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MELPHALAN SPANLASTICS FOR ORAL ADMINISTRATION – FORMULATION AND DEVELOPMENT

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ABSTRACT

The main aim of the present study was to optimize the composition of spanlastics containing Melphalan for oral administration with a view to achieve sustained release. Melphalan loaded spanlastics were prepared by Ethanol Injection Method using non-ionic surfactants (Span 40 & Span 60) and edge activators (Tween 20, Tween 60, Tween 80, Sodium taurocholate and Sodium deoxycholate) in different ratios like 85:15, 80:20, 75:25, 70:30, 60:40 and 50:50. Spanlastics were characterized for their particle size, surface morphology, zeta potential, entrapment efficiency, *in-vitro* diffusion, drug compatibility studies and stability studies. Spanlastics were prepared using Span 60 as non-ionic surfactants and Sodium taurocholate(STC), Sodium deoxycholate(SDC) as edge activators. This combination 80:20 w/w was found to be the optimized formulation. Scanning electron microscopy revealed spherical shape. The entrapment efficiency and percentage drug release of optimized formulation were found to be 78% and 80% respectively.

KEYWORDS: Spanlastics, Melphalan, Ethanol injection method, Non ionic surfactant, Edge activator.

1. INTRODUCTION

Melphalan, 4-[bis(2-chloroethyl)amine]-L-phenylalanine is an antineoplastic agent, used in the treatment of multiple myeloma, advanced ovarian adenocarcinoma, breast cancer, childhood neuroblastoma. The clinical use of Melphalan has practical disadvantages mainly due to its poor water solubility and poor bioavailability. Therefore, the development of novel carrier for Melphalan was attempted to achieve sustained release.

Cevc and Blume (1992) introduced the first generation of highly deformable elastic liposomes, referred to as transferosomes.[1] Touitou et al. (1997) developed ethosomes, soft vesicular carriers mainly consisting of phospholipids and ethanol.[2] A second generation of elastic vesicles mainly consisting of nonionic surfactant (NIS) was introduced by Van den Bergh in 1999.[3] Recently, a novel surfactant-based nanovesicular elastic carrier system named spanlastics was developed. Spanlastics are advanced drug delivery systems. These are elastic non-ionic surfactant vesicles, composed of spans as vesicle forming nonionic surfactant (NIS) and edge activator (EA).[4]

In the present study Span 60 and Span 40 are used as non ionic surfactants. Elasticity is attributed to the use of edge activators. Edge activators are the surfactant molecules that provide flexibility to the vesicles and improve the solubility of water insoluble drugs. Tween 20, Tween 60, Tween 80, Sodium taurocholate and Sodium deoxycholate are used as edge activators.

The main aim of the current study was to enhance the sustained release of Melphalan. In order to achieve this goal, Melphalan loaded spanlastics were prepared by ethanol injection method, using different edge activators.

2. MATERIALS AND METHODS

2.1 MATERIALS

Melphalan was a kind gift from Aurobindo Pharma Limited, Hyderabad, India. Span 40, Span 60, Tween 20 and NaoH were purchased from SD fine chemical limited, Mumbai, India. Tween 60, Tween 80, Sodium taurocholate and Sodium deoxycholate were purchased from Oxford laboratory, Mumbai, India.

2.2 EXPERIMENTAL

2.2.1 Preparation of Melphalan spanlastics

Melphalan spanlastics were prepared by Ethanol Injection Method. In this method, the chosen edge activator was dissolved in 10ml of water and was injected slowly through a 16-guage needle in to 10ml of organic phase containing Span and drug previously dissolved in ethanol. The resultant solution was stirred on magnetic stirrer at 60°C. The prepared spanlastics formulations were listed in Table 1 and 2.

