

Original Article

Fabrication and Release Kinetics of Liposomes Containing Leuprolide Acetate

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Abstract: The present work is focused on the preparation and *in vitro* release kinetics of liposomal formulation of Leuprolide Acetate. In this work, "Thin Lipid Film Hydration Method" was used for preparation of Leuprolide Acetate loaded liposomes. Prepared liposomal formulations of Leuprolide acetate was evaluated by drug entrapment study, *in-vitro* drug release kinetics and stability studies. The percentage drug entrapment of Leuprolide acetate for F1 and F2 formulations were found to be 78.14 ± 0.67 and $66.70 \pm 0.81\%$ respectively. *In-vitro* drug release study of liposomal formulations had shown zero order release pattern. Regression co-efficient (R^2) value of Zero order kinetics for F1 and F2 formulations were 0.9912 and 0.9676 respectively. After storing formulations for 1 month, stability testing was done at 4°C. It was found that all batches were stable. These liposomal formulations of Leuprolide acetate can be formulated for parenteral application to treat prostate cancer and in women, to treat symptoms of endometriosis (overgrowth of uterine lining outside of the uterus) or uterine fibroids.

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INTRODUCTION

Liposomes are nowadays very effective and useful delivery system for transmission of drug, enzymes, vaccine etc, in human or animal body (Senthilkumar *et al.*, 2021; Bhattacharjee *et al.*, 2019; Budai *et al.*, 2007). Researches are taking place to develop vesicular formulations of different drugs (Das *et al.*, 2017; Malakar *et al.*, 2012; Malakar *et al.*, 2014). Liposomes are comprised of at least one phospholipid layer which has an inner aqueous compartment (Bhattacharjee *et al.*, 2020). Liposome structure consists of polar head group (hydrophilic) and non-polar tail groups (lipophilic). The lipophilic group is long hydrocarbon chain (Singha Roy *et al.*, 2018). Liposomes are eliminated by simple degradation without creating any toxic effect and also provide significant advances as compared to other Nano Drug Delivery Systems (Moghimpour *et al.*, 2015; Rathod *et al.*, 2010). Both kinds of drugs like hydrophilic and lipophilic can be entrapped within liposomes for treating different ranges of diseases (Bozzuto *et al.*, 2015; Xing *et al.*, 2016; Lee *et al.*, 2017; Pal *et al.*, 2010). Drug entrapment takes place within aqueous or lipid bilayer (Amin *et al.*, 2018). Cancer is still a complex and difficult disease to treat (Das *et al.*, 2020; Panigrahi *et al.*, 2020). According to WHO, cancer is the second leading death causing disease globally (Quagliarini *et al.*, 2020). In recent years, prostate cancer has become most common type worldwide (Bray *et al.*, 2018; Pikala *et al.*, 2019). According to study in the year 2018, 1.3 Million cases of prostate cancer reports were detected from which 3, 59,000 deaths were observed (Das *et al.*, 2020). This study is focused on formulation and evaluation of liposomal formulation of Leuprolide Acetate for parenteral administration.

Leuprolide Acetate, a nonapeptide, is a strong agonist for receptor that releases gonadotropin hormone. It is applied in the treatment of prostate cancer as well as other diseases like Alzheimer's disease, polycystic ovary syndrome, etc (Wilson *et al.*, 2007). Leuprolide Acetate is mostly preferred for androgen deprivation therapy (ADT) and has been on market for more than 30 years (Hoda *et al.*, 2017). Suppression of testosterone levels in serum is worthwhile for most of the instances (Berges *et al.*, 2006). Leuprolide Acetate also used in women to treat symptoms of endometriosis (overgrowth of uterine lining outside of the uterus) or uterine fibroids.

In the current research work, formulation of liposomes of Leuprolide acetate was prepared by using soya lecithin (L- α -Phosphatidylcholine) and cholesterol. Lecithin can be prepared

from egg yolk and various oils seeds, such as flax, cotton seed, corn germ, sunflower seed, rapeseed and soybeans (Mourad *et al.*, 2010; Ghosh *et al.*, 2020). Hydration of Phospholipids in aqueous solutions is important to form the closed liposome structure (Amin *et al.*, 2018). Another excipient cholesterol increases rigidity of phospholipid bilayer by increasing the viscosity of lipid bilayer. It can also reduce the permeability of membrane and stabilizes the lipid bilayer by improving the rigidity of liposomes (Lee *et al.*, 2017; Pal *et al.*, 2010).

