Available online at www.sciencedirect.com

ScienceDirect

Dyes and Pigments 79 (2008) 170–175

DYES and PIGMENTS

www.elsevier.com/locate/dyepig

Absorption and emission spectral shifts of rose bengal associated with DMPC liposomes

Cheng-Chung Chang [a], Yu-Tsai Yang [b], Jen-Chang Yang [b,c], Hong-Da Wu [b], Tsuimin Tsai [b,c,*]

[a] Department of Chemistry, National Chung-Hsing University, Taichung, Taiwan
[b] Graduate School of Dentistry, Taipei Medical University, Taiwan
[c] Graduate Institute of Biomedical Materials and Engineering, Taipei Medical University, 250 Wu Xing Street, Taipei 110, Taiwan

Received 17 December 2007; received in revised form 4 February 2008; accepted 12 February 2008
Available online 21 February 2008

Abstract

Rose bengal is a water-soluble xanthene dye that is currently used in ophthalmology for the diagnosis of dry eyes. Although the dye is also a potential photosensitizer for photodynamic therapy of tumors, owing to insufficient lipophilicity and tumor accumulation, the clinical application of rose bengal in photodynamic therapy has been hampered. Liposomal encapsulation was seen as a promising approach to overcome these disadvantages, to which end, the spectral properties of the dye in the presence of materials for liposome preparation were studied. The presence of phospholipid influenced the spectral properties of the dye, probably due to the establishment of an equilibrium between monomeric and dimeric forms of the dye, since the photophysical properties of rose bengal depend strongly on its environment. The liposomal encapsulation of the dye generates stronger emission than the free form of the colorant; increased lipid:dye ratio further enhances this emission.
© 2008 Elsevier Ltd. All rights reserved.

Keywords: Rose bengal; Liposome; Photosensitizer; Absorption; Emission; Spectrum

1. Introduction

Photodynamic therapy (PDT) is an evolving modality for the treatment of superficial tumors by utilizing photosensitizing agents that are able to photochemically eradicate malignant/abnormal cells. Various photosensitizers used in PDT include the merocyanines, xanthenes, phthalocyanines and hematoporphyrin dyes, where the first two are mainly used in basic research and the last two for clinical treatment [1–3].

Rose bengal (RB) is a xanthene photosensitizer with a high absorption coefficient in the visible region of the spectrum and a tendency to transfer electrons from its excited triplet state, producing long-lived radicals [4,5]. RB has been exploited as a promising sensitizer in waste water treatment due to its water solubility, absorption in the visible region, good quantum yield of singlet oxygen, and inexpensiveness [6–8]. However, the low lipid solubility of RB has limited its capacity to cross biological barriers such as the cell membranes, and thus limits its clinical application. In addition, it has been reported that in vitro culture of rabbit corneal epithelial cells in the presence of 0.1% RB would result in cell detachment and cell loss [9], and this is due to the intrinsic toxicity associated with RB probably due to its high polarity. Liposomes [10] have been developed as drug carriers for many years to modify the solubility of drugs with extreme polarity (either polar or non-polar), to help target the drug to its therapeutic sites, or to alter the release profile of the therapeutic agent. Liposomes have been shown to enhance the clinical effects of photosensitizers by reducing their toxicity and protecting them from metabolism and immune responses [11–13]. For better clinical results, it is necessary to have a suitable pharmaceutical formulation to supply the delivery of therapeutic or diagnostic agents. With the aim of improving the

* Corresponding author. Graduate Institute of Biomedical Materials and Engineering, Taipei Medical University, 250 Wu Xing Street, Taipei 110, Taiwan. Tel.: +886 02 27361661x5203; fax: +886 02 27390082.
E-mail address: tmtsai00@tmu.edu.tw (T. Tsai).

0143-7208/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dyepig.2008.02.003

lipophilicity and decreasing the cytotoxicity of RB, liposomal delivery could be a better choice.

