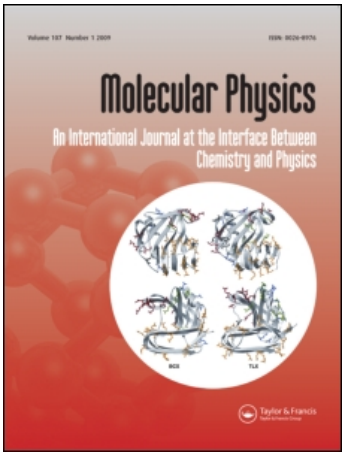

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RESEARCH ARTICLE

Interaction of dipalmitoyl phosphatidylcholine (DPPC) liposomes and insulin

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Insulin, a peptide that has been used for decades in the treatment of diabetes, has well-defined properties and delivery requirements. Liposomes, which are lipid bilayer vesicles, have gained increasing attention as drug carriers which reduce the toxicity and increase the pharmacological activity of various drugs. The molecular interaction between (uncharged lipid) dipalmitoyl phosphatidylcholine (DPPC) liposomes and insulin has been characterized by using Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction. The characteristic protein absorption band peaks, Amide I (at about 1660 cm^{-1}) and Amide II band (at about 1546 cm^{-1}) are potentially reduced in the liposome insulin complex. Wide-angle x-ray scattering measurements showed that the association of insulin with DPPC lipid of liposomes still maintains the characteristic DPPC diffraction peaks with almost no change in relative intensities or change in peak positions. The absence of any shift in protein peak positions after insulin being associated with DPPC liposomes indicates that insulin is successfully forming complex with DPPC liposomes with possibly no pronounced alterations in the structure of insulin molecule.

Keywords: insulin; DPPC; liposomes; FTIR; X-ray diffraction; interaction

1. Introduction

Insulin, the most important regulatory hormone in the control of glucose homeostasis [1], has well-defined properties and delivery requirements. Classical subcutaneous administration of insulin is far from being an ideal method of treatment as insulin does not immediately reach the liver following injection. Other limitations to this route include hypoglycemia (due to a narrow therapeutic window), peripheral hyperinsulinaema [2], lipoatrophy, lipohypertrophy [3,4], obesity associated with intensive therapy [5], insulin neuropathy and insulin presbyopia. This mode of administration can cause psychological stress leading to poor patient compliance. There are an increasing number of diabetics, across the globe, which needs daily multiple injections throughout their life span. Therefore, improved insulin delivery could significantly influence the treatment of diabetes and the quality of life of these individuals. To reduce the injection frequency and toxicity of subcutaneous administered protein drugs, it is necessary to develop safe and sustained injectable delivery systems.

Over the past few decades significant research has been carried out to develop oral formulations using various drug delivery approaches. These include emulsions [6,7], particulate carriers [8–13], colon targeted systems [14], vesicular carriers such as liposomes [15,16], use of permeation enhancers [17,18] and protease inhibitors [19,20], protein–ligand conjugates [21,22], bioadhesive carriers [23,24] and hydrogels [25,26]. These strategies show some improvements in delivery, however, are not sufficient to result in the development of a commercially viable product in use today.

Liposomes have been studied as sustained drug delivery systems [27]. Liposomes have advantages over other delivery systems, since they are biodegradable, non-toxic and non-immunogenic. Liposomes, which are lipid bilayer vesicles, have gained increasing attention as drug carriers which reduce the toxicity and increase the pharmacological activity of various drugs.

Liposomes have been presented as an interesting alternative for administration of biomolecules through several mucosal surfaces [28], since they are versatile and tend to be relatively innocuous (produced with natural and biodegradable compounds), and also provide protection to the encapsulated material [29–32]. Their organized structure (an aqueous core encapsulated within one or more phospholipid bilayers) permits the association of drugs to both the aqueous and lipid phase and drug release can usually be controlled, depending on the bilayers number and composition [29,30].

