Liposomes in polymer matrix. Stability of liposomes in PEG 400 and PEG 8000 solutions.

Magdalena Bajgrowicz[1,2], Jerzy Detyna[2], Marek Langner[1]

[1]Institute of Biomedical Engineering and Instrumentation, Wroclaw University of Technology, Poland
(magdalena.bajgrowicz, marek.langner)@pwr.wroc.pl
[2]Institute of Material Science, Wroclaw University of Technology, Poland
jerzy.detyna@pwr.wroc.pl

Abstract. Integration of liposomes in polymer matrix is a new concept of controlled drug delivery system. Those kind of materials allow to control dosage of applied drug and time of its release. Certain types of liposomes are unstable, so they require structural support. The main aim of the paper is to investigate how polymers influence aggregation of liposomes. Two different polymers were used for this purpose: polyethylene glycol 400 (PEG 400) and polyethylene glycol 8000 (PEG 8000). Considered liposomes were formed from phospholipon 90G. Viscosity of each sample was measured using Ubbelohde viscometer. 100 nm liposomes were prepared using thin film hydratation method followed by extrusion and subsequently mixed with polymer solutions. Molar concentration of liposomes in each sample was equal to 1,3 mM. The polydispersities of samples were measured using DLS method (Malvern ZetaSizer Nano), at temperature 25℃. It has been shown that above certain polymers concentrations liposomes aggregate due the crowding effect. Those concentrations for PEG 600 and PEG 8000 are equal to 6 % and 1.1%, respectively.

Keywords: liposomes, hydrogel, PEG.

1 Introduction

Recently, researchers have been working on new ways of drug delivery to treat microbial and fungal infections. It results from the fact that some of biologically active substances are unstable, insoluble in water and/or incapable of targeted delivery [1]. One of very promising solutions to those problems is drug delivery system based on liposomes [2,3,4]. Entrapping active ingredient inside of this nano-vesicles presents a number of advantages: increased stability and bioavailability, decreased toxicity, modulation of the release rate and a prolonged time of drug systemic retention [5]. Appropriate dosage of the antibiotic is very important in the healing process because when too small it may

not be sufficient, while too high it may increase the systemic adverse effects. Prolonged and insufficient dosage may result with the development drug resistance of bacteria in wound and decrease the effectiveness of the pharmacotherapy (bacteria in the wound are able to produce biofilm reducing effectiveness of antibiotics) [6,7]. That is why it is necessary to develop materials, which will ensure the desire time profile and quantity of the released active ingredient [8,9,10].

To achieve prolonged drug release, it is advantageous to incorporate the drug containing liposomes into a polymer matrix. Type and concentration of the polymer forming the matrix may influence the stability of liposomes, that is why the dependence between rheological properties of polymer and stability of liposomes, evaluated with the capacity to aggregate, must be measured. It has been shown previously that liposomes are compatible with derivatives of acrylic acid [11]. Carbopol 940 at concentrations ranging from 0.5% to 2% are commonly used [12]. Additionally, it has already been demonstrated that the performance of hydrophilic polymers such as Carbopol 940 is superior to other ones [5]. Since PEG (Polyethylene Glycol) is a flexible, hydrophilic polymer with low toxicity, it can be considered as proper substance to create polymer matrix in which liposomes can be incorporated and topically applied. In order to ensure that liposomes are suspended in the polymer matrix the capacity of the polymer to induce the liposome aggregation should be determine.

2 Materials and methods

2.1 Materials

Liposomes were prepared from phosphatidylcholine (Phospholipon 90G). To prepare hydrogels, two polymers were used: polyethylene glycol (PEG) 400 and PEG 8000. Both polymers were purchased from Sigma Aldrich Company.

2.2 Liposomes preparation and characterization

Liposomes with nominal lipid concentration of 2,6 mM were prepared using the thin film hydration followed by the extrusion methods, specifically phospholipon 90G was dissolved in chloroform to create concentrated 100 mg/ml lipid solution. The organic solvent was removed by evaporation under a

stream of nitrogen. As a result a thin lipid film was deposited on a wall of a flask. Next, distilled water was added (5 ml of water for each 100 l of lipid solution), lipid film swelled and formed vesicles (lipid film hydratation). Obtained vesicles were multilayered and heterogeneous in size, that is why they were subjected to the final size unification process. For this purpose, the technique of dimensional calibration was used. The liposomes were down-sized to 100 nm by extrusion through polycarbonate filter (fig. 1 a). Liposome stock solution was stored in a refrigerator. Polydispersity of samples was determined by Dynamic Light Scattering (DLS) method using Malvern ZetaSizer Nano ZS90 (fig. 1 b) at temperature 25℃. All measurements were repeated 3 times.

