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PHOTODEGRADATION OF NIMODIPINE AND FELODIPINE IN MICROHETEROGENEOUS SYSTEMS

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ABSTRACT

The photochemical behavior of nimodipine (NIMO) and felodipine (FELO), photolabile drugs widely used as antihypertensive calcium channel blockers, is studied in constrained media. Specifically, we are interested in the kinetic analysis of 4-aryl-1,4-dihydropyridine photodegradation processes when they are incorporated in biological-mimicking systems like micelles or liposomes. In order to establish if the nature of the head of surfactant (ionic or nonionic) could be important modulating the photo-reactivity of these drugs, we studied the photodegradation of NIMO and FELO incorporated in micelles formed with sodium dodecyl sulfate (SDS, anionic), dodecyl-pyridinium chloride (DPC, cationic) and mono lauryl sucrose ester (MLS, nonionic) as surfactants. Additionally, the results of the photodegradation of these compounds in liposomes were also included. The results clearly indicate that both dihydropyridines studied, NIMO and FELO, are located near to the interface, but the surface charge of micelles does not affect neither, the photodegradation rate constant nor the photodegradation products profile. The absence of singlet oxygen generation in micellar media is consistent with the proposition of these 4-aryl-1,4-dihidropyridines located near to the interface of the micelle, where a polar environment is sensed. In addition, the ethanol preferential location on membranes and dihydropyridine enhanced photodegradation by alcohol presence are interesting results to consider in future research.

INTRODUCTION

Nimodipine (NIMO) and felodipine (FELO) are calcium channel blockers (second-generation 4-aryl-1,4-dihydropyridines), widely used as antihypertensive drugs, whose chemical structures are shown in Figure 1.[1,2] Their pharmacological actions consist in blocking the entrance of extracellular calcium into vascular and cardiac muscles with different selectivity, diminishing blood pressure, associated with their vasodilator action.[3,4] Other biological activities such as the ability of scavenge reactive oxygen species (ROS) have been also attributed to them.[5,6] Unfortunately, they are photolabile and have been related to skin photodamage.[7-9]

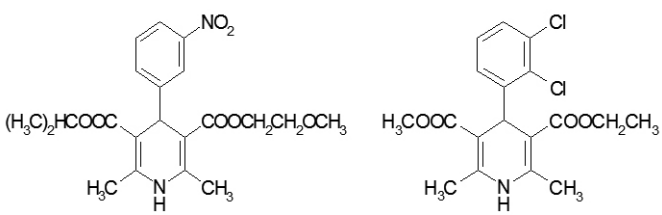

Figure 1. Chemical structures of NIMO and FELO.

There are relatively few reports about the mechanism of photochemical behavior of 4-aryl-1,4-dihidropyridines.[10-14] For NIMO and FELO, we have previously stablished that their photodegradation pathway mainly involves their lowest singlet excited states.[15] The zwitterionic biradical intermediate generated by the fast intramolecular electron transfer yields a pyridine derivative as main photoproduct by an intermolecular proton transfer to the solvent.[16]

In our preceding study we have analyzed the photophysics and photochemistry of both 4-aryl-1,4-dihydropyridines (NIMO and FELO) in homogeneous media.[15] In the present work, considering their reported lipophilicity[17] and a photostabilizing effect when they are incorporated into organized microenvironments,[18] we were interested in the study of the behavior of these drugs in micro-organized media.

Most of chemical processes occurring in biological systems take place confined within small regions in the order of nanometers.[19] The physical chemical properties of these nano-environments, like polarity, viscosity, and pH among others, are usually very different from those in a bulk medium.[20-23] Inside these small regions, the reactants will be closer and probably with specific orientations, these considerations and the local properties of media can exert intense influence on the structure, reactivity, and dynamics of the confined chemical species.[24,25] There are several reports indicating that photochemistry in organized microheterogeneous media can differ markedly from that in any homogeneous fluid medium.[26,27] Additionally, microheterogeneous systems are frequently employed as useful tools to mimic the extremely efficient chemical processes in the biological systems.[28]

Specifically in this work, our main concern is the kinetic analysis of 4-aryl-1,4-dihydropyridine photodegradation processes when they are incorporated into biological-mimicking systems like liposomes or micellar media. As the nature of the head of surfactants (charged or neutral) defines microenvironment physical-chemistry properties, the ionic or non-ionic character could be important in modulating the photo-reactivity of these drugs. We studied the photodegradation of NIMO and FELO incorporated to micelles formed with different kinds of surfactants: sodium dodecyl sulfate (SDS, anionic), dodecylpyridinium chloride (DPC, cationic) and mono lauryl sucrose ester (MLS, nonionic), whose chemical structures are shown in Figure 2. With the aim to extend the study to systems closer to biological membranes, the results of the photodegradation of these compounds in synthetic membranes (liposomes) of dipalmitoylphosphatidylcholine (DPPC) were also included.

