hydrochloric acid (HCl) and *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) were purchased from Merck KGaA, Darmstadt, Germany. Tris(hydroxymethyl)aminomethane (Tris) and 2-(*N*-morpholino)ethanesulfonic acid (MES) were supplied from Sigma Chemical Co., St. Louis, MO, USA. Human blood was withdrawn from a healthy male volunteer and was processed with Na-heparate. Deionised water was employed and all other materials were used without further purification.

2.2. Preparation and physicochemical characterisation of liposomes

The liposomes were prepared by the thin film hydration method (Lasic, 1993b), sized down by brief sonication and sequential extrusion, followed by dialysis against isosmotic and isoprotic glucose solution. The following liposomal physicochemical parameters were determined: osmolality, intensity-weighted hydrodynamic diameter, gel-to-liquid crystalline phase transition temperature (T_m), zeta potential and effective Gd concentration (C_{eff}). C_{eff} is defined as the Gd concentration in the total sample volume. Further experimental details have been described elsewhere (Lokling et al., 2003a).

2.3. In vitro relaxometry

The relaxation measurements were performed at 37 °C and 0.47 T (Minispec PC-120b, Bruker GmbH,

Rheinstetten, Germany). The T_1 relaxation times were obtained by the inversion recovery method. The r_1 at any experimental condition was calculated as previously reported (Lokling et al., 2003a). The relaxometric behaviour of the various liposomal systems was studied both in buffer and blood according to previously described procedure (Lokling et al., 2003a). Briefly, the pH–r_1 dependence was investigated after incubation in isosmotic 50 mM MES buffers (pH range 4.4–8.3) and in MES/HEPES (250 mM)-buffered human whole blood (pH range 5.7–8.2). The pH range for MES buffer could be extended to p$Ka \pm 2$ due to the low buffering capacity of the liposomes and the extensive dilution of the dispersions. The relaxometric behaviour of some of the liposomal systems was also studied after 20-min incubation in human whole blood, followed by pH adjustment with 250 mM MES/HEPES buffers and further incubation. As a control, an isotonic NaCl solution (pH 7.4) was added to one of the vials instead of buffer.

2.4. Experimental design

A reduced three-level factorial design with the two design variables mol% DPSG (A) and PE chain length (B) was used to study the properties of PE/DPSG liposomal GdDTPA-BMA. The PE chain length was restricted to even numbers in the range 14–18 (14, DMPE/DPSG; 16, DPPE/DPSG; 18, DSPE/DPSG). The design, including the experimental points, is schematically shown in Fig. 1. The design solves the following equation:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 AB + \beta_4 A^2 + \beta_5 B^2 + \beta_6 AB^2 \quad (1)$$

where Y is the measured response, β_0 is a constant, β_1–β_6 are regression coefficients and A and B are mol% DPSG and PE chain length, respectively, as described above. Seven different liposomal formulations, with duplicates of the centre point, were prepared and characterised, giving a total of eight batches. The liposome batches were produced in a random order. All other production variables were kept at the same level.

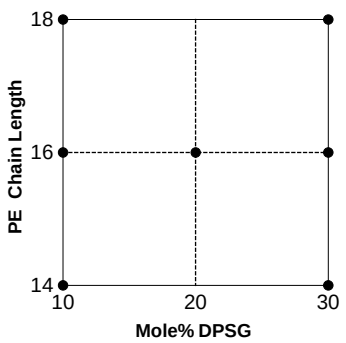

Fig. 1. Schematic presentation of the reduced three-level factorial design with the two variables mol% DPSG and PE chain length, including the experimental points (●).

2.5. Data analysis

A sigmoidal dose–response equation (four-parameter logistic equation) was fitted to the relaxometric profiles using GraphPad Prism 3.02 (GraphPad Software Inc., San Diego, CA, USA) software:

$$r_1 = r_1^{\min} + \frac{r_1^{\max} - r_1^{\min}}{1 + 10^{(\mathrm{pH}_{50} - \mathrm{pH})S}} \quad (2)$$

where $r_1^{\min}$ and $r_1^{\max}$ are the r_1 at the bottom and top plateau of the sigmoidal curve, respectively, pH_{50} is the pH value at which r_1 is the average of $r_1^{\min}$ and $r_1^{\max}$ and S describes the steepness of the curve.

