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# FORMULATION AND EVALUATION OF MELPHALAN LOADED NIOSOME FOR CANCER TREATMENT

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## 1. ABSTRACT

Cancer treatment with conventional chemotherapy drugs often faces challenges such as poor solubility, rapid metabolism, systemic toxicity, and non-specific distribution. Melphalan, an alkylating agent widely used in multiple myeloma and other cancers, is clinically effective but suffers from reduced stability and limited bioavailability. To address these drawbacks, the present work focused on developing melphalan-loaded niosomes as a novel nanocarrier system. The formulations were prepared using the thin film hydration technique with cholesterol and non-ionic surfactants. They were further analyzed for particle size, surface charge, drug entrapment efficiency, pH stability, compatibility studies, and in vitro release behavior. The optimized niosomal formulation exhibited nanoscale vesicles with uniform distribution, high entrapment efficiency, and stable zeta potential. FTIR analysis confirmed no significant drug–excipient interactions. The release profile demonstrated sustained drug release over an extended period, with kinetics indicating a diffusion-controlled mechanism. The findings suggest that melphalan-loaded niosomes can enhance drug stability, provide controlled delivery, reduce systemic side effects, and thereby improve therapeutic efficacy in cancer management.

**KEYWORDS:** Melphalan, Niosomes, Cancer therapy, Sustained release, Nanocarrier, Drug delivery.

## 1. INTRODUCTION

Cancer is one of the most serious global health problems, ranking among the leading causes of death. Chemotherapy remains an important treatment option, but the effectiveness of conventional drugs is often limited due to rapid degradation, poor solubility, lack of specificity, and severe systemic toxicity. These limitations reduce therapeutic success and increase side effects, which highlights the need for safer and more effective drug delivery strategies.

Melphalan, a bifunctional alkylating agent, is widely prescribed for multiple myeloma and other malignancies. It acts by crosslinking DNA and inhibiting replication, ultimately leading to cancer cell death. However, melphalan therapy suffers from major drawbacks including instability in aqueous solutions, low oral bioavailability, rapid clearance, and damage to normal healthy cells. These disadvantages reduce the overall efficiency of treatment and restrict its clinical use.

Novel carrier systems have been investigated to overcome such challenges. Vesicular systems such as liposomes and niosomes are of particular interest because of their ability to encapsulate both hydrophilic and lipophilic drugs, provide sustained drug release, and reduce unwanted toxicity. Among these, niosomes—vesicles prepared from non-ionic surfactants with cholesterol—have gained considerable attention due to their low cost, stability, biocompatibility, and ability to improve drug targeting. Niosomes can protect drugs from enzymatic degradation, prolong their circulation, and enhance their accumulation at tumor sites via the enhanced permeability and retention (EPR) effect.

The present research was designed to formulate and evaluate melphalan-loaded niosomes with the objective of improving drug stability, entrapment efficiency, and sustained release. The prepared formulations were assessed for particle size, zeta potential, compatibility, and drug release profile. It is expected that such a delivery system would reduce systemic side effects while enhancing the therapeutic potential of melphalan in cancer treatment.

## 2. MATERIALS AND METHODS

### 2.1 Materials

Melphalan hydrochloride (SHILPA MEDICARE LIMITED), Tween 60 (NICE chemicals), Tween 80(NICE chemicals), Span 60 (LOBA chemie), Span 80(LOBA chemie), Cholesterol (s.d.fine- CHEM LTd), Methanol (s.d.fine-CHEM LTd).

### 2.2 Equipment and softwares used

The experimental work was carried out using standard laboratory instruments and analytical tools. A magnetic stirrer (REMI 1MLH) was employed for uniform mixing of solutions, while a pH meter (Elico Li 127) was used to monitor the pH of the formulations. Vesicle preparation and size reduction were assisted by a probe sonicator (LABSONIC) and a bath sonicator (Leela Sonic Ultrasonicator). Drug quantification was performed using a UV–Visible spectrophotometer (JASCO V-530 UV 1600), and in vitro drug release studies were conducted with a dialysis membrane (Himedia, MWCO 50 kDa). Compatibility studies were carried out using FTIR spectroscopy (Shimadzu FTIR-8400S). Separation of entrapped and unentrapped drug fractions was achieved with a centrifuge (REMI R-8C Laboratory Centrifuge).

For analysis and data interpretation, different software tools were used. Adobe Photoshop CC2019 (v21.0.2) was applied for graphical editing, while ChemDoodle (v8.0.1) assisted in chemical structure representation. ImageJ (v1.53n) was utilized for particle image analysis, and OriginPro 2021 (v9.8.0.200) was employed for statistical evaluation, graph plotting, and kinetic model fitting of release data.

### 2.3 Methods

#### 2.3.1 Construction of standard curve

**Preparation of stock solution of melphalan hydrochloride**

A 10mg melphalan hydrochloride was accurately weighed and transferred into a 100 mL standard flask. It was dissolved in 50 mL methanol and make up to 100 mL with Phosphate buffer saline pH 6.4.

5mL of this solution was transferred into a 50 mL standard flask and make up to volume with Phosphate buffer saline pH 6.4. the final stock solution was containing 10 µg/mL of melphalan.

**Selection of analytical wavelength**

The stock solution was scanned over the UV wavelength range between 400 nm to 200 nm. The λ max of melphalan hydrochloride was found and further analysis was performed based on the selected analytical wavelength mentioned in Fig 1.

**standard graph of melphalan hydrochloride**

The prepared stock solution was further diluted to get the different concentrations to determine the linearity range. Linearity was obtained in the concentration between 2-10 µg/mL. The standard sample was analysed at 260.5 nm. The absorbance of the various concentration is as mentioned in Table 3 and Fig 2.

