PROPRANOLOL LIPOSOMES: FORMULATION, CHARACTERIZATION, AND IN VITRO RELEASE

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The objective of our study was to formulate different types of liposomes containing Propranolol HCl (Pr), e.g. Multilamellar Vesicles (MLVs), Large Unilamellar Vesicles (LUVs), and Small Unilamillar Vesicles (SUVs) using various phospholipids. MLVs were prepared by freezing thawing method. The freeze- thawing method was chosen because of its simplicity and absence of organic solvents. LUVs were prepared by extrusion of the prepared MLVs through polycarbonate membrane. SUVs were prepared by sonication of the prepared MLVs using probe tip sonicator. The amount of Pr entrapped was determined using HPLC and the entrapment efficiency of the prepared MLVs was calculated. Among the liposome formulations, Phospholipone 90H liposomes showed the highest encapsulation percentage (90.11 %) compared to distearoyl Phosphatidylcholine (DSPC) (88.31%), mixture of distearoyl Phosphatidylcholine (DSPC) and dimyristoyl phosphatidyl choline (DMPC) in a ratio of 1:1 (71.33%) and dimyristoyl phosphatidyl choline (DMPC) (45.68%). The particle size was analyzed by Laser Diffraction Analyzer (LDA). It was found that the particle size was 6.590, 0613 and 0.453 nm for MLVs, LUVs and SUVs respectively. The in vitro release of Pr from the different liposomes was determined using dialysis method. It was found that the release of Pr from the prepared liposomes depends not only on the type of liposomes but also on the fatty chain length.

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Liposome science and technology is one of the fastest growing scientific fields. This is due to several advantageous characteristics of liposomes such as ability to incorporate not only water soluble but also lipid soluble agents, specific targeting to the required site in the body and versatility in terms of fluidity, size, charge and number of lamellae. Liposomes are spherical vesicles composed of one or more phospholipid bilayers (in most cases phosphatidylcholine). Lipophilic drugs can be incorporated within the lipid bilayers while hydrophilic drugs are solubilized in the inner aqueous core[1-2]. Liposomes have been extensively studied as a drug delivery system and can improve biological efficacy and reduce side effects of drugs [3].

Liposomes are biocompatible hollow nanoparticles covered by a lipid bilayer. They can be used as carrier for material entrapped inside the lumen and at the surface in cell-biological and medical applications [4]. Many anticancer drugs have shown improved therapeutic properties when administered in a liposome-encapsulated form [5].

Propranolol (Pr) is a non-selective beta blocker mainly used in the treatment of hypertension. It was the first successful beta blocker developed. Pr is rapidly and completely absorbed, with peak plasma levels achieved approximately 1–3 hours after ingestion and it has a

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variable bioavailability due to extensive first-pass metabolism. So to avoid these disadvantages, it is important to encapsulate Pr in liposomes.

The majority of liposome preparation techniques involve the application of volatile organic solvents (mainly chloroform, ether, or methanol), as a first step, to dissolve or solubilise the lipids. These solvents not only affect the chemical structure of the entrapped substance but will also remain in the final liposome formulation and contribute to toxicity and influence the stability of the vesicles [6-7]. In general, residual solvents in pharmaceuticals, known as organic volatile impurities (OVIs), have no therapeutic benefits but may be hazardous to human health as well as the environment [8]. In order to avoid these problems, a freeze-thawing method for fast production of liposomes was performed. The freeze-thawing method has the advantage that it is a simple method.

1. Materials and methods

1.1 Materials

Phospholipon 90H® (Ph 90H) was purchased from Phospholipid GmbH, Germany. Propranolol was purchased from Sigma-Aldrich Chemie GmbH, Germany**.** 1,2-Dimyristoyl-*sn*-Glycero-3-Phosphocholine (DMPC) and 1,2-Distearoyl-*sn*-Glycero-3-Phosphocholine (DSPC), were purchased from Avanti Polar Lipids, Germany. Sodium dihydrogen Phosphate monohydrate GR was purchased from E. Merck, Germany. Phosphate Buffer Saline (PBS-DULBECCO'S (1X), pH 7.4, was purchased from Biochrom, Germany. Triton® X-100 was purchased from Merck KGaA, Germany. Acetonitril HPLC grade was obtained from Fisher Scientific, UK. All the chemical reagents were of analytical grade.