Table 1: Various Span40-based formulations of Spanlastics

S.No.	Formulation Code	Drug (mg)	NIS:EA	Ethanol	Water
I. Formulations with Span 40 and Tween 20					
1	A1	1	85:15	10	10
2	A2	1	80:20	10	10
3	A3	1	75:25	10	10
4	A4	1	70:30	10	10
5	A5	1	60:40	10	10
6	A6	1	50:50	10	10
II. Formulations with Span 40 and Tween 60					
7	B1	1	85:15	10	10
8	B2	1	80:20	10	10
9	B3	1	75:25	10	10
10	B4	1	70:30	10	10
11	B5	1	60:40	10	10
12	B6	1	50:50	10	10
III. Formulations with Span 40 and Tween 80					
13	C1	1	85:15	10	10
14	C2	1	80:20	10	10
15	C3	1	75:25	10	10
16	C4	1	70:30	10	10
17	C5	1	60:40	10	10
18	C6	1	50:50	10	10
IV. Formulations with Span 40 and (Sodium taurocholate and Sodium deoxycholate)					
19	D1	1	85:15	10	10
20	D2	1	80:20	10	10
21	D3	1	75:25	10	10
22	D4	1	70:30	10	10
23	D5	1	60:40	10	10
24	D6	1	50:50	10	10

NIS=Non ionic surfactant; EA=Edge Activator.

Table 2: Various Span 60-based formulations of Spanlastics

S.No.	Formulation Code	Drug (mg)	NIS :EA	Ethanol	Water
V. Formulations with Span 60 and Tween 20					
1	E1	1	85:15	10	10
2	E2	1	80:20	10	10
3	E3	1	75:25	10	10
4	E4	1	70:30	10	10
5	E5	1	60:40	10	10
6	E6	1	50:50	10	10
VI. Formulations with Span 60 and Tween 60					
7	F1	1	85:15	10	10
8	F2	1	80:20	10	10
9	F3	1	75:25	10	10
10	F4	1	70:30	10	10
11	F5	1	60:40	10	10

12	F6	1	50:50	10	10
VII. Formulations with Span 60 and Tween 80					
13	G1	1	85:15	10	10
14	G2	1	80:20	10	10
15	G3	1	75:25	10	10
16	G4	1	70:30	10	10
17	G5	1	60:40	10	10
18	G6	1	50:50	10	10
VIII. Formulations with Span 60 and (Sodium taurocholate and Sodium deoxycholate)					
19	H1	1	85:15	10	10
20	H2	1	80:20	10	10
21	H3	1	75:25	10	10
22	H4	1	70:30	10	10
23	H5	1	60:40	10	10
24	H6	1	50:50	10	10

3. EVALUATION OF SPANLASTICS

3.1 Fourier Transform Infrared (FTIR) analysis

The drug excipient compatibility study was determined by FTIR (Fourier Transform Infrared Spectroscopy). Samples were scanned in the range from 400- 4000cm^{-1}. The IR spectrum of the pure drug was compared with IR spectrum of combination of drug and excipients to check the interactions.

3.2 Surface morphology

The morphology of spanlastics was determined by scanning electron microscopy (SEM) using HITACHI S-3700N Instrument. SEM gives a three dimensional image of the globules. The samples were examined at suitable accelerating voltage 15kV.

3.3 Particle size measurement and Zeta potential

The particle size and zeta potential of Melphalan spanlastics were measured by Particle size analyzer and Zeta sizer (HORIBA SZ 100). For the measurement, 100µl of formulation was diluted with appropriate volume of PBS pH 7.4. The vesicle diameter and zeta potential were determined.

