Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 4, 2012

Research Article

# DESIGN AND DEVELOPMENT OF LIPOSPHERES FOR CONTROLLED DELIVERY FOR ANTIMALARIAL DRUGS

**SATHEESH BABU NATARAJAN[a,b]*, PRABAKARAN L[c], GAYATHRI RAJARAM[d], ARUN GANESH KUMAR[a], SUNDARESWARA KUMAR C[e], ARTHI I[a]**

[a]Karpagam University, Coimbatore, India, [b]Faculty of Pharmacy, Lincoln University College, Malaysia, [c]R.R. College of Pharmacy, Bangalore, India, [d]Karpagam College of Pharmacy, Coimbatore, India, [e]Advent – Research Centre of Ajantha Pharma Ltd, India.
Email: satheeshbabumpharm@gmail.com

*Received: 16 Sep 2011, Revised and Accepted: 13 Oct 2011*

**ABSTRACT**

Lipospheres have gained the most attention to deliver the drugs in the body with improved loading, controlled release, specific tissue targeting, bioavailability enhancement, feasibility of large scale production, increased stability and cost effectiveness. The aim of this study was to prepare lumefantrine loaded lipospheres by melt dispersion technique using three various lipids (stearic acid, Cetosteryl alcohol, cetyl alcohol). The effect of various stabilizers and surfactants with different concentrations were also carried out for the selected batch of lipospheres. The formulated lipospheres have been characterized for the particle size, loading efficiency and *in vitro* release and the result was found that the lipospheres containing cetyl alcohol is significantly better results than those of other batches. Lipospheres under appropriate experimental conditions can entrap hydrophobic drugs can control the release of the encapsulated drugs and may also allow a reduction in dosage and a decrease in toxicity.

**Keywords***:* Lipospheres, Lumefantrine, Stearic acid, Cetosteryl alcohol, Cetyl alcohol, Loading efficiency.

## INTRODUCTION

In recent years, Lipid colloidal carriers systems have attracted growing interest concerning site specific drug delivery, especially in malarial parasites, *mycobacterium* tuberculosis, HIV and cancer chemotherapy[1-2]. The objective of drug targeting is to increase the drug concentration at the desired site of action with a simultaneous decrease at non target sites, the rationale being the enhancement of therapeutic efficacy with a simultaneous reduction of unwanted side effects. During the last decade, several approaches have been investigated to develop submicron-sized drug-delivery systems.

The lipospheres system represent a new type of particulate dispersion of solid spherical particles consisting of a hydrophobic core compound such as triglycerides or fatty acid derivates surrounded by a layer of lipids, which have their diameter between 0.1- 100 µm[3]. The lipospheres system has been used for the controlled delivery of various drugs including ibuprofen[4], flurbiprofen[5], piroxicam[6], nimodipine[7], bupivacaine[8], pilocarpine[9], carbamazipine[10], amphotericin B[11], and glipizide[12]. They have also been successfully used as carriers for vaccines and adjuvant[13]. Lipospheres have several advantages[3] such as enhanced physical stability due to avoidance of coalescence[14], high dispersability in an aqueous medium, low cost of ingredients, ease of preparation and scale up, high entrapment of hydrophobic drugs, Controlled particle size, Reduced mobility of incorporated drug molecules responsible for reduction of drug leakage, circumvention of instabilities due to interaction between drug molecules and emulsifier film and extended release of entrapped drug. Static interface facilitates surface modification of carrier particles after solidification of the lipid matrix.

The present work was aimed to develop a new delivery system for lumefantrine (lipophilic drug) by encapsulating the drug into a lipospheres carrier, which may reduce the dose, side effects and toxicity.

## MATERIALS AND METHODS

### Materials

Lumefantrine was obtained from Orchid chemicals and Pharmaceuticals (Chennai, India). Cetosteryl alcohol, Stearic acid, Poly vinyl alcohol, Cetyl alcohol and pure Pectin were purchased from Lobachemi Pvt. Ltd (Mumbai, India). Phospholipid PHOSPHOLIPON 85 G was obtained as a gift sample from Lipoid, (Germany). Cholesterol, Tween 40 and Tween 80 were purchased from SD Fine- Chemicals Pvt. Ltd (Mumbai, India). All other materials used in this study were of analytical reagent grade.