MATERIALS AND METHODS:

Materials:

Leuprolide Acetate was received as a gift from Sun Pharma, India. L- α -Phosphatidylcholine (soy-lecithin) and cholesterol, these two excipients were procured from Yarrow Chem, India. From Loba Chemie Pvt. Ltd. Chloroform was procured.

Preparation of Liposomes containing Leuprolide Acetate by "Thin Film Hydration Method:

Thin Lipid Film Hydration Method was employed for preparing liposomal formulation containing Leuprolide Acetate. For preparing formulation of F1 and F2, drug (Leuprolide Acetate) and lipid were used at 1:5 and 1:7 molar ratios respectively. Mixtures containing Leuprolide Acetate, specific molar ratio of Cholesterol-Lecithin were dissolved in 5 ml of chloroform with sonication (Sun *et al.*, 2018). The freshly prepared organic solution was transferred into a round bottom flask and rotated on water bath at approximately 40°C, until the mixture had been completely dried till the smell of chloroform disappeared. Depending on the lecithin concentration, drying time may vary. It took almost 40 minutes for completion of drying. Phosphate buffer of pH 6.8 was incorporated in the round bottom flask followed by vigorous shaking formed a yellowish-white suspension. Throughout the process temperature was maintained at 38°C. The suspension was sonicated by using a Sonicator for 1 hour. The suspension was cooled to room temperature and permitted to stand overnight (Das *et al.*, 2021). On the next day, suspension was taken out for centrifugation process to separate the liposome precipitates. Liposomes were dried under rotary vacuum dryer and examined.

Determination of drug content:

In a centrifuge tube 5 ml of liposomal suspension of Leuprolide Acetate was taken and centrifuged (Remi Motors, India) at 3500 RPM for 1 hour (Ghosh *et al.*, 2020). After completion of centrifugation process, the supernatant solution (A) was

separated and the pellets were dissolved in 3 ml of ethanol by sonicating for 15 minute. This process was required for dissolving excess lecithin. Phosphate buffer pH 5.8, 7 ml, was added to the centrifuge tube followed by centrifugation at 4000 RPM for 1 hour. The clear supernatant solution (B) was taken out and measured by UV-Spectrophotometer (ELICO SA165) at 283 nm by using phosphate buffer pH 5.8 as a blank solution. Dilution was done for solution A with phosphate buffer (pH 5.8) and absorbance was measured by UV-Vis Spectrophotometer (ELICO SA165) at 283 nm. (Das et al., 2020) The total amount of drug present in the liposomal suspension (mg/ml) is the sum of amount of the drug present in solution A and amount of drug entrapped in liposome structure.

Percentage of drug entrapment efficiency and Particle size

Percentage of drug entrapment was determined by using the ratio of entrapped drug amount (mg) in formulation and the total amount of drug (mg) used in the formulation, which may be expressed by the following formula.

$$\text{Entrapment efficiency (\%)} = \frac{\text{Amount of drug entrapped in liposome}}{\text{Total amount of drug initially taken}} \times 100\%$$

Determination of particle size

Particle sizes were measured by optical microscopy method. One drop of the prepared liposome was homogeneously spread onto a glass slide. The samples were observed under an optical microscope of 100X magnification fitted with an eye-piece micrometer.

In vitro drug release study:

Liposomal suspension of Leuprolide Acetate was kept for overnight in refrigerator at 4°C. The liposomal suspension (0.5 ml) was taken in a dialysis membrane (Himedia Laboratories Pvt. Ltd.) that separated the liposomal suspension from the receptor medium. The temperature during this procedure was maintained at 37±0.5°C. Drug release study was continued for 3 hours. Samples were withdrawn from the receptor chamber at every 15 minutes intervals followed by measuring the absorbance for each samples at 283 nm against receptor medium taken as blank.

In vitro Drug Release Kinetics:

Various kinetic models were applied to analyze *in vitro* drug release kinetics (Sunitha et al., 2014; Haghirsadat et al., 2018; Das et al., 2013; Hasnain et al., 2020). In case of zero order kinetics, $F = K_0t$, equation represents F is the fraction of drug release in time t, where K_0 is the zero order rate constant.

