Bulletin of the Chemists and Technologists of Macedonia, Vol. **19**, No. 2, pp. 133–137 (2000)
ISSN 0350 – 0136
UDC: 577.115 : 615.281

GHTMDD – 359
Received: June 6, 2000
Accepted: November 7, 2000

Short communication

BIOPHARMACEUTICAL CHARACTERIZATION OF POVIDONE-IODINE LIPOSOMES

Katerina Goračinova, Ljiljana Klisarova, Liljana Makraduli, Emilija Fredro-Kumbaradži, Marija Glavaš, Kristina Mladenovska

Faculty of Pharmacy, The „Sv. Kiril & Metodij" University, Vodnjanska 17, MK-1000 Skopje, Republic of Macedonia

Different formulations of multilamellar liposomes were prepared from Soya lecithin and poly(1-vinyl-2-pyrrolidone)iodine complex by a mechanical method, vortexing the phospholipid dispersion in water. Biopharmaceutical evaluation and antimicrobial efficacy studies were performed in order to evaluate liposome's potential for use as a sustained release depo with efficient and prolonged antimicrobial action, compared to PVP-I solution. Varying the drug/phospholipid ratio during the preparation of the liposomes, different efficacy of PVP-I encapsulation was achieved. Lower concentrations of PVP-I in the lecithin dispersion for liposome preparation resulted with higher incorporation efficacy. Dissolution test results point that total drug release (%) from the series within 24 hours was 45.08 ± 1.53, 36.15 ± 1.65, 22.54 ± 1.96, 19.98 ± 1.05 for series A, B, C and D, respectively. The *in vitro* microbiological testing demonstrated good antimicrobial efficiency of PVP-I liposome dispersions against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans.* Also prolonged antimicrobial action, compared to PVP-I solution was noticed.

Key words: liposomes; biopharmaceutical characterization; PVP-I; antimicrobial efficacy; sustained release depo

INTRODUCTION

In recent years liposomes have been increasingly explored as novel drug delivery systems. For many years these delivery systems were investigated as parenteral drug carrier systems for anticancer, antibiotic, antifungal agents. Dermal liposome preparations have been exploited by cosmetic industry, with hundreds of products on the market. Liposome formulations for topical drug delivery were introduced in 1980 and since then have attracted considerable interest and generated speculative claims concerning their potential utility both as a drug carrier and reservoir for controlled release of drugs within various layers of the skin (1–3). In general these formulations could be more effective and less toxic than conventional drug formulations. Literature survey points to efficacy of the liposome formulations in treatment of a number of dermatological diseases and disorders such as psoriasis, mycoses, idiopathic hirsutism and cutaneous infections. Depending on size, composition and surface characteristics, liposomes interact specifically with biological structures, so, some aims like penetration enhancement, facilitation of drug delivery to skin and mucosal membranes, intracellular uptake by endocytosis could be achieved (4). The excellent tolerability, lack of immunogenicity of liposomes, and a possibility to create moist molecular film for the wound environment may provide additional benefit from the PVP-I liposome formulation. The literature reports point that liposomes are enriched at the wound bottom for direct action against infection and support of wound healing. Literature survey on *in vivo* experimental studies point to efficacy, tolerability and a good quality of wound healing (deep dermal wounds) with PVP-I liposome preparations (3, 5). Electron-microscopic studies indicate that PVP-I liposomes attach directly to bacterial cells, thus possible facilitating the attack against the microorganisms (6).

EXPERIMENTAL

Materials and methods

Different formulations of multilamellar liposomes (MLV) were prepared from Soya lecithin and poly(1-vinyl-2-pyrrolidone) iodine complex by a mechanical method, vortexing the phospholipid dispersion in water (4). The preparation was carried out with a constant volume of Soya lecithin dispersion in water containing different concentrations of PVP-I (A 7 %, B 3.5 %, C 1.75 % and D 1 %). The prepared samples were kept at 4 °C.

The size distribution of the prepared liposomes was evaluated using laser light scattering equipment (Fritsch particle sizer analysette 22) after dilution with distilled water (7).