### Methods

### Preparation of lipospheres by melt dispersion technique

The lipid was melted at ~70°C in which lumefantrine (100mg) was dispersed and then emulsified into an external aqueous phase (~70°C) containing a suitable surfactant. The emulsion was mechanically stirred and heated to the same temperature as that of the melted lipid phase. The milky formulation was then rapidly cooled to about 4-8°C by immersing the formulation flask in a cool ice bath with continuous the agitation to yield a uniform dispersion of lipospheres. The obtained lipospheres were then washed with distilled water and isolated by filtration through a filter paper and dried.

Different batches of lipospheres (AF1- AF15) containing various lipids (stearic acid, Cetostearyl alcohol, cetyl alcohol) incorporated with lumefantrine (100mg) by melt dispersion technique were prepared and the results were shown in Table1.

### Effect of Surfactants

The effect of surfactants (cholesterol, phospholipid) on the optimized lipospheres containing 500mg of cetyl alcohol was studied using different batches and the results were shown in Table 2.

### Effect of Co-surfactants

The effect of co-surfactants (Tween 40, Tween 80) on the optimized lipospheres containing 500mg of cetyl alcohol was studied using different batches and the results were shown in Table 3.

### Percentage yield

The yield of lipospheres (% w/w) was calculated according to the formula[5] (Veerappan and Reddy et al., 2010).

$$\% \text{ yield} = W_{\text{Liposphere}} / [W_{\text{Lumefantrine}} + W_{\text{Excipients}}] \times 100$$

### Microscopic evaluation

The particle sizes of the formulated lipospheres of different batches were determined by optical microscopy. The projected diameters of 200 lipospheres from each batch were determined and their results were calculated. Morphology of the lipospheres was examined by scanning electron microscopy[5].

**Loading efficiency**

Lipospheres of 25-mg in each drug-lipid ratio were finely powdered in a mortar and transferred to a 25-ml volumetric flask and the volume was adjusted with saline phosphate buffer pH 7.4. The mixture was sonicated for 30 min and filtered and the samples were measured in UV Spectrophotometer at 235 nm. Corresponding concentrations were calculated from a previously constructed standard curve that was linear up to 10 µg/ ml. Finally, the loading efficiency of the lipospheres was determined as the ratio of the actual amount to the theoretical amount of lumefantrine in the lipospheres[7].

**X-Ray Diffraction studies [13-14]**

X-ray diffraction measurement was performed by X-ray diffraction (XRD, 2 Theta 10–80$^0$) on a with a copper anode (Cu K α radiation, 40 kV, 30 mA, $\lambda$ = 1.54060 Å), using X'celerator as a detector (PANalytical, X' per PRO). The sample was mounted into a specific device before the measurement by XRD. The data used were typically collected with a step width of 0.017$^0$ and a count time of 15.5 s. The graph was plotted in 2 theta angle Vs intensity count.

***In vitro* release studies**

The dissolution of Lumefantrine from the prepared lipospheres was monitored using USP XXV paddle II apparatus. The Amount of the lipospheres equivalent to 100 mg of Lumefantrine was dispersed into the dissolution medium. The dissolution media was 900 ml of saline phosphate buffer pH 7.4 maintained at 37 ±0.5° C and rotating at 50 ±1 rpm. Sodium lauryl sulphate (0.5%, w/w) was added to the dissolution medium to improve the wettability of the lipospheres. The 5-ml aliquots were withdrawn at pre-determined time intervals and the withdrawn samples were replaced with fresh dissolution medium. The samples were then analysed to calculate the drug release from each of the formulations[7].

**RESULTS AND DISCUSSIONS**

Lumefantrine loaded lipospheres were prepared by melt dispersion technique using different lipids such as stearic acid, Cetostearyl alcohol and cetyl alcohol for improved entrapment and controlled drug release. Melt dispersion technique is commonly used for the preparation of lipospheres because this method was considered with the aim to possibly reduce the toxicity caused by the organic solvents.

