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ORIGINAL PAPER

# The influence of low power microwave on the properties of DPPC vesicles

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**Abstract** The effect of microwave exposure on liposome at non-thermal level are studied. Dipalmitoyl phosphatidylcholine (DPPC) liposomes were exposed to 950 MHz at power densities of 2.5 mW/cm$^2$, which is equivalent to specific absorption rate (SAR) of 0.238 W/K. The interaction of microwave with liposomes was investigated by membrane solubilization measurements using a non-ionic detergent, octylglucoside (OG), as well as Fourier transform infrared (FTIR) spectroscopy and flow activation energy measurements. The amount of detergent needed to completely solubilize the liposomal membrane was increased after exposure of liposomes to microwave irradiation, indicating an increased membrane resistance to the detergent and hence a change in the natural membrane permeation properties. In the analysis of FTIR spectra the symmetric and antisymmetric $CH_2$ (at 2070 cm$^{-1}$) band and the C═O (at 1640 cm$^{-1}$) stretching bands were investigated after liposomal exposure to microwave irradiation. It is clearly shown from the flow activation energy measurements, that low-power microwave induce changes in the liposomes deformability (decreases the liposome fluidity and increases the liposome rigidity). Finally it could be concluded that low-power microwave of 950 MHz induced structural and functional changes in liposomes as a membrane model system.


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## Introduction

Microwave radiations are now utilized in many fields of our life. They represent the main tool of communications especially in cellular phones which widely spread all over the world. This has led to increase the concern about the possible initiation of brain cancer, as the antennas of these phones lies along the head during use [1,2]. Such concern of the cancer fear had based on a series of recent publications. It was found by Repacholi et al. [3] that exposure to radio frequency (RF) radiation may increase the incidence of lymphoma in mice. Also, Lai and Singh [4,5] have suggested that relatively low-level exposure to RF radiation can cause DNA strand breaks in rat brain cells. Tynes et al. [6] showed that workers whose jobs were assumed to result in exposure to RF radiation did not have an elevated risk of brain cancer, but did have an increased risk of leukemia [7—10].

The potential health hazards associated with exposure to microwave radiation have received much attention in view of numerous documented observations confirming that microwave radiation can affect biological systems [11—14]. The simplest and most widely accepted explanation for such effects has been given in terms of macroscopic heating of the tissues due to the absorption and consequent thermal dissipation of the radiation [15]. However, other studies showing biological effects of low-level microwave radiation raise the question whether or not non-thermal effects could be involved as well [16,17]. The possibility of non-thermal effects arises from the oscillatory similitude between the microwave and living organism. Therefore non-thermal (low intensity) biological effects must be considered for regulation of microwave exposure. It has been suggested that under such circumstances, biological membranes and, more specifically, phospholipids in natural membranes may represent, the major sites of interaction with microwave radiation [18—22].

The study of non-thermal effects of microwave is considered one of the main issues for revising standards [23]. The possibility that the pulsed, low intensity microwave currently used in GSM mobile telephony can exert subtle, non-thermal influences on a living organism arises because microwave have properties other than the intensity that is regulated by safety guidelines. This microwave radiation has certain well defined frequencies, which facilitate its discernment by a living organism, and via which the organism can, inturn be affected [24].

The aim of the present study is to investigate the influence of low power microwave of 950 MHz, as that used in telecommunication especially in cellular telephone transmitter stations, on some structural and functional properties of liposomes which represent a good cell membrane model. The choice of the liposome specimens enabled us to irradiate them for a long duration in which the temperature could be fixed. Our main objective of this study is to eliminate the microwave heat effect, as claimed by many authors to be the only responsible of its effect. The liposome properties were studied by FTIR spectroscopy, membrane solubilization and flow activation energy measurements.

## Materials and methods

L-$\alpha$-Dipalmitoyl phosphatidylcholine (DPPC) specified 99% pure, and non-ionic detergent octylglucoside, (OG) were purchased from Sigma (St. Louis, Mo, USA). Tris ultra pure buffer was obtained from ICN Biomedicals Inc., Ohio, USA ICN Biomedicals Inc., Ohio, USA. Organic solvents (chloroform and ethanol) were of analytical grade and obtained from Merck (Heliopolis, Cairo, Egypt).