3.4 Entrapment efficiency

For the determination of Entrapment efficiency, the unentrapped drug was first separated by centrifugation at 1500RPM for 60min and supernatant was collected. The collected supernatant was estimated using UV Visible Spectrophotometer at 259nm.

$$\% \text{ Entrapment Efficiency} = \frac{[\text{Total drug}] - [\text{Free drug}]}{\text{Total drug}} \times 100$$

3.5 In-vitrodiffusion Study

In-vitro diffusion studies were carried out using Franz-diffusion cell. The membrane was soaked for 24h in 0.1N HCl. The donor compartment was covered with dialysis membrane. The receptor compartment was filled with 7.4 pH phosphate buffer. The prepared formulation was introduced into dialysis membrane in the donor compartment. The whole assembly was maintained at 37°C and the speed of stirring was controlled. 3ml aliquot of drug sample was withdrawn from receptor compartment at specified time intervals 1, 2, 3, 4, 5, 6, 7 and 8h and replaced with fresh medium to maintain sink condition. The samples were analyzed spectrophotometrically at 259nm.

3.6 Stability Studies

Stability studies were conducted on optimized formulation according to ICH guidelines. The spanlastics suspension was kept in sealed ampoule and stored at 25°C±2°C/60±5% RH and 40°C±2°C/75±5% RH for 3months. Samples were withdrawn periodically (intervals 30, 60, 90 days) and analyzed for physical appearance and entrapment efficiency.

4. RESULTS AND DISCUSSION

4.1. Fourier Transform Infrared (FTIR) analysis

The results showed that there was no chemical interaction or changes between Melphalan spanlastics and melphalan pure drug. Melphalan spanlastics showed same characteristic absorption bands as that of melphalan.

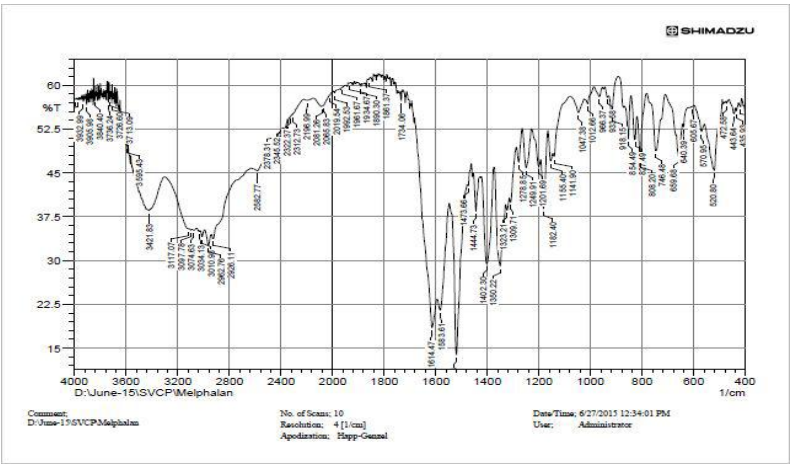

Figure 1: FTIR image of melphalan

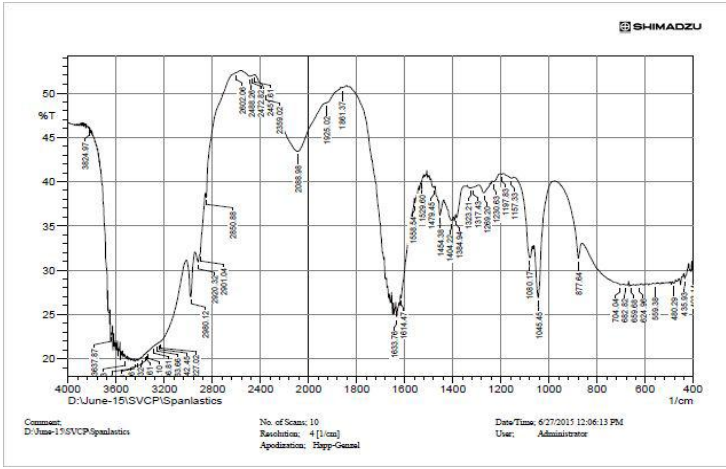

Figure 2: FTIR image of melphalan spanlastics suspension

4.2 Surface Morphology

The three dimensional image of globules was shown in Fig. 3. The image indicates that the obtained spanlastics have a smooth surface and are spherical in shape.

