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Formulation Development and Evaluation of Paclitaxel Loaded Solid Lipid Nanoparticles Using Glyceryl Monostearate

A. Dinda, I. Biswal, P. Chowdhury, R. Mohapatra
School of Pharmaceutical Sciences, SOA University, Bhubaneswar, Odisha-03, India.

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ABSTRACT

Solid Lipid Nanoparticles (SLNs) are important because of their size and stability. SLNs have been reported as an alternative drug delivery device to traditional polymeric nanoparticles. SLNs are in submicron range (50-1000nm) and are composed of physiologically tolerated lipid components. At room temperature the particles are in solid state. They are made up of bio-compatible and bio-degradable materials capable of incorporating lipophilic and hydrophilic drugs. Paclitaxel is a Di-terpenoid Pseudo-alkaloid having anti-neoplastic activity particularly against primary epithelial, ovarian carcinoma, Breast cancer, Colon Cancer, Brain Cancer, Lungs cancer and AIDs Related Kaposi's Sarcoma. Paclitaxel is an effective drug against Aggressive Cancer's because it adversely affect the process of cell division by preventing restructuring. The present study is to investigate the probability of incorporating paclitaxel in SLNs using Glyceryl Mono-stearate (GMS) as a lipid matrix, poly-oxy ethylene (Brij 97) as a surfactant, soya-lecithin as a co-emulsifier. Paclitaxel loaded SLNs are prepared by Solvent emulsification and evaporation method using ultra sonication and optimization of critical process variables were carried out to develop stable SLNs. The average particle size of SLNs was found to be 63nm ± 5.77 with Poly dispersity index (PDI) 0.272 ± 0.02 and entrapment efficiency was found 94.58%. The stability studies and zeta potential were performed at refrigerated temperature (2-8 ^{O}C) indicating no significant increase in particle size after one month storage. In-vitro release studies showed initial burst release followed by controlled release for 48hrs (about 73%). The release profile was fitted into Higuchi's model (r^2=0.9774). The drug diffuses from SLNs at a comparatively slower rate as the distance for diffusion increases.

INTRODUCTION

Paclitaxel is a diterpenoid pseudo-alkaloid and was isolated in early 1960s from the bark of Pacific Yew (*Taxus brevifolia*; family Taxaceae), one of the geographical Varieties of Yew (Wani *et al.*, 1971). Palitaxel has anti-neoplastic activity particularly against primary epithelial ovarian carcinoma, Breast cancer, Colon Cancer, Brain Cancer, Lungs cancer and AIDs Related Kaposi's Sarcoma (Spencer *et al.*, 1994; Alshowaier *et al.*, 2009). Paclitaxel induces programmed cell death (apoptosis in cancer cells by binding to an apoptosis stopping protein called Bcl-2 (B-cell leukemia 2) and thus arresting its function (Jordan *et al.*, 2009; Saville *et al.*, 1995). It interferes with the normal function of microtubule growth. One of the major problems entailed in using paclitaxel arises from its very low solubility in water due to its extreme hydrophobic character.

** Corresponding Author*
Agnimitra Dinda, Department of Pharmaceutics School of Pharmaceutical Sciences, SOA University Bhubaneswar, Odisha-03, India
Email: agnimitrapharmaindia@gmail.com

The present study is to investigate the possibility of incorporating Paclitaxel SLNs for colloidal therapeutic systems (Katja *et al.*, 2003). As a vehicle for delivery of Paclitaxel, SLNs would have the advantage of being constituted of biocompatible components such as lipids (Muller *et al.*, 2006). The main aim was to incorporate paclitaxel, developed as SNLs formulation using GMS as lipid matrix, Brij 97 (Polyoxy ethylene 10 oleyl ether) as surfactant and Soya-lecithin as co-emulsifier.

MATERIALS

Glyceryl monostearate (GMS), Kymphasol, Brij 97 - Sigma, U.S.A; Paclitaxel- Dr. Reddy's Laboratories Limited, Hyderabad; Lecithin, Soya (99%) - Himedia; Dialysis membrane (Mol. Wt. Cut Off 12000) – Sigma Aldrich (U.S.A); Potassium Di-hydrogen ortho-phosphate and Di-sodium hydrogen ortho-phosphate, sodium chloride and Glucose-SD fine chem. Ltd., Mumbai; Methanol, chloroform, Acetonitrile - Merck, India; Dulbecco's Modified Eagle's medium - Sigma, U.S.A.; Fetal Bovine Serum- Gibco, U.S.A.