Photomicrography of the liposome dispersions was conducted by polarizing microscope (Nikon E-800) using the technique of Nomarsky and phase contrast.

The efficacy of PVP-I encapsulation in the prepared liposomes was quantified spectrophotometrically by measuring the quantity of the available-iodine, extracted with chlorophorm from the supernatant ($\lambda = 510$ nm, UV/VIS spectrophotometer, Perkin Elmer, Lambda 16) after centrifugation of the prepared liposome dispersion (6000 rpm, 20 min).

The release rate studies were conducted by incubating of PVP- I liposomes at 32 °C in distilled water as dissolution medium, in several sealed test tubes placed in automatic shaker (Haake SWB 20). Release samples from the test tubes were taken at determined time intervals and centrifuged to obtain clear supernatant for measurement of the released PVP-I (8, 9).

For evaluation of the antimicrobial efficacy of the prepared formulations against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans,* microbiological *in vitro* suspension tests was conducted. The trypticase soy broth suspensions of microorganisms (the inoculum) were prepared with physiological saline solution according to McFarland's standard (10). Liposome dispersion (5 ml) and bacterial suspension (0.5 ml) were poured into the test tubes. After different time periods one each of these solutions were inoculated into Mueller-Hinton medium (for *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*) and Sabouraud agar (for *Candida albicans*), and incubated for 24 h at 37 °C. Physiological saline solution was used as a control. For quantitative determination of the number of grown bacteria equal volumes of test suspensions were streaked into the medium.

RESULTS AND DISCUSSION

Varying the drug/phospholipid ratio during the preparation of the liposomes, different efficacy of PVP-I encapsulation was achieved in the prepared formulations. All variables in the process of preparation of different formulations were kept constant (volume of the Soya lecithin dispersion, Soya lecithin content, mixing conditions, temperature) except the concentration of PVP-I in the medium. Lower concentrations of PVP-I in the medium resulted in higher incorporation efficacy. So at a concentration of 1 % of PVP-I in the lecithin dispersion for liposome preparation, the efficacy of incorporation was 98 %, and for concentrations 1.75 %, 3.5 % and 7 % the efficacy of incorporation was 90 %, 83 %, and 68 %, respectively.

The particle size distribution showed that the mean liposome diameter corresponded with the size of multilamelar vesicles. The size-frequency distribution curves and cumulative distribution plots for the evaluated series are presented in Fig. 1. The geometric mean diameters were, 3,3 μm ± 1.89, 3,28 μm ± 2.06, 2,64 μm ± 2.23, 2.59 μm ± 2.19 for series A, B, C and D, respectively.

The photomicrographs of the serie D presented in Fig. 2 show that during the preparation procedure liposomes were formed from hydrated phospholipids.

Fig. 3 presents the release profiles of PVP-I from liposomes. Series with higher percent of drug loading showed faster drug release rate (release rate constants are presented in Table 1). To examine the mechanism of release of PVP-I from liposomes, the percentage of release was plotted vs. the square root of time. The release profiles demonstrated linear behaviour with correlation coefficients 0.9734, 0.9640, 0.9590 and 0.9636 for series A, B, C and D, respectively.