Formula for second order release kinetics, $\ln(1 - F) = -K_1t$, where F represents the fraction of drug released in time t and K_1 denoted as first order rate constant.

According to Higuchi model, the equation, $F = K_H t^{1/2}$, here F represents the fraction of drug released in time t and K_H denoted as the Higuchi dissolution constant.

In case of Hixon-Crowell model, equation is $Q^{1/3} = Kt + Q_0^{1/3}$. Where, Q is the drug release amount in time t and Q_0 is the starting value of Q and K represents rate constant.

Another equation represents Korsmeyer-Peppas model is $F = K_p t^n$. Here F denotes the fraction of drug released in time t, Korsmeyer-Peppas rate constant is K_p and n is the release exponent.

Stability Testing:

In Eppendorf's tube the liposomal formulation F1 of Leuprolide Acetate was taken and placed at 4°C in the refrigerator for 1 month. (Roy et al., 2019) After 1 month, the liposomal formulation was analyzed by drug entrapment study, *in-vitro* drug release and colour inspection.

Statistical Analysis:

All the data obtained were expressed as mean ± standard deviation (S.D). Each observation was done in triplicate (n= 3). MedCalc software version 11.6.1.0 was used for simple statistical analysis.

RESULTS AND DISCUSSION:

Drug Content

The drug contents of Leuprolide acetate liposomal formulations were determined, where the % of drug contents were within the range 75.55±1.10 to 81.41±0.85. This result might be attributed to the fact that the viscosity increased with increasing ratio of lecithin to cholesterol used in the preparation.

Drug Entrapment Study:

Drug entrapment for liposomal formulations of Leuprolide Acetate was determined. According to the study of drug entrapment, values for F1 and F2 were 39.07 ± 0.33 and 33.35 ± 0.41 mg/ml respectively. Drug entrapment was decreased with increasing the molar ratio of lecithin to cholesterol. But as a result of increasing molar ratio of lecithin to cholesterol, formulations were also become viscous.

Table 1: Results of Drug entrapment study for formulations of Leuprolide Acetate.

Sl. No.	Formulations	Drug Entrapment (mg/ml)	% Drug Entrapment Efficiency
1	F1	39.07±0.33	78.14±0.67
2	F2	33.35±0.41	66.70±0.81

Mean ± SD; n=3

Particle Size

The mean particle sizes (z-average) of liposomes in liposomal suspension of Leuprolide acetate were within the range 4.95±1.51 to 47.85±2.31µm. The smaller mean particle size (4.95±1.51µm) was noticed in case of formulation F1. With the increment of lecithin amount used in the preparation of liposomal formulations of Leuprolide acetate, the mean particle sizes of liposomes were found to be increased, which might be attributed to the fact of viscosity increment with increasing ratio of lecithin to cholesterol amount used in the preparation.

In-vitro drug release:

Drug release immediately started after coming in contact with the dissolution medium. The result of *in vitro* release study showed 3 hours of sustained release pattern. (Fig.1.) Completion of 3 hours study it was observed that F1 had released a maximum % release of 51.89±0.78% and F2 showed lowest % release at 180 minutes (41.56±1.5%).

In vitro drug release kinetics:

In vitro drug release study reports were plotted against various kinetics models (zero order, first order, Higuchi, Hixon Crowell and Korsmeyer-Peppas model equations) for comparison and to analyze the release kinetics (Guo et al., 2016; Hasnain et al., 2018). The results included in various kinetics models are presented in Table 3. Correlation coefficients of kinetic model equations were compared for liposomal formulation of Leuprolide Acetate. It was observed that all the two formulations were best fitted to zero order model (R^2 Value of F1 and F2 was 0.9912 and 0.9676 respectively) over 3 hours. The data were shown in the Table 2.

Table 2: Results of curve fitting of *in vitro* Leuprolide acetate releasing from the liposomal formulations.

