Egypt. J. Biophys. Biomed. Engng. **Vol. 19 ,** pp25-31 (2018)

Biophysical Studies on the Effect of Gamma Rays on Liposomes

S.A. Ali[a], A.A. Mohamed[*b], R.M. Elshazly[c], M.H.Gaber[a, d], A.M. Kany[c]
[a]Biophysics Department, Faculty of Science, Cairo University, [b]Badr University in Cairo, Badr City, [c]Physics Department and Biophysics branch, Faculty of Science, Al-Azhar University, [d]British University in Egypt, El Sherouk City, Suez Desert Road, Cairo, Egypt.

LIPOSOMES are vesicular structures made of lipids that are formed in aqueous solutions, which can be used as models to study the cell membrane. In the present study the effects of gamma (γ) rays on dipalmitoyl-phosphatidylcholine (DPPC) liposomes were studied by transmission electron microscopy (TEM), Fourier transform infrared (FTIR) spectroscopy, as well as dynamic light scattering (DLS) and viscosity measurements. The DPPC liposomes were exposed to three different doses 40, 80, and 120Gy which emitted from ^{60}Co gamma rays source with a dose rate of 9 kGy/h. The DLS measurements confirmed the mono-dispersity of all samples. TEM results revealed that there is a change in morphology and size of liposomes, which is in a good agreement with the increase in viscosity measurements. FTIR measurements showed significant changes in the characteristics bands of DPPC liposomes confirming the effect of γ-rays on the main groups such as CH_2 bending vibrations and the symmetric and antisymmetric PO_2^- stretching vibrations at 1090 and 1220 cm^{-1} respectively. In addition to the shifting of the OH stretching vibrations from 3439 cm^{-1} to 3453 cm^{-1}due to the 120 Gy exposure. The spectral changes seem to be due to some sort of water loss and molecular conformational changes due to ionization and formation of freeradicals which affect the head groups of the DPPC liposomes leading to lipid lateral diffusion enhancing the fusion of small vesicles to form larger structures.

Keywords: Liposomes, DPPC, Gamma rays, Characterization.

Introduction

Ionizing radiation has been widely used in medical application as a tool in diagnostic imaging, nuclear medicine and radiotherapy. Living organisms exposed to radiation can undergo tissue deformity and suffer damages which are a major safety concern. However, there are few studies that investigated the effects of gamma radiation on biological system components, in particular phospholipids. In this study, dipalmitoyl-phosphatidylcholine (DPPC) liposome was used because it is the most abundant phospholipids and can be found almost everywhere in the body and functions to protect liver, fight toxicity and infection in body system[1].

Gamma irradiation has been found to be effective in cleaving the molecular chains ascribed to the decay processes. This is related to the free radicals formed at the primary stage of gamma irradiation, which extends with changes in its chemical composition in addition to its physiological functions [2].

Liposomes are vesicular structures made of lipids that are formed in aqueous solutions. Structurally, they resemble the lipid membrane of living cells[3,4]. Therefore, they have been widely investigated, since the 1960s, as models to study the cell membrane, and as carriers for delivery of bioactive agents[5], in different areas of research including diagnostic imaging[6], and also used in cosmetics and tissue engineering[7].

The liposomes may be affected directly by irradiation or through radicals generated by radiolysis of water[8]. These radicals have unlimited direct effect to damage the liposomal membranes however, the indirect effect of irradiation is considered the most important[9, 10]. There are many examples of radiation-induced

*Corresponding author :ahmedbiophysics2013@gmail.com
DOI: 10.21608/EJBBE.2018.3319.1018

peroxidation of unsaturated lipids dispersed as liposomes [11,12].

In the present study, the effect of different doses of gamma rays on DPPC liposomes was studied by measuring FTIR, size distribution by dynamic light scattering (DLS), transmission electron microscopy (TEM) and viscosity to detect different changes on the molecular level of DPPC liposomes.