1.2 Methods

1.2.1 Preparation of liposomes

1.2.1.1 Preparation of Multilamellar Liposomal Vesicles (MLVs)

MLVs were prepared by the freeze-thawing method [9]. First, multilamellar liposomes were produced by adding 100 mg lipids (Phospholipon 90H, Distearoylphosphatidylcholine (DSPC) and dimyristoylphosphatidylcholine (DMPC), or a mixture of DSPC and DMPC (1:1) to 2 ml of the drug solution (19 mg/mL) in phosphate buffered saline (PBS); the dispersion was rotated for 30 min (10 rpm) and then vortexed intermittently for 30 min (2 min vortexing, 3 min interval in a waterbath at 68 °C), followed by six freeze-thaw cycles: 3 min liquid nitrogen and 6 min waterbath at 68 °C. Liposomes were washed two times to remove unincorporated material by redispersing in 10 mL iso-osmolar phosphate buffer and centrifugating (Eppendorf centriguge 5804R, Germany) at 10,000 g for 30 min. The last pellet was then resuspended with equal volume of PBS for in vitro release**.**

1.2.1.2 Preparation of Large Unilamellar Vesicles (LUVs)

LUVs were prepared by extrusion of the prepared MLVs 11 times through 0.1 µm polycarbonate membrane (Nucleopore Pleasanton, CA) mounted in the mini-extruder fitted with 1 mL Hamilton syringes (Hamilton, Reno, NV). The extrusion was performed at 62 ºC. An odd number of passages were performed to avoid contamination of the sample by large vesicles which might not have passed through the filter [10].

1.2.1.3 Preparation of Small Unilamellar Vesicles (SUVs)

SUVs were prepared by sonication of the prepared MLVs using probe tip sonicator at 1 cycle and 20% amplitude for 15 min (UP 200S, dr. hielscher GmbH, Germany). After sonication, a transparent suspension of SUVs was obtained. The SUV solution was then placed in a 63 °C water bath for 90 min (annealing step). A 100,000g centrifuge spin for 15 min (Eppendorf centriguge 5804R, Germany) was then used to pellet larger lipid aggregates. The clear supernatant was withdrawn for use in experiments [11].

1.2.2 Characterization of the prepared liposomes

1.2.2.1 Separation of free Pr

The free Pr was removed by washing the prepared MLVs liposomes twice with buffer (PBS) and centrifuged (Eppendorf centriguge 5804R, Germany) at 11 000 rpm for 30 min.

1.2.2.2 Determination of % Entrapment Efficiency (%EE)

A volume of 50 µL of the prepared liposomes before washing was taken in a standard flask, and 2 ml of 10% w/v Triton X-100 was added, placed in a 63 °C water bath for 5 min for complete release of the drug from the liposomes, then vortexed. The volume of the solution was made up to 10 ml, and Pr content was measured by HPLC at 294 nm. The same above procedures were done with liposomal dispersion after washing and removing the free Pr (as mentioned in separation of free Pr). The % of entrapment efficiency of Pr was determined as in the following equation

$$\% EE = (AD_a/AD_b) \times 100$$

Where; AD_a is the amount of drug in liposome after washing and AD_b is the amount of drug in liposome before washing

1.2.2.3 Determination of the liposomal particle Size

The mean diameter and particle size distribution of the prepared liposomes were determined using a laser diffraction particle size analyzer. Laser diffraction was performed using a Beckman-Coulter LS 13 320 (Beckman-Coulter, U.S.A.) configured with the universal liquid module. Run conditions were as follows: sample refractive index, 1.33; circulation speed, 50%; measurement time, 90 seconds; and carrier fluid, water. The sample is put in the receptacle of the sampler unit for dispersion and delivery to the flow cell. Volume distributed was plotted using a computer program supplied by the manufacturer.