Kinetic Model	Regression Co-efficient	
	F1	F2
Zero order model	0.9912	0.9676
First order model	0.9703	0.9451
Higuchi model	0.9416	0.8950
Hixon-Crowell model	0.9792	0.9536
Korsmeyer-peppas model	0.7741	0.7242

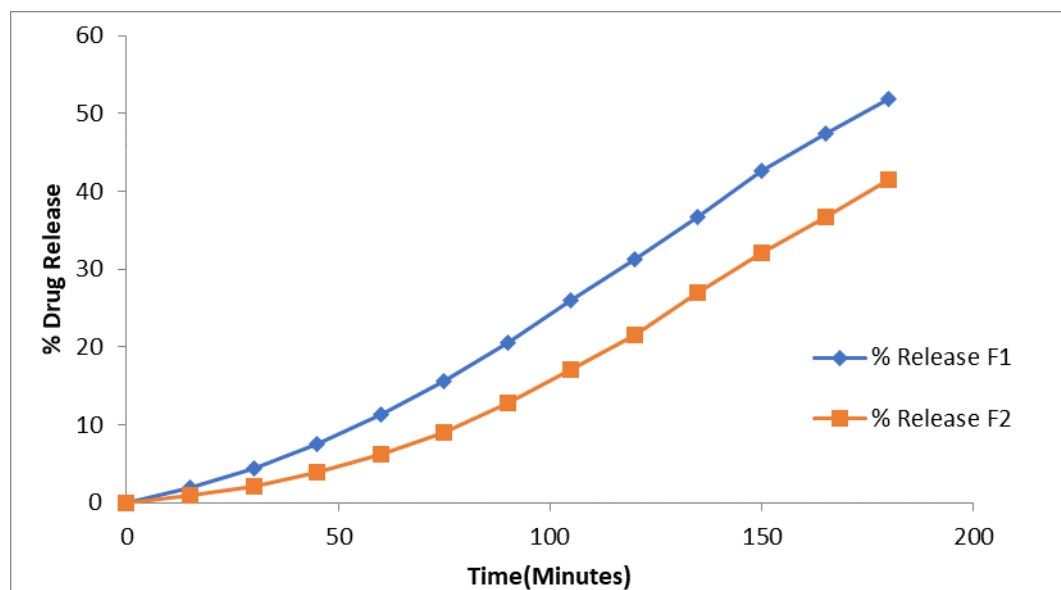


Fig. 1. *In vitro* drug release profile of F1 and F2 liposomal formulations of Leuprolide Acetate.

Table 3: Results of stability testing.

Time	Batch	Colour	Drug content	Percentage of drug entrapment Efficiency	Percentage of drug released in 3hrs.	Changes in physical properties
1 Month	F1	Yellowish white	38.74±0.33	77.48±0.53	50.85±1.13	No Changes

Mean ± SD; n=3

Stability:

Formulation F1 prepared with 1:5 of drug-lipid molar ratio was selected for stability study because of better drug entrapment and drug release pattern and stored for 1 month at 4°C in refrigerator then further analyzed for colour changes, drug entrapment study and *in vitro* drug release study. The colour of the suspension was same as that at the starting, yellowish white. There was no alteration observed for drug entrapment study. *In vitro* release study for 3 hours duration was performed and the percentage release at 180 minutes was 50.85 %. So, there was no significant change occurred during 1 month storage of liposomal formulation of Leuprolide Acetate. Therefore, results revealed that liposomal formulations of Leuprolide Acetate were physically stable. The data shown in the table 3.

CONCLUSION:

In this study, liposomal formulations of Leuprolide Acetate were prepared by thin lipid film hydration method by using lecithin (L- α -Phosphatidylcholine) and cholesterol. The percentage drug entrapment of Leuprolide Acetate for F1 and F2 were 78.14 ± 0.67 and 66.70 ± 0.81 % respectively. The highest percentages of drug release (at 180 minutes) achieved by F1 was 51.89 ± 0.78 %. It was detected that high lecithin: cholesterol ratio makes suspension more viscous so percentage drug entrapment and percentage drug release were decreased. During drug release study, sustained release patterns were observed till 3 hours. Liposomal formulations of Leuprolide Acetate best fitted to zero order release kinetics where the R^2 Value of F1 and F2 were 0.9912 and 0.9676 over 3 hours respectively. After one month stability study at 4°C of F1 batch was found stable enough. However, long term stability study is required for the future development of these liposomal formulations.