It has been reported that RB tends to aggregate in solution at high concentration ($>5 \times 10^{-5}$ M), and such phenomenon restricts the widespread use of RB in solution since the formation of aggregate impaired the photochemical response [14–17]. The existence of different phases or microphases may obscure the interpretation of spectroscopic data because aggregation can take place in more than one phase. Furthermore, heterogeneous systems are normally complicated by the occurrence of light scattering. The nature of the interaction between dye and solvent and between dye and another existing solid/semisolid surface not only determines the partition equilibrium of the dye but also the degree of aggregation. The aggregation process is especially favored in aqueous suspension due to the highly hydrophobic core of the dye. Theoretically, RB would be encapsulated in the hydrophilic core of the liposome rather than locating at the lipid bilayers. Therefore, the aggregation phenomenon of RB in the liposome is assumed to be similar to that in the aqueous solution. In addition, the location and intensity of RB absorption bands were reported to be a function of the solvent composition [17,18], thus selection of the solvent is a very important step in the quantification of RB, especially for a suspension system.

Incorporation of hydrophobic photosensitizers into liposomes has shown a tendency towards forming various aggregation states, which are especially favored by certain solvents [19]. Hydrophobic photosensitizers may aggregate when incorporating into the lipid bilayers, and prevention of both spectral shifting and fluorescence quenching related aggregation seems to be the trend in the development of a better photosensitizer. To date, quantification of the photosensitizer is still heavily relied on spectroscopic methods, however, researchers have become aware that quantitation of the liposomal encapsulated photosensitizer may be hindered due to the formation of aggregates. More reports about the formation of photosensitizer aggregates incorporating in liposomes have been focused on hydrophobic porphyrins. Since hydrophilic photosensitizers may also be incorporated into a liposome, the potential of forming aggregates should be explored. In this study, the absorbance, emitting spectra and fluorescence quantum yield of RB suspension in the water, 0.9% NaC1, ethanol, lipid, empty liposome and encapsulated into the liposome were investigated.

2. Experimental

2.1. Materials

Rose bengal (RB) and dimyristoylphosphatidylcholine (DMPC) were purchased from Sigma (St Louis, MO, USA) and Aventi (Alabaster, AL, USA), respectively. NaCl was purchased from Riedel-de-Häen® (Seelze, Germany), and Sephadex cartridge pre-packed with G-50 silica gel was obtained from Pharmacia Biotech (Uppsala, Sweden). Absolute ethanol was used immediately after open and experiments were performed on freshly prepared samples, though suspensions were found to be stable. A Milli-Q system (Academic, ZMQS60001) was used to purify H_2O, and all other chemicals were analytical reagents obtained from Sigma.

2.2. Methods

2.2.1. Preparation and characterization of liposomal RB

RB was encapsulated in liposomes by reversed-phase evaporation and extrusion (filter pore size = 100 nm). Briefly, 10 μmole DMPC lipids were dissolved in 200 μl chloroform. After layered at the bottom of a test tube by evaporating the chloroform solvent, RB dissolved in 1 ml 0.9% NaCl buffer was added to the tube and the mixture was vortexed for 3 min at 25 °C. The formed multilamellar vesicles (MLVs) dispersion was then passed through a 100 nm diameter polycarbonate membrane for 11 times to form the small unilamellar vesicles (SUVs). The untrapped free RB and lipids were removed by size exclusion chromatography using a Sephadex G-50 column. The final solution on suspension of liposomal RB was in 0.9% (w/v) NaCl and stored at 4 °C. Size distribution was measured with dynamic light scattering using a particle sizer (Coulter N4 Plus Submicron, Beckman Coulter). The amount of RB encapsulated in the liposome was determined by spectrofluorimetry on a Fluorolog®-3 fluorimeter (excitation: 550 nm, emission: 572 nm) after disruption of the liposomes with absolute ethanol. The RB standards were measured using the same condition. The lipid concentration of liposome was determined by the Bartlett assay [20].

2.2.2. Preparation of RB mixed with lipid or empty liposome

Lipids (50 μmole) were dissolved in 1 ml chloroform as the lipid stock solution. The lipid mixed with RB in 0.9% NaCl was made with 2 μl lipid stock and 998 μl 0.9% NaCl containing RB, and the final concentration of chloroform is 0.2%. Empty liposomes were prepared and characterized similar to that described in Section 2.2.1, and the only difference is that the hydration buffer contains no RB in it.