The data from the design were evaluated by partial least squares regression using Modde 5.0 (Umetri AB, Umeå, Sweden) software. The significance of the estimated effects was tested by analysis of variance. A p-value less than 0.05 was considered significant. The accuracy of the models was described by the correlation parameter R^2. R^2 is the fraction of the data explained by the model and values close to 1 indicate a good model.

3. Results and discussion

3.1. Experimental design

The relaxometric properties of DPPE/DPSG liposomal GdDTPA-BMA, comprising 20 mol% DPSG have previously been investigated (Lokling et al., 2003a). Altering the mol% DPSG and PE chain length in the liposomal bilayer may affect the relaxometric

properties. Therefore, in the present study, a factorial design was employed to study analogues of the above-mentioned formulation (Fig. 1). PE chain length was restricted to even numbers in this study and, consequently, odd-even effects (Lasic, 1993a) could not be neglected. DPSG was used in all the formulations, since 1,2-dimyristoyl-*sn*-glycero-3-succinate (DMSG) and 1,2-distearoyl-*sn*-glycero-3-succinate (DSSG) were not commercially available. The DPPE/DPSG liposomes were investigated at both low and high level of DPSG due to the identical lipid chain lengths. As a consequence, the design comprised a higher resolution in the direction of PE chain length, resulting in a reduced three-level factorial design.

3.2. Physicochemical properties

The physicochemical properties of the dialysed liposome formulations are shown in Table 1. The liposome diameter and C_{eff} for the DPPE/DPSG (20 mol% DPSG) are given as the mean of two batches and the interval denotes the lowest and highest value. For the purpose of comparison, the different liposomal formulations were prepared with comparable size (Table 1). The zeta potential could be expected to increase (in absolute value) as a function of mol% DPSG, due to the anticipated corresponding increase in surface charge density. However, no systematic variation in the zeta potential was observed. No indication of PE and DPSG immiscibility was found for any of the formulations, as evidenced by differential scanning calorimetry. The T_m increased for the DMPE/DPSG liposomes and decreased for the DSPE/DPSG liposomes by increasing the amount of DPSG. This effect is due to the positive influence of lipid chain length on the T_m, i.e. the hydrocarbon chains in DPSG melt at a higher and lower temperature than in DMPE and DSPE, respectively. Increasing the DPSG content in DPPE/DPSG liposomes led to a slight decrease in T_m, indicating that DPSG may affect the lipid chain packing.

3.3. pH-relaxivity profiles in buffer and buffered human whole blood

Fig. 2 shows the pH–r_1 dependence at 37 °C for selected liposomal formulations after 20-min incubation in isosmotic MES buffers (Fig. 2A) and in MES-buffered human whole blood (Fig. 2B). The remaining pH–r_1 profiles are omitted for the purpose of simpler visualisation, but their trends (which are similar) are discussed below. Some of the relaxometric profiles displayed a sigmoidal shape with a minimum plateau at high pH and a clear tendency for a maximum plateau at low pH, as exemplified with the DSPE/DPSG (10 mol% DPSG) system in buffer (Fig. 2A). However, most of the relaxometric profiles did not show a maximum plateau in the investigated pH range, such as the DPPE/DPSG (30 mol% DPSG) formulation in buffer (Fig. 2A) and the DPPE/DPSG (10 mol% DPSG) system in blood (Fig. 2B). It is likely to believe that the latter type of profiles would reach the maximum level below the investigated pH interval. Lower pH values were, however, not explored due to the high buffer capacity of blood. Therefore, and in order to compare the data quantitatively, a sigmoidal dose–response equation (Motulsky, 1999) was fitted to each pH–r_1 profile as shown in Fig. 2. This type

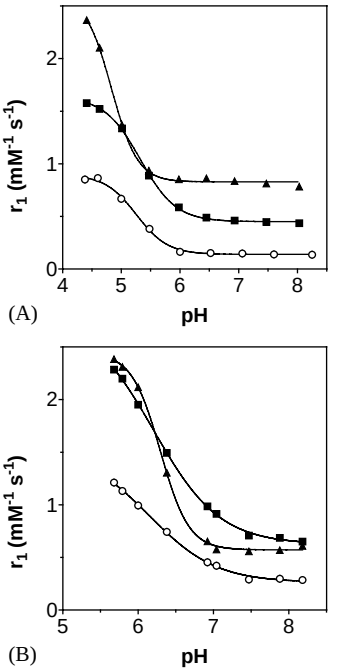

Fig. 2. pH–r_1 dependence of DPPE/DPSG liposomal GdDTPA-BMA containing 10 (■) and 30 (▲) mol% DPSG, and DSPE/DPSG liposomal GdDTPA-BMA containing 10 mol% DPSG (○) in MES buffer (A) and in MES-buffered human whole blood (B) at 37 °C. Data points are given as the arithmetic mean of duplicates (20-min incubation, 0.47 T). The solid lines are the sigmoidal curve fit for each data set.