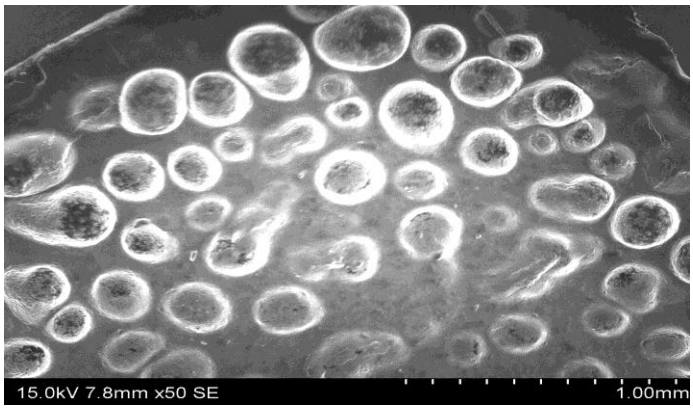

Figure 3: SEM photograph of spanlastic formulation

4.3 Particle size measurement and Zeta potential

The particle size distribution of spanlastics was characterized. The particle size of optimized spanlastics formulation was found to be 234nm. The particle size was found to depend on the HLB value of the non ionic surfactants used. The formulations prepared using STC and SDC showed higher particle size compared to formulations using Tween 80 and Tween 60. With the increase in the HLB value, the vesicle size also was found to increase as shown in Table 3. Zeta potential of optimized formulation was found to be -18.4mv as shown in Fig. 5. This value indicated that optimized formulation was stable.

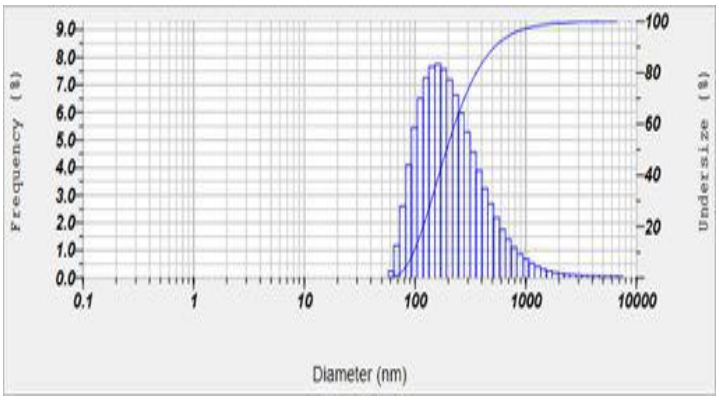

Figure 4: particle size analysis

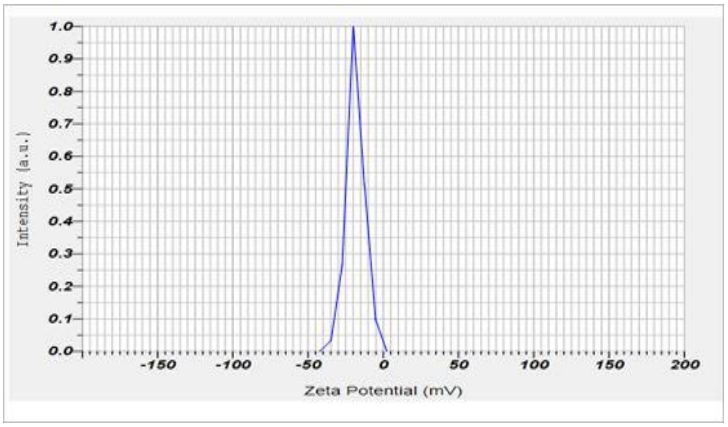

Figure 5: zeta potential

Table 3: Particle size of Spanlastics based on HLB value

Edge activator	HLB value	Particle size (nm)
Sodium tauro cholate and Sodium deoxy cholate	16 and 26	234
Tween 20	16.7	210
Tween 60	14.9	195
Tween 80	15	200

HLB= Hydrophilic Lipophilic Balance.