Material and Methods

Materials

L-α-Dipalmitoyl phosphatidylcholine (DPPC) specified 99 % pure was purchased from Sigma (St. Louis, MO, USA). Organic solvents (chloroform and ethanol) were of analytical grade and obtained from Merck and were without surface impurities and used without further purification. Tris buffer, molecular weight (121.14) was purchased from BDH limited poole (England). Water was triple distilled and then ultra-purified by a Millipore system (Mill-Q system).

Preparation of liposomes

Liposomes were prepared by the thin film method[13]. DPPC (50 mg) was dissolved in chloroform to ensure a homogeneous mixture of lipids in a rounded bottom flask. The organic solvent (chloroform) was evaporated gradually by rotary evaporation to obtain a thin film of lipids on the sides of the flask. The lipid film is thoroughly dried to remove residual organic solvent by placing the flask on a vacuum pump. Multilamellar vesicles were formed by adding an aqueous solution of 10 mM Tris buffer and NaCl (145 mM) (pH 7.4) to the flask and with vigorous shaking at a temperature 45°C above the phase transition temperature of the lipid.

Irradiation setup

The DPPC liposomes in 4 ml glass vials were exposed at ambient temperature, to 40, 80, and 120Gy emitted from ^{60}Co gamma-ray source using a gamma-ray cell at dose rate of 9 kGy/h. The DPPC liposomes, before gamma-ray exposure, was used as a control material.

Particle size distribution

The mean diameter of DPPC liposomes and the polydispersity (% Pd) of the distribution were determined by dynamic light scattering (DLS) using a Zeta-sizer Nano-series (Malvern Instruments, UK). The refractive index and viscosity of pure water were used as calculation parameters and each sample was measured 3 times using the unimodal model for size distribution. The results values of mean diameter of the liposomes and polydispersity are expressed as a mean ±SD. Size distribution was recorded as a function of volume at 25°C.

Transmission electron microscopy (TEM)

DPPC liposomes were analyzed on negative stain electron microscopy using a JEM 1230 electron microscope (Jeol, Tokyo, Japan). A drop of each liposomal suspension was applied to copper coated with carbon grid, and the excess was drawn off with filter paper. An aqueous solution of ammonium molybdate (1% w/v) was used as a negative staining agent. Staining was followed by a 2 minutes wait at room temperature, removal of the excess solution with a filter paper, and it was then examined under the electron microscope.

Viscosity measurements

Samples of pure DPPC liposomes were analyzed at 25^{o}C by using Anton Paar- Rheometer, Model MCR 302 (Anton Paar, Austria), a 2.5 cm plate and plate system was used at 25± ± 0.1^{o}C and shear rate from 42 to 210 s^{-1}. The flow curves were plotted between shear stress (N/m^2 or Pa.) and shear rate (s^{-1}) for each sample. Plastic viscosity and yield stress were investigated from the linear fitting of flow curves by using power law model.

FTIR spectroscopy

FTIR spectra of samples of DPPC liposomes deposited in KBr disks were recorded on a Jasco FT/IR 460 plus (Japan) spectrometer. The scanning was done in the range 400–4,000 cm^{-1} with speed of 2 mm/s at a resolution of 4 cm^{-1} at room temperature. The band width was measured at 50% of height of the peaks.

Result and Discussion

Particle size distribution

The DLS size distribution of DPPC liposomes, as a function of volume at 25°C, was unimodal and relatively narrow which is shown in Fig.1. The calculated mean diameter for the control was 805.4 nm at 55% of volume and 55.12 nm at 45%.The calculated mean diameter at doses 40, 80, and 120 Gy were 887.6, 656.7, and 3087 nm, respectively. The mean value of polydispersity (% Pd) of the 4 samples of DPPC liposome's dispersions was 0.531%. Therefore as a rule of thumb, samples with % Pd ≤ 20% are considered monodisperse[13].