**Percentage yield**

The percentage yield of the formulated lipospheres was calculated and it was found that the lipospheres containing stearic acid (AF1- AF5) has a low yield in a range of 20.8±1.2 % to 28.8±0.4% than that of cetosteryl alcohol (AF6- AF10) with a yield in a range of 34.5±1.0% to 44.4±0.8%. High yield has been obtained for formulation containing Cetyl alcohol (AF11- AF15) in a range of 57.0±1.2% to 68.3±0.6%. The percentage yield was found in the lipospheres formulated by using various stabilizers (cholesterol, phospholipid) was found to be 47.3±1.2% to 43.0±1.6% (BF1- BF4) and 45.5±1.3% to 46.7±0.5% (BF5- BF8) respectively. Prominent yield was produced in the formulation containing cetyl alcohol (68.3±0.6%) due to high lipophilicity of the excipient. There was no significant change in the % yield by using stabilizers.

**Particle size**

The particle size distribution of lipospheres containing stearic acid (AF1- AF5) produces spherical particle with a size range of 140.2±1.0µm to 143.5±0.3µm with irregular surface. In case of cetosteryl alcohol (AF6- AF10) spherical particles are been produced with a size range of 88.3±0.2µm to 104.4±1.2µm with rough surface. Smooth spherical particles have been produced with a size range of 44.5±1.3µm to 83.5±1.4µm in formulation (AF 11- AF15) containing cetyl alcohol. The particle size of formulated lipospheres by using various stabilizers (cholesterol, phospholipid) was found to be 85.0±1.7µm to 117.0±1.4µm and 92.0±0.3µm to 119.0±0.6µm respectively. As the lipid concentration is increased size of the lipospheres also increased. The formulations (CF1-CF8) containing various co-surfactant (Tween 40, Tween 80) does not produce any lipospheres due to the formation of foams. From the characterized batches shown (Table. 4) it was observed that formulation AF15 (83.5±1.4µm) are found to be within the limits and the size distributions graph are shown in Fig1.

**Loading efficiency**

Formulation (AF1- AF5) containing stearic acid showed a loading efficiency of 30.6± 1.3% to 41.0± 0.5% and formulation (AF6- AF10) with cetostearyl alcohol showed a loading of 33.2± 1.5% to 48.4± 0.8%. High percentage of loading has been observed in formulation containing cetyl alcohol (AF 11- AF 15) was 45.0± 1.3% to 71.8± 1.2%. Loading efficiency of lipospheres with stabilizers (cholesterol, phospholipid) showed 37.8± 0.7% to 38.7± 1.6% (BF1- BF4) and 36.8± 1.7% to 40.9± 1.4% (BF5- BF8). Encapsulation efficiency was found maximum in formulation containing cetyl alcohol. Cholesterol and phospholipid showed no influence in the drug loading.

The loading efficiency of the formulated lipospheres was determined for all batches and their results are tabulated. The lipospheres containing stearic acid (AF1- AF5) has a low loading of 30.6± 1.3% to 41.0± 0.5% than that of cetosteryl alcohol (AF6- AF10) has a loading of 33.2± 1.5% to 48.4± 0.8%. Whereas high loading has been observed in the formulation containing cetyl alcohol (AF11- AF15) was 45.0± 1.3% to 71.8± 1.2%. The loading efficiency of formulated lipospheres by using various stabilizers (cholesterol, phospholipid) was also calculated and it was found that the lipospheres containing phospholipid (BF1- BF4) has 37.8± 0.7% to 38.7± 1.6%, cholesterol (BF5- BF8) has 36.8± 1.7% to 40.9± 1.4% of drug loading. The result showed in Table 4 that maximum loading efficiency was found in formulation containing cetyl alcohol (71.8± 1.2%).

**Morphological characterization**

The morphological characterization of the optimized lipospheres in terms of loading and particle size (AF15) were examined by scanning electron microscope with suitable magnifications (low magnification 400x, high magnification 1600x). It revealed that the optimized lipospheres formulation were more or less spherical with a rough surface were shown in Fig 2(a). Numerous drug crystals were clearly evident when examined under high magnification shown in Fig 2 (b).