1.2.3 In-Vitro release of Pr from the prepared liposomes

In vitro release of Pr from MLVs, LUVs and SUVs was carried out using dialysis bag. In this method the dialysis bags (SERVAPOR® dialysis tubing, MWCO 12.000-14.000, pore diameter ca. 25 A, SERVA Electrophoresis GmbH, Germany) were soaked before use in PBS pH 7.4 at room temperature for 12 hours to remove the preservative. The dialysis bags were tied at one end with a thread. The dialysis bags were then filled with 0.5 mL of the liposomal dispersion. After that the bags were tied at the other end and were tested for leakage. The final length of the bag after tying was 3.5 ± 0.2 cm. The dialysis bags were placed immediately into the release medium containing 25 mL of phosphate buffer (PBS) kept at 37 ± 0.2 °C and shaked at 200 rpm. Timing was started as soon as the bags were immersed into the release medium. At 0.5, 1, 2, 4, 6, 12, 24, 48, 72, 168, 336, 504, and 672 hr, 1 mL was sampled from the release medium and the volume of the samples was replaced by the fresh buffer and shaking was continued. The samples were analyzed for Pr by HPLC. All release experiments were performed in triplicate.

2. Results and discussion

2.1 Determination of the % Entrapping Efficiency (%EE) of MLVs

Fig. 1 shows the effect of lipid composition on EE of Pr in MLVs. The % EE of Pr was calculated using the previous mentioned equation. The highest Entrapment Efficiency was found with Ph 90H liposomes with a value of 90.11 while the lowest Entrapment Efficiency was found in DMPC liposomes that had a value of 45.68. This may due to the difference in acyl chain length and in the phase transition temperature (Tc) where Ph has longer acyl chain and higher Tc (54 °C) than DMPC which has the short acyl chain and the low Tc (23 °C). These findings are in agreement with Anderson and Omri, who stated that the increase in acyl chain length is directly proportional to % encapsulation efficiency of inulin [12]. Also A. Manosroi, et. al found that vesicles obtained from the long alkyl chain (C18) give higher % entrapment efficiency than those obtained from the shorter alkyl chain (C12) [13].

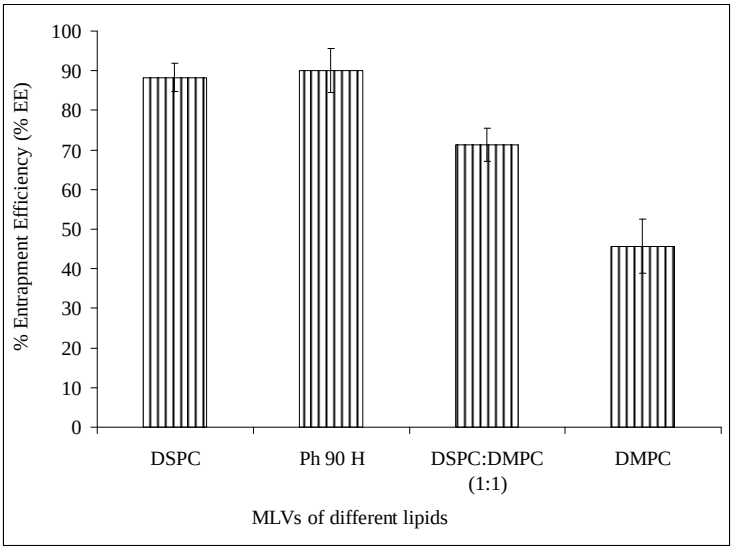

Fig. (1): % of Entrapment Efficiency (% EE) of Pr in the prepared MLVs liposomes composed of different lipids

2.2 Determination of the liposomal particle Size

Fig. 2(A-C) shows the size distribution obtained from laser light scattering. The range of diameters of particles of MLVs, LUVs and SUVs prepared using Ph 90H as a lipid were 6.590, 0.613, 0.453 µm respectively. It was found that the particle size of liposomes increases as the lipid chain length increases. These results are in accordance with that found by Gortzi, et al. [14].