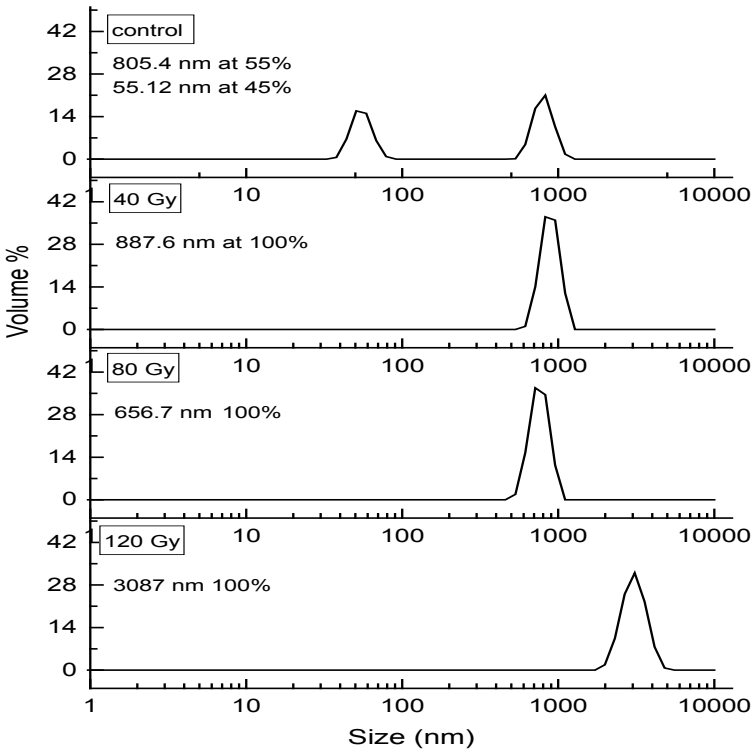

Fig.1. Mean diameter of DPPC liposome at different doses of gamma rays.

Transmission Electron Microscopy

Surface morphological studies on the shape of prepared samples using transmission electron microscopy indicated that the control sample has a regular spherical shape while the irradiated ones lose such regularity gradually as the dose increases and the smaller vesicles started to aggregate and fuse together to form larger vesicles at the final dose of 120Gy, as shown in Fig.2.

FTIR spectroscopy

FTIR spectroscopy is primarily well-suited for studying conformational order in phospholipids' acyl chains[14,15]. With this technique, it is possible to monitor subtle changes in the structure of the lipid assemblies either in acyl chains or in head-groups region by analyzing the frequency and the band-width changes of the vibrational modes of the different functional groups. These changes can be used to extract information about various physicochemical processes taking place in the systems[16,17].

The FTIR scans were performed separately for the four different liposome batches (control and the exposed samples, 40, 80, and 120 Gy).

Fig. 3. shows the full FTIR spectrum of DPPC liposomal samples. As shown from the figure, the spectrum displays the main characteristic bands of DPPC vesicles [18].

The 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, where the OH stretching and bending vibrations at 3470 and 1640 cm^{-1}, respectively. In addition to the symmetric and antisymmetric PO_2^- stretching vibrations at 1090 and 1220 cm^{-1}, respectively, were apparent. These findings are in good accordance with the data reported in the literature [18, 19].

Comparing the FTIR scans of irradiated samples with the control, one notices that as the dose of gamma rays increases there is a change in the liposome bands that disappears of others: as in CH_2 bending vibrations and the symmetric and antisymmetric PO_2^- stretching vibrations at 1090 and 1220 cm^{-1}, respectively. In addition to the shifting of the OH stretching vibrations from 3439cm^{-1} to 3453 cm^{-1} of the control and 120 Gy irradiated liposomal sample respectively, as shown in Fig. 3.