**X- Ray Diffraction studies**

X-Ray diffraction is a means of identifying crystalline compounds. It can be particularly useful when these compounds are very fine-grained components or mixtures. The X-Ray spectrum of lumefantrine, cetyl alcohol and formulation were determined using X-Ray Diffractometer. The phase compositions of the samples prepared at different parameters were similar due to both the precursor slurry and the drying temperature were controlled at the identical conditions. Best formulation batch was selected to do the XRD test, as shown in Fig. 3. The XRD scan of plain lumefantrine showed intense peaks of crystallinity. Diffractogram of lumefantrine showed high intensity peaks between 2θ of 20-30$^0$ values demonstrating the crystalline nature of drug. No intense peaks were observed in diffractogram of cetyl alcohol which indicates amorphous nature. The XRD pattern of formulation exhibited halo pattern with less intense and denser peaks compared to plain lumefantrine.

***In vitro* release studies**

*In vitro* release for the formulated lipospheres containing lumefantrine of various batches has been carried out for 8 h and their results were calculated. The percentage release of lipospheres containing stearic acid (AF1- AF5) has its maximum release of 29.565±1.0% (AF5), cetosteryl alcohol (AF6- AF10) showed a maximum release of 41.868±0.9% (AF10), and cetyl alcohol (AF11- AF15) has a release of 68.418±1.0% (AF15) at the end of 12 h. The *in-vitro* release of the formulated lipospheres containing various stabilizers (phospholipid, cholesterol) has also been carried out in which the lipospheres containing phospholipid (BF1-BF4) has its maximum release of 26.184±1.0%, lipospheres containing cholesterol (BF5- BF8) has its maximum release of 39.726±1.2% (BF8) at the end of 12 h. From the characterized batches it was found that formulation AF15 showed prominent result (Fig.4).

**Table 1: Formulation of lumefantrine incorporated lipospheres**

| Batches | Composition | | | | | |
|---|---|---|---|---|---|---|
| | Lipids | | Surfactant | | Co-Surfactant | Description |
| | Stearic acid (mg) | Cetosteryl alcohol (mg) | Cetyl alcohol (mg) | PVA (mg) | Pectin (mg) | |
| AF1 | 100 | - | - | 50 | 2 | AP |
| AF2 | 200 | - | - | 50 | 2 | AP |
| AF3 | 300 | - | - | 50 | 2 | AP |
| AF4 | 400 | - | - | 50 | 2 | AP |
| AF5 | 500 | - | - | 50 | 2 | AP |
| AF6 | - | 100 | - | 50 | 2 | AP |
| AF7 | -- | 200 | - | 50 | 2 | AP |
| AF8 | - | 300 | - | 50 | 2 | AP |
| AF9 | - | 400 | - | 50 | 2 | AP |
| AF10 | - | 500 | - | 50 | 2 | AP |
| AF11 | - | - | 100 | 50 | 2 | DP |
| AF12 | - | - | 200 | 50 | 2 | DP |
| AF13 | - | - | 300 | 50 | 2 | DP |
| AF14 | - | - | 400 | 50 | 2 | DP |
| AF15 | - | - | 500 | 50 | 2 | DP |

AP: Aggregated spherical particles; DP: Discrete spherical particles.

**Table 2: Effect of Surfactant with various concentrations**

| Batches | Composition | | | | |
|---|---|---|---|---|---|
| | Lipids | Surfactant | | Co-surfactant | Description |
| | Cetyl alcohol (mg) | Phospholipid (%) | Cholesterol (%) | Pectin (mg) | |
| BF1 | 500 | 0.25 | - | 2 | DP |
| BF2 | 500 | 0.50 | - | 2 | AP |
| BF3 | 500 | 0.75 | - | 2 | AP |
| BF4 | 500 | 1.0 | - | 2 | AP |
| BF5 | 500 | - | 0.25 | 2 | DP |
| BF6 | 500 | - | 0.50 | 2 | AP |
| BF7 | 500 | - | 0.75 | 2 | AP |
| BF8 | 500 | - | 1.0 | 2 | AP |