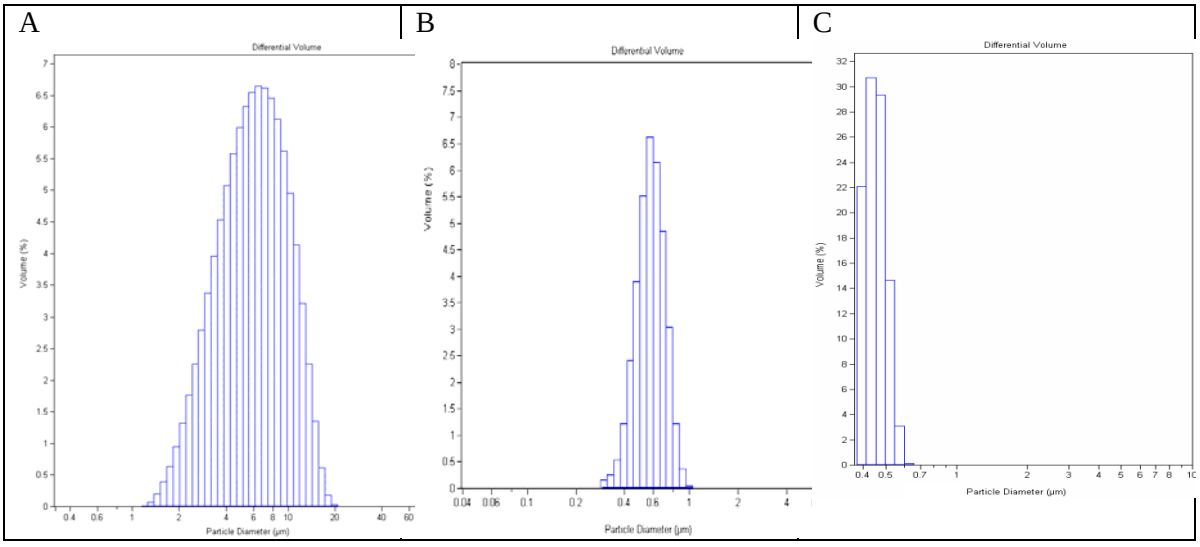

Fig. 2: Particle size distribution of the prepared liposomes A: MLVs, B: LUVs, C: SUVs

2.3 In vitro drug release from liposome dispersions

2.3.1 In vitro release of Pr from MLVs

Fig. 3 exhibits release profiles of free Pr and Pr MLVS formulations with different lipids in phosphate buffer (PBS, pH 7.4). The results were illustrated by plotting the percentage cumulative amount released of Pr versus time (hr). From the release profiles, it was found that the free drug diffuses completely (100 %) through the membrane into the release medium in about one hour. Regarding the formulations containing DSPC, Ph 90H, and DMPC, they exhibited an initial

burst drug release during the first four hours (22.08, 15.18 and 15.00 % respectively) followed by a plateau for over 336 hours. It means that either the hydration process of the liposomes in the semi permeable bag or the release of Pr from liposomal dispersion after the hydration slows down the overall release. Similar results were previously reported by Byung-Nak, et al., 1995 [15]. The interactions between Pr and the lipid may slow down the drug release or hamper its complete dissolution. This was in consistent with Nathalie, et al., 2004 [16]. Although DMPC has short acyl chain and the low Tc (23 °C), the release of prop was slow and constant up to 504 hours and this might due to sorption of Pr in DMPC MLVs. These results are in accordance with that found by Jan, et al., 2007 [17], who reported that the amounts of prop were sorbed in DMPC vesicles at pH 7 at 37 °C, where the vesicles are in a liquid-crystalline state and at 4 °C, i.e., in the gel state, the amounts sorbed were slightly less than half of those found in the liquid-crystalline. But in case of DSPC/DMPC, there was high increase of the drug up to 12 hours and after that the drug release was slow till reaching 100 % after 504 hours. This might due to interaction between DSPC and DMPC leaving Pr free into phase boundaries between solid and fluid domains. Regarding Ph 90H MLVs, the sorption of Pr vesicles are stronger than in DMPC vesicles.