METHOD OF PREPARATION

Paclitaxel loaded SLNs were prepared by, using glyceryl monostearate (GMS) as lipid, soya-lecithin as co emulsifier and brij 97 as surfactant. Solvent emulsification and evaporation method was selected to prepare SLNs due to convenience of lab scale equipments and suitability of the method. In this method, accurately weighed amounts of 60mg lipid; 3mg drug and 20 mg co-emulsifier were dissolved in 1 ml of organic solvent, chloroform. In a 50ml beaker 10 ml of 1.0% brij 97 solution is taken. Then organic phase was added to this 10ml of brij 97 solution and homogenized at 12400 rpm for 3 min, in order to get a coarse O/W emulsion (Beija *et al.*, 2012). Further this coarse emulsion was subjected to ultra-sonication for 10 min using a probe sonicator at 45% amplitude. During sonication, due to solvent emulsification and evaporation SLNs were precipitated and settled down. Thus, the paclitaxel loaded SLNs were formed. The formulations were made in triplicate for further characterization. While optimizing one variable (both in process and formulation) other variables were kept constant.

EVALUATION

Determination of Drug Entrapment Efficiency

The total content of drug present in the formulation was determined by dissolving 50µl of formulation in 950 µl of methanol. Then this solution was injected to HPLC and the Paclitaxel content was estimated using calibration equation in methanol (Anil *et al.*, 2002). Total content of the drug was found to be 2.46 mg and unentrapped drug was 0.165mg and entrapment efficiency was calculated by using the equation given below, the formulation has shown 94.58% entrapment efficiency.

Entrapment Efficiency (EE) = $(W_{\text{total drug added}} - W_{\text{free drug}} / W_{\text{total drug added}}) \times 100$

In-vitro Release Studies of Paclitaxel from SLNs Formulation

The drug from the optimized formulation (Paclitaxel in SLNs) was monitored by dialysis method. The dialysis membrane was soaked in double distilled water for 12 hrs before using for release study. The dialysis was carried out at room temperature using dialysis membrane with molecular weight cut off 12,000 and 25 ml of PBS-7.4 was used as sink solution. An aliquot of 2ml, equivalent to 600 µg of drug was placed in the dialysis bag. The concentration of drug was analysed at various time points during the dialysis process by HPLC method as described above. To study release kinetics, data obtained from in-vitro release studies were plotted in various kinetic models; zero order and first order. To evaluate the mechanism of drug release from Paclitaxel SLNs, data was plotted in Higuchi's model and Korsmeyer equations (Peltier *et al.*, 2006). The release of drug from the SLNs can be influenced by the lipid matrix, surfactant concentration and production parameters.The surfactant concentration is optimised as 1.55% in the present study. The drug release profile was affected by other parameters such as lipid nature, solubility of drug in lipid and partition co-efficient. Release studies were carried out for 48hrs from the percent cumulative amount release data, it is observed that, about 73% of drug was released from the optimised Paclitaxel loaded SLNs.

Size and Size Distribution

Smaller particles have higher surface area/volume ratio, which makes it easier for the encapsulation drug to be released from the SLNs via diffusion and surface erosion and also have the added advantage for the drug loaded SLNs to penetrate into, and permeate through the physiological barriers.

It was reported in the literature that smaller SLNs would have greater ease of entry and durability in the tumours (Hamid *et al.*, 2006). It was suggested that large particles (<5 µm) would be taken up via the lymphatic's and small particles (<500 nm) can cross the membrane of epithelial cells through endocytosis (Anil *et al.*, 2002).

Surface Charge

Zeta potential is a key factor to evaluate the stability of colloidal dispersion through the electrostatic repulsion between the particles. It is an important factor to determine their interaction in-vivo with cell membrane, which is usually negatively charged. In addition, from Zeta potential measurement, we can roughly know the dominant component of particle's surface. High absolute value of zeta potential indicates high surface charge of SLNs, which leads to strong repellent interactions among the SLNs in dispersion and thus high stability.