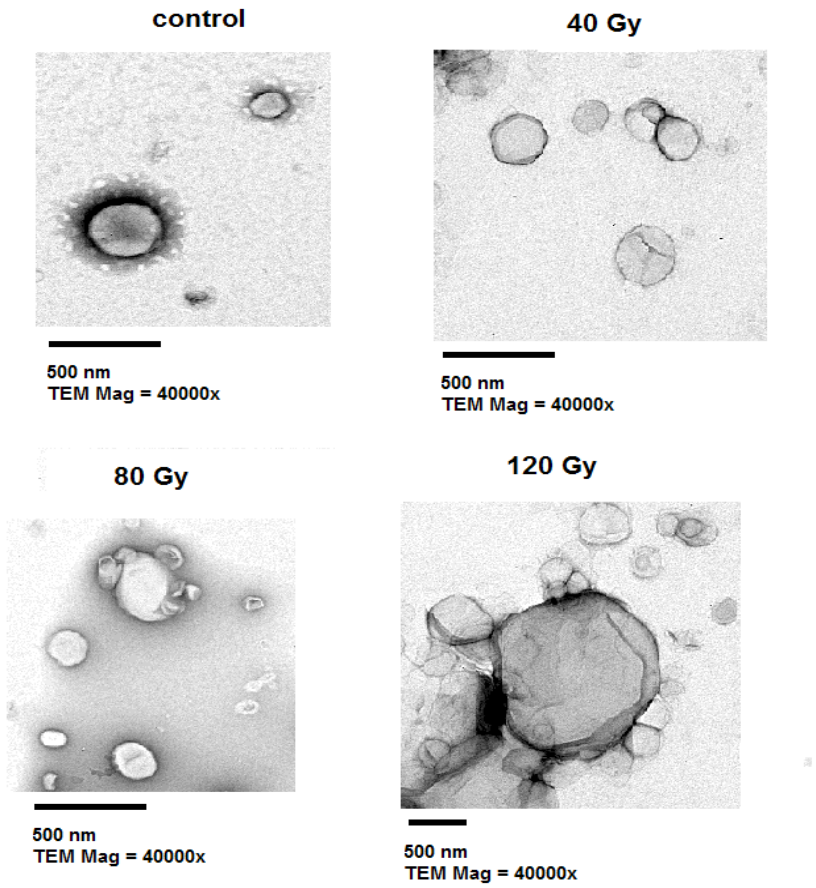

Fig.2.TEM images revealing shape,structure,and sizes of DPPC liposomes at different radiation doses.

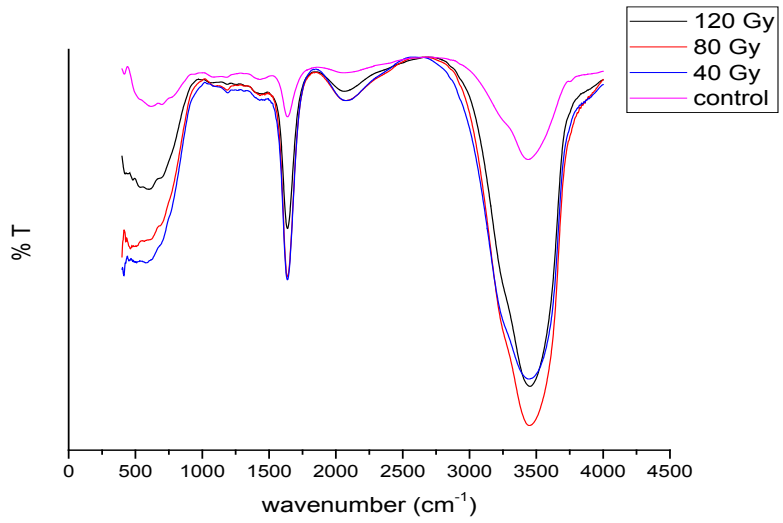

Fig.3. FTIR transmission spectra of DPPC liposomes at different doses.

The spectral changes found as a result of gamma rays irradiation may be due to some sort of water loss and molecular conformational changes due to ionization and formation of free radicals which affects the head groups of the DPPC liposomes as indicated from the change occurred for the symmetric and antisymmetric PO_2^- vibrations and CH_2 bending vibrations. Such molecular conformational changes may affect the lipid molecules that lead to the lipid lateral diffusion which in turn enhances the fusion of the small liposomal vesicles to form larger structures [20].

Viscosity measurements

The rheological properties of liposomes were measured to study the effect of gamma ray on DPPC liposome at different doses of gamma rays. Fig. 4 shows the flow curves for DPPC liposomes at different doses (0, 40, 80, and 120Gy).