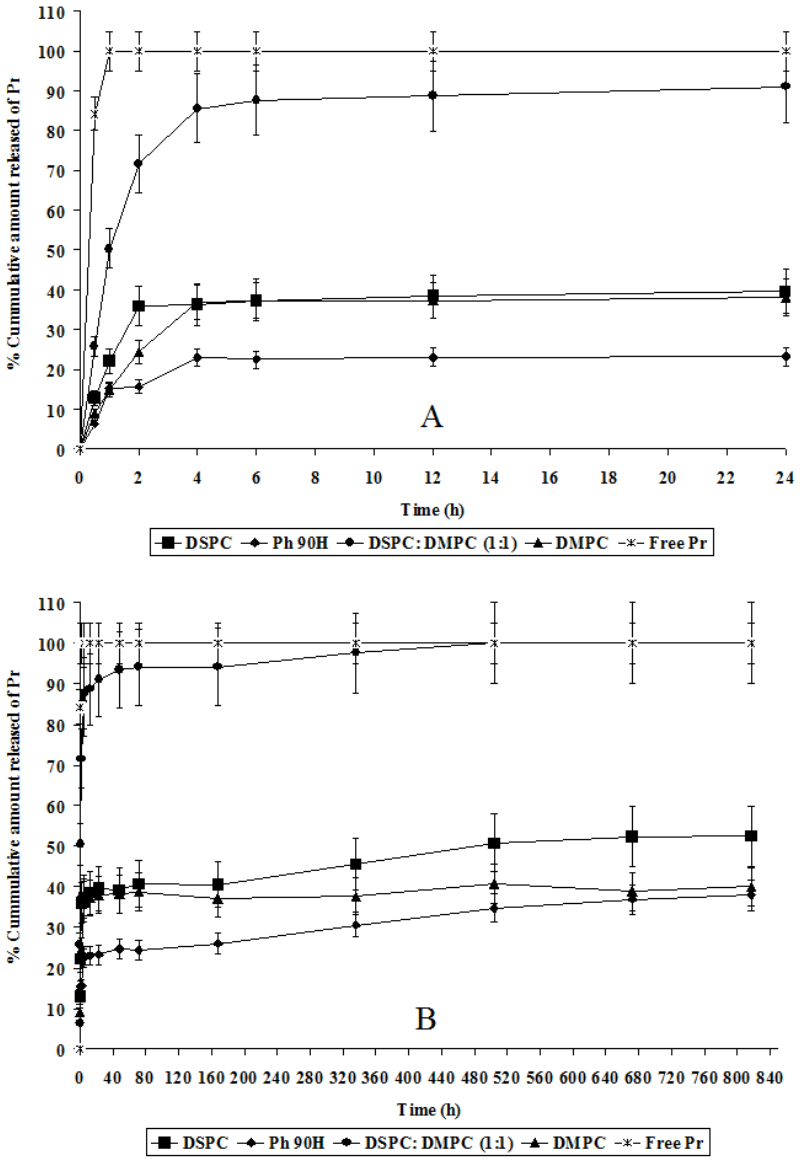

Fig.3: In vitro release of Pr from MLVs in phosphate buffer pH 7.4 using dialysis bag. Fig. A shows release up to 24 hours while Fig. B shows release up to 840 hours

2.3.2 In vitro release of Pr from LUVs

The release profiles of prop from LUVs formulations with different lipids, in pH 7.4 phosphate buffer are shown in fig. 4. The results were illustrated by plotting the percentage cumulative amount released of Pr versus time (h). From fig. 4, it was found that the greatest cumulative percentage of drug release value was exhibited by DSPC and Ph 90 H (100%), followed by DSPC: DMPC (1:1) (80.09%) and DMPC (71.31%) after 6 hours. This might due to sorption of Pr in DMPC vesicles at pH 7 at 37 °C. The same results were found by Saveyn et al. [18].