2.2.3. Spectroscopic analysis

Studies on absorption and fluorescence spectra of RB were carried out in water, 0.9% NaCl, absolute ethanol, 0.9% NaCl mixed lipid or empty liposome and encapsulated in liposomes suspension in 0.9% NaCl. Absorption spectra were measured with a Beckman COULTER DU800 spectrophotometer. Fluorescence spectra were obtained on a Fluorolog®-3 luminescence spectrometer.

2.2.4. Fluorescence quantum yield (Φ_F)

The fluorescence quantum yields of RB in different solvents were measured using the ratio method described by Eaton equation [21], utilizing RB in ethanol as a standard ($\Phi_{\text{Fstandard}} = 0.05$) [18]

$$\Phi_{\text{F}(X)} = \Phi_{\text{Fstandard}}\left(\frac{A_{\text{standard}}}{A_{\text{sample}}}\right)\left(\frac{F_{\text{sample}}}{F_{\text{standard}}}\right)\left(\frac{n_{\text{sample}}}{n_{\text{standard}}}\right)^2$$

where F_{sample} and F_{standard} are the measured fluorescence for the sample and standard, respectively, A_{standard} and A_{sample}

Table 1
The composition of the different formulations of RB used in this study

Solvent	Lipid (μM)	RB (μM)
Water	0	2
0.9% NaCl	0	2
Ethanol	0	2
Lipid[a] (RB + lipid)	0.1	2
Empty liposome[a] (RB + empty liposome)	0.1	2
Enacapsulated in liposome[a] (liposomal RB)	0.1	2

[a] Suspension medium: 0.9% NaCl.

are the measured absorbance, n_{sample} and $n_{standard}$ are the refractive index of the solvent used for the sample and standard, respectively, and $\Phi_{Fstandard}$ is the fluorescence quantum yield of the standard.

3. Results and discussion

Table 1 summarizes the composition of the different formulations of RB used in this study. The size of the liposomes prepared with or without encapsulating RB was all around 80–100 nm. For all liposomal RB, the encapsulation efficiency was above 80%. Fig. 1 shows the absorption and emission spectra of RB in the 450–700 nm range in various molecular environments. The λ_{max} values and the shape of the spectra strongly depend on the solvents: water 548.5 (shoulder 511.2) nm, 0.9% NaCl 548.9 (510.8) nm, ethanol 558.0 (518.9) nm, lipid 563.5 (522.6) nm, empty liposomes 563.9 (522.2) nm and liposomal RB 563.9 (523.5) as shown in Table 2. The relative intensity of shoulder to the peak is usually used as a measure of the aggregation of RB in solution [22]. Larger ratio value (absorption of longer wavelength to shorter wavelength) indicates lesser aggregation. The ratios of the intensities of these two bands are 0.9% NaCl (3.20), water (2.88), ethanol (3.33), lipid (3.91), empty liposomes (3.85) and liposomal RB (3.45). Corresponding to the absorption condition, under 548 nm excitation, the fluorescence quantum

Table 2
The absorption position and fluorescence quantum yield of RB in various molecular environments (RB: 2 μM; Ex: 548 nm)

	Absorption (nm)			Fluorescence (Quantum yield)[d]
	λ_1	λ_2[b]	Intensity ratios (λ_1/λ_2)	
0.9% NaCl	548.9	510.8	3.20	565.8 (0.005)
Water	548.5	511.2	2.88	565.8 (0.006)
EtOH[a]	559.0	518.9	3.33	573.2 (0.050)
Free lipid[c]	563.5	522.6	3.91	579.6 (0.059)
Free liposome[c]	563.9	522.2	3.85	579.4 (0.063)
Liposomal RB	563.9	523.5	3.45	579.2 (0.060)

[a] Quantum yield reference.
[b] λ_1: Max, λ_2: shoulder.
[c] Mixing solution directly.
[d] Excited wavelength (548 nm).

yield (Φ_F) can be normalized based on the RB in ethanol condition ($\Phi_F = 0.05$); fluorescence emission spectra of RB yielded significant patterns of solvent effect on RB. Compared to aqueous solutions, these emission spectra were intensities-enhancement and red-shifted in ethanol and in lipid or liposome series; 0.9% NaCl 565.8 ($\Phi_F = 0.005$) nm, water 565.8 ($\Phi_F = 0.006$) nm, ethanol 573.2 ($\Phi_F = 0.050$) nm, lipid 579.6 ($\Phi_F = 0.059$) nm, empty liposomes 579.4 ($\Phi_F = 0.063$) nm and liposomal RB 579.2 ($\Phi_F = 0.060$) nm. Since longer wavelength of light tends to penetrate deeper into the biological tissue, and higher fluorescence quantum yield is preferential to generate more efficient PDT result, the above finding indicates that in the presence of DMPC (whether in its lipid solution form or as liposomes with or without encapsulating RB), the fluorescence spectra red-shifted and generated higher quantum yield.