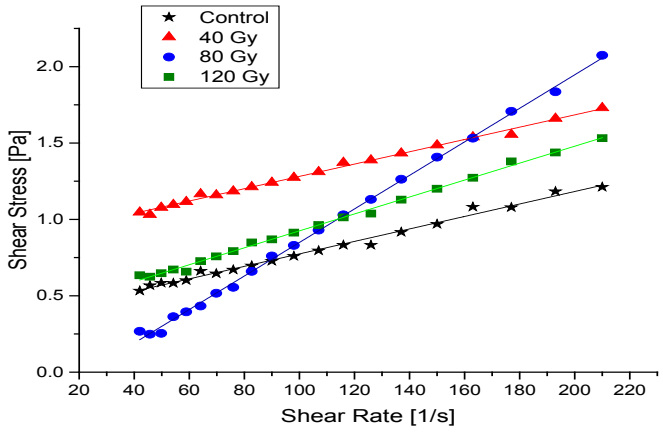

Fig.4. Flow curves for DPPC liposomes at different doses (0, 40, 80, and 120Gy).

The rheological properties of the different irradiated liposomal samples can be described by the power law model [21].

$$\sigma = k\,\gamma^{n}$$

Where σ is the shear stress, k is the consistency index, γ is the shear rate, and n is the flow behavior index.

Curve fitting the data of the different irradiated liposomal samples to the power law model yields the rheological parameters as shown in Table 1:

The consistency index k is an indication of the viscous nature of liposomes, and the flow behavior index n is a measure of departure from Newtonian flow[21].

TABLE 1. Rheological Parameters of DPPC liposomes at different doses of gamma ray.s.

Sample	Plastic viscosity (cP)	Consistency index, *k* (Pa.s.)	n
Control	04.08	0.078±0.08	0.504±0.01
40 Gy	04.03	0.316±0.04	0.309±0.01
80 Gy	10.98	1.518±0.15	1.363±0.033
120 Gy	05.50	0.067±0.07	0.577±0.017

For the control and the 40 and 120 Gy irradiated samples, the flow index, n were calculated to be 0.5, 0.31, and 0.57 and the consistency index, k values were 0.078, 0.316, and 0.067 Pa.s., respectively. It is clear that liposome suspensions exhibited a pseudo-plastic behavior because the values of the flow index n <1. However, at 80 Gy, n and k values were 1.363 and 1.518 Pa.s., respectively. A flow behavior index equal to 1.363, a value significantly greater than 1.0, is a numerical indication of a large shear-thickening effect and hence an increase in the sample viscosity.

Figure 5 shows the plastic viscosity of DPPC liposomes that increased after gamma irradiation, where it is increased from 4.08 to 10.98 cP at 80 Gy then decreased again to 5.5 cP at 120 Gy.

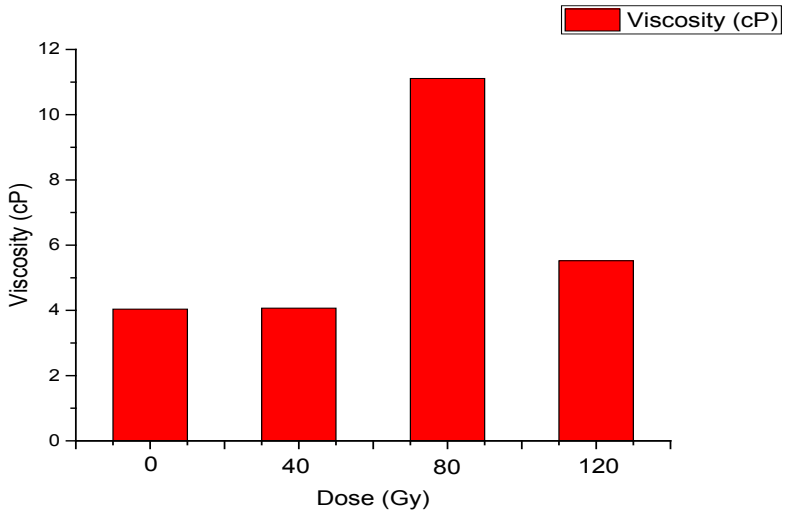

Fig. 5. Effect of viscosity of DPPC liposomes with different doses of gamma ray.

