INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com ISSN : 0976-4550

Volume-7, Issue-2, April-June-2016 Coden IJABFP-CAS-USA Copyrights@2016

Received: 23rd Feb 2016 *Revised: 26th Mar 2016* *Accepted: 27th Mar 2016*

Research Article

DEVELOPMENT AND CHARACTERIZATION OF LIPOSOMES CONTAINING GLATIRAMER ACETATE FOR MULTIPLE SCLEROSIS

Renata P Camargo-Mascarenhas[1], Aline F Xavier[1], Daiana K Deda[2], Ariana de S Moraes[1], Lívia F Rodrigues[1], Marystela Ferreira[1], Gisela BRF da Silva[1,3], Fábio L Leite[1*]

[1]Departamento de Física, Química e Matemática, Universidade Federal de São Carlos, 18052-780 Campus, Sorocaba/SP, Brazil

[2]Instituto de Química, Universidade de São Paulo, 05508-000 campus São Paulo/SP, Brazil

[3]Departamento de Fármacos e Medicamentos, Laboratório de Tecnologia Farmacêutica, Faculdade de Ciências Farmacêuticas – UNESP, 14801-902 campus de Araraquara/SP, Brazil

ABSTRACT: The frequent administration of glatiramer acetate in the treatment of subcutaneous multiple sclerosis causes undesirable local reactions. This study suggests this drug incorporation in liposomes so that certain benefits may be achieved. Three types of liposomes were developed:
(1) With lipid dipalmitoyl phosphatidylglycerol (DPPG)
(2) With the lipid palmitoil phosphatidylglycerol (POPG)
(3) With a mixture of these lipids (DPPG/POPG)
The physicochemical characterization was performed. An atomic force microscopy revealed that the liposome with DPPG showed well-defined oval and irregular vesicles while the liposome with POPG presented vesicle fusion and film formation on the surface of mica. The liposome with DPPG/POPG was opaque, with an intense aggregation of vesicles. The diameter analysis showed that all liposomes formed large multilamellar vesicles. The polydispersity of all types of liposomes showed high values, while the zeta potential was negative. The encapsulation efficiency was greater for the liposome GLAM + DPPG/POPG, followed by GLAM+DPPG and less for GLAM+POPG. Based on the results obtained in our work, it is believed that the liposome produced from DPPG had promising results, representing a new possibility to be explored. It might improve quality of life and consequently treatment compliance by glatiramer acetate users.

Key words: Liposome; Glatiramer acetate; Multiple sclerosis; Diameter; Atomic force microscopy; Encapsulation efficiency

*Corresponding author: Fábio Lima Leite, Departamento de Física, Química e Matemática, Universidade Federal de São Carlos, 18052-780 campus Sorocaba/SP, Brazil, E-mail: fabiolimaleite@gmail.com

INTRODUCTION

The first case of multiple sclerosis (MS) was described by Jean Charcot Martin in 1868. However, only after 122 years the first drugs to treat specific disease were created (Piehl F, 2014).

Multiple sclerosis is a chronic inflammatory, demyelinating, autoimmune, neurodegenerative neurological disease, defined by repeated episodes of neurological dysfunction remission variable (Costa CCR, et al., 2005; Noseworthy JH, et al., 2000). In addition, multiple sclerosis may affect the mental life of the patient, being depression very common (Mendes MF, et al., 2003) due to adverse reactions to the most common treatment drugs (Moreira MA, et al., 2002) (glatiramer acetate, (Saguil A, et al., 2014; Ben-Nun A, et al., 2014; Soares Almeida LM, et al., 2006; Lebrun C, et al., 2011) interferon beta (Piehl F, 2014; Saguil A, et al., 2014) and glucocorticoids such as methylprednisolone).

Glatiramer acetate (GLA) appeared in 1996. It was indicated for cases of relapsed MS outbreaks, administered subcutaneously with daily doses. In addition, GLA is one of the most widely used drugs for the treatment of MS. It also has a therapeutic potential for the treatment of other autoimmune diseases such as rheumatoid arthritis and Crohn's disease (an inflammatory bowel disease) (Ben-Nun A, et al., 2014). The continued use of GLA subcutaneously usually causes undesirable local reactions that affect about 60% of drug users (Soares Almeida LM, et al., 2006; Lebrun C, et al., 2011), the most common being localized lipoatrophy (Lebrun C, et al., 2011; Soos N, et al., 2004). The formation of on-site nodules, bruises and abscesses may occur (Soares Almeida LM, et al., 2006; Beer K, et al., 2011). Another common reaction is contact dermatitis caused by the drug and, depending on its intensity, it may be necessary to discontinue treatment (Haltmeier S, et al., 2011).

Faced with all difficulties mentioned, the pain of every application is one of the main reasons for discontinuation treatment (Beer K, et al., 2011; Devonshire V, et al., 2011, Jongen PJ, et al., 2011). Although GLA is a very effective drug, adherence to treatment may be impaired by the existence of these undesirable effects. In an attempt to solve this problem, many studies are being developed such those regarding the reduction in the administration volume; (Anderson G, et al., 2010) development of pharmaceutical formulations that allow an oral (Teitelbaum D, et al., 2004, Croxford JL, et al., 2009, Teitelbaum D, et al., 1999, Maron R, et al., 2002) or nasal administration (Graça JS, et al., 2015); devices facilitating self-injection (Maron R, et al., 2002, Bayas A, 2013, Gross Y, et al., 2013); and reduction in the frequency of applications (Achiron A, 2009; Comi G, et al., 2011, Khan O, et al., 2012, Teva, 2013). Of all cited studies, only two presented promising results (Haltmeier S, et al., 2011, Anderson G, et al., 2010). One considered nasal (Duchi S, et al., 2013) administration and the other is the reduction of the frequency of administrations (Teitelbaum D, et al., 2004).