Table 1
Physicochemical properties of various liposomal GdDTPA-BMA formulations

Liposome composition	Liposome diameter (nm)[a]	Zeta potential (mV)[a]	T_m (°C)	C_{eff} (mM)
DMPE/DPSG				
10 mol% DPSG	122	−47.5	50.9	14.1
30 mol% DPSG	115	−45.8	52.3	11.3
DPPE/DPSG				
10 mol% DPSG	116	−46.4	64.9	14.8
20 mol% DPSG	113 ± 2[b]	−47.4	64.4	13.5 ± 1.0[b]
30 mol% DPSG	110	−46.1	64.1	11.4
DSPE/DPSG				
10 mol% DPSG	128	−45.6	75.0	14.8
30 mol% DPSG	113	−45.8	73.5	11.1

[a] Measured in isosmotic glucose solution (pH 8.4, 25 °C).
[b] Intervals denotes the lowest and highest values for two batches.

of curve has been used to describe a wide range of binding phenomena and pH titrations. The correlation coefficients, R^2, for all the models were found acceptable (>0.99 for all). The software adjusted the r_1^{max} to best fit the experimental data for the profiles without a maximum r_1 plateau. Consequently, the r_1^{max} value is rather uncertain for such profiles, which also applies for the r_1^{max}-correlated parameter pH_{50}. For the purpose of comparison, the experimental maximum in r_1 ($r_1^{max,E}$) was used instead of r_1^{max}. Furthermore, the pH at which the r_1 was 20% of $r_1^{max,E}$ relative to r_1^{min}, i.e. pH_{20}, was calculated from the sigmoidal curve fits and Eq. (2). The pH_{20} should be a useful descriptor for the pH at which the different formulations "switch on". Tables 2 and 3 show the relevant curve fit parameters for the different liposomal formulations after incubation in buffer and buffered blood, respectively.

3.3.1. *Minimum plateau r_1 at high pH (r_1^{min})*

The r_1^{min} (Tables 2 and 3) was significantly lowered compared to that of non-liposomal GdDTPA-BMA for all the liposomal formulations, most probably due to an exchange limited relaxation process. Fig. 3 shows the response surfaces derived from the results obtained in the reduced three-level factorial design with r_1^{min} as response after buffer (Fig. 3A) and blood incubation (Fig. 3B). Increasing the PE chain length from 14 to 18 carbon atoms gave a significant reduction in r_1^{min} both in buffer and blood (Fig. 3). The bilayer water permeability most likely decreased as a function of bilayer thickness. Therefore, the exchange limited relaxation process should be more pronounced in the liposomal membranes with the longest lipid chains and the r_1^{min} is expected to be lower (Fossheim et al., 1999). The r_1^{min} in buffer increased with increasing mol% DPSG

Table 2
Curve fit parameters for various liposomal GdDTPA-BMA formulations in MES-buffered media (20-min incubation, 37 °C)

Liposome composition	r_1^{min} ($mM^{-1}\,s^{-1}$)	$r_1^{max,E}$ ($mM^{-1}\,s^{-1}$)	pH_{20}	R^2	$r_1^{max,E} - r_1^{min}$ ($mM^{-1}\,s^{-1}$)
DMPE/DPSG					
10 mol% DPSG	0.75	2.33	5.67	0.99	1.58
30 mol% DPSG	1.14	2.99	5.03	0.99	1.85
DPPE/DPSG					
10 mol% DPSG	0.45	1.58	5.78	0.99	1.13
20 mol% DPSG[a]	0.43 ± 0.01	1.71 ± 0.04	5.60 ± 0.07	0.99	1.28 ± 0.05
30 mol% DPSG	0.83	2.37	5.18	0.99	1.54
DSPE/DPSG					
10 mol% DPSG	0.14	0.85	5.64	0.99	0.71
30 mol% DPSG	0.86	2.85	5.24	0.99	1.99

[a] Arithmetic mean of duplicates ± S.E.M.