4.4 Entrapment efficiency

Entrapment efficiency of selected formulations was in the range of 65 to 78%. Highest entrapment efficiency (78%) was found in H2 formulation. H2 formulation contains a Span 60 and combination of Sodium taurocholate and Sodium deoxycholate in the ratio of 80:20. High entrapment efficiency of Span 60 may be due to the solid nature, hydrophobicity and high-Phase transition temperature. The entrapment efficiency was found to increase when the Non ionic surfactant: Edge activator ratio was increased to 80:20. When the proportion of NIS and EA was further increased (85:15), it was interesting to note that the entrapment

efficiency decreased indicating that beyond an optimum concentration, the NIS showed drug leakage and aggregate formation.

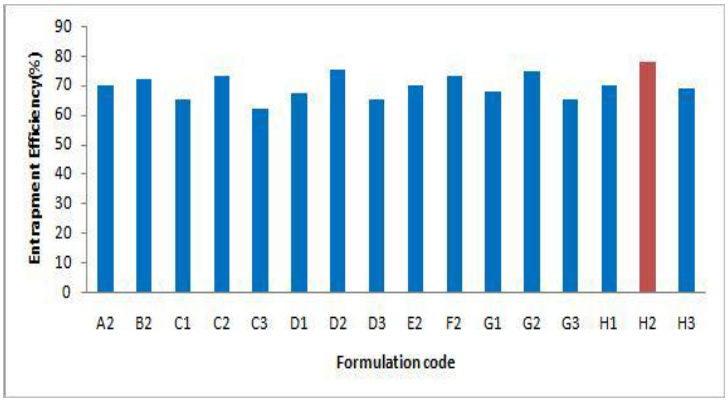

Figure 6: entrapment efficiency of selected formulations

Table 4: Entrapment efficiency and percent drug release of selected formulations

S.No.	Formulation code	%EE	% DR
1	A2	70±1.33	69±2.65
2	B2	72±0.97	74±2.41
3	C1	65±0.56	66±4.53
4	C2	73±2.43	70±4.53
5	C3	62±1.34	63±3.75
6	D1	67±2.34	68±.276
7	D2	75±0.76	77±3.56
8	D3	65±1.56	64±2.76
9	E2	70±2.65	72±2.65
10	F2	73±2.54	74±2.41
11	G1	68±1.43	70±4.53
12	G2	74.5±2.34	75±5.23
13	G3	65±2.65	65±3.75
14	H1	70±2.32	71±.276
15	H2	78±2.54	80±3.56
16	H3	69±2.65	68±2.76

EE=Entrapment Efficiency, %DR= percentage drug release.

4.5 I*n-v*itro diffusion Studies

The percentage drug release of span 40-based spanlastics was in the range of 63 to 77%, while that of span 60-based formulations was in the range of 65 to 80%. H2 formulation showed highest % drug release of 80% in 8h. The Rate of drug release was influenced by the concentration of non ionic surfactant and edge activator concentration as shown in Fig. 8, Fig. 9 and Fig. 10. It was observed that drug release was increased with an increasing the

amount of non ionic surfactant. Concentration of edge activator also affects drug release from spanlastics.