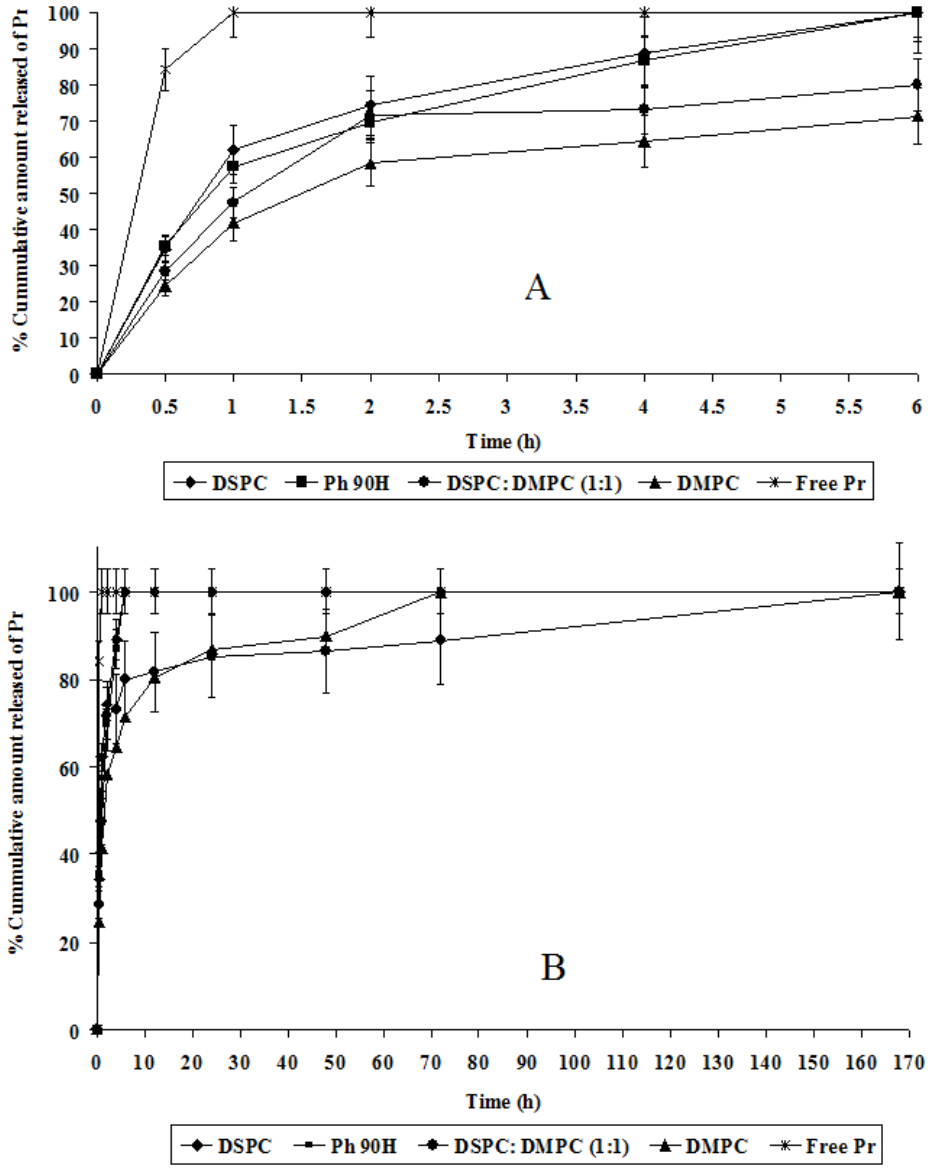

Fig.4: In vitro release of Pr from LUVs in phosphate buffer pH 7.4 using dialysis bag. Fig. A shows release up to 6 hours while Fig. B shows release up to 170 hours

2.3.3 In vitro release of Pr from SUVs

Fig. 5 exhibited the release of Pr from different SUVs in pH 7.4 phosphate buffer. It was found that the greatest % cumulative percentage of Pr released value was exhibited by Ph 90H (100%), followed by DSPC (96.145%), DSPC: DMPC (1:1) (93.02%) and DMPC (59.48%) after 4 hours.