**Compatibility study (FT-IR analysis)**

KBr method, 300 mg of KBr and 3 mg melphalan HCL (and with polymers) placed in mortar 60 sec grind with pestle then placed into the hydraulic laboratory press, Press in vacuo at 15,000 pounds for 6 mins. The prepared pellet was analysed by using Shimadzu FTIR-8400S, then from FTIR graph, functional groups were interpreted and shown in Fig 3,4 and Table 4.

**Synthesis of Niosomes**

3 different Niosomal formulations such as F1, F2 and F3 containing melphalan hydrochloride were synthesised by ultrasonication method.in this study,10 mg of melphalan hydrochloride added to 20mL phosphate buffer saline 7.4 pH at 50mL beaker and were mixed by magnetic stirrer (REMI 1MLH) at 250 RPM. After the drug was solubilized in phosphate buffer, then added the required amount of surfactant and cholesterol, according to Table 7. This mixture was sonicated by probe sonicator (LABSONIC ULTRASONIC HOMOGENIZER) for 25 min and maintained the probe temperature at 50oC until to get a niosomal suspension (pulse mode range - 50sec sonication and 10sec pause).

**Table 1: Composition of melphalan niosomal suspention.**

| Formulation code | Drug (mg) | Surfactant (mg) | Cholesterol (mg) |
|---|---|---|---|
| F1 | 10 | 10 | 10 |
| F2 | 10 | 20 | 10 |
| F3 | 10 | 30 | 10 |

**2.3.2 Characterization of niosomes Transmission Electron Microscopy**

Niosome imaging was done under confocal microscope with Laser excitations of 100 and 3000 nm. Finally, the imaging process was done under confocal microscope and its process under the image J software.

**Measurement of Particle size**

The particle size of 3 different noisomal formulation was measured by particle size analyzer (Malvern version 7.13). For the measurement, 100 µl of the formulation was diluted with an appropriate volume of ethanol and phosphate buffer solution pH 7.4 in 2:8 Ratio and the vesicle diameter of all formulations were determined.

**ζ-potential**

A 1ml of each niosomal formulation was diluted to 10ml with water, 5 mL of this diluted sample was transferred to a cuvette and the zeta potential was measured at Malvern Zeta-sizer.

**pH**

The pH of all 3 noisomal formulation ware measured by using digital pH meter and their values were recorded.

**Entrapment Efficiency**

A 20 mL of each noisomal formulations were centrifuged at 4500-5000 rpm in a 20 mL centrifuge tube for 30 mins and the supernatant solution was discarded and 20 mL phosphate buffer saline 7.4 was added in a centrifuge tube containing niosomal formulation and this procedure was repeated for 3 times. The resultant Purified niosomal formulations was diluted with 1:10 (v/v) (niosomal formulation: methanol) and placed in a bath sonicator for 10 mins. The quantification of the cargo molecules was analysis by JASCO V-530 UV 1600 UV- visible spectrophotometer at 261 nm. The amount of entrapped drug and drug loading capacity calculated from the equation.

$$Entrapment\ Efficiency(EE) = \frac{amount\ of\ drug\ entrapped}{total\ amount\ added} \times 100$$

***In-vitro* release studies**

*In-vitro* release of 3 different formulations were investigated using dialysis method. This method was carried out by using Himedia dialysis membrane 50 with the molecular weight cut-off range from 12000-14000 Daltons which has the capacity of holding 1.61 mL/cm. The dialysis bag (donor compartment) was soaked in warm water for 30 mins for removal of glycerol and then thread was used to close the dialysis bag on both sides to prevent the leakage of formulation during drug release study. The purified each niosomal suspension was placed in the 12 cm dialysis bag and closed with thread. Dialysis bag was placed in 250 mL of phosphate buffer saline pH 7.4 (receptor compartment). The medium was stirred by magnetic stirrer at 50-150 rpm in 37oC. at each one-hour interval and 3 mL of sample was withdrawn and makeup to 10 mL with phosphate buffer saline 7.4 pH and replaced the same volume of fresh medium to maintain the sink conditions. determined the absorbance at 261 nm wavelength by using JASCO V-530 UV 1600 UV-visible spectrophotometer.

**Following steps are used to find out the percentage drug release**

**Step 1**

$$concentration = \frac{absorbance}{slope}$$

**Step 2**

$$concentration \times dilution\ factor$$

$$dilution\ factor = \frac{initial\ volume + final\ volume}{initial\ volume}$$

**Step 3**

$$in\ 250mL = \frac{concentration \times receptor\ volume}{1000mL}$$

**Step 4**

$$cumulative\ drug\ release = in\ 250mL + cumulative\ drug\ release$$

1st one cumulative value only exceptional.

**Step 5**

$$\%drug\ release = \frac{cumulative\ drug\ release}{amount\ of\ drug\ taken} \times 100$$

***In-vitro* Release Kinetics**

Drug release kinetics of 3 different melphalan loaded niosomal formulations were calculated by using a software Microsoft Office Excel Add – In. The *In-vitro* drug permeation data of obtained formulation was fitted to zero order kinetics (cumulative amount of drug released versus time), first order kinetics (log cumulative percentage of drug remaining versus time), Higuchi model (cumulative percentage drug release versus log time) to assess the kinetic modeling of drug release and the model with higher correlation coefficient (i.e higher R2) was considered to be best fit model.

## 3. RESULT AND DISCUSSION

**Selection of analytical wavelength**

The prepared stock solution was further diluted and scanned for λmax and it was found to be maximum absorption 260.5 nm (λmax) as shown in Fig 1.