DECLARATION OF COMPETING INTEREST:

No conflict of interest declared by authors.

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REFERENCES

- Amin, S.G., Shah, D.A., Dave, R.H. (2018) Formulation and evaluation of liposomes of fenofibrate prepared by thin film hydration technique. *International Journal of Pharmaceutical Science Research*. 9, 3621-3637.
- Berges, R., & Bello, U. (2006) Effect of a new leuprorelin formulation on testosterone levels in patients with advanced prostate cancer. *Current Medical Research and Opinion*. 22(4), 649-655.
- Bhattacharjee, A., Das, P. J., Dey, S., Nayak, A. K., Roy, P. K., Chakrabarti, S., Marbaniang, D., Das, S. K., Ray, S., Chattopadhyay, P., Mazumder, B. (2020) Development and optimization of besifloxacin hydrochloride loaded liposomal gel prepared by thin film hydration method using 3² full factorial design. *Colloids and Surfaces A: Physicochemical Engineering Aspects*. 585, 124071.
- Bhattacharjee, A., Das, P.J., Adhikari, P., Marbanjang, D., Pal, P., Ray, S., Mazumder, B. (2019) Novel dry delivery systems for ocular therapy: with special reference to liposomal ocular deliver. *European Journal of Ophthalmology*. 29(1), 113-126.
- Bozzuto, G., Molinari, A. (2015) Liposomes as nanomedical devices. *International Journal of Nanomedicine*. 10, 975-999.
- Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L. A., Jemal, A (2018) Global cancer statistics, 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA: A Cancer Journal for Clinicians. 68, 394-424.
- Budai, L., Hajdu, M., Budai, M., Grof, P., Beni, S., Noszal, B., Klebovich, I., Antal, I. (2007) Gels and liposomes in optimized ocular drug delivery: studies on ciprofloxacin formulations. *International Journal of Pharmaceutics*. 343, 34-40.
- Das, B., Nayak, A. K., Nanda, U. (2013) Topical gels of lidocaine HCl using cashew gum and Carbopol 940: preparation and *in vitro* skin permeation. *International Journal of Biological Macromolecules*. 62, 514-517.
- Das, B., Sen, S.O., Maji, R., Nayak, A. K., Sen, K. K. (2017) Transfersosomal gel for transdermal delivery of risperidone: formulation optimization and *ex vivo* permeation. *Journal of Drug Delivery Science and Technology*. 38, 59-71.