DP: discrete spherical particles; AP: aggregated spherical particles

**Table 3: Effect of Co-surfactants with various concentrations**

| Batches | Composition | | | | |
|---|---|---|---|---|---|
| | Lipid | Surfactant | Co-surfactants | | Description |
| | Cetyl alcohol (mg) | PVA (mg) | Tween 40 (%) | Tween 80 (%) | |
| CF1 | 500 | 50 | 0.1 | - | F |
| CF2 | 500 | 50 | 0.2 | - | F |
| CF3 | 500 | 50 | 0.3 | - | F |
| CF4 | 500 | 50 | 0.4 | - | F |
| CF5 | 500 | 50 | - | 0.1 | F |
| CF6 | 500 | 50 | - | 0.2 | F |
| CF7 | 500 | 50 | - | 0.3 | F |
| CF8 | 500 | 50 | - | 0.4 | F |

F: Foams.

**Table 4: Characterization of lumefantrine loaded lipospheres**

| Batch | Percentage yield (%) | Particle size (µM) | Loading efficiency (%) |
|---|---|---|---|
| AF1 | 20.8±1.2 | 140.2±1.0 | 30.6±1.3 |
| AF2 | 23.9±0.5 | 131.7±1.0 | 31.3±1.4 |
| AF3 | 27.2±0.3 | 128.0±1.0 | 35.6±1.9 |
| AF4 | 28.1±0.6 | 115.2±1.5 | 39.3±0.7 |
| AF5 | 28.8±0.4 | 143.5±0.3 | 41.0±0.5 |
| AF6 | 34.5±1.0 | 88.3±0.2 | 33.2±1.5 |
| AF7 | 38.5±0.7 | 89.7±0.8 | 37.7±1.2 |
| AF8 | 40.8±1.5 | 93.0±2.0 | 42.3±1.8 |
| AF9 | 44.7±1.4 | 97.2±1.5 | 45.4±1.9 |
| AF10 | 44.4±0.8 | 104.4±1.2 | 48.4±0.8 |
| AF11 | 57.0±1.2 | 44.5±1.3 | 45.0±1.3 |
| AF12 | 59.6±1.5 | 58.3±0.7 | 52.6±0.9 |
| AF13 | 65.4±1.0 | 66.9±0.6 | 59.7±1.1 |
| AF14 | 68.3±0.6 | 73.2±1.2 | 65.8±1.0 |
| AF15 | 70.0±1.5 | 83.5±1.4 | 71.8±1.2 |
| BF1 | 47.3±1.2 | 85.0±1.7 | 37.8±0.7 |
| BF2 | 44.6±1.4 | 96.3±1.0 | 38.0±0.5 |
| BF3 | 43.4±1.0 | 106.0±1.5 | 34.8±0.4 |
| BF4 | 43.0±1.6 | 117.0±1.4 | 38.7±1.6 |
| BF5 | 45.5±1.3 | 92.0±0.3 | 36.8±1.7 |
| BF6 | 45.7±1.8 | 105.0±0.5 | 40.6±1.5 |
| BF7 | 43.9±1.6 | 112.0±0.6 | 42.0±1.7 |
| BF8 | 46.7±0.5 | 119.0±0.6 | 40.9±1.4 |