On the other hand, as biological substrates are able to react with reactive oxygen species (ROS), it is important to determine the capacity of these drugs to generate transients such as singlet oxygen, $O_2(^1D_g)$.[29] Moreover, singlet oxygen can react with the same drug (self-sensitized photooxidation), yielding other photoactive species or toxic products, with concomitant reduction of its pharmacological effect. We have previously demonstrated that the singlet oxygen generation quantum yields for NIMO and FELO are significantly dependent on the polarity of its surrounding media,[15] thus the measurements of this parameter in microheterogeneous systems will give us information about its location in the constrained media.

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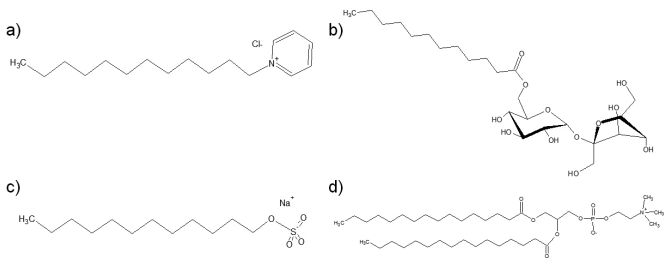


Figure 2. Chemical structures of surfactants: a) dodecyl-pyridinium chloride (DPC) b) mono-lauryl sucrose ester (MLS); c) sodium dodecyl sulfate (SDS); d) dipalmitoyl phosphatidylcholine (DPPC).

2. EXPERIMENTAL SECTION

2.1. Drugs and Reagents

Nimodipine (1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid 2-methoxyethyl 1-methylethyl ester) and felodipine (4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethylpyridine-3,5-pyridinedicarboxylic acid ethyl methyl ester) (Sigma) were used as received. 9,10-dimethylanthracene (DMA), Octadecylamine (ODA) from Aldrich were used without further purification. All solvents (Merck) were of spectroscopic or HPLC grade. Water was purified and deionized using a Waters Milli-Q system. 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) (Sigma) was used as received. Sodium dodecyl sulfate (SDS), from Merck, and dodecylpiridinium chloride (DPC), from Aldrich, were recrystallized twice from acetone. The sucrose monoester b-D-fructofuranosyl-6-*O*-lauryl-a-D-glucopyranoside (MLS) was synthesized and isolated as described in literature [30].

2.2. Apparatus and Procedures

UV-VIS absorption spectra and steady state kinetic experiments were performed in an Agilent 8453 spectrophotometer.

Micellar solutions were prepared adding a quantity of surfactant five times over the critic micellar concentration to milliQ water and sonicating during 30 minutes. Thus, micellar solution concentrations were SDS 40 mM, DPC 75 mM and MLS 1.75 mM. Incorporation of the drugs was made adding solid compound to the micellar solutions until saturation of the microaggregates.

Photolysis experiments were done with $1x10^{-4}$ M solutions (3 mL) of the compounds in several selected solvents, micellar or liposome air-equilibrated solutions in a 10 mm fluorescence quartz cell. The solutions were irradiated with a Black Ray UV lamp with a 366 nm filter. The radiant flux was determined as described in the literature.[31]

A Hewlett-Packard Gas Chromatograph model 5890 series II equipped with a NPD detector and a Hewlett-Packard Ultra-2 capillary column were used to monitor substrate consumption.

Singlet oxygen generation was checked with time-resolved phosphorescence experiments using phenalenone as reference (f_D = 0.98 in acetonitrile, f_D = 0.93 in benzene and f_D = 0.97 in ethanol),[32] by comparing the response of the detector extrapolated to zero time at low laser power, the latter adjusted with a glan-thompson polarizer. Optically matched solutions of drug and actinometer in the same solvent, were excited by the third harmonic (355nm, ca. with 28mJ per pulse as maximum power) of the Nd:YAG laser.