In water solution, with or without NaCl, the spectra show neither significant wavelength shifts nor relevant modifications in shapes. The only difference is the magnitude of the absorption coefficient, probably due to aggregation with respect to the intensity ratios in difference. In DMPC lipid, liposome or encapsulated in liposomes conditions, absorption and

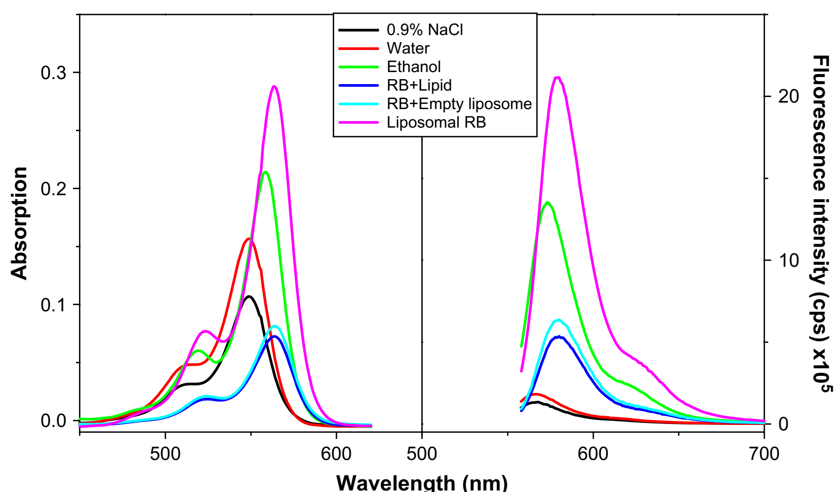

Fig. 1. Absorption and emission spectra of RB (2 μM) in the 450–700 nm range in the following molecular environments: dissolved in water; dissolved in 0.9% aqueous NaCl; dissolved in ethanol; dissolved in 0.9% aqueous NaCl containing lipid; dissolved in 0.9% aqueous NaCl containing empty liposomes; encapsulated in liposomes and dispersed in 0.9% aqueous NaCl.

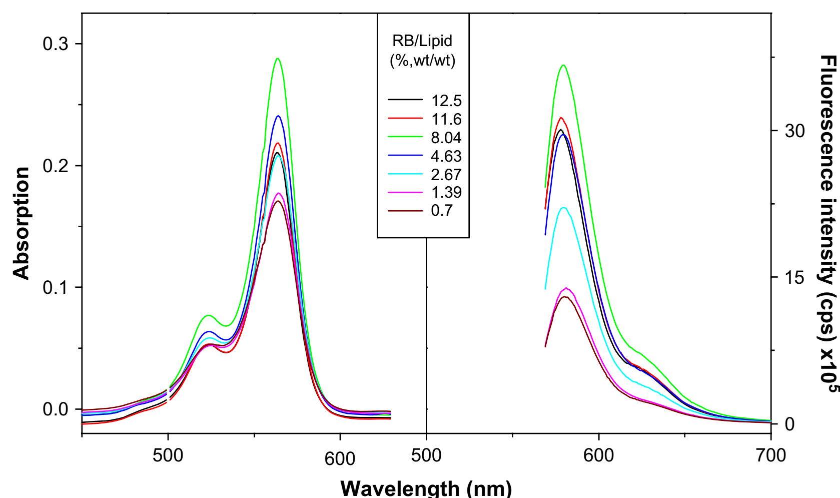

Fig. 2. The absorption and fluorescence spectrum of liposomal RB at different weight of RB and lipid ratio. (RB: 2 μM, Ex: 560 nm.)

emission patterns of RB are shifted to higher wavelengths with variable of degree between λ_{max} and shoulders. The intensity ratios (Table 2) of the two bands corresponding to the dimer and monomer species are almost constant in directly solution-mixing condition, with lipids and liposomes. Nevertheless, after normalization, the quantum yields of RB in those lipid or liposome conditions always keep in constant with respect to the similar maximum emission wavelength. Hence, we assume that RB should be at the similar environment either when encapsulated into the liposomes or when in associated with lipids or liposomes.