Although there are many attempts to improve the formulations, in all studies the drug was used in its free form, that is, no Nano encapsulation technique was used. In the database Web of Science, there is only one study on glatiramer acetate encapsulation in Nano carriers - incorporation in Nanolipodendrosome (Afzal E, et al., 2013) and the filing of a patent on the microencapsulation of the drug (Wang B, et al., 2013). Thus, the incorporation of GLA in a Nano carrier, such as liposomes, is an alternative to be explored and may offer a new perspective. Some benefits can be achieved, such as a reduction in the frequency of administration or the possibility of administration by an alternate route such as oral, nasal or intradermal.

Liposomes are a type of Nano carriers consisting of spherical vesicles from 25 to 2,500 nanometers (Khan O, et al., 2012) comprised of natural or synthetic phospholipids (Vemuri S, et al., 1995; Sharma A, et al., 1997). They are able to incorporate lipophilic, hydrophilic and amphiphilic drugs (Torchilin VP, 2006). Liposomes are very similar to biological membranes (Graça JS, et al., 2015), which have a lipid bilayer. The similarity with biological membranes provides liposomes with biomimetic characteristics (Xiang TX, et al., 2006) that facilitate the permeation and the transport of drugs through the cell membrane, and protect the drug from degradation by the reticuloendothelial system (Gregoriadis G, 1995).

One of the major advantages of Nano systems used for treatment and diagnosis of disorders of the central nervous system (CNS) is the possibility of allowing the drug to surpass the blood-brain barrier (BBB). BBB serves as a protective barrier to the CNS, preventing the passage of pathogens such as viruses and bacteria (Kanwar JR, et al., 2012). These functions act as a selective membrane allowing only lipophilic molecules or molecules with a molecular weight below 400-600 Da. The high selectivity and efficiency in protecting CNS prevents that many drugs surpass it, limiting the diagnosis and the treatment of CNS disorders (Kanwar JR, et al., 2012). With nanotechnology, it is possible to encapsulate a drug or a diagnostic agent that typically would not exceed the BBB, making it possible for this drug to reach the CNS (Nance EA, et al., 2012; Modi G, et al., 2009). Nanoneurobiophysics is an area of research which studies mechanisms of demyelinating and neurodegenerative diseases and development of new methods based on nanotechnology for development of diagnosis and treatment of these diseases, thus, the development of liposomes containing glatiramer acetate is in this area of research (Leite FL, et al., 2015).

In this context, studies on the use of liposomes for the treatment of CNS diseases such as MS showed a higher bioavailability of the drug and reduced inflammatory infiltration and preservation of the myelin sheath (Avnir Y, et al., 2016; Linker RA, et al., 2008). The association with certain substances, such as glutathione or transferrin, may guide the delivery in the CNS, favoring the passage through the BBB (Gaillard PJ, et al., 2012). In addition to pharmacokinetic improvements, in some cases encapsulation in liposomes may offer some pharmacological advantages, such as an increased solubility and a consequent reduction in the administration volume (when the drug becomes more soluble, a lower volume of solvent is needed to solubilize it).

Some drugs, such as Doxil, used in the treatment of ovarian cancer, are already incorporated in liposomes. They are sold in the market and there are many studies on the incorporation of this drug into a release system (Modi G, et al., 2009, Teli MK, et al., 2010, Devalapally H, et al., 2007, Patlak M, 2010, Re F, et al., 2012). Regarding the incorporation of peptides, liposomes represent an extensive area of research that has been developed for parenteral, oral, intranasal, transdermal and ocular administration (Du AW, et al., 2014). However, there are not many studies on the incorporation of GLA in liposomes.

GLA has a similar structure to the myelin sheath (Ben-Nun A, et al., 2014; Teitelba D, et al., 1971; Schrempf W, et al., 2007; Aharoni R, 2013). It comprises a random mixture of synthetic polypeptides, which consists of four amino acids: L-alanine, L-lysine, L-glutamine and L-tyrosine in the ratios 4.2: 3.4: 1.4: 1, respectively (Teitelba D, et al., 1971; Schrempf W, et al., 2007; Aharoni R, 2013). The average molecular mass is between 5 and 9 kDa. The acetate group on the molecule ensures the solubility of the drug in water. Due to complexity and variability of the polypeptide mixture, the mechanism of action is still not sufficiently explained, i.e., the random mixture of amino acids seems to provide multiple forms of action (Avnir Y, et al., 2016). The main objective of this work was to develop a nano carrier for glatiramer acetate seeking to offer better conditions for patient adherence to the treatment of MS.

EXPERIMENTAL

Drug

The Teva laboratory provided the product (a mixture of glatiramer acetate, water and mannitol) in 28 pre-filled syringes, each containing water for injection (1 mL), mannitol (40 mg) and glatiramer acetate (20 mg). The syringes were put in a plastic tube, with a total volume of approximately 28 mL of solution.

Lyophilization

The total sample volume (glatiramer acetate, water and mannitol) remained for 24 hours in a freezing process. It was then freezed for 32 hours and the water was removed. In this process, 1.33 g of a white powder was obtained. Its composition was glatiramer acetate (33.33%) and the mannitol excipient (66.67%). This mixture was called "GLAM".