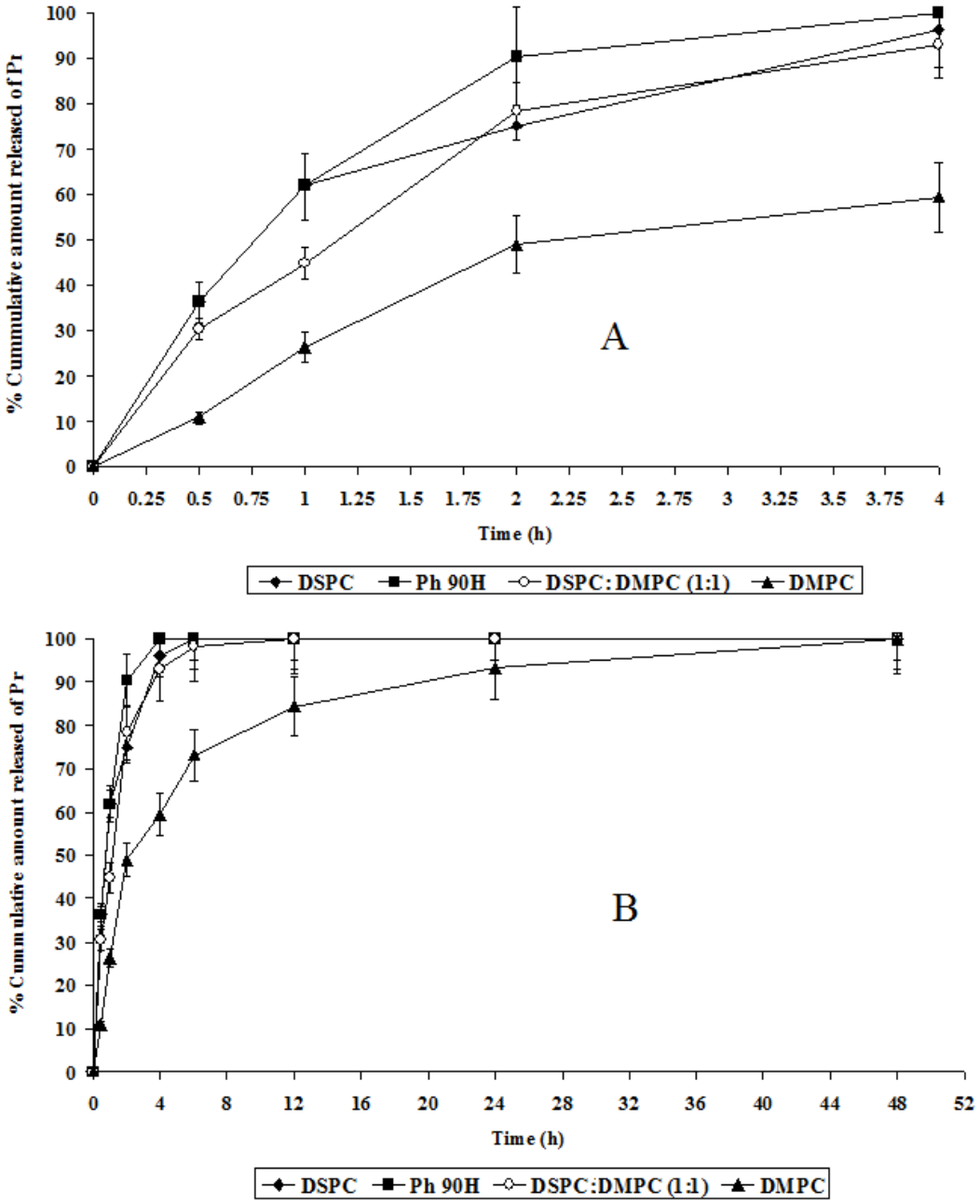

Fig.5: In vitro release of Pr from SUVs in phosphate buffer pH 7.4 using dialysis bag. Fig. A shows release up to 4 hours while Fig. B shows release up to 52 hours

2.3.4 Comparison of in-vitro release of Pr from liposomes of different lipids and vesicles

Fig. 6 shows the pattern of Pr release from liposomes of different lipid types and different vesicles. The release of Pr was improved from MLVs to LUVs to SUVs. This behaviour could be a consequence of a decreasing surface area to volume ratio from SUVs to LUVs to MLVs. Moreover, SUVs possess only a single bilayer, which could have been altered more easily than those of multilamellar vesicles because of the back diffusion of buffer in the donor compartment. These are in agreement with that found by [18].