Fig. 1. a) Extruder used for the size calibration, b) ZetaSizer Nano ZS90.

a b

2.3 Polymer matrixes preparation and characterisation

13 samples with PEG 400 solutions and 7 samples with PEG 8000 solutions were prepared by mixing polymers with water in desire proportions. Concentrations of samples were as follow: for PEG 400: 0.1, 0.3, 0.6, 1.2, 1.8, 2, 3, 4, 6, 9, 15 % and for PEG 8000: 0.2, 0.6, 1.2, 1.8, 2, 3, 4 %.
Viscosities of those samples were measured with Ubbelohde K/60 viscometer (fig. 2). Viscosity was calculated from the following relation:

$$h = K \cdot t \quad (1)$$

where K = 0,003259 mm^2/s^2 - viscometer constant parameter and t is a time needed for a certain volume of the liquid to flow through the capillary.

Fig. 2. Ubbelohde K/60 viscometer

2.4 Preparation of liposome/polymer mixtures.

Suspensions were prepared by adding 0,5 ml of liposome solution (2,6 mM) to 0,5 ml polymer solutions at various concentrations and gently mixed. Liposomes polidyspersity of prepared samples was than measured with Nano Zeta Sizer (DLS method). Measurements have been carried out in a temperature of 25° C and repeated three times.

3 Results

3.1 Viscosities of PEG solutions

Viscosity of different polymer solutions was measured using Ubbelohde viscometer. Obtained values are presented in Table 1 and Table 2.

Table 1. Viscosity of solutions containing PEG 8000.

$C_{PEG8000}$ [%]	η [mPa·s]	Δη [mPa·s]
0,1	0,91	0,04

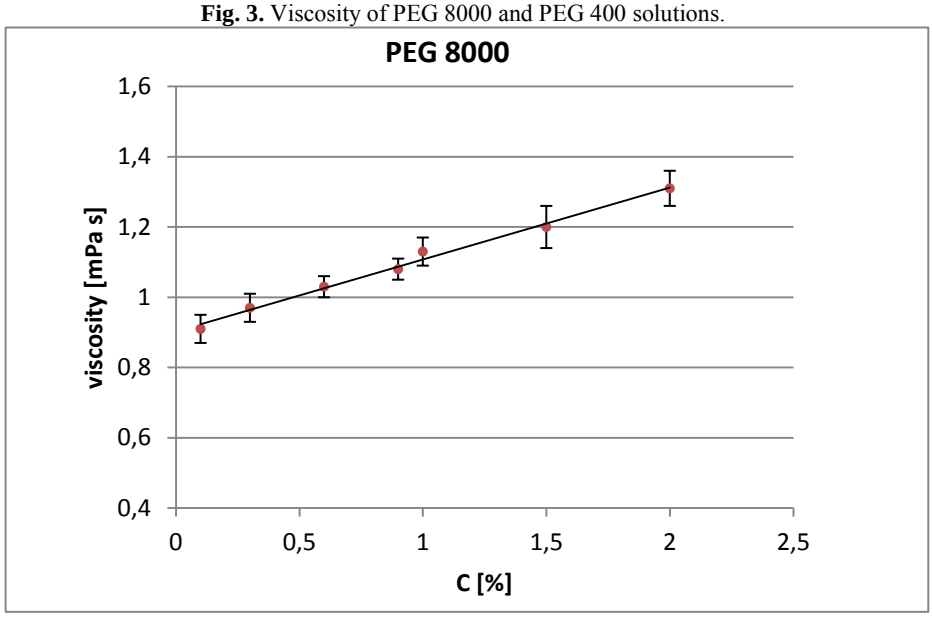
0,3	0,97	0,04
0,6	1,03	0,03
0,9	1,08	0,03
1,0	1,13	0,04
1,5	1,20	0,06
2,0	1,31	0,05

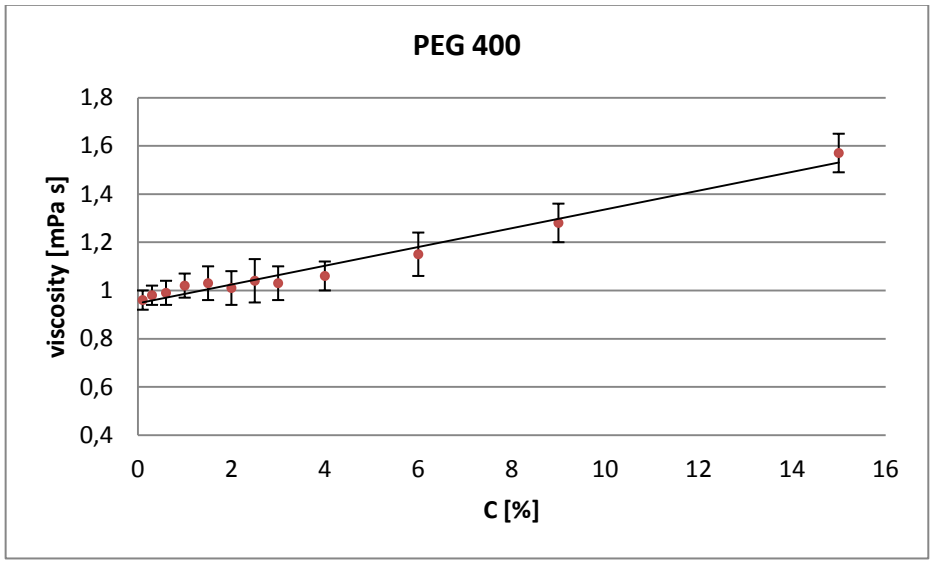
Table 2. Viscosity of solutions containing PEG 400.