Characterization of glatiramer acetate (GLA) by UV-vis and fluorescence

The tyrosine in the glatiramer acetate molecule was used as a parameter to characterize the drug. A scanning between 200 and 290 nm in buffered solutions containing GLA was performed at different concentrations, since tyrosine is within this absorbance range (Brasil, 2010). All samples were analyzed in duplicate. These solutions were analyzed by a UV-vis spectrophotometer Genesis 6 to determine the maximum absorbance value of tyrosine in GLA. The absorbance value observed in the UV-vis was used for excitation in a spectrofluorimeter Shimadzu RF-5301PC equipped with a xenon lamp and a quartz cuvette, optical path 10 nm. The experimental parameters were spectrofluorimeter λEX = 275 nm, λEM = 280-400 nm and 1.5 nm slit.

Liposome preparation containing a GLAM mixture

The preparation of liposomes was made according to the hydration of the lipid film described by Lima et al. (Lima EM, et al., 2002) The chosen lipids were DPPG (dipalmitoyl phosphatidylglycerol - CAS 200880-41-7) and POPG (palmitoil phosphatidylglycerol glycerol - CAS268550-95-4), with transition temperatures 41°C and -2°C, respectively, and both negatively charged (Avanti-Polar-Lipids, 2014). They were acquired from Avanti Polar Lipids.

Three liposome formulations were prepared from the mixture of phospholipids with GLAM: (1) GLAM + DPPG, (2) GLAM + POPG, and (3) GLAM + DPPG/POPG in the ratio 1:1. The phospholipids were weighed and each dissolved in a round bottom flask at a concentration of 1.0 $mmol.L^{-1}$ in chloroform and methanol.

The volatile phase was extracted by evaporation with nitrogen gas in a rotary motion to form a thin lipid film on the walls of the flasks. The films were hydrated with a PBS buffer solution at 0.1 $mol.L^{-1}$ (pH 7.4) containing GLAM in a concentration of approximately 0.05 $mg.L^{-1}$ of glatiramer acetate. Then, the flasks were subjected to an ultrasound bath (Unique MAXCLEAN model 750), under a frequency of 25 KHz for 2 hours to form the vesicles. The heating was not turned on and the temperature was monitored throughout the procedure, with a variation from 20°C at the beginning of the process to 47°C at the end of the process.

Liposomes Characterization techniques

Fluorescence Spectroscopy

Three liposome formulations were subjected to analysis by fluorescence spectroscopy to verify whether encapsulation in the vesicles affected the tyrosine fluorescence band.

Atomic Force Microscopy (AFM)

The morphology and diameter of the vesicles were assessed by atomic force microscopy (AFM) using a Bruker V Nano scope with an AN-CSG01 tip. The samples (in triplicate) were placed on the surface of muscovite mica. Micas were cleaved and 40 µL of each liposomal formulation were deposited in it. The samples were left resting exposed to air for 24 hours to partially dry the formulations. After this period, we proceeded to read the samples in "Tapping" to avoid damage to the vesicles.

Dynamic light scattering

The size distribution and the loading of liposomes were verified by dynamic light scattering and zeta potential measurements in a Nano Trac 252 (Micro Trac Inc.). This assay aimed to evaluate the size of the vesicles obtained in the production of liposomes, the zeta potential and the polydispersity of these vesicles.

Encapsulation efficiency (%EE)

The three liposomal formulations (concentration of approximately 0.05 $mg.L^{-1}$ of glatiramer acetate) were subjected to an encapsulation efficiency test in order to determine the percentage of encapsulated/adsorbed drug on the surface of the vesicles. The initial fluorescence characterization of formulations was performed and then 2 mL of each formulation were added in Amicom Ultra filter - 4 Millipore devices with a porosity of 100 kDa. The devices containing the samples were centrifuged in a Sorvall Biofuge ultra Stratos at 5,000 G for 20 minutes at 10°C. The liquid resulting from this centrifugation was analyzed and the free drug was quantified. The percentage of incorporated GLA was calculated from the equation below: (Ohlweiler OA, 1976)

$$\%EE = \frac{T_0 - F_0}{T_0} \times 100$$

Where:

%EE = encapsulation efficiency of GLA in percentage
T_0 = fluorescence intensity drug present in the initial formulation (spectrofluorimeter reading)
F_0 = free drug fluorescence intensity detected in the supernatant collected in centrifuge tubes (spectrofluorimeter reading)

RESULTS AND DISCUSSION

Buffered solutions with different concentrations of GLAM were analyzed by UV-vis spectroscopy. The analysis by UV-vis spectroscopy is a widely used technique for quantification, because there is a wide variety of organic and inorganic species capable of absorbing energy in the UV and Vis regions. Those that do not have this characteristic may be converted by appropriate chemical treatments (Sun LM, et al., 2012). The method enables choosing a wavelength range in which only the considered species acts as an absorbent. This makes the technique specific and precise. The tyrosine present in the GLA molecule was used as a parameter for drug characterization. According to the literature, the maximum tyrosine absorbance occurs at 280 nm (Brasil, 2010). In the GLAM mixture, there was a band at 275 nm (Figure 1), which was assigned to the tyrosine present in the glatiramer acetate. This offset for the band is stated in the Lambert-Beer law and may occur because of an interaction between the compounds of mannitol and the glatiramer acetate mixture, or by tyrosine linked to other amino acids of the molecule (Sun LM, et al., 2012). The spectroscopy obtained in this analysis is characteristic of compounds that present transition electrons n to the excited state π*, which generally have a higher absorbance (200-700 nm) (Cueva EA, 2005, Cienfuegos F, et al., 2000).