Table 3
Curve fit parameters for various liposomal GdDTPA-BMA formulations in MES-buffered human whole blood (20-min incubation, 37 °C)

Liposome composition	r_1^{min} ($mM^{-1}\,s^{-1}$)	$r_1^{max,E}$ ($mM^{-1}\,s^{-1}$)	pH_{20}	R^2	$r_1^{max,E} - r_1^{min}$ ($mM^{-1}\,s^{-1}$)
DMPE/DPSG					
10 mol% DPSG	1.05	1.52	6.30	0.99	0.47
30 mol% DPSG	1.26	1.53	6.04	0.99	0.27
DPPE/DSPG					
10 mol% DPSG	0.62	2.28	6.95	0.99	1.66
20 mol% DPSG[a]	0.43 ± 0.03	2.03 ± 0.06	6.63 ± 0.08	0.99	1.60 ± 0.03
30 mol% DPSG	0.57	2.38	6.52	0.99	1.81
DSPE/DPSG					
10 mol% DPSG	0.26	1.21	6.92	0.99	0.95
30 mol% DPSG	0.56	2.24	6.65	0.99	1.68

[a] Arithmetic mean of duplicates ± S.E.M.

(Fig. 3A), which could be explained by corresponding higher bilayer permeability. The same tendency was observed in blood (Fig. 3B), but the effect was not significant. The R^2 values for the models in buffer and blood were 0.94 and 0.92, respectively. No significant increase in the r_1^{min} could be observed after further incubation of the liposomes up to 2 h in buffer (data not shown). Only a moderate increase in the r_1^{min} could be observed after analogous incubation in blood (data not shown), demonstrating low extent of leakage from the liposomes.

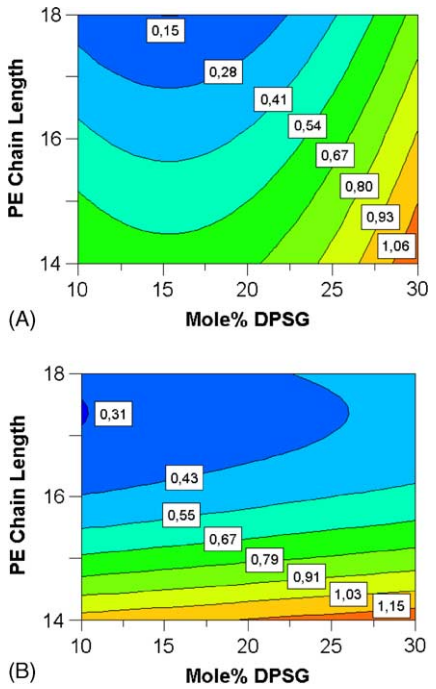

Fig. 3. The response surfaces from the reduced three-level factorial design showing the r_1^{min} for PE/DPSG liposomal GdDTPA-BMA as a function of PE chain length and mol% DPSG after incubation in MES buffer (A) and in MES-buffered human whole blood (B). The PE chain length was restricted to even numbers (14, DMPE/DPSG; 16, DPPE/DPSG; 18, DSPE/DPSG).

3.3.2. *Relaxometric response* ($r_1^{max,E} - r_1^{min}$)

Leakage of contrast agent from aggregated liposomes is most likely the reason for the increase in r_1 towards $r_1^{max,E}$ when lowering the pH. The relative increase in r_1 is an important feature for the pH-sensitive systems. A good descriptor for the pH–r_1 response should therefore be $r_1^{max,E} - r_1^{min}$ (Tables 2 and 3). Fig. 4 shows the response surfaces derived from the results obtained in the reduced three-level factorial design with $r_1^{max,E} - r_1^{min}$ as response after buffer (Fig. 4A) and blood incubation (Fig. 4B). There were major differences between the two response surfaces.

After incubation in buffer, the $r_1^{max,E} - r_1^{min}$ increased significantly as a function of mol% DPSG. The increase was more pronounced for a PE chain length of 18 carbon atoms than for 14 (Fig. 4A). The leakage process from aggregated liposomes seemed to be more effective in bilayers with the highest content of DPSG. There was also a tendency for the $r_1^{max,E} - r_1^{min}$ to decrease as a function of PE chain length. Such a chain length dependency could be due to a slower leakage process from aggregated liposomes comprising the thickest bilayers. Bilayer fusion processes, for example, are reported to decrease with