Liposome preparation

Dipalmitoylphosphatidylcholine with octadecylamine (9:1), at a desired concentration, were dissolved in chloroform. The organic solvent was evaporated under nitrogen stream and the dry lipid films were maintained 2 h under reduced pressure to remove solvent traces. Multilamellar vesicles were generated by the addition of phosphate buffer pH 7.4 at 60°C to the dried lipid film. The dispersion was vortexed vigorously and carefully frozen using a liquid nitrogen bath for 5 min and thawed in a water bath held at 60°C for the same period of time. This cycle was repeated five times. Multilamellar vesicles generated as such were previous term extruded through polycarbonate filters with 200 nm pores. NIMO-liposome standard solutions were prepared by addition of small volumes (5 or 10 uL) of a standard stock solution of NIMO in ethanol to DPPC liposome solution (3 mL). The mixture was homogenized in a vortex shaker and heated in a bath at 43°C for 30 min. Then the solution was slowly cooled up to 20°C and stored. In addition, drugs were also incorporated adding solid compounds to the liposome solutions, using an ultrasonic bath until saturation of the liposomes. The NIMO loaded MLVs were frozen and stored at −22 °C and just thawed before the extrusion procedure. The same protocol was used with FELO.

High-performance liquid chromatography (HPLC)

The HPLC system Waters consisted of a Waters 600 controller, helium degasser, column thermostat, quaternary pump and a Waters 996 photodiode array detector. Chromatographic analysis was performed using a ODS Hypersyl (5 mm-particle size, 20 cm × 4.6mm i.d.) column from Hewlett Packard. All experiments were carried out at column temperature of 20°C. The mobile phase consisted of a 49:1:50 v/v solution of water-glacial acetic acid-ethanol with isocratic elution at a flow-rate of 0.7 ml/min. The diode array detector was operated at 358 nm with 4 nm of bandwidth. Injection volume was set at 20 mL.

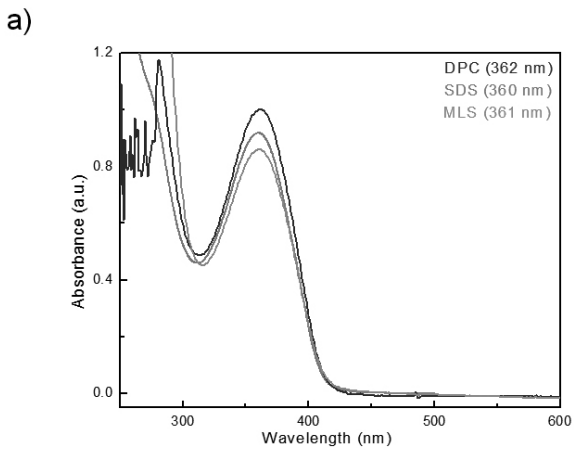
Preparation of NIMO loaded liposome samples for HPLC analysis

Samples of NIMO and FELO loaded liposomes for HPLC injections were prepared by diluting 300 mL of the liposome solution with 400 mL of ethanol in a conical plastic tube. The mixture was shaked in a vortex mixer for 2-3 min, left in rest by 10 min and then centrifuged at 4000 g during 30 min. Supernatant again centrifuged at 4000 g during 30 min, it was finally separated and employed for HPLC injections.