### Preparation of liposomes

The lipids must first be dissolved and mixed in chloroform to assure a homogeneous mixture of lipids. The organic solvent should be removed by rotary evaporation yielding a thin lipid film on the sides of a round bottom flask. The lipid film is thoroughly dried to remove residual organic solvent by placing flask on a vacuum pump. Hydration of the dry lipid film is accomplished simply by adding Tris-buffered saline (10 mM Tris and 140 mM NaCl at pH 7.4) to the container of dry lipid and agitating at temperature above phase transition temperature of the lipid. Multilamellar vesicles (MLV) are formed of final lipid concentration of 5.45 mM.

## Membrane solubilization

The solubilization of DPPC multi-lamellar liposomes of 0.68 mM was followed at room temperature by continuous addition of non-ionic detergent octylglucoside (OG) and monitored by measuring their optical density (OD) at 400 nm using UV/visible spectrophotometer (Jenway 6405, Barloworld Scientific, Essex, UK). The OG solution was continuously added at a constant rate to the cuvette, equipped with a paddle stirrer (to ensure a uniform detergent concentration throughout the sample) and containing 2 ml of liposomes solution. During OG injection, OD of the mixed solution was measured. From the data obtained, OG concentrations were calculated using the formula of Paternostre et al. [25].

$$[D_t] = \frac{[D_i]}{V_t} \times V_a$$

Where, $D_t$ is the total detergent concentration in the cuvette in mM,

$D_i$ is the initial detergnet concentration before addition to the sample in mM,

$V_a$ is the added volume of the OG to the cuvette in ml and $V_t$ is the total volume of the sample in the cuvette in ml.

Consequently, OD profiles of solubilized liposomes were plotted as a function of total detergent concentration.

### Microwave exposure system

Microwave generator (Model HP 83712B, HP, USA) with a horn antenna was used. Samples were exposed to 950 MHz at power densities 2.5 mW/cm$^2$, which is equivalent to specific absorption rate (SAR) of 0.238 W/K. The SAR can be approximately calculated according to the formula [26]:

$$SAR = \frac{\sigma}{\rho}|E_{RMS}|^2 [mW/g],$$

where $E_{RMS}$ is the root mean square value of the electrical field, $\sigma$ is the mean electrical conductivity of the samples and $\rho$ is the mass density. The selected exposure times were 1 h and 2 h. Aqueous solutions of DPPC were exposed for different exposure times. The spectrophotometric, rheometric and FTIR measurements for the different samples were performed after the end of exposure.

### Activation energy measurement

The effect of temperature on the liposome viscosity has been measured by Brookfield rheometer (Model DV IIICP, Boland) using CP.40 cone-plate geometry (cone angle = 0.8 and diameter = 2.4 cm). The rheometer was connected to Brookfield circulating water bath. The range of temperature is from 25 to 48 °C. The applied shear rate was uniform over the different measurements carried out.

The effect of temperature on the samples viscosity has been described by the Arrhenius type equation [27]:

$$\eta = \eta_o e^{(Ea/RT)}$$

Where $\eta$ is the viscosity, $\eta_o$ is the viscosity at reference temperature, $E_a$ is the flow activation energy <energy of activation needed to initiate the flux among molecules>, $R$ is the universal gas constant <$a$ constant relative to the molecular weight and volume of the fluid>, and $T$ is the absolute temperature. From the slope of the plot of ln $\eta$ versus 1000/T (Fig. 2), the $E_a$ for the flow was determined for different exposure times at 950 MHz.