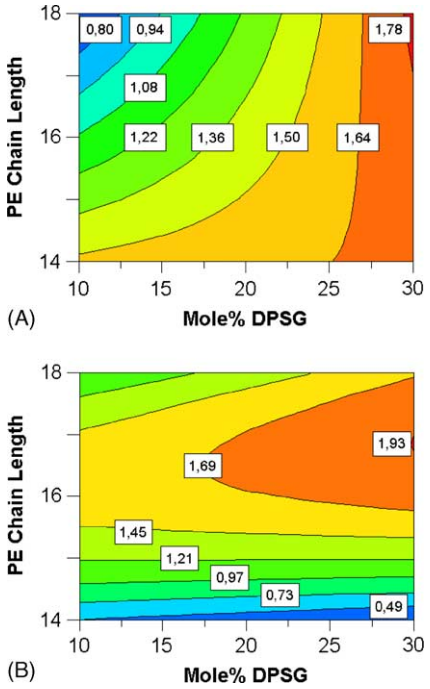

Fig. 4. The response surfaces from the reduced three-level factorial design showing the relaxometric response ($r_1^{max,E} - r_1^{min}$) for PE/DPSG liposomal GdDTPA-BMA as a function of PE chain length and mol% DPSG after incubation in MES buffer (A) and in MES-buffered human whole blood (B). The PE chain length was restricted to even numbers (14, DMPE/DPSG; 16, DPPE/DPSG; 18, DSPE/DPSG).

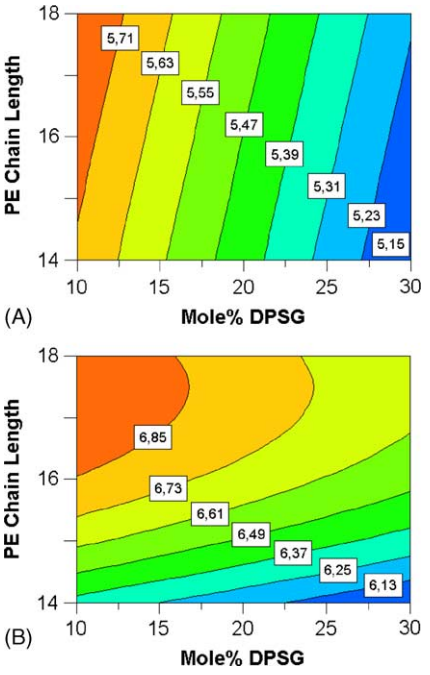

Fig. 5. The response surfaces from the reduced three-level factorial design showing the pH_{20} for PE/DPSG liposomal GdDTPA-BMA as a function of PE chain length and mol% DPSG after incubation in MES buffer (A) and in MES-buffered human whole blood (B). The PE chain length was restricted to even numbers (14, DMPE/DPSG; 16, DPPE/DPSG; 18, DSPE/DPSG).

bilayer thickness (Gaber and Sheridan, 1982). The R^2 value for the model was 0.87.

After incubation in buffered blood, the situation was different (Fig. 4B). The effect of mol% DPSG was relatively small, whilst the effect of PE chain length was complex. The $r_1^{max,E} - r_1^{min}$ displayed the lowest level for a PE chain length of 14 carbon atoms (Fig. 4B), contrary to the situation in buffer (Fig. 4A). This phenomenon could be due to interaction of the DMPE/DPSG bilayers with amphiphilic blood components. Insertion of amphiphilic blood components into the bilayer and diminishment of the pH-sensitivity has for instance been reported for liquid crystalline pH-sensitive liposomes (Liu and Huang, 1989). The $r_1^{max,E} - r_1^{min}$ was higher for PE chain lengths of 16–18 carbon atoms than the corresponding response in buffer, except for DSPE/DPSG liposomes comprising 30 mol% DPSG showing a moderate decrease. The R^2 value for the model was 0.99.

For MRI purposes, caution should be exercised when evaluating the relaxometric response. It is desirable that the difference between the "off" and "on" MR signal is as large as possible, but also that the "off" contribution is low. A compromise could therefore be necessary. For example, even if the $r_1^{max,E} - r_1^{min}$ for the DSPE/DPSG (30 mol% DPSG) formulation were higher than for the DSPE/DPSG system comprising 10 mol% DPSG, the latter could be a better candidate, due to the significantly lower "off" level.