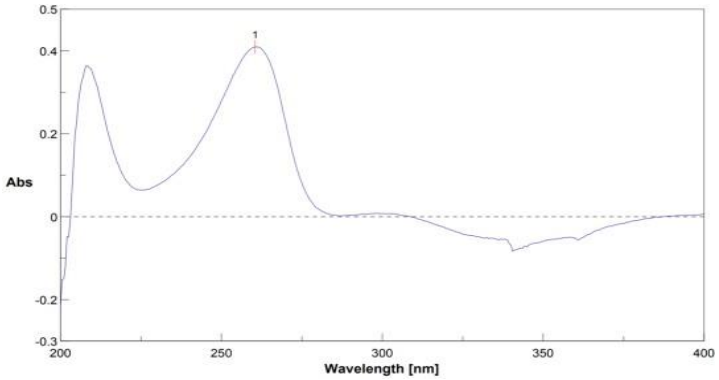


**Fig 1: spectra of melphalan measured using UV spectrophotometer.**

**Table 2: Analytical wavelength of melphalan.**

| S. No | Wavelength | Absorbance |
|---|---|---|
| 1 | 260.5 | 0.40977 |

**Construction of standard curve**

**Table 3: Melphalan concentration for calibration graph.**

| S. No | Concentration (µg/mL) | Absorbance (260.5nm) |
|---|---|---|
| 1 | 2 | 0.149 |
| 2 | 4 | 0.2857 |
| 3 | 6 | 0.4301 |
| 4 | 8 | 0.5588 |
| 5 | 10 | 0.6918 |

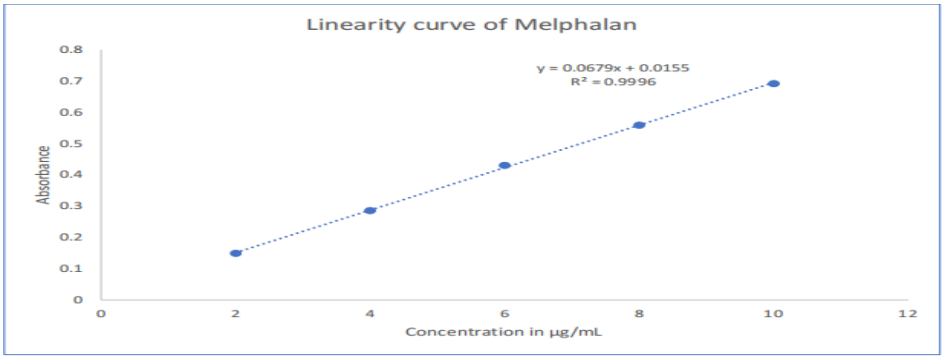


**Fig 2: Standard graph of melphalan.**

Straight light equation y = 0.0679x+0.0155 Correlation coefficient r2 = 0.9996

**Infrared spectral analysis**

The compatibility between the melphan and span 60 was evaluated by using the FT-IR matching approach.

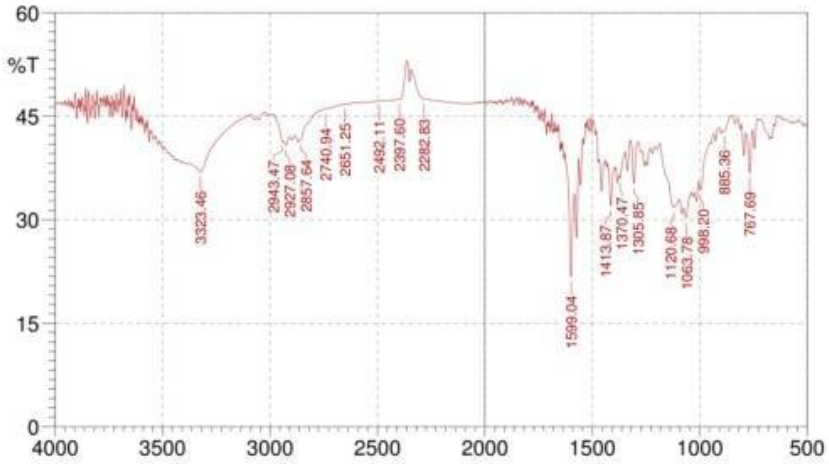


**Fig 3: IR spectrum of pure drug – melphalan.**

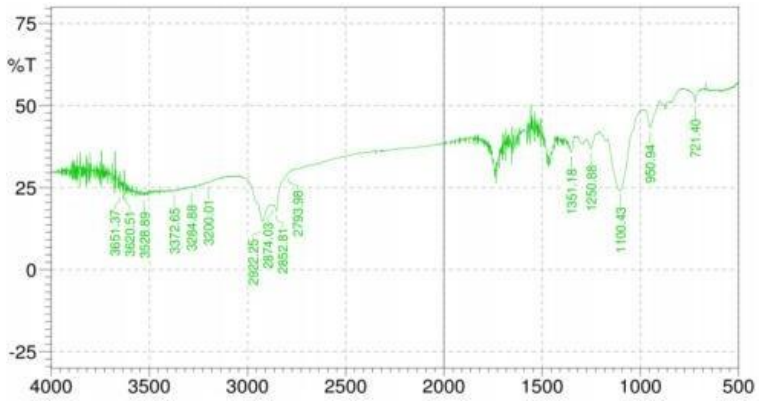


**Fig 4: IR spectrum of melphalan with span 60 Table 4: FT-IR Spectral analysis.**

| Peak assignment functional group | Frequency range (cm-1) | Wave number (cm-1) | |
|---|---|---|---|
| | | Spectrum position of IR in pure melphalan (cm-1) | Spectrum position of drug and span 60 (cm-1) |
| C=C (stretch aromatic) | 1450-1700 | 1599 | - |
| Tertiary amine | No peak will be seen | No peak | No peak |
| -C=O-OH | 2800-3400 | 3323 | 3284 |
| -sp2 C-H | 700-900 | 885 | - |
| -sp3 C-H (bend) | 2700-3400 | 1370 | 1351 |

FT-IR studies revealed that there is no appearance of a new peak and disappearance of existing peaks, which indicated that there is no interaction between the drug and span 60.

**pH**

The pH values of all the prepared formulations ranged from 7.25 to 7.45. which probably would not produce any irritation to the body.