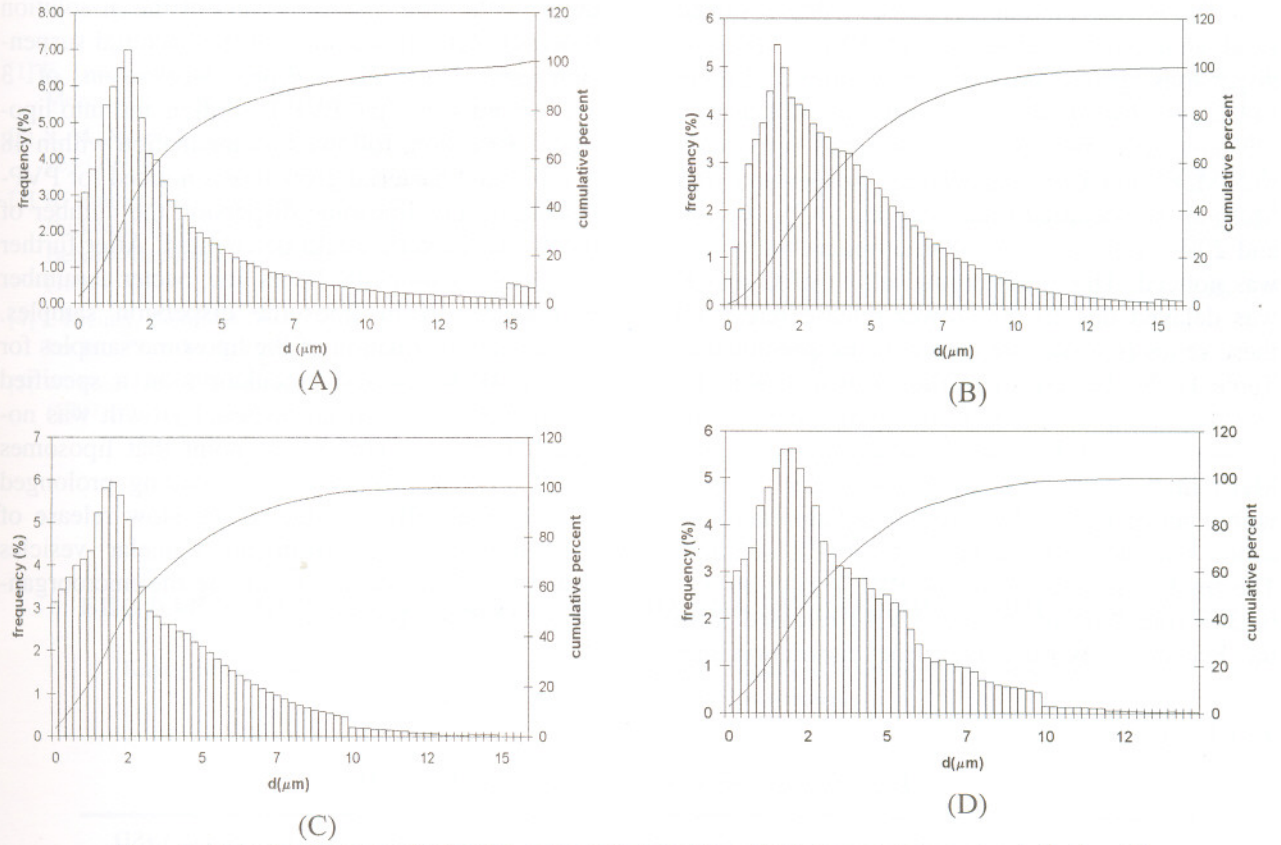

Fig. 1. Particle size-frequency distribution curves and cumulative distribution plots for series A, B, C and D

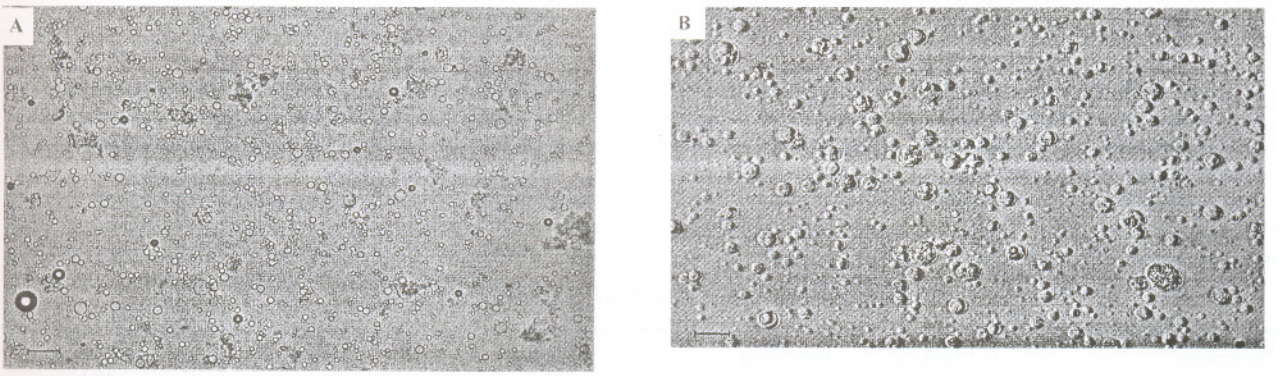

Fig. 2. Photomicrographs of serie D (A – phase contrast, bar = 16.67 μm; B – Nomarsky, bar = 10 μm)