- Das, S. S., Alkahtani, S., Bharadwaj, P., Ansari, M. T., ALKahtani, M.D.F., Pang, Z., Hasnain, M. S., Nayak, A. K., Aminabhavi, T. M. (2020) Molecular insights and novel approaches for targeting tumor metastasis. *International Journal of Pharmaceutics*. 585, 119556.
- Das, S., Samanta, A., Mondal, S., Roy, D., Nayak, A. K., (2021) Design and release kinetics of liposomes containing abiraterone acetate for treatment of prostate cancer. *Sensors International*. 2, 100077.
- Ghosh, G. (2020) Cancer detection of cancer: focus on antibody coated metal and magnetic nanoparticle-based biosensors. *Sensors International*. 1, 100050.
- Guo, Y., Shen, L., Lu, Y., Li, H., Min, K., Li, L. (2016) Preparation of rutin liposome drug delivery systems and evaluation on their in vitro antioxidant activity. *Chinese Herbal Medicines*. 8, 371-375.
- Haghiralsadat, F., Amoabediny, G., Helder, M. N., Naderinezhed, S., Sheikha, M. H., Forouzanfar, T., et al. (2018) A comprehensive mathematical model of drug release kinetics from nanoliposomes, derived from optimization studies of cationic PEGylated liposomal doxorubicin formulations for drug-gene delivery. *Artificial Cells, Nanomedicine, and Biotechnology*. 46, 169-177.
- Hasnain, M. S., Rishishwar, P., Ali, S., Alkahtani, S., Tabish, M., Milivojevic, M., Ansari, M. T., Nayak, A. K. (2020) Formulation and ex vivo skin permeation of lidocaine HCl topical gels using dillenia (*Dillenia indica* L.) fruit gum. *Revista Mexicana de Ingeniería Química*. 19, 1465-1476.
- Hasnain, M. S., Rishishwar, P., Rishishwar, S., Ali, S., Nayak, A. K. (2018) Extraction and characterization of cashew tree (*Anacardium occidentale*) gum; use in aceclofenac dental pastes. *International Journal of Biological Macromolecules*. 116, 1074-1081.
- Hoda, M. R., Kramer, M. W., Merseburger, A. S., Cronauer, M. V. (2017) Androgen deprivation therapy with Leuprolide acetate for treatment of advanced prostate cancer. *Expert Opinion on Pharmacotherapy*. 1, 105-113.
- Lee, Y., Thompson, D.H. (2017) Stimuli responsive liposomes for drug delivery. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*. 9(5).
- Malakar, J., Basu, A., Nayak, A.K. (2014) Candesartan cilexetil microemulsions for transdermal delivery: formulation, in-vitro skin permeation and stability assessment. *Current Drug Delivery*. 11, 313-321.
- Malakar, J., Sen, S.O., Nayak, A. K., Sen, K. K. (2012) Preparation, optimization and evaluation of trasferosomal gel for transdermal insulin delivery. *Saudi Pharmaceutical Journal*. 20, 355-363.
- Moghimpour, E., Salami, A., Monjezi, M. (2015) Formulation and evaluation of liposomes for transdermal delivery of celecoxib. *Jundishapur Journal of Natural Pharmaceutical Products*. 10, 1-6.
- Mourad, A.M., De Carvalho Pincinato, E., Mazzola, P. G., Sabha, M., Moriel, P. (2010) Influence of soya lecithin administration on hypercholesterolemia. *Cholesterol*. 1, 1-5.
- Pal, D., Nayak, A.K. (2010) Nanotechnology for targeted delivery in cancer therapeutics. *International Journal of Pharmaceutical Sciences Review and Research*. 1, 1-7.
- Panigrahi, B. K., Nayak, A. K. (2020) Carbon nanotubes :an emerging drug delivery carrier in cancer therapeutics. *Current Drug Delivery*. 17, 558-576.
- Pikala, M., Burzynska, M., Maniecka-Bryla, I. (2019) Epidemiology of mortality due to prostate cancer in poland, 2000-2015. *International Journal of Environmental Research and Public Health*. 16(16), 2881.
- Quagliarini, E., Di Santo, R., Pozzi, D., Caracciolo, G. (2020) Protein corona-enabled serological tests for early stage cancer detection. *Sensors International*. 1, 100025.
- Rathod, S., Deshpande, S. G. (2010) Design and evaluation of liposomal formulation of pilocarpin nitrate. *Indian Journal of Pharmaceutical Science*. 72, 155-160.
- Roy, D., Das, S., Samanta, A. (2019) Design and in-vitro release kinetics of liposomal formulation of acyclovir. *International Journal of Applied Pharmaceutics*. 11, 61-65.
- Senthilkumar, K. L., Ezhilmuthu, R. P. & Praveen, P. (2021) Preparation and characterization of nabumetone liposomes. *International Journal of Life Sciences Biotechnology and Pharma Research*. 1, 81-86.
- Singha Roy, A., Das, S., Samanta, A. (2018) Design, formulation and evaluation of liposome containing isoniazide. *International Journal of Applied Pharmaceutics*. 10, 52-56.
- Sun, V., Zhang, N., Li, A., Xu, W. Z. (2008) Preparation and evaluation of N3-O-toluyfl-uouracil-loaded liposomes. *International Journal of Pharmaceutics*. 353, 243-250.
- Sunitha, S., Thirupathi, A., Vijaya, K. (2014) Preparation and evaluation of liposome entrapped hydrogel complex system of itracozole for enhanced transdermal permeation. *Journal of Pharmaceutical and Scientific Innovation*. 3, 25-29.
- Wilson, A. C., Meethal, S. V., Bowen, R.L., Atwood, C. S. (2007) Leuprolide acetate: a drug of diverse clinical applications. *Expert Opinion on Investigational Drugs*. 16(11), 1851-1863.
- Xing, H., Hwang, K., Lu, Y. (2016) Recent developments of liposomes as nanocarriers for theranostic applications. *Theranostics*. 6, 1336-1352.