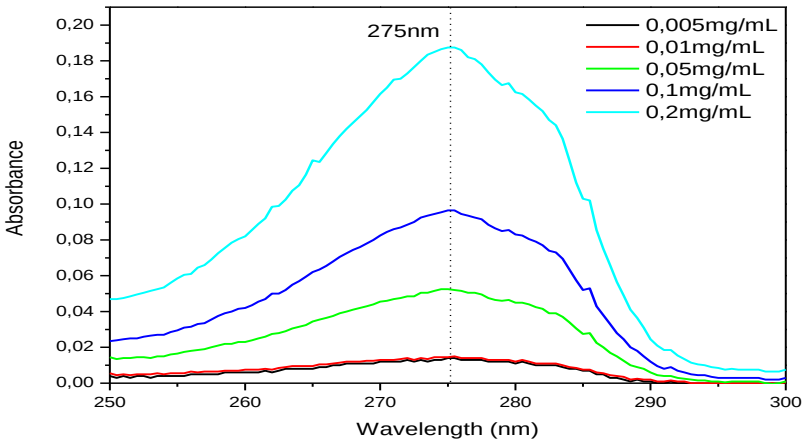

Figure 1: UV-vis spectroscopy of GLAM in different concentrations, Tyrosine at 275 nm.

Based on the tyrosine absorbance band shown in Figure 1, the choice of the excitation wavelength used in the spectrofluorometer was 275 nm. In the literature, the emission band is at 310 nm (Azevedo JCRD, et al., 2008). In the samples, a band was observed at 313 nm (Figure 2). The shift of the emission band from 310 to 313 nm may be justified because it is a mixture of two compounds (mannitol and glatiramer acetate) and because tyrosine is linked to other amino acids present in the drug molecule (alanine, lysine and glutamine) suffering interactions resulting in changes in fluorescence measurements (Sun LM, et al., 2012).

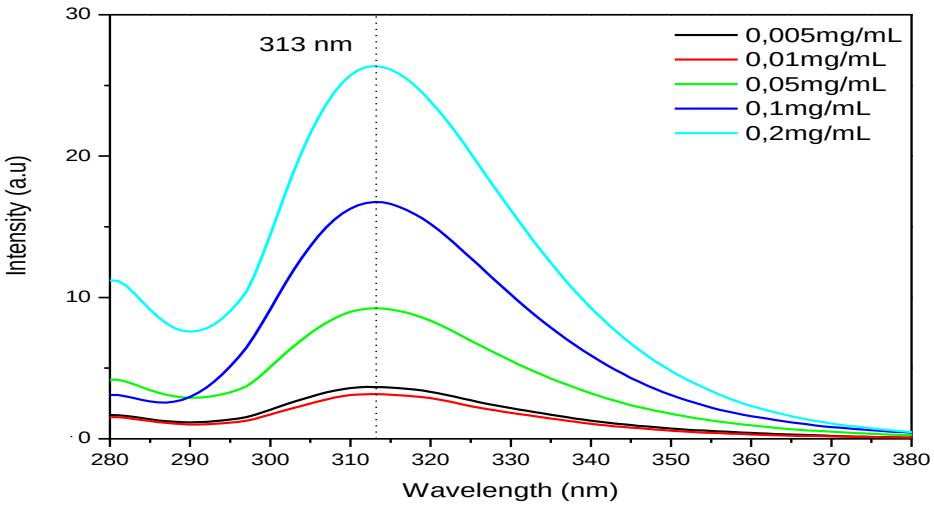

Figure 2: Fluorescence spectroscopy of GLAM at different concentrations, Tyrosine at 313 nm.

Fluorescence spectra of liposomes showed an increase in band intensity compared to the buffered solution of free GLAM (Figure 3, black curve). This is because the liposome when encapsulated offers a protected microenvironment where the molecules are distant from each other, with the deactivation of energy transfer between molecules. The loss of power provides a higher probability of energy decay in electrons, from an excited state to a flat state. This is reflected in the increase in fluorescence intensity (Farrapo Xavier AC, et al., 2013).

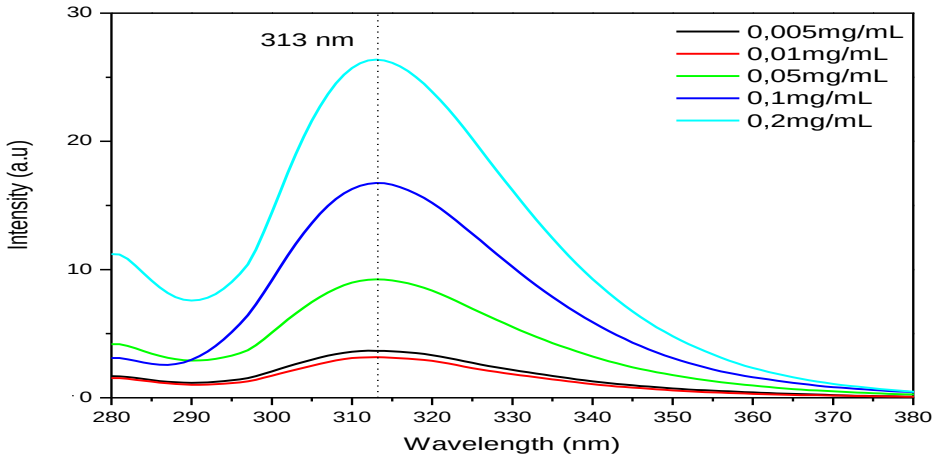

Figure 3: Fluorescence spectra of liposome formulations and GLA solution of 0.05 mg L^{-1}.

After the 24-hour period of drying in air, the liposome samples GLAM+DPPG, POPG+GLAM and GLAM+DPPG/POPG were analyzed with AFM. The AFM technique was widely used to evaluate nano carrier because can reveal the morphology and providing the diameter of these nano systems. The DPPG liposomes showed oval and irregular vesicles (Figure 4A and 4B) with a diameter of 350 ± 50 nm (Figure 4C).