3. RESULTS AND DISCUSSION

3.1. Absorption properties and media effect.

The high lipophilicity reported for 4-phenyl-1,4-dihydropyridine derivatives,[17] allows to expect that they have a great affinity for hydrophobic microenvironments. This proposal can be proved when absorption spectra of these compounds dissolved in different media are observed (Fig. 3a). Absorption spectrum of saturated water solution of NIMO only shows a weak absorption band in the UV region (362 nm, $e = 6x10^3$ $M^{-1}s^{-1}$), while saturated micellar solutions present the relatively intense characteristic absorption band centered at around 360 nm ($e = 7x10^3$ $M^{-1}cm^{-1}$) atributted to the dihydropyridine chromophore. As the extinction coefficients are fairly solvent dependent ($e \sim 6.5x10^3$ $M^{-1}s^{-1}$ in organic solvents), the low intensity of NIMO absorption in water, can be ascribed to its low solubility. Indeed, the partition coefficients (K_p) were determined for NIMO and FELO between the different micellar solutions and water with Scott equation which relates the dependence of absorption with the micelle concentration.[33] The values determined for Log K_p are in the order of 2.8 for NIMO and 4.1 for FELO, confirming that these compounds are completely incorporated to micelles. The dihydropyridine chromophore absorption band is sensitive to the media as can be seen in Fig. 3b, being red-shifted as the solvent polarity is increased from benzene to ethanol. A difference of more than 10 nm is found between the maximum in micellar media when compared with an apolar solvent like benzene. The UV-Vis absorption spectra of 4-phenyl-1,4-dihydropyridine in micellar media does not depend on the nature of the head of surfactant. The position of absorption bands for NIMO or FELO incorporated to micelles formed with different kinds of surfactants like sodium dodecyl sulfate (SDS, anionic), dodecyl-pyridinium chloride (DPC, cationic) and mono lauryl sucrose ester (MLS, nonionic), remains the same. The bathochromic displacement of the absorption band in micellar media indicates that the compounds are incorporated and are located at the interface of the micelle in the region closer to where the water molecules can still penetrate.[34,35]



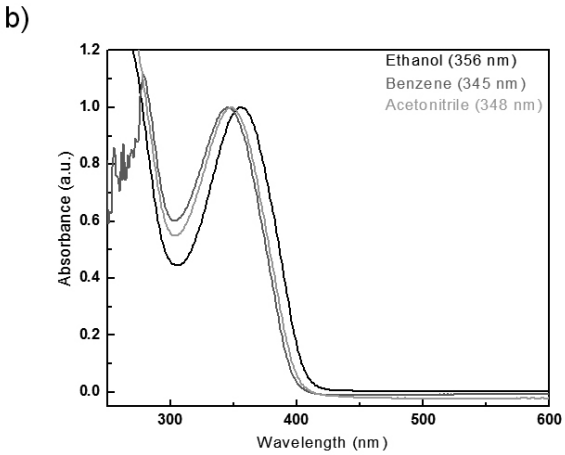


Figure 3. Absorption spectra of NIMO a) in micellar solutions; b) in homogeneous solvents.

3.2. Generation of Singlet Oxygen by NIMO and FELO in micellar media.

In a previous report,[15] we found that NIMO and FELO do no generate singlet oxygen in polar solvents like acetonitrile or ethanol, but in non-polar solvents, for example benzene, the generation quantum yield, F_D, being low, it shows measurable values of 0.085 and 0.003, for NIMO and FELO respectively. In the present word, we observe that when these compounds are incorporated into a micellar media, no phosphorescence signal of singlet oxygen is detected using the same experimental conditions that those employed for benzene as solvent. This behaviour is consistent with the proposition that the studied 4-aryl-1,4-dihidropyridines are located near to the interface of the micelle, where the polarity is dominated by the interaction of the water molecules and the heads of surfactants, resulting in a media with porperties similar to polar solvents.[34]

3.3. Media effect on the photodegradation kinetics.

The first order kinetic rate constants and quantum yields of NIMO and FELO photodegradation processes were obtained following the comsumption of the drugs in micellar solutions, after irradiation with UV light (365 nm). These results were compared with the values obtained in homogeneous solvents of different polarities (Table 1). The photodegradation rate constants found for both compounds in all micellar solutions were lower than the ones determined in benzene or ethanol.

Table 1. First Order Kinetic Constants, k, and Quantum Yields, F, of photodegradation of 1,4-Dihydropryridines.

	$k_{photodeg}$ (10^{-2} min^{-1})		$F_{photodeg}$ (10^{-3} molec./ abs. phot.)	
Medium	NIMO	FELO	NIMO	FELO
sodium dodecyl sulfate micelles (SDS)	5.75	0.37	2.86	0.22
dodecyl-pyridinium chloride micelles (DPC)	4.00	0.25	2.80	0.11
mono lauryl sucrose ester micelles (MLS)	5.50	0.30	1.40	0.12
DPPC liposomes with ethanol	26.4	0.48	10.00	0.27
DPPC liposomes without ethanol	5.28	0.16	5.00	0.12
acetonitrile	0.20	0.07	0.20	0.08
benzene	12.5	0.19	6.30	0.07
ethanol	25.0	0.33	11.30	0.14

k and F errors are within 10% for all results.