### FTIR measurements

FTIR measurements are carried out using a single beam Fourier Transform Infrared Spectrometer, FTIR-430, Jascow, Japan. The wave number range for this instrument is from 7800 to 400 $cm^{-1}$ and its signal to noise ratio at a resolution of 4 $cm^{-1}$ is $10^4$: 1 at 2200 $cm^{-1}$. The FTIR spectra of the samples are obtained in the spectral range from 4000 to 400 $cm^{-1}$ with a scanning speed of 2 mm/s and a resolution of 4 $cm^{-1}$. The number of scans is set to "Auto". Lyophilized liposome samples are mixed with appropriate amount of KBr powder and gently ground in a special mortar for 30 s. The mixture is then pressed into homogenous disk at a pressure of 5 tons/$cm^2$ using a hydraulic pressing system (Riken Power, Riken Seiki Co LTD. Japan). The infrared measurements are performed immediately after preparing the discs. Data are refined (using a 7-point Savitzky-Golay smoothing). Deconvolution was conducted in order to resolve overlapping bands using Jasco software (Spectra Manager 1.3). This technique yields spectra that have much narrower bands and is able to distinguish closely spaced features. The instrumental resolution is not increased, but the ability to differentiate spectral features can be significantly improved.

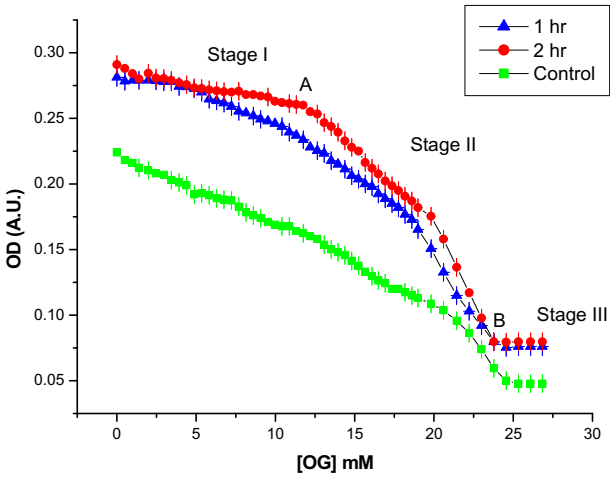


**Figure 1** Changes of the optical density at 400 nm of DPPC liposomal samples as a function of the detergent concentration, before and after exposing to microwave irradiation of 950 MHz. Lipid concentration of 0.68 mM ($n$ = 3).

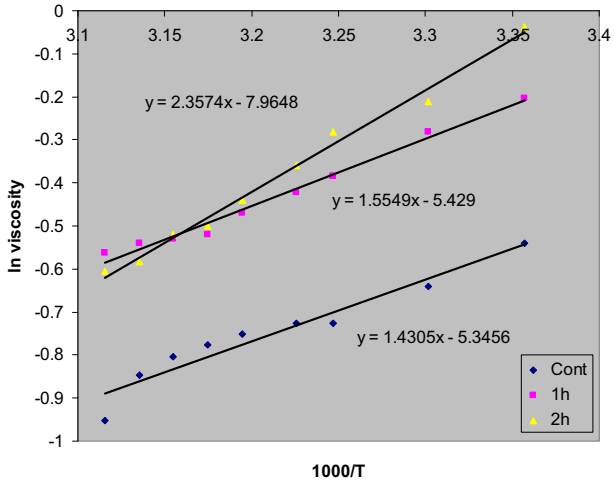


**Figure 2** Arrhenius plots for liposomal samples before and after exposure to 950 MHz.

## Results and discussion

### Solubilization

Nonionic detergents are widely used as molecular tools in membranology. In particular, they are essential in the solubilization and reconstitution of integral membrane proteins. The solubilization of liposomal membrane by the non-ionic detergent octylglucoside (OG) was proceed to exhibit three stages of transition, from the vesicular form to mixed micellar form, namely, stages I, II and III (Fig. 1). At first, detergent monomers insert into the membrane bilayer and start to solubilize lipids in stage I until point A. As detergent concentration increased, the detergent molecules began to be incorporated within the membrane bilayer. The increase in detergent concentration caused a corresponding increase of detergent molecules incorporated within the bilayer leading to the complete solubilization of the membrane (stage II until point B) and formation of mixed micelles [28,29]. Stage III started after point B.