C_{PEG400} [%]	η [mPa·s]	Δη [mPa·s]
0,1	0,98	0,04
0,3	0,98	0,04
0,6	0,99	0,05
0,9	1,00	0,05
1,0	1,02	0,05
1,5	1,03	0,07
2,0	1,01	0,07
2,5	1,04	0,09
3,0	1,03	0,07
4,0	1,06	0,06
6,0	1,15	0,09
9,0	1,28	0,08
15,0	1,57	0,08

For both polymer (PEG 8000 and PEG 400) linear correlation between concentration of polymer and viscosity was observed (fig. 3).

Fig. 3. Viscosity of PEG 8000 and PEG 400 solutions.





3.2 Polydispersity index determination.

Polydispersity index (PdI) is a parameter which define how homogeneous is a sample. High PdI inform about the fact that there are population of particles with different sizes in the sample. For homogeneous sample PdI should be lower than 0,1.

Fig. 4. The correlation between PdI of liposome suspension as a function of PEG 8000 concentration.

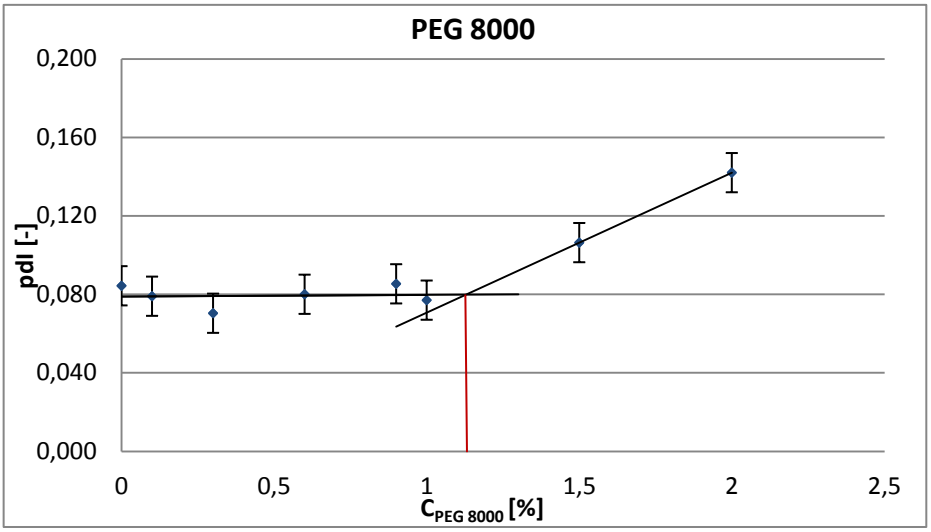
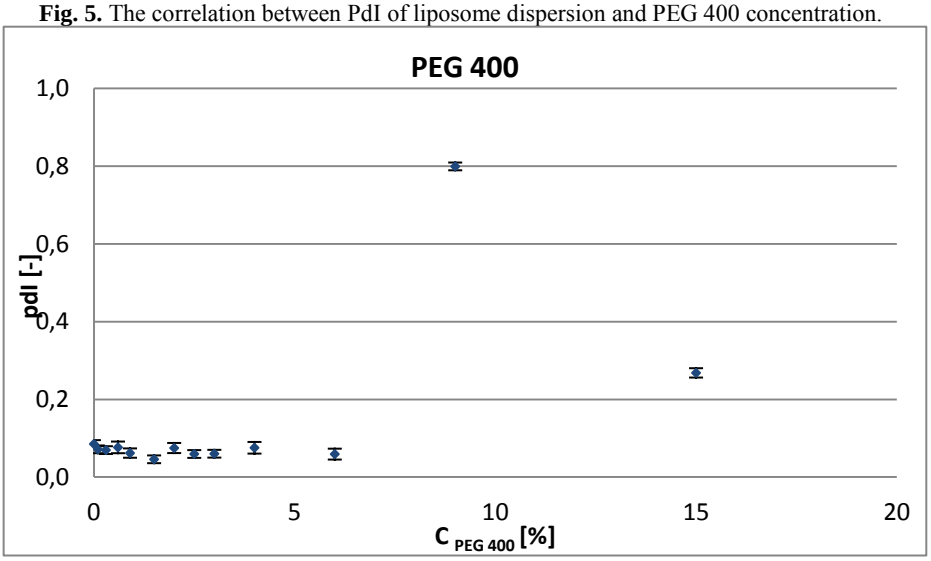


Fig. 5. The correlation between PdI of liposome dispersion and PEG 400 concentration.