Fig. 2 depicts the fluorescence spectra of liposomal RB containing the same amount of RB in association with different concentrations of lipid under 560 nm excitation. Increasing the lipid concentration in the liposome suspended aqueous solution produces a gradual increase in the intensity of the fluorescence signal, similar to that observed by Ricchelli et al. [23]. The presence of fluorescence signals seems to indicate that the absorption peak at 563 nm can be ascribed to the presence of the monomeric RB, as further confirmed by the fluorescence quantum yield under normalization of absorbance intensities, compared to that obtained for the monomer at the RB concentration in ethanol with quantum yield $\Phi_F = 0.05$ [18]. Of interests are about the spectra data of RB in lipid and empty liposome as shown in Fig. 1 and Table 2. Free RB has the opportunity to be encapsulated in lipid or empty liposome in these solutions, and the higher intensity ratios indicated that hydrophilic RB goes through the bilayer and stays in the hydrophilic cavities mainly as monomeric form but not as dimer form. This is interesting since one would expect to see the liposomal encapsulated RB form dimers or larger aggregates as they usually are in the hydrophilic environment. It is assumed that majority of the dimeric forms of RB generally come from the preparation of RB during the encapsulation process.

There is little doubt about DMPC lipid aggregates spontaneously and becomes vesicles even at very low concentration in aqueous solution. Whereas, it appears that the concentration of lipid concentration plays a relevant role in encapsulating RB. In general, addition of lipid results in the red shift of the absorption in favor of the species absorbing at ∼563 and ∼523 nm. The increased concentration of the lipid seems mainly to decrease the existence of dimeric RB in the inner cavity and, consequently, perturb the fluorescence quantum yield (Table 3 and Fig. 3). Since the photophysical RB properties relevant for PDT are mainly associated with the presence

Table 3
The intensity ratios (λ_1/λ_2) and fluorescence quantum yield of liposomal RB at different weight ratio of RB and lipid

(RB/LP)[a]	Absorption (nm)			Fluorescence (Quantum yield)[b]
	λ_1	λ_2	Intensity ratios (λ_1/λ_2)	
0.7	563.5	523.1	3.24	580.6 (0.045)
1.39	563.9	523.5	3.42	581.0 (0.047)
2.67	563.9	523.5	3.57	579.3 (0.062)
4.63	563.9	523.5	3.78	579.3 (0.071)
8.04	563.5	522.6	4.01	579.3 (0.079)
11.6	563.9	523.1	4.03	578.4 (0.083)
12.5	563.5	523.1	3.97	577.8 (0.081)

[a] Weight percentage of RB over lipid (%).
[b] Excited wavelength (560 nm).

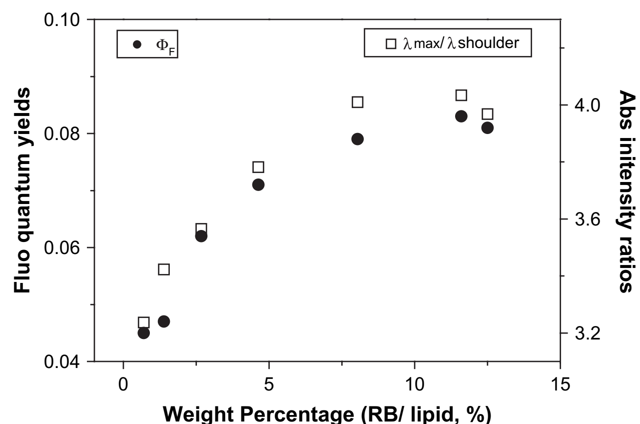

Fig. 3. The intensity ratios ($\lambda_{max}/\lambda_{shoulder}$) and fluorescence quantum yield of liposomal RB at different weight ratio of RB and lipid.