In general, particles could be dispersed stably when absolute value of Zeta potential was above -30 mV due to the electric repulsion between the particles (Feng *et al.*, 2004). In the present work Zeta potential of Paclitaxel loaded SLNs was found to be -24.4 mV.

Powder X-ray Diffractometry (P-XRD)

Powder –XRD studies were performed to characterize the state of the drug and lipid modification. Characterization of degree of lipid crystallization and lipid modification is helpful in understanding the drug incorporation (Venkateshwarlu e al, 2004). Powder –XRD studied of Paclitaxel, GMS, lyophilized formulation (SLNs) and blank Lyophilized SNLs were performed. Powder –XRD pattern of Paclitaxel exhibits sharp peaks at 2θ-scattered angles 21.4, 15.7, 14.6, 9.9, 7.8, 7.1, which indicate crystalline nature of drug. Lipid, glyceryl monostearate (GMS) shows sharp peak at 2θ scattered angles 16.6, 4.6 and 3.8. There were less intensity characteristic peaks for Paclitaxel in lyophilized formulation (SLN). This suggests that paclitaxel was not in crystalline form in SLNs.

Transmission Electron Microscopy (TEM)

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) are very useful in determining shape and morphology of lipid nanoparticles and allow determination of particle size and distribution. TEM determines the particle size with or without staining. SEM uses electrons transmitted from the

specimen surface, while TEM uses electrons transmitted through the specimen. TEM allows visualization of SLNs after freeze fracturing and freeze substitution. From TEM studies, Size of SLNs was found to be 70nm.

Stability Studies of Optimized Formulation

Based on the results of optimization studies of all processes and formulation variables, stable SLNs were prepared using optimized formula and kept for stability studies for one month at refrigerated temperature (2^0 C-8^0 C). The particle size evaluation can be used to predict the stability of the formulations (Table No. 3). Generally smaller particle size provides a better stability. Furthermore, difference in particle sizes with time would be strong evidence to the stability of SLNs. The mean values of particle size were compared with these obtained at time zero. Particle size and Zeta potential were measured at time intervals of 1, 3, 5, 10, 15, 20, 25 and 30 days. There *was* no significant change in the mean particle diameter at the storage temperature after one month of SLNs production. Percentage of difference in size was calculated after stability study. It was observed that the total increase in size was 9.64%. Though there were subjected to statistical analysis employing unpaired t-test and p-values. Zeta potential values were almost constant indicating the stability of the formulation.

RESULTS AND DISCUSSION

Solvent emulsification and evaporation method was used to prepare Paclitaxel loaded SLNs. Optimization of process and formulation parameters resulted in the production of Paclitaxel loaded SLNs with particle size 75 nm ± 5.77 (Fig.4) with PDI 0.272 ± 0.02 and entrapment efficiency of 94.58% of 3 mg drug loading. From TEM studies, Size of SLNs was found to be 70nm.
In-vitro release studies shows initial burst release followed by controlled release for 48hrs (Fig.2). Initial rate of release was high up to 8hrs.And almost remained constant after 10hrs. From the cumulative % drug release data, it was observed that about 73% of the drug was released from optimised Paclitaxel loaded SLNs. It was found that the in-vitro drug release of the Paclitaxel loaded SLNs was best explained by first order equation as the plot showed highest linearity (R^2 = 0.9747) followed by zero order (R^2 =0.9413). So the release rate constant is concentration dependent. The release data was fitted into Higuchi's model (R^2 = 0.9774). So the drug diffuses at a comparatively slower rate as the distance for diffusion increases, which is referred to as square root kinetics (Higuchi's kinetics). Mechanism of drug release is explained by Korsmeyer-Pappas equation indicating a good linearity (R^2 = 0.9762). The release exponent 'n' was 0.5, which appears to indicate non-Fickian diffusion and may indicate that the drug release is controlled by more than one process, diffusion followed by erosion. Intensity of pure lipid peaks was decreased after lyophilisation of samples. This reduces intensity indicating the decreased crystallinity of lipid, which favours successful drug incorporation. Lipids of less ordered crystal lattices favour successful drug inclusion, compared to those prepared by using highly ordered crystal-packing. P-XRD patterns of pure drug (Fig.5), lipid and formulation indicated that Paclitaxel is dispersed in SLN formulation is an amorphous state. The stability studies were performed at refrigerated temperature (2-8^0 C) and indicated no significant increase in particle size and zeta potential after one month of storage.