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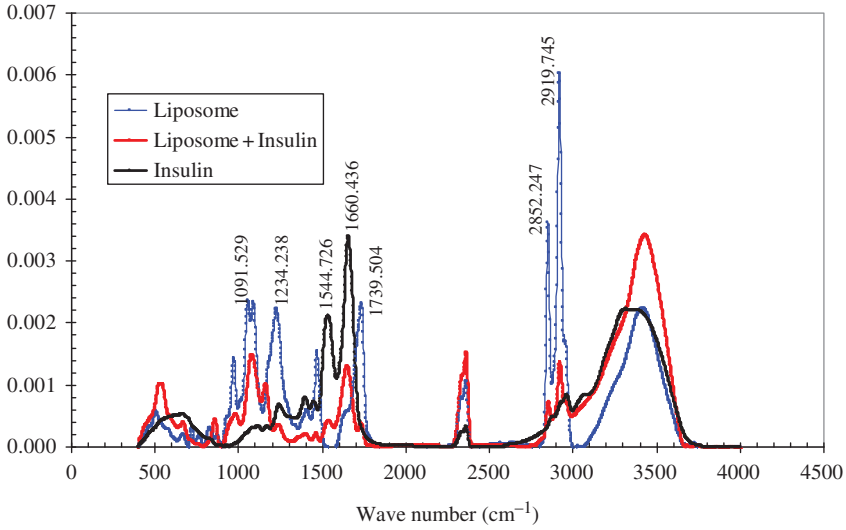

Figure 1. FTIR absorption spectra of different samples.

In order to evaluate liposomes as a safe and sustained injectable delivery systems for proteins, insulin was chosen as a model of protein drug since it is one of the most widely used protein drugs and it has a short half-life in circulation after intravenous administration [33].

The most common phospholipid used in liposomal drug carriers is phosphatidyl choline [34]. Liposomes of pure dipalmitoylphosphatidylcholine (DPPC) have been widely used as membrane model systems to study the effects of molecules of biological interest (like hormones, vitamins and ions) on membrane properties [35,36].

There is a limited knowledge about the interaction between insulin and DPPC (as uncharged lipid) liposomes. Some investigators suggested that the interaction between anionic liposomes and insulin may be hydrophobic in nature [37]. So, the aim of this study was to determine the structural integrity of insulin upon entrapment in uncharged DPPC liposomes. Because of their large application on the protein physical stability analysis [38], Fourier transform infrared (FTIR) and X-ray diffraction have been used in this study to examine the insulin structure and interactions between insulin and uncharged DPPC lipid after its entrapment.

2. Materials and methods

2.1. *Materials*

L-α-Dipalmitoyl phosphatidylcholine 'DPPC' in powder form of purity 99% and sephadex G-75 were purchased from Sigma (St. Louis, MO, USA). Recombinant human insulin crystals were purchased from VACSERA, Egypt. The purity of the protein was greater than 98.8%. Double distilled deionized water was used throughout the study. All other reagent and solvents were of analytical grade and were used without further purification.

2.2. *Preparation of liposomes*

The liposomes used in this work were prepared from DPPC using reverse phase evaporation technique [39]. Reverse phase evaporation of liposomes, involves (1) drying down lipids (2) addition of volatile solvent (3) addition of water (4) sonication to clarity (5) evaporation of the volatile solvent. The lipids were first dissolved and mixed in chloroform/ethanol (5:1, v/v) to assure a homogeneous mixture of lipids. The organic solvent was removed by rotary evaporation yielding a thin lipid film on the sides of a round bottom flask. The lipid film was thoroughly dried to remove residual organic solvent by placing flask on a vacuum pump overnight. Hydration of the dry lipid film was accomplished simply by adding an aqueous medium to the container of dry lipid and agitating at temperature above phase transition temperature of the lipid. The sample is sonicated to clarity. Then, the solvent is evaporated under a stream of nitrogen.

For liposomes encapsulated with insulin samples, 10 mg insulin powder was dissolved in 5 mL sterile water and then was added to the dried lipid flask and agitating at temperature above phase transition temperature of the lipid. The non-entrapped insulin was