Fig. 4 shows the first order kinetic fit for NIMO photodecomposition in the different media. The highest values for the kinetic parameters were obtained in ethanol, and they have been interpreted in terms of the direct participation of solvent molecules in the mechanism pathway. Thus, when solvent is able to accept a proton from the zwiterionic biradical intermediate involved, the rate constant increases notably.[15, 16] In this way, if 4-aryl-1,4-dihydropyridines are incorporated near to the interface of the micelle, the microenvironment sensed would be relatively polar due to the presence of water molecules nearby, however they could not interact easily with the intermediate probably because the preferential interaction involves the surfactant heads and its presence hinders the approach of the solvent.

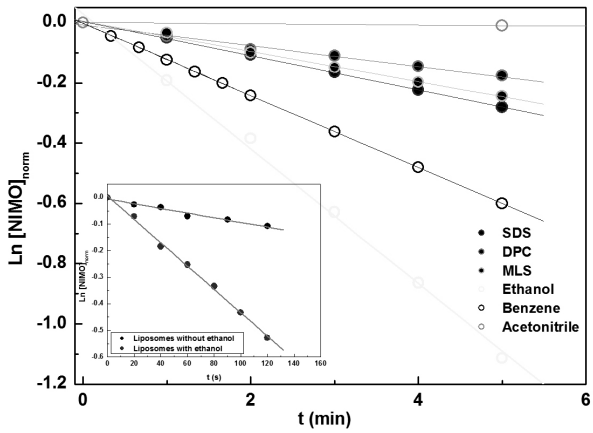


Figure 4. First order plot for the photodegradation of NIMO in different media: in homogeneous solvents (open circles) and in micellar solutions (filled circles). The inset shows the NIMO photodegradation in liposomes (DPPC) in the presence (blue circles) and absence (black circles) of ethanol.

For comparison with other more complex microheterogeneous systems, the compounds were also incorporated into extruded dipalmitoylphosphatidylcholine liposomes (DPPC). The partition constants were evaluated for both compounds, finding that near one hundred percent of the 4-phenyl-1,4-dihydropyridines were incorporated to the compartimentalized system. Two procedures of incorporation were employed with liposome solutions, adding aliquots of the drug dissolved in ethanol or by direct drug solubilizing from solid state. We found a dramatic difference between the photodegradation rates for each procedure as can be appreciated in the inset of Fig. 4. The quantum yields for NIMO photodecomposition in both cases were 0.010 and 0.005, respectively. These values are similar to those obtained using ethanol and benzene as solvents, which could be interpreted in terms of the location of the compounds and what kind of media they are sensing. When DPPC liposomes are loaded with 4-phenyl-1,4-dihydropyridines in solid state, they are incorporated near to the interface in a microenvironment with a moderate polarity. However, when liposomes are loaded with compounds dissolved in ethanolic solutions, probably NIMO and FELO are located in the same region but, at the same time, ethanol molecules are able to locate near to the substrates. Indeed, according to nuclear Overhauser spectroscopy determinations in literature, ethanol locates in the bilayer at the interface, mainly around phosphate moieties, blocking the water diffusion.[34, 36] The proximity of ethanol molecules could permit the proton transfer from the zwitterionic intermediate to them, promoting the photodegradation reaction. These results, ethanol preferential location on membranes and dihydropyridine enhanced photodegradation by alcohol presence are such an interesting topic to be considered in future research.

Photodegradation process for 4-aryl-1,4-dihydropyridines seems to be very dependent on the capacity of molecule to stabilize the zwitterionic intermediate. When NIMO and FELO are compared, the presence of the electron acceptor nitro group facilitates the charge separation, situation less favored when two chlorine atoms are the substituents. When the zwitterionic form is favored, the media has a noticeable effect (for FELO, where photodegradation process is less favored, the media does not show any noticeable influence). For NIMO, the solvent molecules modulate photodegradation pathway not only stabilizing charged intermediates, but also providing a proton acceptor to yield products.

In microorganized media, like micelles, the charge at the surface will promote different orientations for the zwitterionic intermediate involved, see

Fig. 5, but measured consumption quantum yields, in both ionic cases (SDS and DPC), are similar, indicating that proton removal has the same efficiency, despite orientation, proton is equally accessible.