CONCLUSION

In conclusion, Paclitaxel loaded solid lipid nano-particles were prepared by solvent emulsification and evaporation method. In-vitro characterization was carried out, to evaluate the stability and release characteristics. Drug release from SLNs is dependent on the diffusion of drug through lipid matrix an in-vivo degradation of lipid matrix. In contrast to polymeric nanoparticles, lipid nanoparticles can be degraded by lipase in blood and allowed to release drug. The diffusion velocity of the drug to the SLNs surface is very slow; thus, sustained release is obtained. Further in-vivo studies in animal models are needed to prove the enhanced cytotoxicity and pharmacokinetics of Paclitaxel loaded solid lipid nanoparticles.

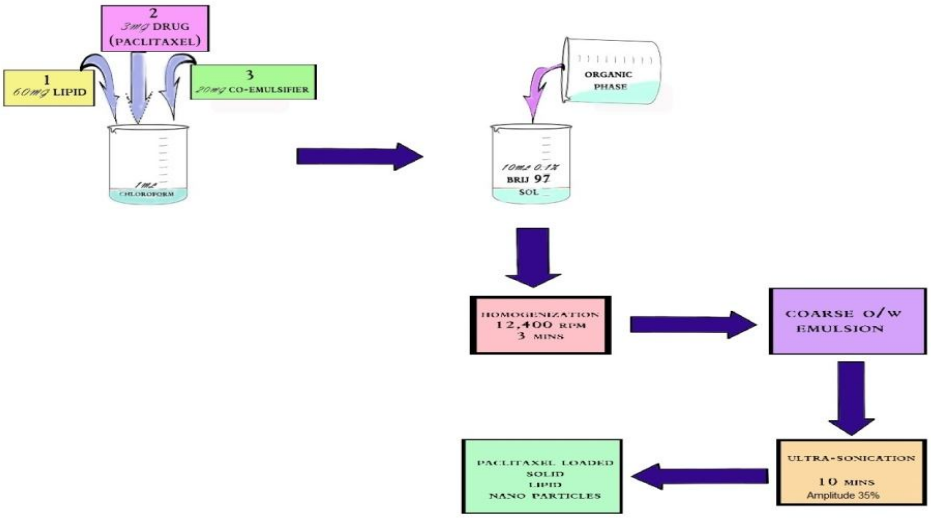

Fig.1: Graphical Representation of Method of Preparation of Paclitaxel Loaded SNL.

Table. 1: Formulation Parameters.

Ingredients	Amount used for optimization	Average Size (nm)	Polydispersity Index (PDI)	Zeta Potential (mV)	Entrapment Efficiency(EE)
Brij 97(%w/v)	0.5 %	181 ± 5.57	0.548±0.05	-31.5 ± 2.52	90.36 ± 0.44
	1 %	78 ± 7.00	0.394 ± 0.13	-25.7 + 2.50	86.41 ± 5.86
	1.5 %* †	63 ± 5.77	0.272 ± 0.02	-26.8 ± 1.44	94.58 ± 1.72
	2 %	86 ± 6.03	0.384 ± 0.10	-28.6 ± 1.11	92.68 ± 2.47
GMS(mg)	30	55 ± 3.01	0.328 ± 0.04	-25.0 ± 3.24	67.32 ± 4.32
	60* †	61 ± 3.73	0.277 ± 0.03	-26.8 ± 1.55	94.91 ± 1.84
	90	84 ± 4.66	0.303 ± 0.08	-30.9 ± 2.14	93.80 ± 3.05
Co-emulsifier (mg)	10	74 ± 5.67	0.371 ± 0.05	-27 ± 1.92	90.08 ± 2.64
	20* †	61 ± 3.73	0.268 ± 0.02	-25.4 ± 1.04	94.44 ± 1.73
	30	59 ± 2.36	0.337 ± 0.06	-26.2 ± 1.72	89.64 ± 3.27
Paclitaxel(mg)	1	55 ± 2.52	0.335 ± 0.05	-30.3 ± 2.13	74.87 ± 3.51
	3* †	61 ± 3.73	0.268 ± 0.02	-25.4 ± 1.04	94.44 ± 1.73
	6	407 ± 11.15	0.365 ± 0.10	-26.1 ± 1.90	93.81 ± 5.00
	* Statistical Significant with 10mg.P<0.05		† Statistical Significant with 30mg.P<0.05		