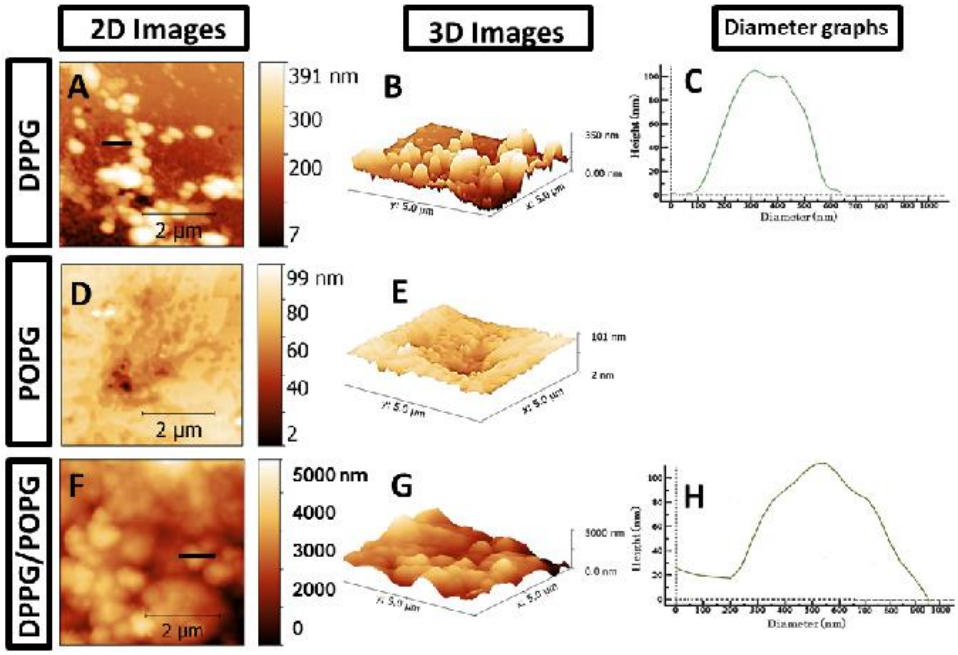

Figure 4: AFM images of liposomes produced with GLAM+DPPG (A, B and C), GLAM+POPG (D and E) and GLAM+DPPG/POPG (F, G and H).

The POPG liposomes had film formation on the surface of mica (Figure 4D and 4E) due to the fusion of vesicles because the transition phospholipid temperature (-2°C) (Avanti-Polar-Lipids, 2014) is lower than that of the sample temperature (25°C). Because of the formation of film, it was not possible to obtain the diameter of these vesicles by AFM. On the other hand, the images obtained from GLAM+POPG/DPPG liposomes showed the formation of large liposomes with an intense aggregation of the vesicles (Figure 4F and 4G) and diameter 500 ± 80 nm (Figure 4H). The larger diameter may be due to aggregation of the vesicles, which was observed in AFM images and subsequently confirmed by the DLS test.

The DLS test provides important information on vesicle diameter, polydispersity and zeta potential of systems. The diameter of vesicles may be influenced by interactions between the encapsulated substance and the phospholipid, and by the technique used for the production of the vesicles. Size and number of bilayers influence the release profile of drug from liposomes. For example, size influences half-life time, whereas number of bilayers may determine the quantity of drug to be incorporated (Akbarzadeh A, et al., 2013; Patil YP, et al., 2014).

The results obtained for the diameter of the vesicles showed that all liposomal formulations were larger than 100 nm, being therefore large vesicles (Table 1). As the preparation technique (lipid film hydration) favors the formation of multiple layers (Akbarzadeh A, et al., 2013; Patil YP, et al., 2014), liposomes obtained were probably from the type "MLV" (multilamellar vesicles). The diameter values presented by the liposome formulations GLAM+DPPG and GLAM+DPPG/POPG in the DLS test were similar to values found in the analysis with AFM (Figure 4C, 4H and Table 1).

With regard to the polydispersity, values inferior or equal to 0.3 represent ideal measurement conditions, which correspond to a homogeneous suspension of particles with a low agglomeration rate and with only one size distribution (Malvern-Instrument Easier, 2007; Dos Santos EP, et al., 2013; Kuelkamp IC, et al., 2009). The high values presented in this assay (Table 1) indicate agglomeration or heterogeneity in vesicle size.

The zeta potential is used to detect the magnitude of repulsive interactions among colloidal particles and to determine colloid stability (Casals E, et al., 2003). It can be defined as the charge existing between a single particle and its associated ions in a surface. Negative zeta potential values found in this assay (Table 1) demonstrate negatively charged phospholipids that were used in the production of liposomes (Florence AT, et al., 2003).

Table 1: DLS measurements of liposomal formulations (n=3).

	Diameter (nm)	**Polydispersity P/d**	**Zeta Potential PZ (mV)**
GLAM+DPPG	403.0 ± 27.8	0.6 ± 0.1	-32.5 ± 0.9
GLAM+POPG	242.5 ± 34.4	0.5 ± 0.3	-19.2 ± 0.5
GLAM+DPPG/POPG	738.9 ± 136.3	0.6 ± 0.1	-24.0 ± 8.3

The encapsulation efficiency was the mechanical separation of the free drug (5-9 kDa) from drug encapsulated by centrifugation (Ankley GT, et al., 2009) with the support of a membrane with 100 kDa pores.

From the quantification of the drug by fluorescence spectra, from collected supernatants, the encapsulation efficiency of each formulation was determined (Figure 5). The highest efficiency (85.2%) was obtained by formulating GLAM+DPPG/POPG. Then, 81.7% of the liposomes produced from the mixture GLAM+DPPG. A lower encapsulation efficiency was obtained (73.2%) from GLAM+POPG liposomes.