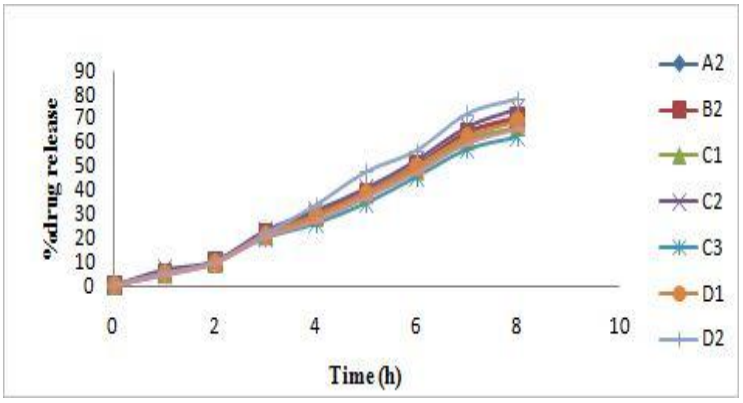

Figure 7: Cumulative percent drug release of selected span 40 formulations

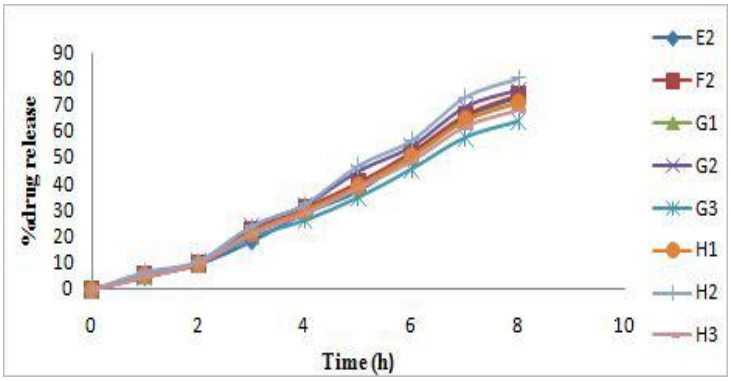

Figure 8: Comparative percent drug release of selected span 60 formulations

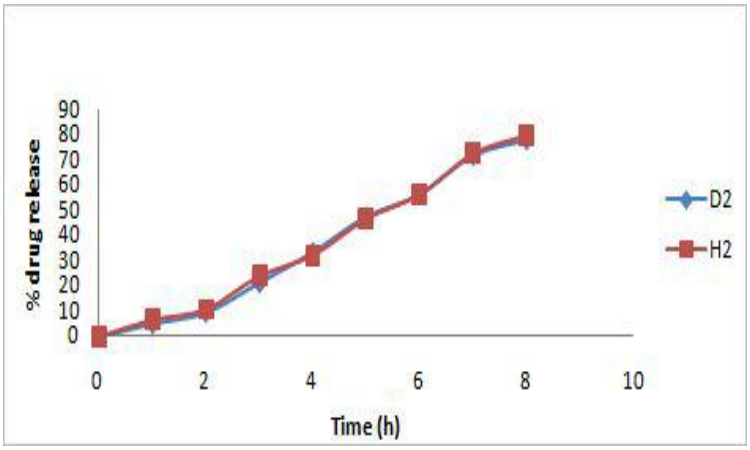

Figure 9: Comparative drug release of span 40 and span 60 formulations

4.6 Stability Studies

It was observed that there was no change in the physical appearance of the formulation. The drug content was analyzed and there was marginal difference between the formulations kept

at different temperatures as shown in Table 5. Spanlastics suspension retained good stability throughout the study.

Table 5. Stability studies

Optimized Formulation	Storage conditions	Physical appearance	Percentage entrapment efficiency			
			Initial	1 month	2 months	3 months
H2	25°C±2°C/60±5% RH	Clear solution	80%	79.78%	78.67%	77.86%
	40°C±2°C/75±5% RH	Clear solution	80%	79.69%	78.20%	77.36%

5. CONCLUSION

Sustained release spanlastics were successfully formulated using Span 40, Span 60 as non ionic surfactants and Tween 20, Tween 60, Tween 80, Sodium taurocholate and Sodium deoxycholate as edge activators. Prepared spanlastics showed good entrapment efficiency and sustained release up to 8h. The stability studies indicate that the optimized formulation was stable without physical and chemical degradation. Melphalan loaded spanlastics could be a promising dosage form to improve sustained release of Melphalan by oral route.

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