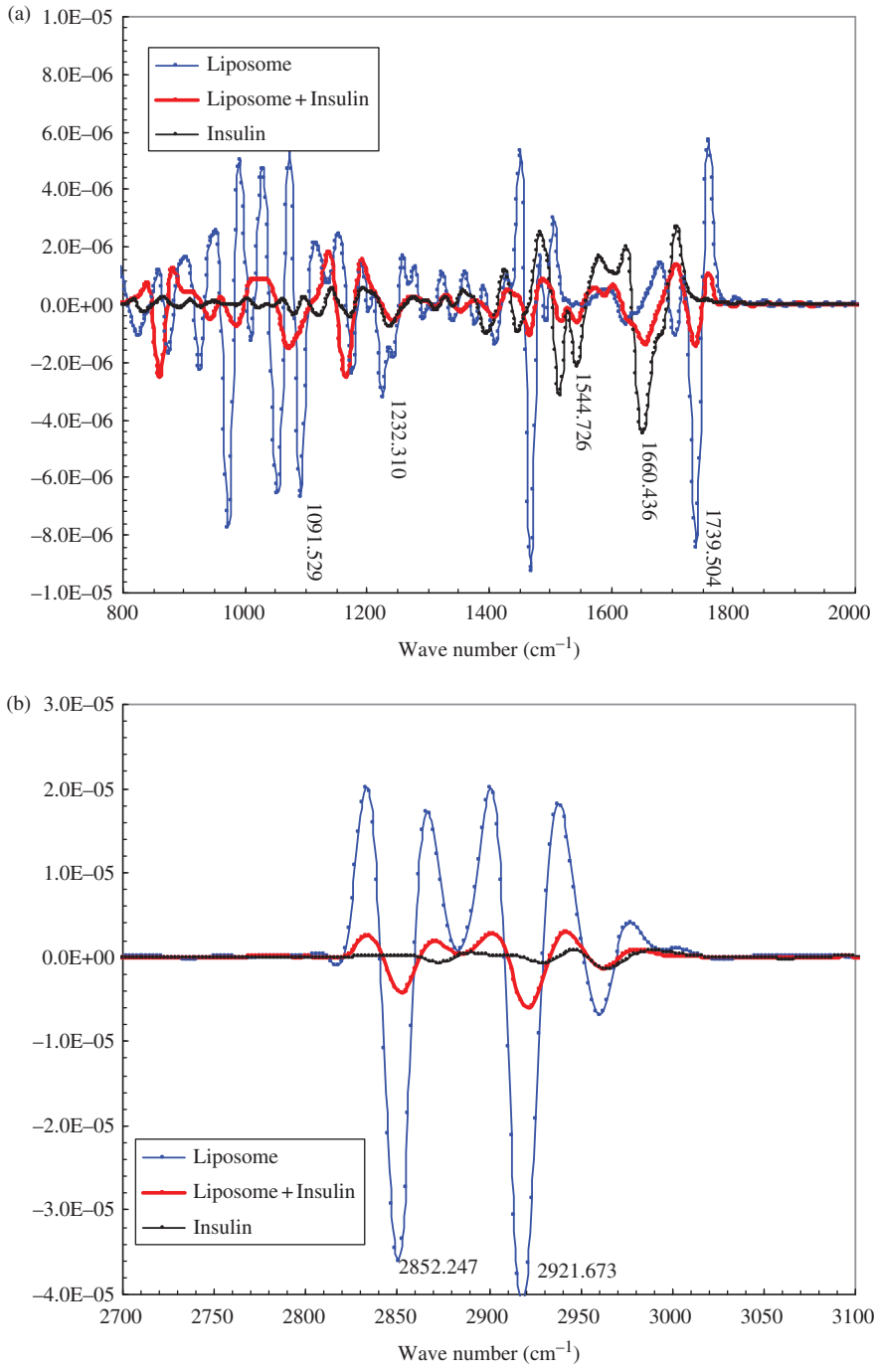

Figure 2. (a) Second derivative of the area normalized, and (b) difference FTIR spectra of different samples.

separated using gel chromatography column of sephadex G-75.

2.3. *Characterization of DPPC and insulin interaction by FTIR*

FTIR spectra of lyophilized samples of DPPC liposomes and DPPC liposomal encapsulated insulin, deposited in KBr disks were measured using NICOLET 6700 FTIR Thermo scientific spectrometer, UK in the range of 400–4000 cm^{-1} at 2 mm/s and a resolution of 4 cm^{-1} at room temperature The resultant spectra were smoothed with a seven-point Savitsky Golay smooth function to remove the noise [40–42]. After area normalization, the second-derivative spectra were obtained using the derivative function