For samples with low concentration of PEG ($C_{PEG\ 8000} \leq 1\%$, $C_{PEG\ 400} \leq 6\%$) polidyspersity is lower than 0.1. It means that the population of liposomes are uniform with the respect to their sizes. Increased concentration of PEG caused increase of polydispersity. This fact indicates that liposomes changed their sizes and sample is no longer stable. The increased liposome size is interpret as the appearance of aggregates. To determine concentration limit at which

the destabilization of liposomes occurs for PEG 8000 solutions the experimental data was approximated with two straight lines (Fig. 4). Equations determining those lines were calculated using the linear regression method. X coordinate of the point at which the two lines intersect corresponds to the value of the critical concentration of polymer. In the case of PEG 8000, critical concentration was determine to be $C_{PEG8000} = 1{,}11\%$. Critical concentration of PEG 400 is $C_{PEG\ 400} = 6\%$.

4 Discussion

Presented experimental data shows that proper selection of polymer and its concentration are important parameters for the formation of stable liposome formulations, which can be used as a drug delivery system. Both tested polymers (PEG 400 and PEG 8000) in certain concentration ranges serves as a suitable matrices in which liposomes can be incorporated without aggregation. Too high concentration of the polymer causes the destabilization of liposomes. In such case, the material ceases to perform its function as well defined drug carrier system. For PEG 8000 critical concentration is 1,1%, and for PEG 400 it is 6%. Below this values liposome are stable, their size and shape are unchanged.

Viscosities of PEG 8000 and PEG 400 at critical concentrations were very similar (table 3). This indicates, that stability of liposomes may depend on viscosity of solution.

Table 3. Viscosities of PEG solutions at critical concentrations.

	$C_{critical}$ [%]	Ƞ [mPa·s]
PEG 8000	1,11	$1{,}15 \pm 0{,}06$
PEG 400	6	$1{,}15 \pm 0{,}09$

5 Acknowledgements

Works presented in the paper was possible thanks to the support by Institute of Biomedical Engineering and Instrumentation at Wroclaw University of Technology.

6 References

1. Nosanchuk J. D.; "Current status and future of antifungal therapy for systemic mycoses"; Recent Patenton Anti-Infecive Drug Discovery; 1(1):75-84, (2006)
2. Liu Y., Liang D.; „Hydrogel integrated with liposome: A two-stage drug delivery system"; Journal of Controled Release, 2011
3. Suri A., Campos R., Rackus D.G.; „Liposome – doped hydrogel for implantable tissue"; Soft Matter, 2011
4. Hosny K.M.; „Optimalization of gatifloxacin liposomal hydrogel for enhanced transcorneal permeation"; Journal of Liposome Research, 2010
5. Ortan A.; "Rheological Study of a Liposomal Hydrogel Based on Carbopol"; Romania Biotechnology Letters, Vol 16, No. 1,2011
6. Hurler J., Berg O., Skar M.; „Improved Burns Delivery Systems for Mupirocin"; Wiley Online Library, 2012
7. Bialik-Wąs K., Pielichowski K.; „Polimerowe opatrunki hydrożelowe dla zastosowań biomedycznych"; Wydawnictwo Politechniki Krakowskiej, 2011
8. Tamburic S., Craig, D.Q.M.; "Rheological evaluation of polyacrylic acid hydrogels"; Pharm. Sci; 1 pp. 107–109, (1995).
9. Reza S., Quadir M.A., Haider S.S.; "Comparative evaluation of plastic, hydrophobic and hydrophilic polymers as matrices for controlled – release drug delivery"; Pharm Pharmaceut Sci, 2003
10. Raizada A., Bandari A., Kumar B.; „Polymers in drug delivery: a review"; International Journal of Pharma. Research & Development, 2010
11. Rosa S., Quadir M.A.,Haider S.S.; "Comparative Evaluation of Plastic, Hydrophobic and HydrophilicPolymers, As Matrices for Controlled-Release Drug Delivery"; J. Pharm. Pharmaceut. Sci.; 6(2): 274-91,(2003).
12. Horsemeyer R.W., Gurny R., Doelker E.M., Buri P., Peppas N.A.; "Mechanism of Solute Release from Porous Hydrophilic Systems"; Int. J. Pharm.; 15:25-35, (1983).