Table. 2: Size analysis of SLN formulations in various optimizing parameters.

	Homogenization speed(rpm)			Homogenization time(min)			Sonification amplitude (%)				
	10,400	**11,400**	**12,400**	**2**	**3**	**4**	**30**	**35**	**40**	**45**	**50**
Average particle size (nm)	151 ± 6.66	**124 ± 6.56**	140 ± 5.77	138 ± 1.00	**124 ± 6.56**	148 ± 3.51	98 ± 7.10	**76 ± 2.43**	117 ± 7.64	124 ± 6.56	124 ±6.66
Polydispersity Index (PDI)	0.569	**0.536**	0.538	0.555	**0.536**	0.543	0.328	**0.311**	0.599	0.536	0.537

Table. 3: Effect of Storage Time On SLN Formulations At Refrigerated Temperature (2-8^{0}C).

Day	Average Size (nm)	Zeta potential (mv)
1st day	63.3 ± 5.77	-26.77 ± 1.44
3rd day	62.1 ± 2.38	-24.90 ± 1.56
5th day	64.5 ± 4.95	-27.50 ± 1.51
10th day	65.4 ± 3.47	-23.60 ± 2.03
15th day	67.2 ± 3.13	-22.70 ± 0.85
20th day	67.6 ± 3.73	-24.50 ± 1.64
25th day	69.1 ± 2.03	-22.63 ± 1.34
30th day	69.4 ± 1.90	-24.80 ± 1.10

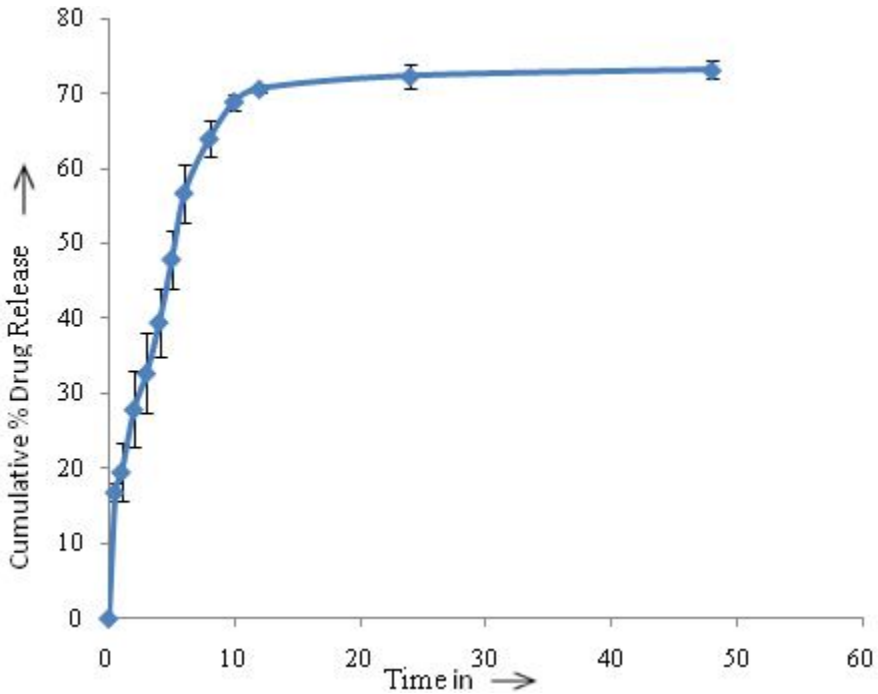

Fig. 2: Drug Release Profile of SLNs.