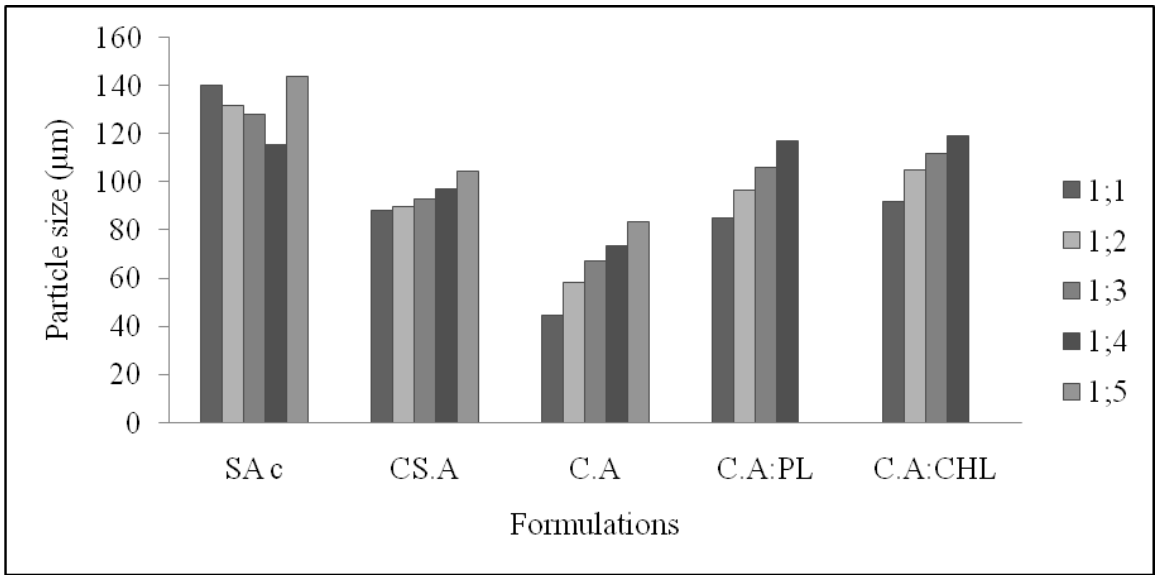


**Fig. 1: Particle size distribution of lumefantrine loaded lipospheres**

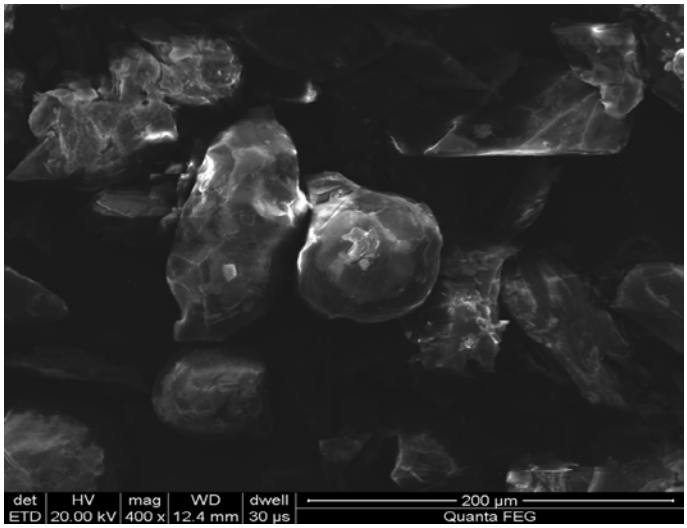


**Fig. 2a: Micrograph of AF15 lipospheres by scanning electron microscope at (400x)**

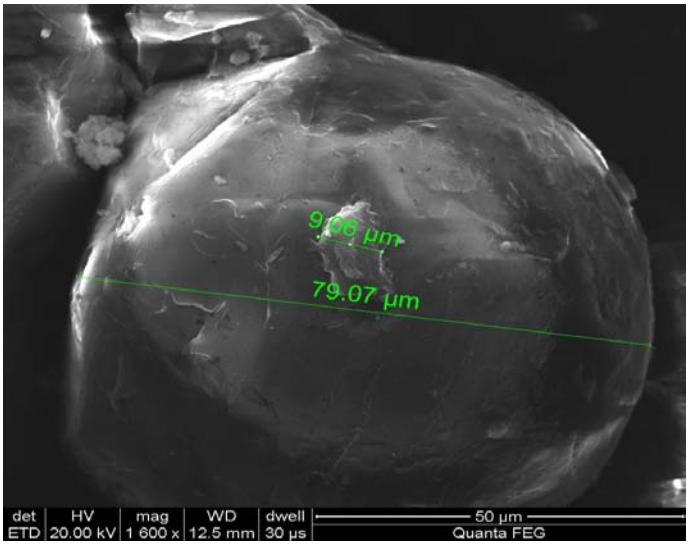


**Fig. 2b: Micrograph of AF15 lipospheres by scanning electron microscope at (1600x)**

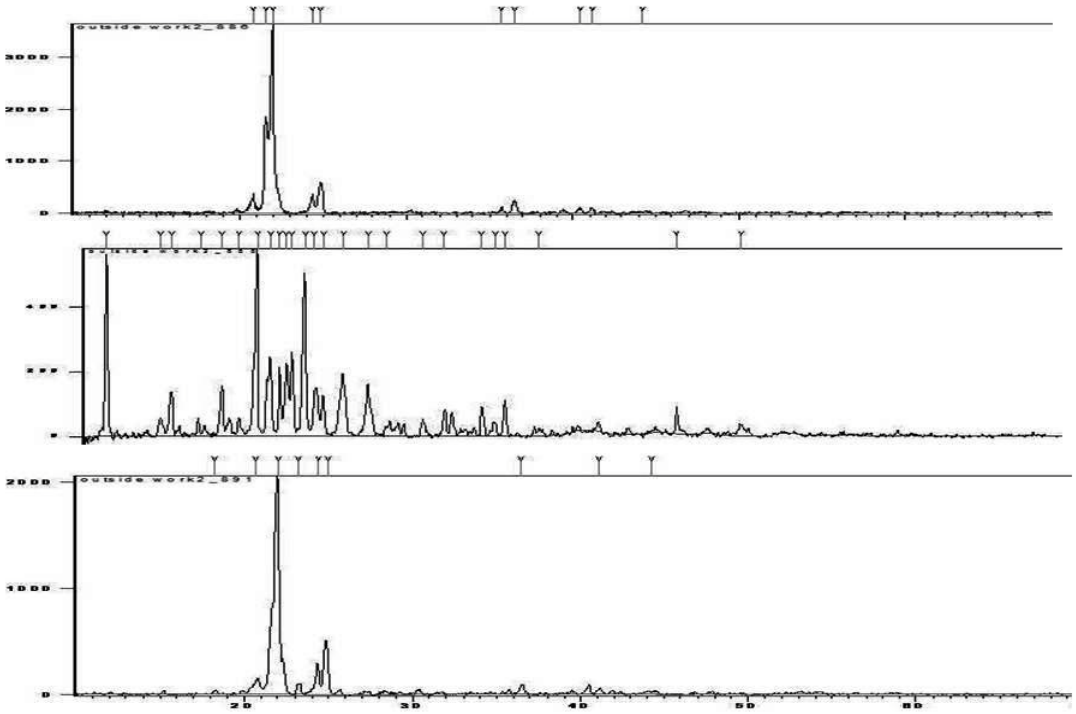

**Fig. 3: XRD graphs of cetyl alcohol, lumefantrine and lumefantrine loaded lipospheres respectively.**

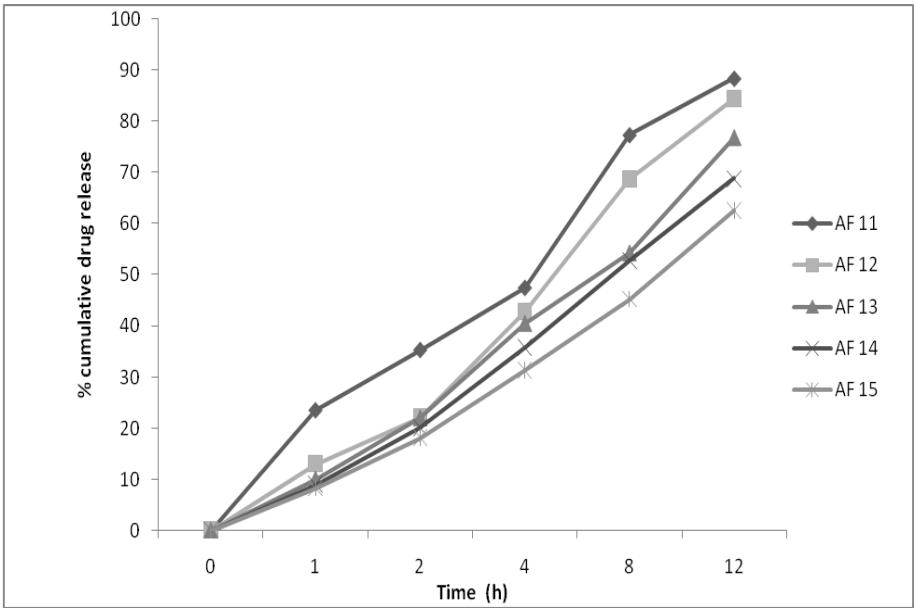


**Fig. 4: *In vitro* release lumefantrine lipospheres (AF11-AF 15) in PBS (pH 7.4)**

## CONCLUSION

Lipospheres can be considered as a promising drug delivery system for delivery of antimalarial drug like Lumefantrine. The Lumefantrine loaded lipospheres prepared by melt dispersion technique, with the use of different lipids, stabilizers, and surfactants influenced the particles shape, size distribution and loading efficiency.