of the monomeric form, the inclusive ability of liposome to modulate the amounts of the monomer and dimer RB in solution turns out to be of particular interest in view of an eventual use in photodynamic therapy. At a concentration ratio [RB]/[lipid] lower than ~7.5% (where the concentration of lipid is 100 nM), fluorescence quantum yields and intensity ratios are linear with increasing lipid concentration; that is, the linear relationship is molar ratio controlled in the range below 7.5% [RB]/[lipid]. Nevertheless, a suitable inclusion ratio of liposomal RB is located at ~10 wt%. In order to overcome the aggregation of RB in water, covalently attached RB to chitosans has been tried [24]. Moczek and Nowakowska found that the major absorption peak of RB in water after linked to chitosan became broader and red-shifted to 560 nm (shoulder peak at 525 nm), and the ratio of the intensities of these two bands is similar to that of RB in water. The results indicated that the shape of the absorption spectrum and the ratio of the absorbance at the maxima are not dependent on polymer concentration range studied (1–0.1 g/L), and the quantum yield of singlet oxygen formed by RB after modified with chitosan was very similar to free RB in water. The conclusion was that RB attached to the chitosan did not decrease the photosensitizing activity of chromophores. Although chitosan can find application as a carrier for RB in water, this has to be done by linking RB via the covalent bonding to chitosan, and the degree of interaction probably would affect the release of RB.

In our liposomal system, a 1.4-fold in the ratio increase of the intensities of these two bands was observed with the liposomal RB (10% wt/wt) compared to that in water. The release rate of RB from the liposomes may be better than the RB covalently associated with chitosan. Effect of the fluorescence quantum yield result by the different dye:lipid ratio indicates that increasing the ratio with decreased fluorescence yield is not only because of the presence of a weakly fluorescence dimer that absorbs a high fraction of the total absorbed light but also due to quenching of monomer emission.

From the results in lipid-free aqueous solution, the fluorescence intensity of the RB in water is much lower compared to that in ethanol at the same concentration; this effect can be viewed in relation with the different balance between monomer and aggregate existing in the solutions of liposome, as shown in Fig. 3. Hence, the binding constants K of RB–LP complex were obtained by means of fluorescence quantum yield data using the Benesi–Hildebrand model [25] and a linear fitting slope and intercept were calculated. The $K = 3.65 \times 10^7\ M^{-1}$ and quantum yield of RB in RB–LP complex is $\Phi_F = 0.0086$ which almost match with the continuous variation method [26,27] (job plot, Fig. 3). In this figure, the shifts and intensities both present a maxima, which allowed for easy determination of K and Φ_F of the complex with no perturbation of dimer and determination of the optimum condition for preparing the RB incorporated in liposomes.

4. Conclusions

Liposomal encapsulation of RB that can be excited at a longer wavelength is of potential interest for PDT since deeper tissue penetration can be reached with longer visible wavelength of light. Spectral studies can be a simple and useful screening gate in the development of a delivery system for photosensitizers for PDT.

Acknowledgements

This work was supported by the grant NSC93-2731-B038-014 from the National Science Council, Taipei, Taiwan.

References

[1] Vena FCB, Turchielo RF, Blais J, Laville I, Pigalio S, Tedesco AC. 5-Aminolevulinic acid ester-induced protoporphyrin IX in a murine melanoma cell line. Lasers in Medical Science 2004;19(2):119–26.

[2] Nunes SMT, Dos F, Sguila S, Tedesco AC. Photophysical studies of zinc phthalocyanine and chloroaluminum phthalocyanine incorporated into liposomes in the presence of additives. Brazilian Journal of Medical and Biological Research 2004;37:273–84.

[3] Turchiello RF, Vena FCB, Maillard Ph, Souza CS, Bentley MVBL, Tedesco AC. Cubic phase gel as a drug delivery system for topical application of 5-ALA, its ester derivatives and m-THPC in photodynamic therapy (PDT). Journal of Photochemistry and Photobiology B: Biology 2003;70:1–6.

[4] Giulivi C, Sarcansky M, Rosenfeld E, Boveris A. The photodynamic effect of rose bengal on proteins of the mitochondrial inner membrane. Photochemistry and Photobiology 1990;52(4):745–51.

[5] Kochevar IE, Lambert CR, Lynch MC, Tedesco AC. Comparison of photosensitized plasma membrane damage caused by singlet oxygen and free radicals. Biochimica et Biophysica Acta 1996;1280(2):223–30.