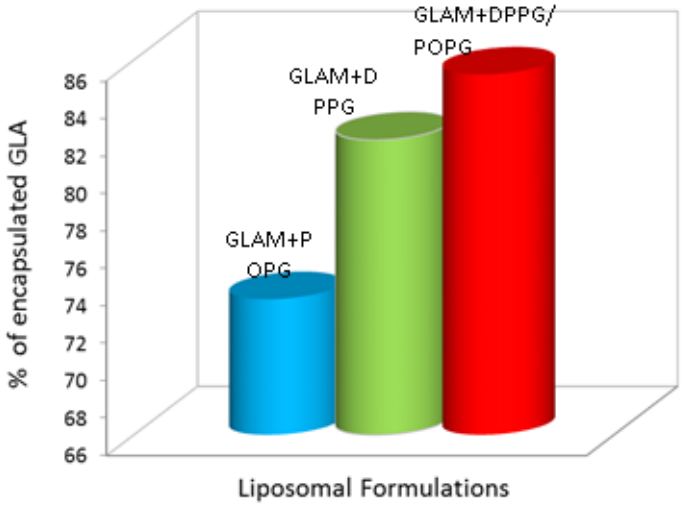

Figure 5: Encapsulation efficiency of GLA in GLAM+DPPG, GLAM+DPPG/POPG and GLAM+POPG liposomes.

Encapsulation efficiency values presented may be explained by two reasons. The first is related to the diameter of vesicles. Liposomes produced with GLAM+DPPG/POPG presented a larger diameter (738.9 nm) if compared to other formulations (GLAM+POPG=242.4 nm and DPPG+GLAM=403.0 nm). It is therefore capable of storing larger amounts of drug. Another plausible explanation for this result is based on the transition temperature of the phospholipid (42°C for DPPG and -2°C for POPG). Since the assay was performed at 10°C, DPPG was still in the solid-crystalline form, whereas POPG had reached the transition temperature, initiating the release of the drug during the spin cycle. Therefore, the high transition temperature of DPPG (42°C) provided these liposomes with a greater temperature stability in which the tests were performed.

CONCLUSION

The results presented in our study are promising, since the liposome produced from DPPG has oval and well-designed vesicles, a suitable diameter and high encapsulation efficiency, leading to believe that further studies on the production of liposomes could be conducted. It is noteworthy that the only study published to date on the production nano carriers containing glatiramer acetate was in 2013 by Afzal et al. Thus, the use of liposomes for encapsulation of this drug is still in an exploratory stage and intense research on the development and characterization of liposomes containing glatiramer acetate are needed to improve conditions and patient compliance in the treatment of MS.

ACKNOWLEDGEMENTS

To TEVA laboratory for providing the drug, to the pharmaceutical company Quemy union, where lyophilization was performed, and to the Research Support Foundation (FAPESP process n° 2013/22141-5 and n° 2014/17519-1).

REFERENCES

Achiron A (2009). Gene Expression Profiles Following One-Year Treatment with Glatiramer Acetate 20mg and 40mg (GA, Copaxone (R)) in Relapsing-Remitting Multiple Sclerosis Patients. Neurology 72: A38-A39.

Afzal E, Zakeri S, Keyhanvar P, Bagheri M, Mahjoubi P, (2013). Nanolipodendrosome-loaded glatiramer acetate and myogenic differentiation I as augmentation therapeutic strategy approaches in muscular dystrophy. International Journal of Nanomedicine 8: 2943-2960.

Aharoni R (2013). The mechanism of action of glatiramer acetate in multiple sclerosis and beyond. Autoimmun Rev 12: 543-553.

Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, (2013). Liposome: classification, preparation, and applications. Nanoscale Res Lett 8: 102.

Anderson G, AL E (2010). Tolerability and safety of novel half milliliter formulation of glatiramer acetate for subcutaneous injection: an open-label, multicenter, randomized comparative study. Journal of Neurology 257: 1917-1923.

Ankley GT, Bencic DC, Breen MS, Collette TW, Conolly RB, (2009). Endocrine disrupting chemicals in fish: Developing exposure indicators and predictive models of effects based on mechanism of action. Aquatic Toxicology 92: 168-178.

Avanti-Polar-Lipids (2014). Phospholipids [Internet]. Available from: http://avantilipids.com.

Avnir Y, Turjeman K, Tulchinsky D, Sigal A, Kizelsztein P, et al. (2016) Fabrication Principles and Their Contribution to the Superior In Vivo Therapeutic Efficacy of Nano-Liposomes Remote Loaded with Glucocorticoids. Plos One: 13.

Azevedo JCRD, Nozaki J (2008). Fluorescence analysis of huic substances extracted from water, soil and sediment of the Patos Lagoon - MS. Química Nova 31.

Bayas A (2013). Improving adherence to injectable disease-modifying drugs in multiple sclerosis. Expert Opin Drug Deliv 10: 285-287.

Beer K, Müller M, Hew-Winzeler AM, Bont A, Maire P, (2011). The prevalence of injection-site reactions with disease-modifying therapies and their effect on adherence in patients with multiple sclerosis: an observational study. Bmc Neurology 10: 11.

Ben-Nun A, Kaushansky N, Kawakami N, Krishnamoorthy G, Berer K, (2014). From classic to spontaneous and humanized models of multiple sclerosis: impact on understanding pathogenesis and drug development. J Autoimmun 54: 33-50.

Brasil (2010). Ministério da Saúde Farmacopeia Brasileira(5th edn), Agência Nacional de Vigilância Sanitária. Brasília 523.