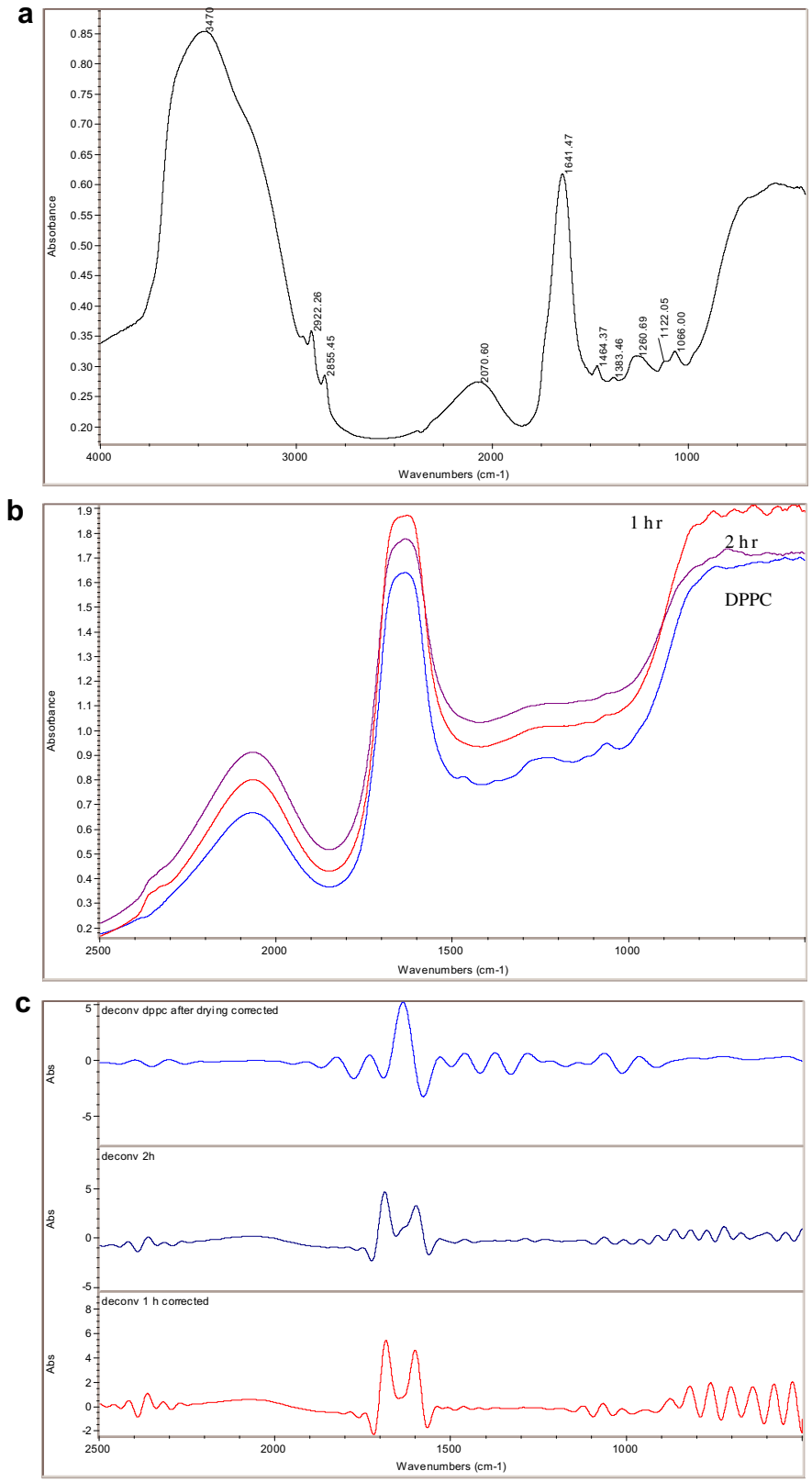


**Figure 3** a) The full FTIR spectrum of DPPC liposomal sample, b) The magnified part (2500—500 $cm^{-1}$) of FTIR spectra of control DPPC and low power microwave exposed DPPC liposome samples for 1 h and 2 h, c) Deconvolution of FTIR spectra of different samples.