Based on the present results of liposomal rheological properties, it can be concluded that gamma irradiation has a great effect on the phospholipids molecules whereas the gamma ray dose increases up to 80 Gy the liposomes plastic viscosity increases but it decreased again when the gamma ray dose increased more as in the case of 120 Gy. There is a direct relationship between the particle size of liposomes and the measured viscosity. The interaction of gamma ray with liposomes enhances molecular conformational changes due to ionization and formation of free radicals which affects the head groups of the DPPC liposomes which in turn leads to lipid lateral diffusion enhancing the fusion of small vesicles to form larger structures.

Conclusion

The effect of ionizing radiation on DPPC liposomes was confirmed by transmission electron microscope (TEM) images, FTIR spectroscopy, and dynamic light scattering (DLS). The gamma rays affect the particle size distribution whereas the dose increases there is a disruption for the liposomal vesicles to form smaller monodsiperse vesicles as shown from dynamic light scattering results (DLS) which in turn affects directly the viscosity measurements whereas the particle size decreases the viscosity increased from 4.08 cP at 0Gy to 10.98 cP at 80Gy and then decreased again to 5.5 cP at 120Gy due to the adhesion of small vesicles to form larger structure and hence the viscosity decreases[22, 23]. Finally, it could be concluded that gamma rays induced structural changes in liposomes as a membrane model system.

References

1. Singleton JA, Ruan M, Stanford JH, Haney CA, Stikeleather LF. Separation and characterization of peanut phospholipid molecular species using high-performance liquid chromatography and fast atom bombardment mass spectrometry. *J. Amer Oil Chem Soc*., **76**, 49-56 (1999).

2. Kim JK, Srinivasan P, Kim JH, Choi J, Park HJ, Byun MW, Lee JW. Structural and antioxidant properties of gamma irradiated hyaluronic acid. *Food Chem*., **109**, 763-770 (2008).

3. Bangham AD, Standish M, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. *J Molec Biol.,* **13**, 238-252 (1965).

4. Sessa G, Weissman G. Phospholipid spherules (liposomes) as a model for biological membranes. *J Lipid Res.*, **9,** 310-318 (1968).

5. Harrington KJ, Syrigos KN, Vile RG. Liposomally-targetted cytotoxic drugs in the treatent of cancer. *J Pharm Pharmacol.,* **54,** 1573-1600 (2002).

6. Matteucci ML, Thrall DE. The role of liposomes in drug delivery and diagnostic imaging: a review. *Vet Radiol Ultrasound*., **41,** 100-107 (2000).

7. Monteiro N, Martins A, Reis RL, Neves NM. Liposomes in tissue engineering and regenerative medicine. *J R Soc Interface*., **11**, 20140459 (2014).

8. Edwards JC, Chapman D, Cramp WA, Yatvin MB. The effects of Ionizing radiation on biomembrane structure and function. *Prog Biophys Molec Biol.,* **43**, 71-93 (1984).

9. Stark G. The effect of ionizing radiation on lipid membranes. *Biochim Biophys Acta.,* **1071**, 103-122 (1991).

10. Kale RK, Sitasawad SL. Radiation induced lipid peroxidation in liposomes. *Radiat Phys Chem.*, **36,** 361-364 (1990).

11. Petkau A, Chelack WS. Radioprotective effect of superoxide dismutase on model phospholipid membranes. *Biochim Biophys Acta*., **433**, 445-456 (1976).

12. **Nakazawa T, Nagatsuka S**. Radiation-induced Lipid Peroxidation and Membrane Permeability in Liposomes. *Int J Radiat Biol*., **38**, 537-544 (1980).

13. New RCR. In liposomes: a practical approach. Oxford, UK: IRL Pres., 33-103 (1989).

14. Mendelsohn R, Moore D. Vibrational spectroscopic studies of lipid domains in biomembranes and model systems. *Chem Phys Lipids*., **96**, 141–157 (1998).

15. Los DA, Murata N. Membrane fluidity and its roles in the perception of environmental signals. Biochim Biophys Acta; 1666: 142-157 (2004).