Casals E, Galán AM, Escolar G, Gallardo M, Estelrich J (2003). Physical stability of liposomes bearing hemostatic activity. Chem Phys Lipids 125: 139-146.

Cienfuegos F, Vaisman D (2000). Análise Instrumental. Rio de Janeiro-RJ: 606.

Comi G, Cohen JA, Arnold DL, Wynn D, Filippi M; FORTE Study Group (2011). Phase III dose-comparison study of glatiramer acetate for multiple sclerosis. Ann Neurol 69: 75-82.

Costa CCR, Fonteles JL, Praça LR, Andrade ÂCO (2005). Adoecimento do portador de esclerose múltipla: percepções e vivências a partir da narrativa de dois casos clínicos. Brazilian Journal in Health Promotion 18: 117.

Croxford JL, Yamamura T (2009). Back to the future for multiple sclerosis therapy: focus on current and emerging disease-modifying therapeutic strategies. Immunotherapy 1: 403-423.

Cueva EA (2005). Efeitos de um antipsicótico e um antidepressivo tricíclico sobre a bomba de sódio e potássio, Na+, Ka+ -ATPpease: estudo através da fluorescência. [dissertação]. [Rio de Janeiro]. Pontifica Universidade Católica: 110.

Devalapally H, Chakilam A, Amiji MM (2007). Role of nanotechnology in pharmaceutical product development. J Pharm Sci 96: 2547-2565.

Devonshire V, Lapierre Y, Macdonell R, Ramo-Tello C, Patti F. (2011). The Global Adherence Project (GAP): A multicenter observational studies on adherence to disease-modifying therapies in patients with relapsing-remitting multiple sclerosis. European Journal of Neurology 18: 69-77.

Dos Santos EP, Barboza JCS (2013). Evaluation of Anionic Hydrolyzed Starch as Stabilizers in Polymeric Nanocapsules for Topical Formulations. Polimeros-Ciencia E Tecnologia 23: 624-629.

Du AW, Stenzel MH (2014). Drug carriers for the delivery of therapeutic peptides. Biomacromolecules 15: 1097-1114.

Duchi S, Ovadia H, Touitou E (2013). Nasal administration of drugs as a new non-invasive strategy for efficient treatment of multiple sclerosis. J Neuroimmunol 258: 32-40.

Farrapo Xavier AC, De Moraes ML, Ferreira M (2013). Immobilization of a loin encapsulated into liposomes in Layer-by-layer films for transdermal drug delivery. Materials Science & Engineering C-Materials for Biological Applications 33: 1193-1196.

Florence AT, Attwood D (2003). Physicochemical Principles of Pharmacy (3rdedn), São Paulo: Edusp: 528.

Gaillard PJ, Appeldoom CCM, Rip J, Dorland R, Van der Pol SMA, (2012). Enhanced brain delivery of liposomal methylprednisolone improved therapeutic efficacy in a model of neuroinflammation. Journal of Controlled Release 164: 364-369.

Graça JS, Ferreira M (2015). Encapsulação de Biomoléculas MS Lipossomos: Aplicações MS Biossensores Enzimáticos e Imunossensores. Revista Virtual de Química. Brasil 7: 1552-1564.

Gregoriadis G (1995). Engineering liposomes for drug delivery: progress and problems. Trends Biotechnol 13: 527-537.

Gross Y, Cabiri O (2013). Inventors; Medimop Medical Projects Ltd., assignee. Activation mechanism e. g. needle activation mechanism, for Copaxone drug delivery device, wearable by patient, has cannula holder coupled to cannula opposite free end and movable along longitudinal axis between retracted positions. United States Patent.

Haltmeier S, Yildiz M, Müller S, Anliker MD, Heinzerling L (2011). Contact dermatitis induced by glatiramer acetate. Mult Scler 17: 1390-1392.

Jongen PJ, Al E (2011). Drug adherence and multidisciplinary care in patients with multiple sclerosis: Protocol of a prospective, web-based, patient-centered, nationwide, Dutch cohort study in glatiramer acetate treated patients. BMC Neurology 11: 40-49.

Kanwar JR, Sun X, Punj V, Sriramoju B, Mohan RR, (2012). Nanoparticles in the treatment and diagnosis of neurological disorders: untamed dragon with fire power to heal. Nano medicine-Nanotechnology Biology and Medicine 8: 399-414.

Khan O, Rieckmann P, Boyko A, Selmaj K, Zivadinov R, (2012). A phase 3 trial to assess the efficacy and safety of glatiramer acetate injections 40mg administered 3 times a week compared to placebo. Multiple Sclerosis Journal 18: 512-513.

Kuelkamp IC, Paese K, Guterres SS, Pohlmann AR (2009). Stabilization of lipoic acid by encapsulation in polymeric nanocapsules designed for cutaneous administration. Quimica Nova 32: 2078-2084.

Lebrun C, Mondot L, Bertagna M, Calleja A, Cohen M (2011). Endermology: a treatment for injection-induced lipoatrophy in multiple sclerosis patients treated with sub cutaneous glatiramer acetate. Clin Neurol Neurosurg 113: 721-724.

Leite FL, Hausen M, Oliveira GS, Brum DG, Oliveira ON Jr (2015). Nanoneurobiophysics: new challenges for diagnosis and therapy of neurologic disorders. Nanomedicine (Lond) 10: 3417-3419.

Lima EM, Oliveira AG (2002). Tissue tolerance of diclofenac sodium encapsulated in liposomes after intramuscular administration. Drug Development and Industrial Pharmacy 28: 673-680.