Figure 1 illustrates the variation of the optical density (OD) of liposomal samples as a function of the detergent concentration before and after exposing to microwave irradiation. The results indicate that the detergent concentration needed to make complete solubilization of the membrane depends on the exposed time of lipid samples to microwave irradiation. Liposomal membrane solubilization results showed the need for higher detergent concentration to solubilize the exposed liposomal membrane than unexposed sample indicating that there is an increasing membrane resistance to the detergent solubilization after microwave irradiation and hence changes in the natural membrane permeation properties. The data indicate that the low power microwave irradiation makes the liposomal membrane more rigid and less soluble by the detergent compared to unexposed samples.

Liposome solubilization studies by non-ionic detergent could give clear idea about their conformational structure. Membrane solubilization of liposomes by detergent 'OG' measured using optical density technique is a simple and fast method for assessing membrane physical state.

## Flow activation energy

The flow activation energy describes the energy of activation needed to initiate the flux among molecules. Using this energy they are able to move against the internal flow resistance which is caused by the friction between the neighboring cells. The change in the flow activation energy can give an idea about the change in the ordered structure of the membrane [30].

Figure 2 shows that arrhenius plots for liposomal samples before and after exposure to 950 MHz. From the slope of the plot of (ln viscosity) versus 1000/T, the flow activation energy for DPPC was 11.89 kJ/mol and it became 12.88 kJ/mol and 19.54 kJ/mol after irradiated with microwave for 1 and 2 h, respectively.

Also, the plastic viscosities were 0.58 cP, 0.82 cP and 0.96 cP for DPPC liposomes; and after liposomal irradiated with microwave for 1 and 2 h respectively, at room temperature.

From the obtained results it is obvious that the flow activation energy and viscosity were increased with increasing the exposure time which indicate the increasing of membrane rigidity and reflect relative disorder in the membrane (DPPC) as a result of microwave irradiation for 1 and 2 h, respectively.

### FTIR

FTIR spectroscopy is inherently well-suited to study conformational order in phospholipid acyl chains [31,32]. With this technique, it is possible to monitor subtle changes in the structure of the lipid assemblies by analyzing the frequency and the bandwidth changes of the vibrational modes investigating the acyl chains and head group region of the lipid molecule before and after exposure to microwave for 1hr and 2 h. These changes can be used to extract information about various physicochemical processes taking place in the systems [33,34].

The FTIR scans were performed separately for the three different liposome batches (control and exposed samples). Care was directed towards any differences or changes in the resultant graphs.

Figure 3a shows the full FTIR spectrum of DPPC liposomal sample. As shown from this figure, the spectrum displays the main characteristic bands of DPPC vesicles, especially those of CH vibrations at 2855 and 2922 $cm^{-1}$; and OH stretching and bending vibrations at 3470 and 1640 $cm^{-1}$, respectively. The other characteristic bands of DPPC such as C═O and C—O were found to be overlapped by the strong absorption bands of OH groups of the hydrated DPPC. In addition, the P═O appears at about 1066 $cm^{-1}$. These findings are in good accordance with the data reported in the literature [29]. Figure 3b shows the magnified part (2500-500 $cm^{-1}$) of the spectra of microwave irradiated DPPC liposomes as compared to the control sample. This figure shows some spectral changes such as broadening and lower wavenumber shifts. To investigate such spectral changes as well as to examine the C═O vibrations, doconvolution was performed for the spectra (Fig. 3c). After deconvolution, the C═O has been found at about 1730 $cm^{-1}$ in the spectrum of the control sample. After microwave irradiation, this peak showed lower intensities together with the appearance of a new peak at 1667 $cm^{-1}$. The intensities of this new peak increased with the increase of exposure time. In addition, the spectra of the exposed samples showed shifts to lower wave numbers as it can be easily seen from the deconvoluted spectra. For samples exposed for 2 h, a new shoulder about 1600 $cm^{-1}$ appeared. The appearance of a new peak at 1667 $cm^{-1}$ and a new shoulder at 1600 $cm^{-1}$ reveal a broadening in this spectral region.