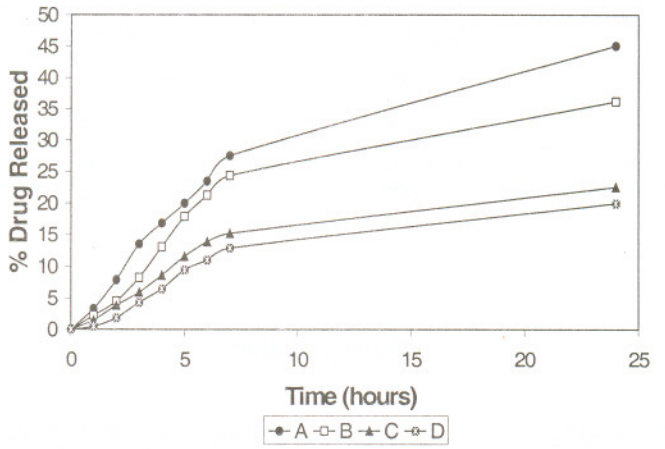

Fig. 3. Dissolution profiles for series A, B, C and D (each point represents the mean, $n = 5$)

The *in vitro* antimicrobial testing demonstrated good antimicrobial efficiency of PVP-I liposome dispersions against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans*. After inoculation of bacterial suspension (0.5 McF) and *Candida albicans* suspension (0.5 McF) at the specified time intervals (40'', 90'', 5' and 20') a rapid and efficient antimicrobial action was noticed. The antimicrobial effect of C and D was delayed due to the slower release rate from these series (release rate constants are presented in Table 1). No bacterial and fungal growth was detected after continuous inoculation of bacterial suspension (0.5 McF) and *Candida albicans* (0.5 McF) in the liposome dispersion every 60 min. within period of 4 h. Also, prolonged antimicrobial action was noticed compared to PVP-I solution. The final concentration of the PVP-I solution was 0.02 % (the concentration of PVP-I released from the liposomes of serie D in the dissolution medium, within the first two hours of the dissolution process). After inoculation of the bacterial suspension and *Candida albicans* suspension of 3 McFarland's into the PVP-I solution and into liposome dispersion, followed by incubation within 48 h, unlimited bacterial growth was noticed for PVP-I solution. For liposome dispersion the number of the grown bacteria could be counted. After further incubation within 48 hours the bacteria number was decreased in liposome dispersion samples. Subsequent incubation of the liposome samples for further 48 hours and inoculation on a specified medium showed that no bacterial growth was noticed (Table 2). This might point that liposomes could serve as PVP-I depo providing prolonged antimicrobial efficacy due to the slow release of the drug substance from multilamelar vesicles and/or specific interaction among the microorganisms and PVP-I liposomes.

Table 1

Release rate constants for series A, B, C and D

Sample	Lecithin/drug ratio	% encapsulated	k % ($h^{-1/2}$)	Total release at 24 h (%) ±SD
A	1:0.4	68.00	9.9582	45.08±1.53
B	1:0.2	83.48	8.3487	36.15±1.65
C	1:0.1	90.36	5.0566	22.54±1.96
D	1:0.05	98.02	4.7656	19.98±1.05

Table 2

Antimicrobial efficacy of PVP-I liposomes (serie D)