[6] Killig F, Stark G, Apell HJ. Photodynamic inactivation of the Na, K-ATPase occurs via different pathways. The Journal of Membrane Biology 2004;200:133–44.

[7] Paulino TP, Ribeiro KF, Thedei Jr G, Tedesco AC, Ciancaglini P. Use of hand held photopolymerizer to photoinactivate streptococcus mutans. Archives of Oral Biology 2005;50:353–9.

[8] Wilkinson F, Helman WP, Ross AB. Quantum yields for the photosensitized formation of the lowest electronically excited singlet state of molecular oxygen in solution. Journal of Physical and Chemical Reference Data 1993;22:113.

[9] Feenstra RP, Tseng SC. What is actually stained by rose bengal? Archives of Ophthalmology 1992;110:984–93.

[10] Caruthers SD, Wickline SA, Lanza GM. Nanotechnological applications in medicine. Current Opinion in Biotechnology 2007;18(1):26–30.

[11] Oku N, Namba Y, Okada S. Tumor accumulation of novel RES-avoiding liposomes. Biochimica et Biophysica Acta (BBA) – Lipids and Lipid Metabolism 1992;1126:255–60.

[12] Hoebeke M. The importance of liposomes as models and tools in the understanding of photosensitization mechanisms. Journal of Photochemistry and Photobiology B: Biology 1995;28:189–96.

[13] Reddi E. Role of delivery vehicles for photosensitizers in the photodynamic therapy of tumours. Journal of Photochemistry and Photobiology B: Biology 1997;37:189–95.

[14] Aguilera OV, Neckers DC. Aggregation of rose bengal ethyl ester induced by alkali metal cations in aqueous solution. Journal of Photochemistry and Photobiology A: Chemistry 1989;47(2):213–22.

[15] Rohatgi KK, Mukhopadhyay AK. Isolation of unique dimer spectra of dyes from the composite spectra of aggregated solutions. Photochemistry and Photobiology 1971;14:551–9.

[16] Aguilera V, Neckers DC. Rose bengal ethyl ester aggregation in aqueous solution. The Journal of Physical Chemistry 1988;92:4286–9.

[17] Xu D, Neckers DC. Aggregation of rose bengal molecules in solution. Journal of Photochemistry and Photobiology A: Chemistry 1987;40:361–70.

[18] Linden SM, Neckers DC. Type I and type II sensitizers based on rose bengal onium salts. Photochemistry and Photobiology 1988;47(4):543–50.

[19] Postigo F, Mora M, De Madariaga MA, Nonell S, Sagrista ML. Incorporation of hydrophobic porphyrins into liposomes: characterization and structural requirements. International Journal of Pharmaceutics 2004;278:239−54.
[20] Barlett GR. Phosphorus assay in column chromatography. Journal of Biological Chemistry 1959;234:466−8.
[21] Eaton DF. International union of pure and applied chemistry organic chemistry division commission on photochemistry. Reference materials for fluorescence measurements. Journal of Photochemistry and Photobiology B: Biology 1988;4:523−31.
[22] Islam SDM, Ito O. Solvent effects on rates of photochemical reactions of rose bengal triplet state studied by nanosecond laser photolysis. Journal of Photochemistry and Photobiology A: Chemistry 1999;123:53−9.
[23] Ricchelli F, Stevanin D, Jori G. Porphyrin−liposome interactions: influence of the physico-chemical properties of the phospholipid bilayer. Photochemistry and Photobiology 1988;48(1):13−8.
[24] Moczek L-ukasz, Nowakowska Maria. Novel water-soluble photosensitizers from chitosan. Biomacromolecules 2007;8:433−8.
[25] Nigam S, Durocher G. Spectral and photophysical studies of inclusion complexes of some neutral 3*H*-indoles and their cations and anions with β-cyclodextrin. The Journal of Physical Chemistry 1996;100:7135−42.
[26] Ladbury JE, Chowdhry BZ. Sensing the heat: the application of isothermal titration calorimetry to thermodynamic studies of biomolecular interactions. Chemistry and Biology 1996;3:791−801.
[27] Keating LR, Szalai VA. Parallel-stranded guanine quadruplex interactions with a copper cationic porphyrin. Biochemistry 2004;43:15891−900.