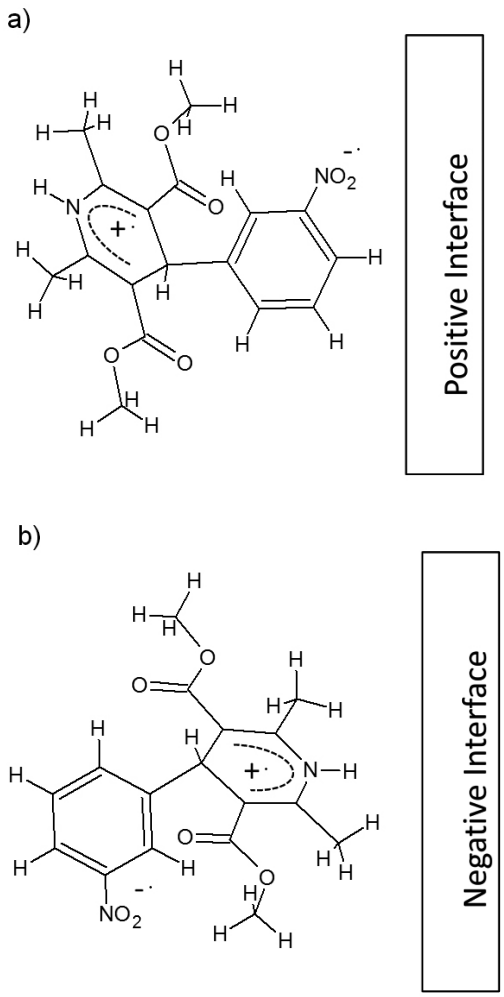


Figure 5. Orientation of zwitterionic biradical intermediate of NIMO in charged micellar interface: a) positive; b) negative.

The magnitude of reaction is directly related with the access of water molecules (or ethanol) to the proximity of acid proton on dihydropyridine ring. The values of $k_{photodeg}$ determined for three types of micelles are relatively high, result in accordance with the easiness of water to penetrate them, but the value determined in DPPC liposomes has the same magnitude, this result is high, considering that this membrane (DPPC) is more closed than micellar structures.

Photodegradation products for both NIMO and FELO (the corresponding 3-nitrophenyl and 2,3-dichlorophenyl pyridine derivatives), were the same in homogeneous and microheterogeneous media, as HPLC analysis shows. Thus, the region where the compounds are located does not modulate the generation of different photoproducts.

4. CONCLUSIONS

Our results show that NIMO and FELO occupy similar location into all studied microorganized systems. However, the head of surfactants does not have mayor influence on the photodegradation rates, despite the different orientation that zwitterionic intermediate must adopt depending on the surfactant charge forming micelle. Neither, any effect on the main photodegradation products, the corresponding pyridine derivatives, was observed. The absence of singlet oxygen generation in micellar media is consistent with the proposition of these 4-aryl-1,4-dihidropyridines are ubicated near to the interface of the micelle, where a more polar environment is sensed. The ethanol preferential location on membranes and dihydropyridine enhanced photodegradation by alcohol presence are interesting results to consider in future research.