16. Lewis RNAH, Mc Elhaney RN. The structure and organization of phospholipid bilayers bilayers as revealed by infrared spectroscopy. *Chem Phys Lipids*., **96**, 9-2 (1998).

17. 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.*, **1668**, 215- (2005)

18. Mady MM, Allam MA. The influence of low power microwave on the properties of DPPC vesicles. *Physica Medica.,* **28**, 48-53 (2012).

19. Mady MM, Mirhane MD, Safaa K, Wafaa MK. Biophysical Studies on chitosan-coated liposome. *Eur Biophys J.*, **38**, 1127-1133 (2009).

20.Jastimi RE, Edwards K, Lafleur M. Characterization of permeability and morphological perturbations induced by Nisin on phosphatidylcholine membranes. *Biophys J.*, **77,** 842-52 (1999).

21.Steffe J. Rheological methods in food process engineering. 2nd ed. USA : Freeman Press, Mitchigan State University., 21 (1996).

22. Pal R. Effect of droplet size on the rheology of emulsions. *AIChE Journal.,* **42**, 3181-3190 (1996).

23. Van der Werff JC, De Kruif CG. Hard-sphere colloidal dispersions: The scaling of rheological properties with particle size, volume fraction, and shear rate. *Journal of Rheology.*, **33**, 421-454 (1989).

(*Received 26*/3/ 2018;
accpted29 / 4/ 2018)

دراسات بيوفيزيائية علي تأثير أشعة جاما على الليبوسومات

سعيد عبدالفتاح طه* ، احمد عبدالمنعم محمد ، رائد محمد الشاذلى*** ، محمد حسنين جابر* و عمرو محمد كانى*****

*قسم الفيزياء الحيوية – كلية العلوم – جامعة القاهرة ، **شعبة الفيزياء الحيوية– قسم الفيزياء – كلية العلوم(بنين) – جامعة الازهر و *** قسم الفيزياء -كلية العلوم – جامعة الأزهر – مصر.

الليبوسومات هي عبارة عن تراكيب حويصلية مصنوعة من جزيئات دهنية التي تتشكل فى المحاليل المائية ، والتي يمكن استخدامها كنماذج لدراسة غشاء الخلية. في هذه الدراسة تم دراسة تأثير أشعة جاما (γ) على الليبوسومات المكونة من (DPPC) بواسطة قياسات المجهر الالكتروني النافذ (TEM)، التحليل الضوئي بالاشعة تحت الحمراء (FTIR) بالاضافة الى تشتت الضوء الديناميكي (DLS) وقياسات اللزوجة . حيث تعرضت الليبوسومات الى ثلاث جرعات مختلفة وهي(١٢٠،٨٠،٤٠ Gy (المنبعثة من عنصر الكوبالت المشع (Co٦٠) بمعدل ٩ KGy/h حيث اكدت دراسات (DLS) تجانس العينات من حيث حجم الجزيئات لجميع العينات كما كشفت نتائج (TEMان هناك تغييرا فى شكل و حجم الليبوسومات و الذي يتوافق مع الزيادة فى اللزوجة للعينات كما هو ظاهر من قياسات اللزوجة وأيضا أظهرت قياسات (FTIR) تغييرات كبيرة فى نطاق الخصائص التركيبية لليبوسومات التي تؤكد تأثير اشعة جاما على المجموعات الرئيسية CH٢ ، PO٢ المتماثلة وغير المتماثلة عند ١٠٩٠ ، ١٢٢٠ سم-١ على التوالى. بالاضافة الى تحرك مجموعة OH من ٣٤٣٩ ١-cm الى ٣٤٥٣ ١-cm وذلك عند التعرض لجرعة ١٢٠ Gy. يبدو أن التغييرات الطيفية ترجع الى نوع من فقدان الماء والتغييرات الجزيئية بسبب التأين وتكوين الشوارد الحرة التي تؤثر على مجموعة رأس الليبوسومات مما يؤدي الى تحركات جانبية للجزيئات الدهنية مما يعزز من إندماج الحويصلات الصغيرة لتكون حويصلات ذات أحجام اكبر.