**Table 5: pH value for niosomal formulations.**

| Formulation code | pH |
|---|---|
| F1 | 7.26 |
| F2 | 7.36 |
| F3 | 7.41 |

**Transmitted electron microscope**

The surface morphology of the 3 different prepared niosomal formulations were observed by ransmission electron microscope (TEM). TEM photographs were given in the Fig 5.

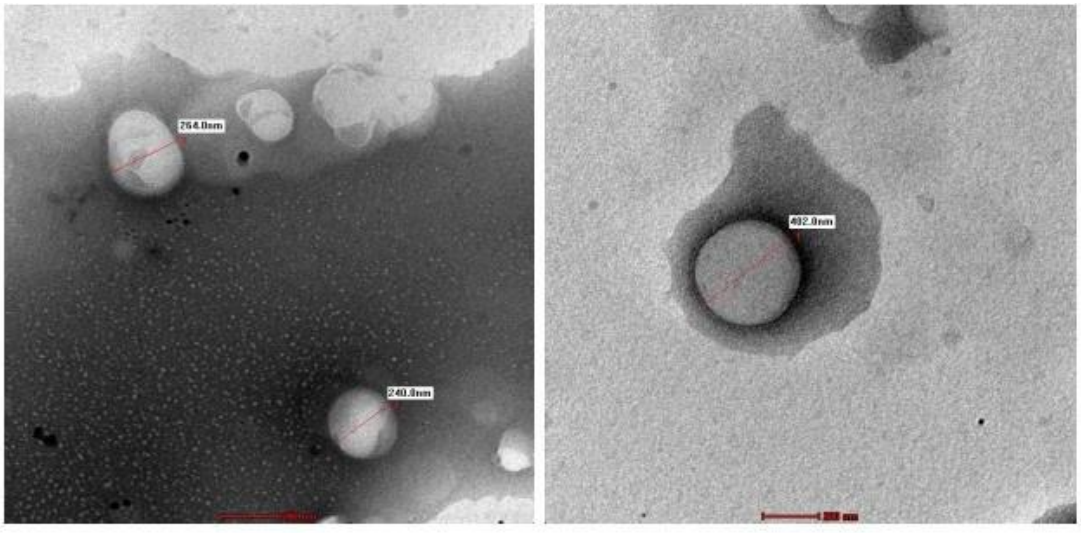


**Fig 5: Transmission electron microscopy of niosomal formulation (F1).**

**Particle size distribution**

The size distribution reports were shown in Fig.8,9 and 10. The size distributions along the mean diameter of the niosomes were measured by Dynamic Light Scattering Particle Size Analyzer (Malvern instruments). Particles of all formulations were in nanosize having a smooth spherical surface. Form formulations F1, F2 and F3 the average particle size was found to be. 278 nm, 684 nm and 2650 nm respectively. As the surfactant concentration increased there was an increase in particle size. The particle size of niosomes composed of span 60 and cholesterol formulation F1 in a 1:1 equimolar ratio was 278 nm, which was the least when compared to other formulations.

The particle size data showed that niosomes produced were of nanosize and had a high polydispersity index which indicates relatively broad particle size distribution for F1 and F3 preparations. F2 which has indicated narrow particle size distribution. The polydispersity index (PI) of formulations F1, F2 and F3 was found to be 1.000PI, 0.253PI and 1.000PI respectively.

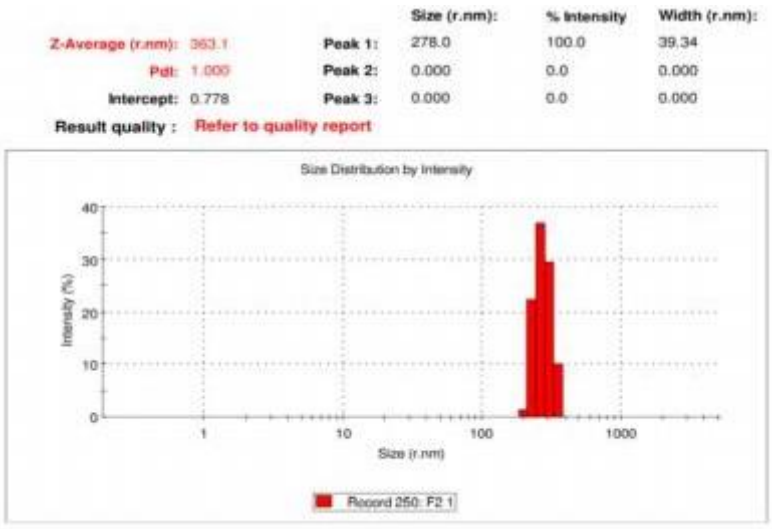


**Fig 6: Particle size distribution of Formulation F1.**


**Fig 7: Particle size distribution of Formulation F3.**

**Zeta potential**

The stability study of the all prepared niosomal formulations were evaluated by measuring the vesicle surface charge (zeta potential) of the niosomes by the zeta meter. The zeta potential reports were shown in Fig.11, 12 and 13. The Zeta potential of all formulated niosomes was in the range of −0.279 to - 4.4 mV which indicates the charge of a particle is neutral & negative.

| | | Mean (mV) | Area (%) | Width (mV) |
|---|---|---|---|---|
| Zeta Potential (mV): -0.279 | Peak 1: | -0.279 | 100.0 | 5.01 |
| Zeta Deviation (mV): 5.01 | Peak 2: | 0.00 | 0.0 | 0.00 |
| Conductivity (mS/cm): 0.722 | Peak 3: | 0.00 | 0.0 | 0.00 |
| Result quality : Good | | | | |