The spectral changes found as a result of microwave exposure may be due to some sort of water lose and some molecular conformational changes. Such conformational changes may be the formation of curved lipid planes that lead to the lipid lateral diffusion occurring in the intermediate motional regime [35].

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## References

[1] Stuchly MA. Biomedical concerns in wireless communications. Crit Rev Biomed Eng 1998;26:117—51.
[2] Moulder JE, Erdreich LS, Malyapa RS, Merritt J, Pickard WF. Vijayalaxmi. Cell phones and cancer: what is the evidence for a connection? Radiat Res 1999;151:513—31.
[3] Repacholi MH, Basten A, Gebski V, Noonan D, Finnie J, Harris AW. Lymphomas in E mu-Pim1 transgenic mice exposed to pulsed 900 MHZ electromagnetic fields. Radiat Res 1997; 147:631—40.
[4] Lai H, Singh NP. Acute low-intensity microwave exposure increases DNA single-strand breaks in rat brain cells. Bioelectromagnetics 1995;16:207—10.
[5] Lai H, Singh NP. Melatonin and a spin-trap compound block radiofrequency electromagnetic radiation-induced DNA strand breaks in rat brain cells. Bioelectromagnetics 1997;18:446—54.
[6] Tynes T, Andersen A, Langmark F. Incidence of cancer in Norwegian workers potentially exposed to electromagnetic fields. Am J Epidemiol 1992;136:81—8.