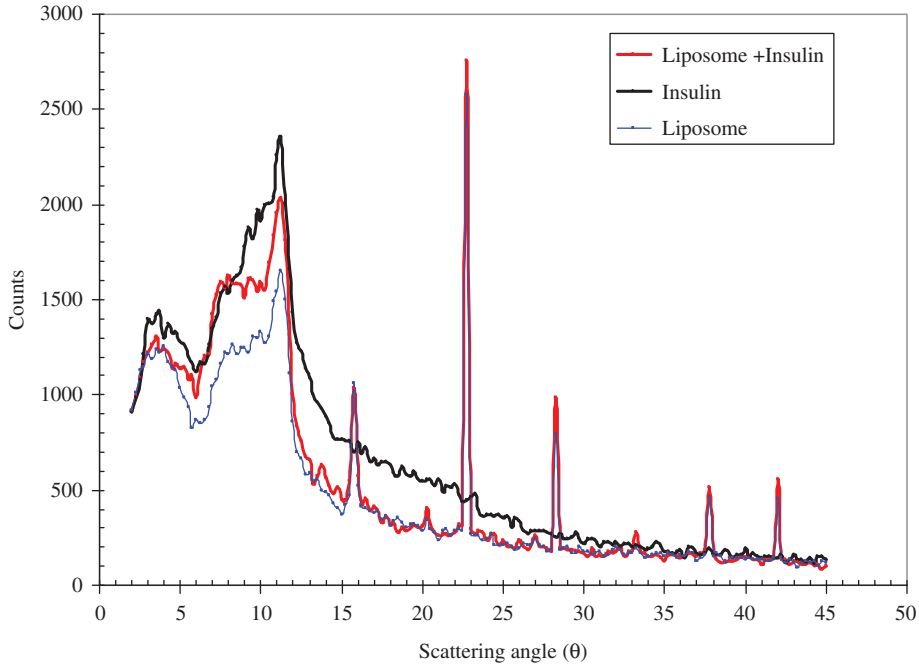

Figure 3. X-ray diffraction patterns of different samples.

of Origin software Version 6.0. The difference between spectra was obtained by subtracting the second-derivative spectra of different samples from control.

2.4. *Characterization of DPPC and insulin interaction by X-ray diffraction*

X-ray diffraction (XRD) patterns of the investigated samples were measured with a Shimadzu Labx-XRD-6000 diffractometer (Kyoto, Japan), using a Cu K_α target operating at 30 kV and 40 mA in a θ–2θ mode. The diffraction angle (θ) ranged from 2° to 45°. Measurements were carried out in a step mode at a step equal to 0.5°.

3. Results and discussion

Figure 1 shows that the characteristic protein absorption band peaks, Amide I (at about 1660 cm^{-1}) due to C=O stretching vibration of the peptide group, and Amide II band (at about 1546 cm^{-1}) due to N–H bending with contribution of C–N stretching vibrations [43–45] are potentially reduced in the liposome insulin complex. This may be attributed to either a restriction on the Amide I and Amide II vibrations due to the nature of the liposome insulin complex or, most probably, due a 'quenching' effect resulting from the association of insulin with the lipids of the liposome. The absence of any shift in protein peak positions after insulin being associated with DPPC liposomes indicates that insulin is complexed with liposomes with possibly no pronounced alterations in the structure of the insulin molecule.

On the other hand, comparing the insulin–liposome complex with empty liposome, one notices that there is a reduction in the absorption of some characteristic liposome bands: the strong bands around 2920 and 2850 cm^{-1} corresponding to the CH_2 antisymmetric and symmetric stretching modes of acyl chains, respectively [46–48], the C = O stretching band at 1740 cm^{-1}, [49], the CH_2 scissoring band near 1470 cm^{-1} [35] and the symmetric and antisymmetric PO_2^- stretching bands near 1090 and 1220–1240 cm^{-1}, respectively [46,49].

In order to further investigate that there is no shift in peak positions in the insulin–DPPC liposomes complex, the second derivative of the area normalized and difference FTIR spectra for empty liposomes, insulin-liposomes complex and free insulin are also plotted (Figure 2(a) and 2(b)). The second derivative spectra is a band narrowing technique [50–52] which would probably reveal any shift in peak position in the liposome insulin complex relative to empty liposome or free insulin. It is clear from Figure 2(a) and (b), that, for almost all absorption peaks of interest there is no shift in peak positions for the liposome insulin complex

compared to liposome or insulin alone. This result further supports the observation that there is probably no detectable structural change in either of liposome or insulin–DPPC liposome complex. Thus the association of insulin with DPPC liposomes and the formation of liposome insulin complex is probably far beyond the induction of a major change that would affect the structure and function of either insulin or liposomal membrane.