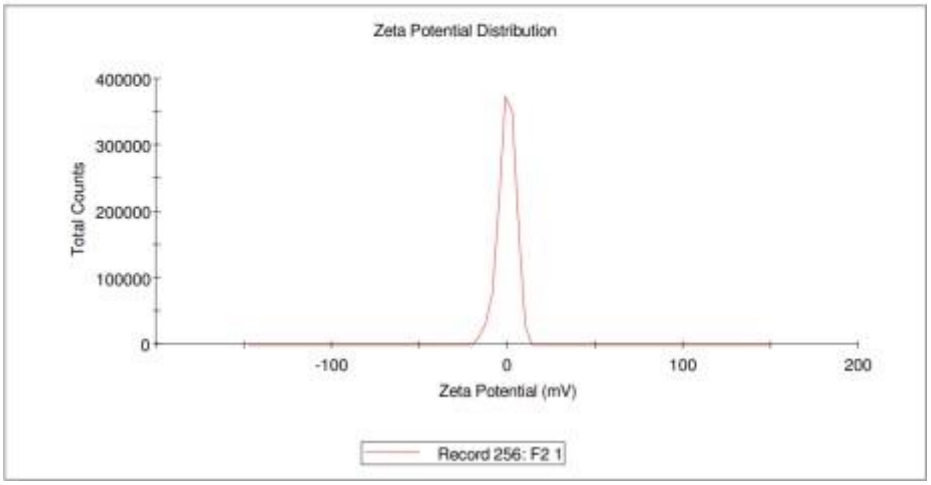


**Fig 8: Zeta potential of Formulation F1.**

| | | Mean (mV) | Area (%) | Width (mV) |
|---|---|---|---|---|
| Zeta Potential (mV): -14.4 | Peak 1: | -14.4 | 100.0 | 5.24 |
| Zeta Deviation (mV): 5.24 | Peak 2: | 0.00 | 0.0 | 0.00 |
| Conductivity (mS/cm): 1.68 | Peak 3: | 0.00 | 0.0 | 0.00 |
| Result quality : Good | | | | |

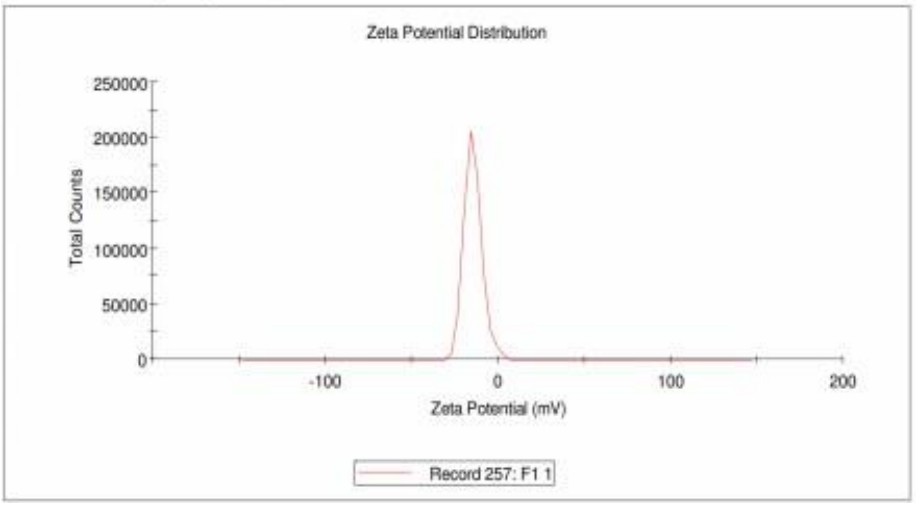


**Fig 9: Zeta potential of Formulation F2.**

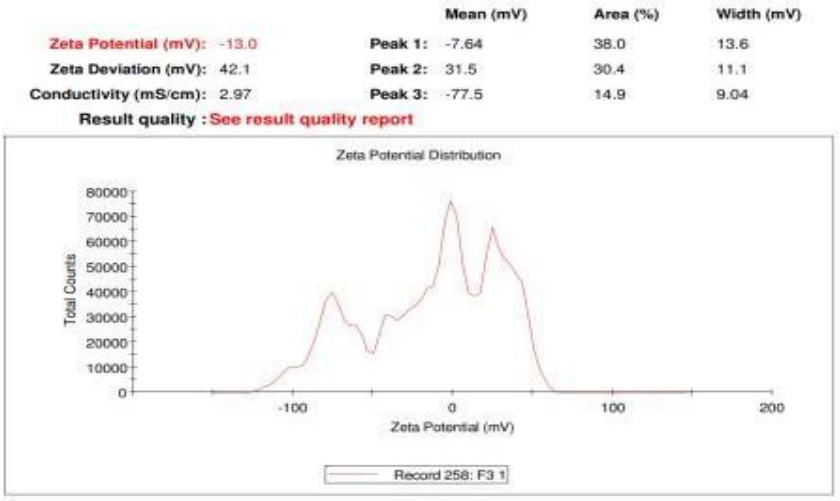


**Fig 10: Zeta potential of Formulation F3.**

**Entrapment efficiency**

All prepared niosomal formulation were tested for percentage entrapment efficiency. The result of percentage entrapment efficiency indicates maximum entrapment has been achieved when niosomal formulation prepared by using span 60. The result is shown in the table 12. the percentage entrapment efficiency for 3 different niosomal formulations were found to be 97.12, 95.02 and 88.06% respectively.

**Table 6: Percentage entrapment efficiency of niosomes.**

| Formulation code | Percentage entrapment efficiency (%) |
|---|---|
| F1 | 97.12 |
| F2 | 95.02 |
| F3 | 88.06 |

***In-vitro* drug release**

The initial cumulative percentage drug release for F1 formulation was 2.5% at 45 mins and increase up to 70.90% by the end of the 8th hour. The total release percentage by the end of 24 hrs was found to be 96.10%.