[7] Hardell L, Sage C. Biological effects from electromagnetic field exposure and public exposure standards. Biomed Pharmacother 2008;62:104—9.
[8] Hardell L, Carlberg M, Söderqvist F, Hansson Mild K, Morgan LL. Long-term use of cellular phones and brain tumours: increased risk associated with use for 10 years. Occup Environ Med 2007;64:626—32.
[9] Hardell L, Hansson Mild K, Carlberg M. Pooled analysis of two case control studies on use of cellular and cordless telephones and the risk for malignant brain tumours diagnosed in 1997e2003. Int Arch Occup Environ Health 2006;79:630—9.
[10] Lai H, Singh NP. Magnetic-field-induced DNA strand breaks in brain cells of the rat. Environ Health Perspect 2004;112(6): 687—94.
[11] Meyer R, Streckert J, Hansen V. Biological effects of radiofrequency fields. Scattering; 2002:508—27.
[12] Louis NH, Sheila AJ, Patrick AM. Radio frequency electromagnetic fields: cancer, mutagenesis, and genotoxicity. Bioelectromagnetics 2003;24(Suppl. 6):S74—100.
[13] Chavdoula Evangelia D, Panagopoulos Dimitris J, Margaritis Lukas H. Comparison of biological effects between continuous and intermittent exposure to GSM-900-MHz mobile phone radiation: detection of apoptotic cell-death features. Mutat Research/Genetic Toxicol Environ Mutagenesis 2010; 700:51—61.
[14] Ferreri F, Pasqualetti P, Curcio G, Del Duca M, Ponzo D, Giambattistelli F, et al. P17—19 Mobile phone emissions and brain excitability in Alzheimer disease. Clin Neurophysiol 2010;121. S207.
[15] Michaelson SM, Lin JC. Biological effects and health implications of radiofrequency radiation. New York: Plenum Press; 1987.
[16] Peinnequin A, Piriou A, Mathieu J, Dabouis V, Sebbah C, Malabiau R, et al. Non-thermal effects of continuous 2.45 GHz microwaves on Fas-induced apoptosis in human Jurkat T-cell line. Bioelectrochemistry 2000;51:157—61.
[17] Moghimi Hamid R, Alinaghi Azadeh, Erfan Mohammad. Investigating the potential of non-thermal microwave as a novel skin penetration enhancement method. Int J Pharmaceutics 2010;401:47—50.
[18] -Moraru Roxana Pologea, Kovacs Eugenia, Iliescu Karina Roxana, Calota Violeta, Sajin Gheorghe. The effects of low level microwaves on the fluidity of photoreceptor cell membrane. Bioelectrochemistry 2002;56:223—5.
[19] Moustafa Yasser M, Moustafa Randa M, . Belacy A, Abou-El-Ela Soad H, Ali Fadel M. Effects of acute exposure to the radiofrequency fields of cellular phones on plasma lipid peroxide and antioxidase activities in human erythrocytes. J Pharm Biomed Anal 2001;26:605—8.
[20] Nittby Henrietta, Brun Arne, Eberhardt Jacob, Malmgren Lars, Persson Bertil RR, Salford Leif G. Increased blood—brain barrier permeability in mammalian brain 7 days after exposure to the radiation from a GSM-900 mobile phone. Pathophysiology 2009;16:103—12.
[21] Jauchem James R. Effects of low-level radio-frequency (3 kHz to 300 GHz) energy on human cardiovascular, reproductive, immune, and other systems: a review of the recent literature. Int J Hyg Environ Health 2008;211:1—29.
[22] Sandblom J, Theander S. The effect of microwave radiation on the stability and formation of gramicidin-A channels in lipid bilayer membranes. Bioelectromagnetics 1991;12:9—20.
[23] Peinnequin A, Piriou A, Mathieu J, Dabouis V, Sebbah C, Malabiau R, et al. Non-thermal effects of continous 2.45GHz microwave on fast-induced apoptosis in human Jurkat T-Cell line. Bioelectrochemistry 2000;51:157.
[24] Hyland GJ. Physics and biology of mobile telephony. Lancet 2000;356:1782.
[25] Paternostre M, Virad M, Meyer O, Ghannam M, Ollivon M, Blumenthal R. Solubilization and reconstitution of vesicular stomatitis virus envelope using octylglucoside. Biophys J 1997; 72:1683—94.
[26] ICNIRP Guidelines. Guidelines for limiting exposure to time-varying electric, magnetic and electromagnetic fields (up to 300 GHz). Health Phys 1998;74:494—522.
[27] Steffe J. Rheological methods in food process engineering. 2nd ed. USA: Freeman Press, Mitchigan State University; 1996. pp 21.
[28] Morandat S, El Kirat K. Solubilization of supported lipid membranes by octyl glucoside observed by time-lapse atomic force microscopy. Colloids Surf B Biointerfaces 2007;55: 179—84.
[29] Mady MM, Mirhane MD, Safaa K, Wafaa MK. Biophysical Studies on chitosan-coated liposome. Eur Biophys J 2009;38:1127—33.
[30] Desouky Omar S, Selim Nabila S, El-Bakrawy Eman M, El-Marakby Seham M. Biophysical Characterization of beta-Thalassemic Red blood cells. Cell Biochem Biophys 2009;55: 45—53.
[31] Mendelsohn R, Moore D. Vibrational spectroscopic studies of lipid domains in biomembranes and model systems. Chem Phys Lipids 1998;96:141—57.
[32] Los DA, Murata N. Membrane fluidity and its roles in the perception of environmental signals. Biochim Biophys Acta 2004;1666:142—57.
[33] Lewis RNAH, Mc Elhaney RN. The structure and organization of phospholipid bilayers as revealed by infrared spectroscopy. Chem Phys Lipids 1998;96:9—21.
[34] Severcan F, Sahin I, Kazanci N. Melatonin strongly interacts with zwitterionic model membranes-evidence from Fourier transform infrared spectroscopy and differential scanning calorimetry. Biochim Biophys Acta 2005;1668:215—22.
[35] Jastimi RE, Edwards K, Lafleur M. Characterization of permeability and morphological perturbations induced by Nisin on phosphatidylcholine membranes. Biophys J 1999;77:842—52.