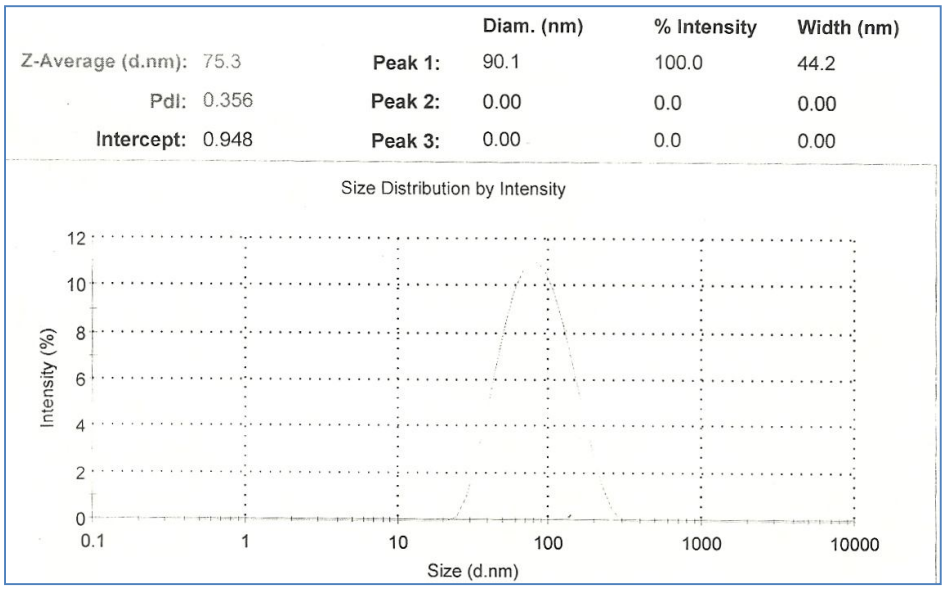

Fig. 3: PDI of prepared SLNs.

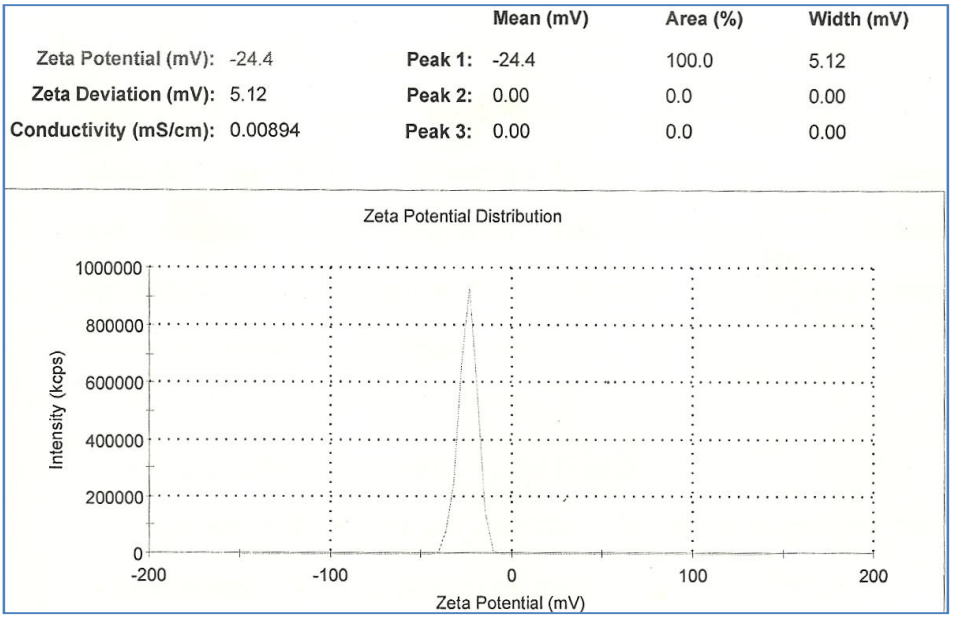

Fig. 4: Zeta Potential of prepared SLNs.