Linker RA, Weller C, Lühder F, Mohr A, Schmidt J, (2008). Liposomal glucocorticosteroids in treatment of chronic autoimmune demyelination: Long-term protective effects and enhanced efficacy of methylprednisolone formulations. Experimental Neurology 211: 397-406.

Malvern-Instrument Easier (2007). Nano Zeta Sizer User Manual. Worcesterchire 270.

Maron R, Slavin AJ, Hoffmann E, Komagata Y, Weiner HL (2002). Oral tolerance to copolymer 1 in myelin basic protein (MBP) TCR transgenic mice: cross-reactivity with MBP-specific TCR and differential induction of anti-inflammatory cytokines. International Immunology 14: 131-138.

Mendes MF, Tilbery CP, Balsimelli S, Moreira MA, Barão-Cruz AM (2003). Depressão na esclerose múltipla forma remitente-recorrente. Arquivos de Neuropsiquiatria 61: 591-595.

Modi G, Pillay V, Choonara YE, Ndesendo VM, du Toit LC, (2009). Nanotechnological applications for the treatment of neurodegenerative disorders. Prog Neurobiol 88: 272-285.

Moreira MA, Lana-Peixoto MA, Callegaro D, Haussen SR, Da GamaPD. (2002). Consenso expandido do BCTRIMS para o tratamento da esclerose múltipla. As evidências para o uso de glicocorticóides e imunomoduladores. Arquivos de Neuropsiquiatria 60: 875-880.

Nance EA, Woodworth GF, Sailor KA, Shih T-Yu, Xu Q, (2012). A Dense Poly(Ethylene Glycol) Coating Improves Penetration of Large Polymeric Nanoparticles Within Brain Tissue. Science Translational Medicine 4: 149.

Noseworthy JH, Wolinsky JS, Lublin FD, Whitaker JN, Linde A, (2000). Linomide in relapsing and secondary progressive MS: part I: trial design and clinical results. North American Linomide Investigators. Neurology 54: 1726-1733.

Ohlweiler OA (1976). Química Analítica Quantitativa. Rio de Janeiro-RJ: Livros Técnicos e Científicos Editora 1039.

Patil YP, Jadhav S (2014) Novel methods for liposome preparation. Chem Phys Lipids 177: 8-18.

Patlak M (2010). Nanotechnology takes a new look at old drugs. J Natl Cancer Inst 102: 1753-1755.

Piehl F (2014). A changing treatment landscape for multiple sclerosis: challenges and opportunities. J Intern Med 275: 364-381.

Re F, Gregori M, Masserini M (2012). Nanotechnology for neurodegenerative disorders. Maturitas 73: 45-51.

Saguil A, Kane S, Farnell E (2014). Multiple sclerosis: a primary care perspective. Am Fam Physician 90: 644-652.

Schrempf W, Ziemssen T (2007). Glatiramer acetate: mechanisms of action in multiple sclerosis. Autoimmun Rev 6: 469-475.

Sharma A, Sharma US (1997). Liposomes in drug delivery: progress and limitations. International Journal of Pharmaceutics 154: 123-140.

Soares Almeida LM, Requena L, Kutzner H, Angulo J, De Sa J, (2006). Localized panniculitis secondary to subcutaneous glatiramer acetate injections for the treatment of multiple sclerosis: A clinicopathologic and immunohistochemical study. Journal of the American Academy of Dermatology 55: 968-974.

Soos N, Shakery K, Mrowietz U (2004). Localized panniculitis and subsequent lipoatrophy with subcutaneous glatiramer acetate (Copaxone) injection for the treatment of multiple sclerosis. American Journal of Clinical Dermatology 5: 357-359.

Sun LM, Zhang CL, Li P (2012). Characterization, Antibiofilm, and Mechanism of Action of Novel PEG-Stabilized Lipid Nanoparticles Loaded with Terpinen-4-ol. Journal of Agricultural and Food Chemistry 60: 6150-6156.

Teitelba D et al. (1971). Supression of experimental allergic encephalomyelitis by a synthetic polypeptide. Israel Journal of Medical Sciences 7: 630.

Teitelbaum D, Aharoni R, Klinger E, Kreitman R, Raymond E, (2004). Oral glatiramer acetate in experimental autoimmune encephalomyelitis - Clinical and immunological studies. Oral Tolerance: New Insights and Prospects for Clinical Application 1029: 239-249.

Teitelbaum D, Arnon R, Sela M (1999). Immunomodulation of experimental autoimmune encephalomyelitis by oral administration of copolymer 1. Proceedings of the National Academy of Sciences of the United States of America 96: 3842-3847.

Teli MK, Mutalik S, Rajanikant GK (2010). Nanotechnology and nanomedicine: going small means aiming big. Curr Pharm Des 16: 1882-1892.

Teva (2013). Teva Announces FDA Acceptance of sNDA for a Higher Concentration Dose of COPAXONE®. Given Three Times a Week [Internet].

Torchilin VP (2006). Multifunctional nano carriers. Adv Drug Deliv Rev 58: 1532-1555.
Vemuri S, Rhodes CT (1995). Preparation and characterization of liposomes as therapeutic delivery systems: a review. Pharm Acta Helv 70: 95-111.
Wang B (2013). Inventors, Hybio Pharm Co Ltd., assignee. Glatiramer acetate microspheres comprise glatiramer acetate, emulsifier, polymer carrier and protective agent, in preset weight ratio. China Patent.
Xiang TX, Anderson BD (2006). Liposomal drug transport: A molecular perspective from molecular dynamics simulations in lipid bilayers. Advanced Drug Delivery Reviews 58: 1357-1378.

ISSN : 0976-4550

INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Email : ijabpt@gmail.com

Website: www.ijabpt.com