3.3.3. pH_{20}

Fig. 5 shows the response surfaces derived from the results obtained in the reduced three-level factorial design with pH_{20} as response after buffer (Fig. 5A) and blood incubation (Fig. 5B). The pH_{20} decreased significantly both in buffer and blood as a function of the mol% DPSG in the liposomal formulations,

which could be explained by principles for colloidal stability (Derjaguin and Landau, 1941; Verwey and Overbeek, 1948). Two charged liposomes approaching each other in a stable dispersion have an energy barrier against aggregation, set up by electrostatic repulsion. The size of this barrier, and with that the stability of the liposome dispersion, is a function of the liposomal surface charge density. This charge density increases as a function of mol% DPSG at high pH, where the acid groups are charged. Consequently, the liposomes with the highest content of DPSG should also have the highest barrier against aggregation and, as a result, the pH–r_1 profiles are expected to be shifted laterally to lower pH values. Similar effects have previously been reported for liquid crystalline pH-sensitive liposomes (Jizomoto et al., 1994). A lateral shift in the pH–r_1 profiles could be diagnostically important. It is well known that the interstitial acidity varies considerably from tumour type to tumour type and depends on the severity of the disease. Modifying the systems with respect to pH_{20} could therefore be necessary for the suitable utility of the liposomal contrast agent.

Increasing the PE chain length gave no significant change in pH_{20} in buffer (Fig. 5A), but in blood the pH_{20} decreased significantly from a PE chain length of 16–14 carbon atoms (Fig. 5B). The lower pH_{20} for the DMPE/DPSG liposomes could be due to bilayer interaction with amphiphilic blood components and a diminished pH-sensitivity, as described above. The R^2 values for the models in buffer and blood were 0.85 and 0.95, respectively.

An important difference between the two response surfaces in Fig. 5 is the significantly higher pH_{20} after incubation in blood (Fig. 5B) compared to incubation in buffer (Fig. 5A). This lateral shift of the pH–r_1 profiles, previously described for DPPE/DPSG (20 mol% DPSG) liposomal GdDTPA-BMA (Lokling et al., 2003a), to higher pH values in blood may, at least partly, be caused by the higher concentrations of cations in blood. An increased counter ion concentration screens the surface charges of the anionic liposomes and, consequently, lowers the barrier against aggregation. The pH value at which the liposomes aggregated and released GdDTPA-BMA should therefore be shifted upwards, an effect which has previously been described for DPPE/palmitic acid liposomal GdDTPA-BMA in buffer (Lokling et al., 2003b). The displaced pH–r_1 profiles in blood are most likely in a more diagnostic relevant pH region than the corresponding profiles in buffer.

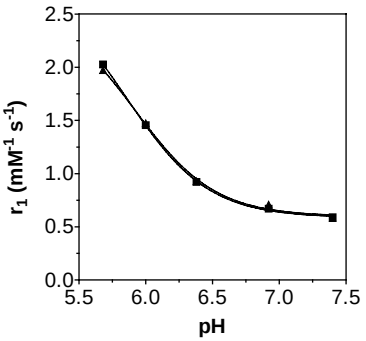

Fig. 6. pH–r_1 dependence of DPPE/DPSG (■) and DSPE/DPSG (▲) liposomal GdDTPA-BMA at 37 °C, both containing 30 mol% DPSG after 20-min incubation in human whole blood, followed by pH adjustment with MES/HEPES buffers (one vial with NaCl solution, pH 7.4) and further incubation for 20 min. Data points are given as the arithmetic mean of duplicates (0.47 T). The lines are for guidance only.

3.4. Effect of pre-incubation in human whole blood

The diagnostic application of intravenously injected paramagnetic liposomes requires minimal leakage of liposome contents and/or diminished pH-sensitivity, prior to reaching the target tissue. The pH–r_1 dependence for the most promising systems, with respect to relaxometric response in buffered blood, was therefore investigated after 20-min pre-incubation in blood, followed by pH adjustment and further incubation for 20 min. Fig. 6 shows the pH–r_1 dependence for two representative liposomal systems. The pH–r_1 profiles were similar to those after incubation in buffered blood and the pH-sensitivity of the liposomes was not markedly diminished after pre-incubation in blood. These findings were in accordance with previously reported results (Lokling et al., 2003a). No significant increase in liposomal r_1 was observed after prolonged incubation at physiological pH (results not shown), demonstrating the low extent of leakage of GdDTPA-BMA at pH 7.4.

4. Conclusions

It was possible to modify the relaxometric response and pH_{20} of PE/DPSG liposomal GdDTPA-BMA

as a function of bilayer composition both in buffer and buffered blood. Furthermore, the liposomal systems displayed minimal leakage of contrast agent after incubation in blood at physiological pH and, equally important, retained their pH-sensitivity after pre-incubation in blood.

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