Further investigations are carried out using wide-angle X-ray scattering measurements of the free insulin, free liposome and insulin–liposome complex (Figure 3). Compared with empty liposome, the insulin–liposome complex still maintains the characteristic liposome DPPC diffraction peaks with almost no change in relative intensities or change in peak positions. At low scattering angles (θ up to 14°) the insulin–liposome complex produces a scattering halo that holds the main features of both insulin and liposome scattering halos with an intensity which is midway between insulin and liposome. The wide-angle X-ray scattering results thus support the observations drawn from the FTIR results in the aspect that there is no evidence of detectable change in the molecular structure of any of insulin or liposome in the insulin–liposome complex.

References

[1] C. Dai, B. Wang and H. Zhao, Colloid Surf. B **41**, 117 (2005).

[2] F.P. Kennedy, Drugs **42**, 213 (1991).

[3] A. Marble, L.P. Krall, R.E. Brandley, A.R. Christlieb and J.S. Soeldner, *Joslin's Diabetes Mellitus*, 12th ed. (Lea and Febiger, Philadelphia, 1985), pp. 843–886.

[4] L. Monaco, G. Gettken and J.H. Silverstein, Clin. Pediatr. **35**, 191 (1996).

[5] M.G. Carlson and P.J. Campbell, Diabetes **42**, 1700 (1993).

[6] Y. Onuki, M. Morishita and K. Takayama, J. Control. Release **97**, 91 (2004).

[7] E. Toorisaka, H. Ono, K. Arimori, N. Kamiya and M. Goto, Int. J. Pharm. **252**, 271 (2003).

[8] T. Morçöl, P. Nagappan, L. Narenbaum, A. Mitchell and S.J.D. Bell, Int. J. Pharm. **277**, 91 (2004).

[9] H. Zhenqing, Z. Zhenxi, Z. Chuanxin and H. Mei, J. Control. Release **97**, 467 (2004).

[10] G.P. Carino, J. Jacob and E. Mathowitz, J. Control. Release **65**, 261 (2000).

[11] H. Chung, J. Kim, J.Y. Um, I.C. Kwon and S.Y. Jeong, Diabetologia **45**, 448 (2002).

[12] F. Cui, K. Shi, L. Zhang, A. Tao and Y. Kawashima, J. Control. Release **114**, 242 (2006).

[13] C. Damage, C. Michel, M. Aprahamian and P. Couvreur, Diabetes **37**, 246 (1988).

[14] H. Tozaki, J. Komoike, C. Tada, T. Maruyama, A. Terabe, T. Suzuki, A. Yamamoto and S. Muranishi, J. Pharm. Sci. **89**, 1016 (1997).

[15] H. Takeuchi, H. Yamamoto, T. Niwa, T. Hino and Y. Kawashima, Pharm. Res. **13**, 896 (1996).

[16] M.A. Kisel, L.N. Kulik, I.S. Tsybovsky, A.P. Vlasov, M.S. Vorob'yov, E.A. Kholodova and Z.V. Zabarovskaya, Int. J. Pharm. **216**, 105 (2001).

[17] A. Fasano and S. Uzzau, J. Clin. Invest. **99**, 1158 (1997).

[18] T. Uchida, Y. Toida, S. Sakakibara, Y. Miyanaga, H. Tanaka, M. Nishikata, K. Tazuya, N. Yasuda and K. Matsuyama, Chem. Pharm. Bull. **49**, 1261 (2001).

[19] A. Yamamato, T. Taniguchi, K. Rikyuu, T. Tsuji, T. Fujita, M. Murakami and S. Muranishi, Pharm. Res. **11**, 1496 (1994).

[20] M.K. Marschűtz, P. Caliceti and A. Bernkop-Schnurch, Pharm. Res. **17**, 1468 (2000).

[21] J.G. Still, Development of oral insulin: progress and current status, Diabetes Metab. Res. Rev. (2002) S29–S37.

[22] C.Q. Xia, J. Wang and W.C. Shen, J. Pharmacol. Exp. Ther. **295**, 594 (2000).

[23] S.A. Uraizee, A.M. Mcphillips, W.A. Ritschel and A. Sakr, Drugs Made Ger. **43**, 41 (2000).

[24] Y. Pan, Y. Li, H. Zao, J. Zheng, H. Xu, G. Wei, J. Hao and F. Cui, Int. J. Pharm. **249**, 139 (2002).

[25] C.T. Musabayane, O. Munjeri, P. Bwititi and E.E. Osim, J. Endocrinol. **164**, 1 (2000).