**Table 7: Drug release data for niosomal formulation F1.**

| Time (hrs) | concentration | concentration x dilution factor | Concentration x receotor volume/1000 mL | Cumulative drug release (mg) | Percentage drug release (%) |
|---|---|---|---|---|---|
| 0.45 | 0.3033 | 1.0008 | 0.2502 | 0.2502 | 2.5 |
| 2 | 1.0073 | 3.3240 | 0.831 | 1.0812 | 10.81 |
| 4 | 2.2415 | 7.3969 | 1.8492 | 2.9304 | 29.30 |
| 6 | 2.4830 | 8.1939 | 2.0484 | 4.9788 | 49.78 |
| 8 | 2.5596 | 8.4466 | 2.1116 | 7.0904 | 70.90 |
| 24 | 3.0544 | 10.0795 | 2.5198 | 9.6102 | 9.6102 |

The initial cumulative percentage drug release for F2 formulation was 3.09% at 45 mins and increase up to 44.17% by the end of the 8th hour. The total release percentage by the end of 24 hrs was found to be 59.59%.

**Table 8: Drug release data for niosomal formulation F2.**

| Time (hrs) | concentration | concentration x dilution factor | Concentration x receotor volume/1000 mL | Cumulative drug release (mg) | Percentage drug release (%) |
|---|---|---|---|---|---|
| 0.45 | 0.3755 | 1.2391 | 0.3097 | 0.3097 | 3.09 |
| 2 | 0.6111 | 2.0166 | 0.5041 | 0.8138 | 8.13 |
| 4 | 1.3091 | 4.2963 | 1.0740 | 1.8878 | 18.87 |
| 6 | 1.3195 | 4.3543 | 1.0885 | 2.9763 | 29.76 |
| 8 | 1.7466 | 5.7637 | 1.4409 | 4.4172 | 44.17 |
| 24 | 1.8689 | 6.1673 | 1.5418 | 5.9590 | 59.59 |

The initial cumulative percentage drug release for F3 formulation was 2.4% at 45 mins and increase up to 45.55% by the end of the 8th hour. The total release percentage by the end of 24 hrs was found to be 64.71%.

**Table 9: Drug release data for niosomal formulation F3.**

| Time (hrs) | concentration | concentration x dilution factor | Concentration x receotor volume/1000 mL | Cumulative drug release (mg) | Percentage drug release (%) |
|---|---|---|---|---|---|
| 0.45 | 0.2945 | 0.9718 | 0.2429 | 0.2429 | 2.4 |
| 2 | 0.9351 | 3.0858 | 0.7714 | 1.0143 | 10.14 |
| 4 | 1.1620 | 3.8346 | 0.9586 | 1.9729 | 19.72 |
| 6 | 1.4904 | 4.9183 | 1.2295 | 3.2024 | 32.02 |
| 8 | 1.6406 | 5.4139 | 1.3534 | 4.5558 | 45.55 |
| 24 | 2.3225 | 7.6645 | 1.9160 | 6.4714 | 64.71 |

The cumulative percentage release of the different niosomal formulation (F1,F2 and F3) is shown in the Fig 11. The formulation F1 had shown the highest drug release of 95% drug at 24 hrs. The formulation F2 and F3 had shown a similar rate of drug release of about 60%. In all the formulations, 40-75% of the drug was released within 10 hrs followed by 70-95% and followed by next 24 hrs.

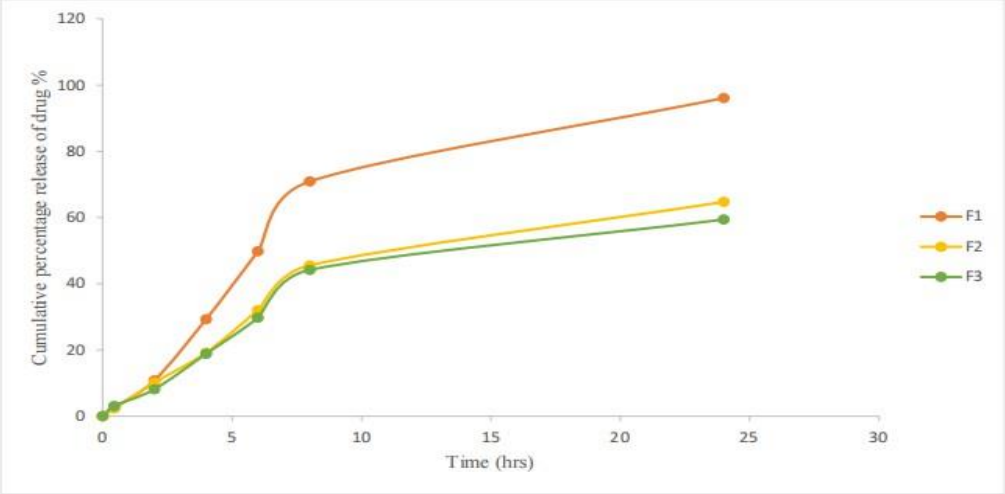


**Fig 11: Comparative cumulative percentage release of different niosomal formulations.**

**Drug release kinetics**

Various models such as Zero-order kinetics (percentage amount of drug release versus time), First-order kinetics (log percentage of drug remaining to release versus time), Higuchi (percentage amount of drug unreleased versus square root of time) and Korsermeyer-Peppas (log percentage of drug released versus log time) were applied to assess the kinetics of drug release from prepared niosome suspensions. The most suited model for drug release was predicted based on regression coefficient i.e. nearer the value of regression towards 1, greater the suitability of the best-fitted release mechanism. In the table, the kinetic parameter for 3 different melphalan loaded niosomal formulations were presented. As clearly indicated in the Table 16, the in-vitro release profile of drugs from all the formulations could be best expressed by Hixson, First order and Higuchi matrix diffusion type Fig 15,16 and 17.