Inoculum	Blank	Inoculum 3 McF		Incubation 48 h		Incubation 48 h		Incubation 48 h	
		PVP-I	D	PVP-I	D	PVP-I	D	PVP-I	D
Pseudomonas aeruginosa	∞	∞	22 × 200	∞	9 × 200	∞	7 × 200	∞	/
Escherichia coli	∞	∞	29 × 200	∞	12 × 200	∞	9 × 200	∞	/
Staphylococcus aureus	∞	∞	61 × 200	∞	32 × 200	∞	12 × 200	∞	/
Candida albicans	∞	∞	56 × 200	∞	19 × 200	∞	17 × 200	∞	/

/ – no growth

CONCLUSION

The prepared PVP-I liposomes showed a potential for use as a sustained release depo. Also, efficient and prolonged antimicrobial action, compared to PVP-I solution was noticed.

REFERENCES

[1] E. Touitou, H. E. Junginger, N. D. Weiner, T. Nagai, M. Mezei, *J. Pharm. Sci.*, **83**, 1189 (1994).

[2] S. M. Moghimi and H. M. Patel, *J. Microencapsul.*, **10**, 155 (1993).

[3] K. Reimer, W. Fleischer, B. Brogmann, H. Schreier, P. Burkhard, A. Lanzendorfer, H. Gumbel, H. Hoekstra, W. Behrens-Baumann, *Dermatology,* 195 (suppl. 2), 93 (1997).

[4] J. Kreuter, *Colloidal Drug Delivery Systems*, Marcel Dekker Inc., New York, 1994.

[5] W. Fleischer, K. Reimer, *Dermatology,* **195** (suppl. 2), 3 (1997).

[6] H. Schreier, G. Erdos, K. Reimer, B. Konig, W. Konig, W. Fleischer, *Dermatology,* **195** (suppl. 2), 111 (1997).

[7] R. H. Müller, *Colloidal Carriers for Controlled Drug Delivery and Targeting*, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 1990.

[8] S. L. Law, T. F. Jang, P. Chang, C. H. Lin, *Int. J. Pharm.*, **103**, 81 (1994).

[9] I. Schumahecher and R. Margalit, *J.Pharm.Sci.*, **86**, 635 (1997).

[10] E. J. Blair, H. E. Lennete, P. J. Truant. *Manual of Clinical Microbiology*, American Society of Microbiology, 1970.

Р е з и м е

БИОФАРМАЦЕВТСКА КАРАКТЕРИЗАЦИЈА НА ЛИПОЗОМИ СО ПОВИДОН-ЈОД

Катерина Горачинова, Љиљана Клисарова, Лилјана Макрадули, Емилија Фредро-Кумбараџи, Марија Главаш, Кристина Младеновска

Фармацевтски факултет, Универзитет „Св. Кирил и Методиј“, Водњанска 17, МК-1000 Скопје, Република Македонија

Клучни зборови: липозоми; биофармацевтска карактеризација; PVP-I; антимикробна ефикасност; продолжено ослободување

Подготвени беа различни формулации на липозоми од соја-лецитин и поли(1-винил-2-пиролидон)јод со користење на механичкиот метод, односно вортексирање на фосфолипидната дисперзија во вода. За да се определи потенцијалната употреба на липозомите како депо за продолжено ослободување на активниот принцип, односно обезбедување на ефикасно и продолжено антимикробно дејство, беше извршена биофармацевтска карактеризација и испитувана антимикробната ефикасност во споредба со истата на раствор на PVP-I. Преку варирање на односот активен принцип/фосфолипид беше постигната различна ефикасност во инкорпорирање на активната супстанција, при што пониските концентрации на PVP-I во лецитинската дисперзија на липозомскиот препарат резултираа со повисока ефикасност на инкорпорирање. Дисолуционите тестови покажаа дека вкупното количество на ослободена лековита супстанција (%) во текот на 24 часа изнесува 45.08 ± 1.53, 36.15 ± 1.65, 22.54 ± 1.96, 19.98 ± 1.05 за сериите A, B, C и D, соодветно. Испитувањата *in vitro* на антимикробното дејство покажаа добра ефикасност спрема *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* и *Candida albicans.* Исто така беше забележано продолжено антимикробно дејство во споредба со дејството на растворот на PVP-I.