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REFERENCES

1. D. J. Triggle, *Drug Development Research* **58**, 5–17, (2003)
2. P. A. Zwieten, *Pharmacology of antihypertensive drugs*. Elsevier: 1984; p 373.
3. B. Ljung, *Journal of Cardiovascular Pharmacology* **15**, S11-S16, (1990)
4. Y. Ono,K. Mizuno,M. Goto,S. Hashimoto,T. Watanabe, *Current Therapeutic Research-Clinical and Experimental* **60**, 392-401, (1999)
5. L. Cominacini,A. F. Pasini,U. Garbin,A. M. Pastorino,A. Davoli,C. Nava,M. Campagnola,P. Rossato,V. Lo Cascio, *Biochemical and Biophysical Research Communications* **302**, 679-684, (2003)
6. G. Supinski,D. Nethery,D. Stofan,A. DiMarco, *Journal of Applied Physiology* **87**, 2177-2185, (1999)
7. J. Ferguson, *Photodermatology Photoimmunology & Photomedicine* **18**, 262-269, (2002)
8. J. F. Silvestre,M. P. Albares,L. Carnero,R. Botella, *Journal of the American Academy of Dermatology* **45**, 323-324, (2001)
9. S. M. Cooper,F. Wojnarowska, *Clinical and Experimental Dermatology* **28**, 588-591, (2003)
10. H. R. Memarian,M. M. Sadeghi,A. R. Momeni, *Indian Journal of Chemistry Section B-Organic Chemistry Including Medicinal Chemistry* **40**, 508-509, (2001)
11. A. Buttafava,A. Faucitano,E. Fasani,A. Albini,A. Ricci, *Research on Chemical Intermediates* **28**, 231-237, (2002)
12. P. De Filippis,E. Bovina,L. Da Ros,J. Fiori,V. Cavrini, *Journal of Pharmaceutical and Biomedical Analysis* **27**, 803-812, (2002)
13. P. Pavez,M. V. Encinas, *Photochemistry and Photobiology* **83**, 722-729, (2007)
14. E. Fasani,D. Dondi,A. Ricci,A. Albini, *Photochemistry and Photobiology* **82**, 225-230, (2006)
15. N. Pizarro,G. Gunther,L. J. Nunez-Vergara, *Journal of Photochemistry and Photobiology: A-Chem* **189**, 23-29, (2007)
16. E. Fasani,M. Fagnoni,D. Dondi,A. Albini, *Journal of Organic Chemistry* **71**, 2037-2045, (2006)
17. I. T. Mak,P. Boehme,W. B. Weglicki, Circulation Research **70**, 1099-1103, (1992)
18. G. Ragno,A. Risoli,G. Ioele,E. Cione,M. De Luca, *Journal of Nanoscience and Nanotechnology* **6**, 2979-2985, (2006)
19. F. H. Quina,E. A. Lissi, *Account of Chemical Research* **37**, 703-710, (2004)
20. R. Barnadas-Rodriguez,J. Estelrich, *Journal of Physical Chemistry B* **113**, 1972-1982, (2009)
21. Y. Avnir,Y. Barenholz, *Analytical Biochemistry* **347**, 34-41, (2005)
22. N. A. Vodolazkaya,N. O. McHedlov-Petrossyan,G. Heckenkemper,C. Reichardt, *Journal of Molecular Liquids* **107**, 221-234, (2003)
23. M. A. Bahri,B. J. Heyne,P. Hans,A. E. Seret,A. A. Mouithys-Mickalad,M. D. Hoebeke, *Biophysical Chemistry* **114**, 53-61, (2005)
24. Y. Tamoto,H. Segawa,H. Shirota, *Langmuir* **21**, 3757-3764, (2005)
25. S. Hashimoto,J. K. Thomas, *Journal of the American Chemical Society* **107**, 4655-4662, (1985)
26. K. Kalyanasundaram, *Photochemistry in microheterogeneous systems*. Academic Press: 1987; p 388.
27. V. Ramamurthy, *Photochemistry in organized and constrained media*. VCH Publishers: 1991; p 875.
28. J. H. Fendler, *Membrane mimetic chemistry: characterizations and applications of micelles, microemulsions, monolayers, bilayers, vesicles, host-guest systems, and polyions*. Wiley: 1982; p 522.
29. C. Schweitzer,R. Schmidt, *Chemical Reviews* **103**, 1685-1757, (2003)
30. N. Becerra,C. Toro,A. L. Zanocco,E. Lemp,G. Günther, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **327**, 134-139, (2008)
31. H. J. Kuhn,S. E. Braslavsky,R. Schmidt, *Pure and Applied Chemistry* **76**, 2105-2146, (2004)

32. R. Schmidt,C. Tanielian,R. Dunsbach,C. Wolff, *Journal of Photochemistry and Photobiology a-Chemistry* **79**, 11-17, (1994)
33. A. Pola,K. Michalak,A. Burliga,N. Motohashi,M. Kawase, *European Journal of Pharmaceutical Sciences* **21**, 421-427, (2004)
34. D. Huster,A. J. Jin,K. Arnold,K. Gawrisch, *Biophysical Journal* **73**, 855-864, (1997)
35. H. J. C. B. a. S.-J. Marrink, *Pure Appl. Chem.* **65**, 8, (1993)
36. S. E. Feller,C. A. Brown,D. T. Nizza,K. Gawrisch, *Biophysical Journal* **82**, 1396-1404, (2002).