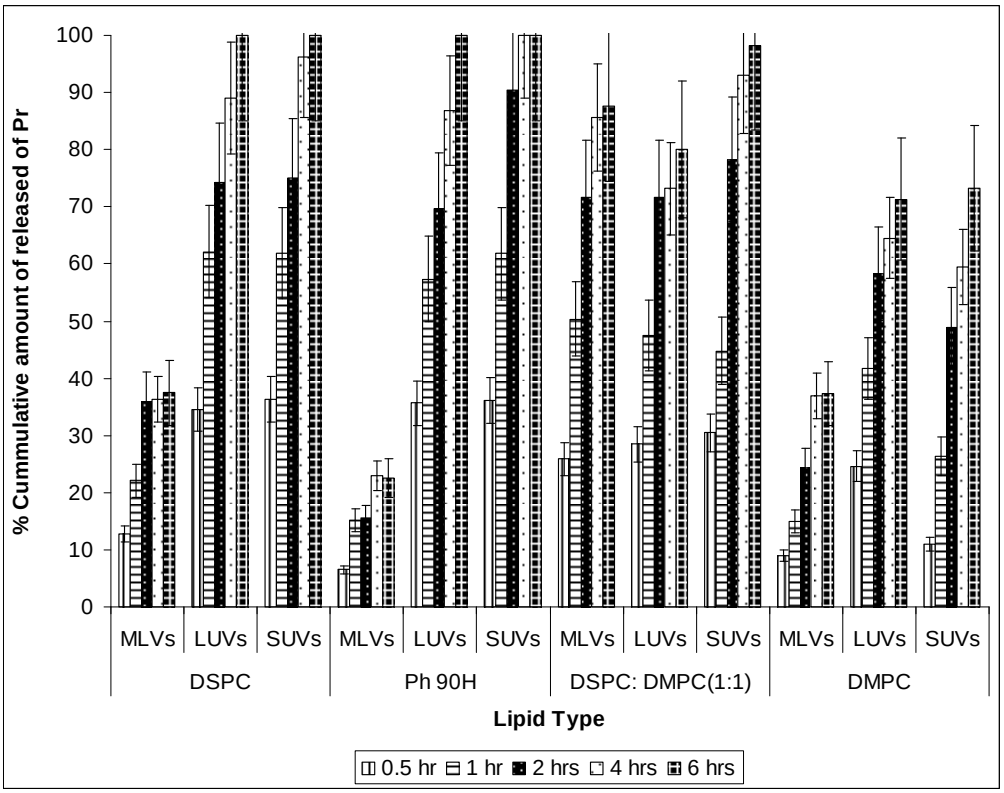

Fig. 6: Release pattern of Pr from liposomes of different lipids and vesicles.

3. Conclusion

In conclusion we successfully obtained liposomes, containing high propranolol concentrations inside the lipid bilayer, by using the freeze thawing method. More interestingly, this method has two additional advantages of avoiding the use of chloroform. We successively prepared different types of liposomes e.g. MLVs, LUVs and SUVs with different lipids e.g. Ph, DSPC, DMPC and 1:1 mixture of DSPC and DMPC. The in-vitro release of Pr from MLVs was carried out by dialysis method. It was found that Pr release was improved from MLVs to LUVs to SUVs.

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References

[1] S. Martins, B. Sarmento, D.C Ferreira. Int J Nanomedicine. **2**(4), 595 (2007).
[2] A.Sezer , J.Akbuga, A. Bas. 2007. Drug Deliv. **14**, 47 (2007).
[3] Norbert Maurer, David B Fenske & Pieter R Cullis. Expert Opin. Biol. Ther. **1**(6), 1 (2001).
[4] M. Rawat, D. Singh, S. Saraf, and S. Saraf. The Pharmaceutical Society of Japan. **128**(2), 269 (2008).
[5] N. Maurer, D. B Fenske, P. R. Cullis. Expert Opin. Biol. Ther. **1(6),** 923 (2001).
[6] S. Vemuri, and C.T. Rhodes. Pharm. Acta Helv. **70**, 95(1995).
[7] R Cortesi, E. Esposito, S. Gambarin, P. Telloli, E. Menegatti, and C. Nastruzzi. J. Microencapsul. **16**, 251 (1999).
[8] Dwivedi, A.M. Pharm. Technol. Europe **14**, 26 (2002).

[9] G. Shazly, T. Nawroth, and P. Langguth. Dissolution Technologies, **15(2)**, 7 (2008).
[10] Y. Hagiwara, H. Arima, Y. Miyamoto, F. Hirayama, and K. Uekama. Chem. Pharm. Bull. **54**(1), 26 (2006).
[11] W. Pitcher and Wray H. Huestis. Biochemical and Biophysical Research Communications. **296**, 1352 (2002).
[12] M. Anderson and A. Omri. Drug Delivery, **11**, 33 (2004).
[13] A. Manosroi, P, Wongtrakul, J. Manosroi, H. Sakai, F. Sugawara, M. Yuasa, M. Abe. Colloids and Surfaces B: Biointerfaces. **30,**129 (2003).
[14] O. Gortzi, S. G. Antimisiaris, P. Klepetsanis, E. Papadimitriou, P. V. Ioannou. European Journal of Pharmaceutical Sciences. **18**, 175 (2003).
[15] B. Ahn, S. Kim, C. Shim. Journal of Controlled Release, **34,** 203 (1995).
[16] N. Ubrich, P. Bouillot, C. Pellerin, M. Hoffman, P. Maincent. Journal of Controlled Release **97**, 291 (2004).
[17] J. Cocquyt, P. Saveyn, P. Meeren, and M. Cuyper. Langmuir, **23**, 1959 (2007).
P. Saveyn, J. Cocquyt, M. De Cuyper, P. Van der Meeren. Langmuir. **24**(12), 6007 (2008).
[18] M. Manconi, C. Sinico, D. Valenti, G. Loy, A.M. Fadda. Int. J. Pharmac. **234**, 237 (2002).