**Table 10: Drug release kinetics.**

| Formulation code | R2 value | | | | |
|---|---|---|---|---|---|
| | Zero order | First order | Higuchi | Korsemeyer- Peppas | Hixson |
| F1 | 0.8949 | 0.9866 | 0.9496 | 0.6343 | 0.9877 |
| F2 | 0.8986 | 0.9512 | 0.9553 | 0.6493 | 0.9359 |
| F3 | 0.9151 | 0.9723 | 0.9642 | 0.6506 | 0.9570 |

Formulation F1 follows Hixson drug release kinetics with R2= 0.9877

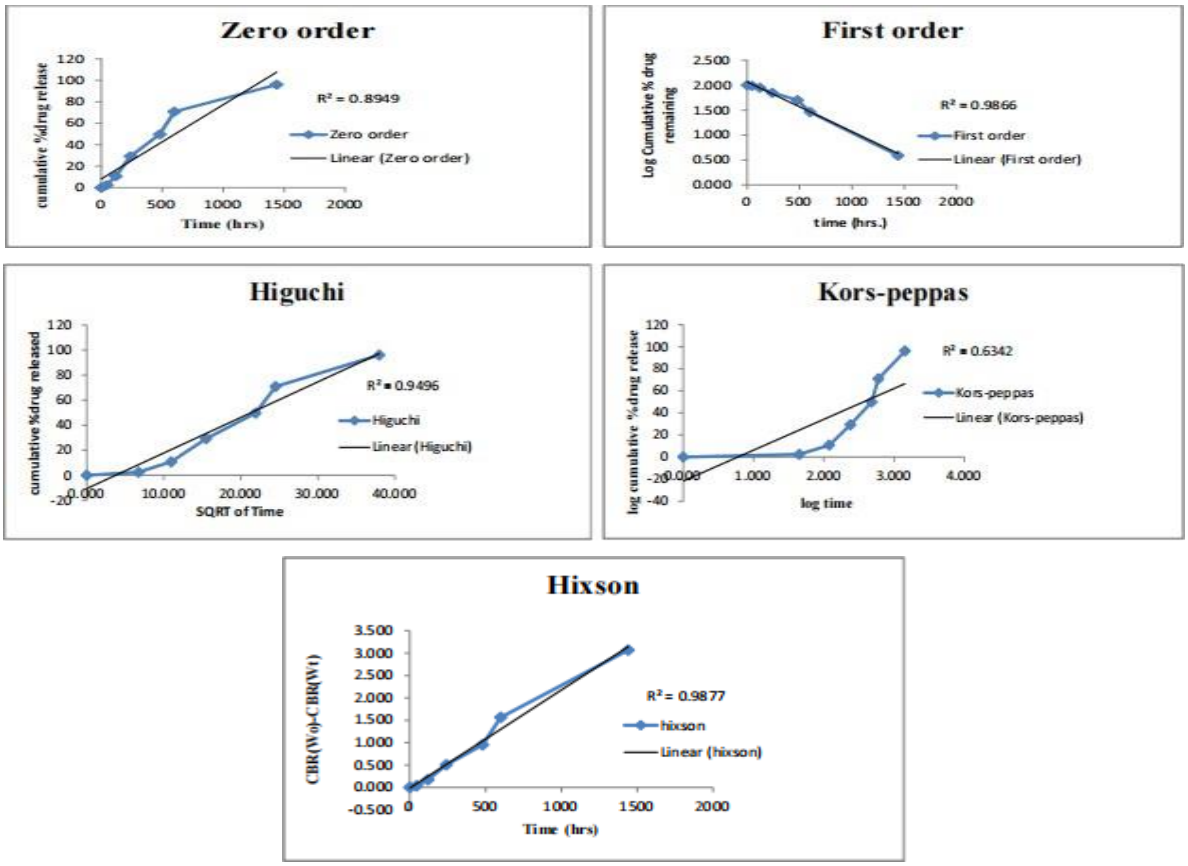


**Fig 12: Drug release rate kinetics of melphalan loaded niosome F1.**

Formulation F2 follows Higuchi drug release kinetics with R2= 0.9553

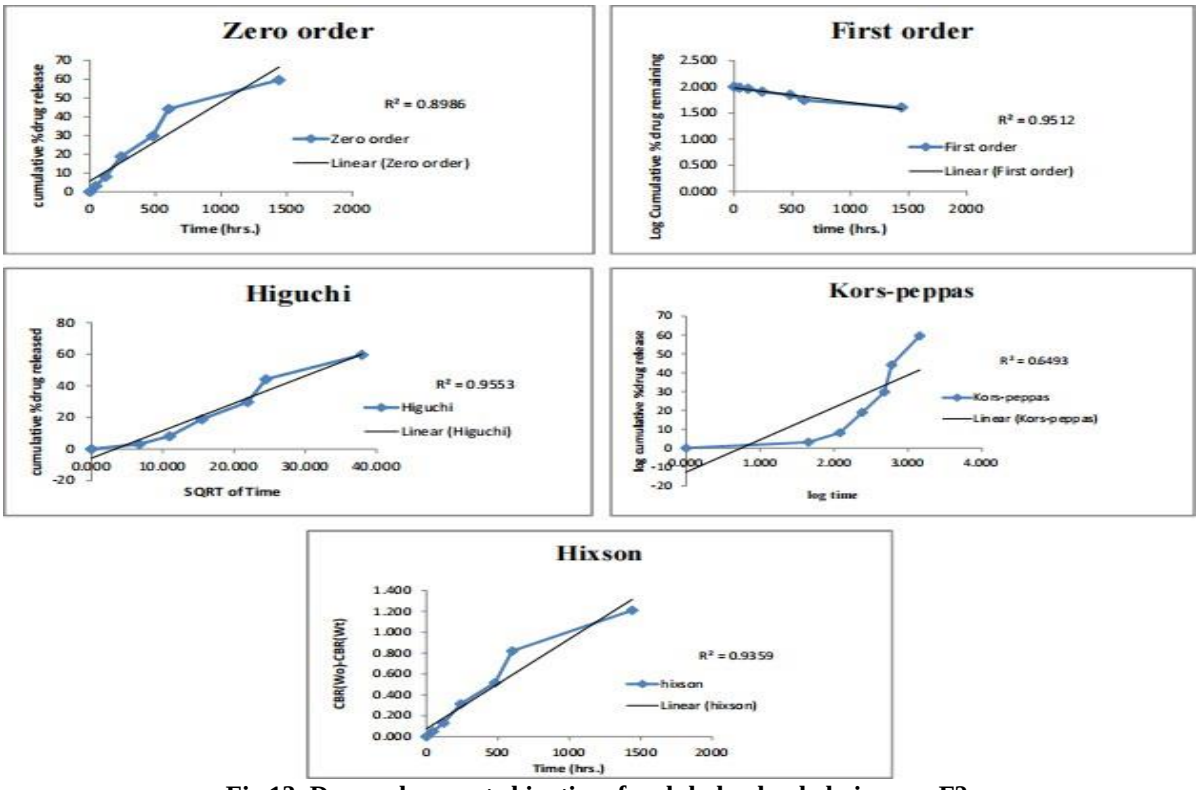


**Fig 13: Drug release rate kinetics of melphalan loaded niosome F2.**

Formulation F3 follows First order drug release kinetics with R2= 0.9723

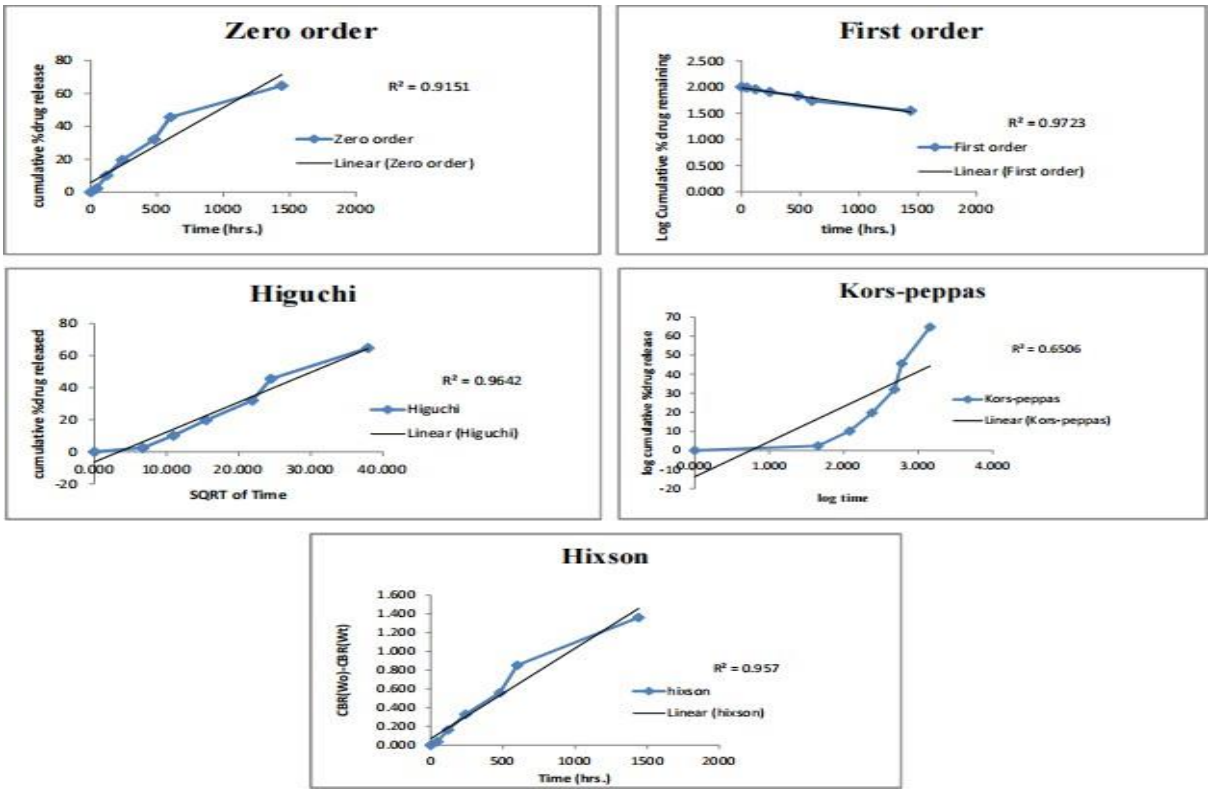


**Fig 14: Drug release rate kinetics of melphalan loaded niosome F3.**

## 4. CONCLUSION

Melphalan-loaded niosomes were formulated using Span 60 and cholesterol by probe sonication to improve drug targeting and reduce systemic toxicity. Compatibility studies confirmed no interaction between melphalan and excipients, and all batches showed acceptable pH values. Particle size was influenced by drug-to- polymer ratios, and TEM confirmed spherical vesicles capable of entrapping drug effectively. Entrapment efficiency ranged from 88.06% (F3) to 97.12% (F1). Zeta potential values varied, with F2 showing the highest (– 14.4) and F1 the lowest (–0.29).

In vitro release studies revealed sustained drug release, with F1 achieving 96.10% release at 24 hours. Release kinetics showed different patterns: F1 followed Hixson–Crowell ($R^2$ = 0.9877), F2 followed Higuchi ($R^2$ = 0.9553), and F3 followed First-order ($R^2$ = 0.9723). Niosomal encapsulation of melphalan improved stability, entrapment, and sustained release while reducing normal cell toxicity, making it a promising carrier for cancer therapy. Further optimization and in vivo studies are needed to support scale-up and clinical application.

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