[26] M. Morishita, T. Goto, N.A. Peppas, J.I. Joseph, M.C. Torjman, C. Munsick, K. Nakamura, T. Yamagata, K. Takayama and A.M. Lowman, J. Control. Release **97**, 115 (2004).

[27] G. Blume and G. Cevc, Biochim. Biophys. Acta **1029**, 91 (1990).

[28] A. Sharma and U.S. Sharma, Int. J. Pharm. **154**, 123 (1997).

[29] H.M. Courrier, N. Butz and T.F. Vandamme, Crit. Rev. Ther. Drug Carrying Syst. **19**, 425 (2002).

[30] C.J. Kirby and G. Gregoriadis, in *Encyclopedia of Controlled Drug Delivery*, edited by E. Mathiowitz (John Wiley and Sons, New York, 1999), p. 461.

[31] M. Mezei, in *Liposomes as Drug Carriers: Recent Trends and Progress*, edited by G. Gregoriadis (John Wiley and Sons, Chichester, 1988), p. 663.

[32] P.J. Mihalko, H. Schreier and R.M. Abra, in *Liposomes as Drug Carriers: Recent Trends and Progress*, edited by G. Gregoriadis (John Wiley and Sons, Chichester, 1088), p. 679.

[33] D.R. Owens, *Human Insulin. Clinical Pharmacological Studies in Normal Man* (MTP Press, Lancaster), p. 19860.

[34] V. Torchilin and V. Weissig, *Liposomes: A Practical Approach*, 2nd ed. (Oxford University Press, New York, 2003).

[35] B. Biruss, R. Dietl and C. Valenta, Chem. Phys. Lipids **148**, 84 (2007).

[36] F. Castelli, A. Raudino and S. Gurrieri, Chem. Phys. Lipids **52**, 129 (1990).
[37] Y. Maitani, Masayuki Nakagaki and Tsuneji Nagai, Int. J. Pharm. **64**, 89 (1990).
[38] L. Nielsen, R. Khurana, A. Coats, S. Frokjaer, J. Brange, S. Vyas, V.N. Uversky and A.L. Fink, Biochemistry **40**, 6036 (2001).
[39] F. Szoka and D. Papahadjopoulos, Proc Natl. Acad. Sci. USA **75**, 4194 (1978).
[40] B. Sarmento, D.C. Ferreira, L. Jorgensen and M. van de Weert, Eur. J. Pharmaceut. Biopharmaceut. **65**, 10 (2007).
[41] Lene Jørgensen, Charlotte Vermehren and Simon Bjerregaard, Int. J Pharmaceut **254**, 7 (2003).
[42] Wei-Guo Dai and Laing C. Dong, Int. J. Pharmaceut. **336**, 58 (2007).
[43] M. Weert, I. van de, E. Haris Parvez and J.A. Hennink Wim, Crommelin Daan, Anal. Biochem. **297**, 160 (2001).
[44] S. Mario, M. Michela, M. Tania, C. Laura, M. Giulia, C. Raffaella, D. Luisa, T. Florian, M. Luca, M. Giovanna, J. Kourie Joseph, B. Orso, S. Deepak, I. Hideyo, A. Kirschner Daniel, G. Forloni and T. Fabrizio, J. Biol. Chem. **278**, 48146 (2003).
[45] N. Antonino, A. Diletta, B. Stefania, L. Marina and S.M. Doglia, Biochem. J **385**, 511 (2005).
[46] F. Severcan, U. Baykal and S. Süzer, Anal. Bioanal. Chem. **355**, 415 (1996).
[47] T. Lefèvre, S Toscani, M. Picquart and J. Dugué, Eur. Biophys. J **31**, 126 (2002).
[48] M. Mady, D. Mirhane, K. Safaa and K. Wafaa, Eur. Biophysics J **38**, 1127 (2009).
[49] N. Toyran and F. Severcan, Spectroscopy **16**, 399 (2002).
[50] O.S. Desouky, W.M. Elshemey and N. Selim, Nucl. Instr. Methods A **607**, 463 (2009).
[51] G. Rudolf, W. Skip, R.H. Callender and W.H. Woodruff, Rian S D.R., Proc. Natl Acad. Sci. USA **94**, 3709 (1997).
[52] G. Stefan, S. Vytautas and W. Roland, J. Mol. Biol. **356**, 497 (2006).