Lipospheres under appropriate experimental conditions can entrap lumefantrine (lipophilic drug) and can control the release of the encapsulated drug. Lipid microspheres appears as ideal carrier system to administer lumefantrine by reducing toxicity, drug tolerance, cost effective and ease of preparation with scale up. The encouraging results obtained in this study could propose lipospheres for future *in vivo* studies, especially in the delivery of antimalarial drug.

## ACKNOWLEDGEMENT

Authors are thankful to Karpagam University, Coimbatore, India and Lincoln University College, Malaysia for the encouragement and facilities provided for the research work.

## REFERENCES

1. Widder KJ, Senyei AE and Sears B, Experimental methods in cancer therapeutics. J. Pharm. Sci 1982; 71: 379.

2. Gupta PK, Drug targeting in cancer chemotherapy: A clinical perspective. Pharm. Sci 1990; 79: 949.
3. Kirsten western and Britta siekmann, Biodegradable colloidal Drug carrier systems Based on solid lipids. Microencapsulation methods and industrial application. 213-254; 1996.
4. Xiang Li Shu-fang Nie, Jun Kong Ning Li, Cheng-yi Ju, Wei-san Pan, A controlled-release ocular delivery system for ibuprofen based on nanostructured lipid carriers. Int. J. Pharm 2008; 363: 177–182.
5. Loganathan Veerappan, Sivaprasada Reddy, MV, Formulation development and evaluation of flurbiprofen lipospheres. International Journal for The Advancement of Science & Arts 2010; 1: 90-95
6. 6.Sunil Prabhu, Maru Ortega, Chan Ma, Novel lipid-based formulations enhancing the in vitro dissolution and permeability characteristics of a poorly water-soluble model drug, piroxicam. Int. J. Pharm 2005; 301: 209–216.
7. Fu-Qiang Hu, Yu Zhang, Yong-Zhong Du, Hong Yuan, Nimodipine loaded lipid nanospheres prepared by solvent diffusion method in a drug saturated aqueous system. Int. J. Pharm 2008; 348: 146–152.
8. Elliot V Hersh, Manoj Maniar, Mark Green, Stephen A Cooper, Anesthetic activity of the lipospheres bupivacaine delivery system in the Rat. Anesth Prog 1992; 39: 197-200.
9. Cavalli R, Morel S, Gasco MR, Chetoni P, Saettone MF, Preparation and evaluation in vitro of colloidal lipospheres containing pilocarpine as iron pair. Int. J. Pharm 1995; 117: 243-246.
10. Nahla S Barakat, Alaa Eldeen B Yassin, In vitro characterization of carbamazipine-loaded precifac lipospheres. Drug Delivery 2006; 13: 95–104.
11. Krystyna Konopka, Luke SS Guo, Nejat Duzgunes, Anti-HIV activity of Amphotericin B-cholesteryl sulfate colloidal dispersion in vitro. Antiviral Research 1999; 42, 197–209.
12. Hagalavadi Nanjappa Shivakumar, Pragnesh Bharat Patel, Bapusaheb Gangadhar Desai, Purnima Ashok, Sinnathambi Arulmozhi, Design and statistical optimization of glipizide loaded lipospheres using response surface methodology. Acta Pharm 2007; 57: 269–285.
13. Collins-Gold LC, Lyons RT, Bartholow LC, Parenteral emulsions for drug delivery. Adv. Drug Deliv. Rev 2010; 5: 189.
14. Domb AJ, Bergelson L, Amselem S, Lipospheres for controlled delivery of substances. Drugs Pharm. Sci 1996; 73: 377-410.