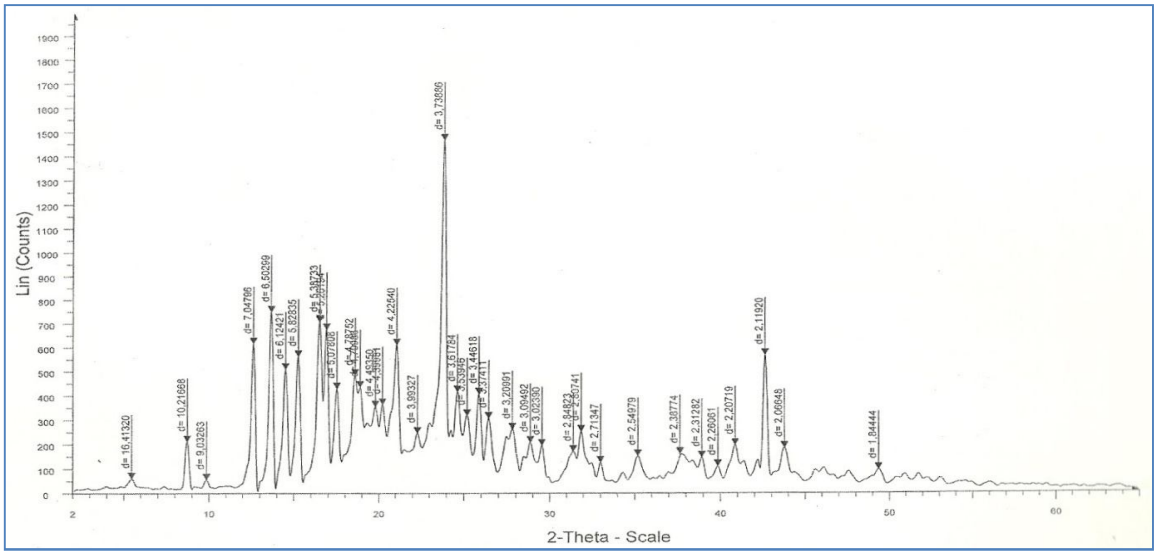

Fig. 5: Powder X-ray diffractometry SLNs.

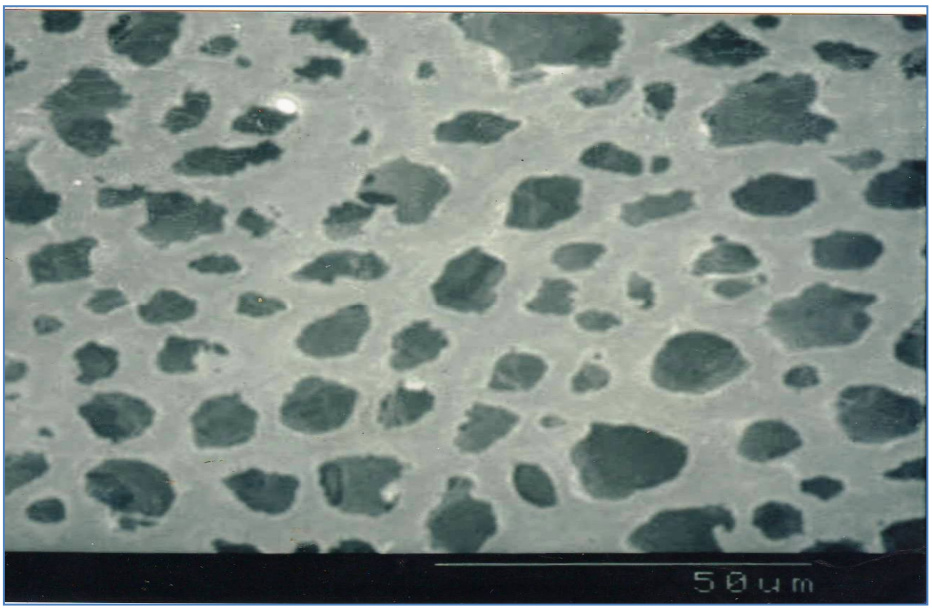

Fig. 6: TEM of prepared SLNs.

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