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Research Article

FORMULATE, OPTIMIZE AND EVALUATE LIPOSPHERES CONTAINING THE ANTIHYPERTENSIVE DRUG VERAPAMIL

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Abstract

This study focused on the formulation, optimization, and evaluation of lipospheres containing the antihypertensive drug Verapamil. The lipospheres were developed using the melt dispersion technique, and various formulations were prepared with different lipid core compositions.The formulations (Fl to F6) varied in the quantities of drug (Verapamil), lipid core components (stearic acid and cetyl alcohol), surfactant (Tween 80), stabilizer (gelatin or pectin), and water. The percentage yields were determined for each formulation, with values ranging from 69.98% to 81.12%.The percentage drug entrapment efficiency was assessed, showing values between 65.58% and 80.22%.

Introduction

Recently, advances in pharmaceutical research is focused on new delivery systems utilizing new devices to achieve modification of delivery time, targeting, as well as improve the invivo solubility and hence bioavailability of poorly soluble drugs. Lipospheres are new type of drug delivery system developed mainly for parenteral system. As most of the recent drugs discovered (nearly 40-45 %) are lipophilic in nature having poor water solubility and less bioavailability. Hence researchers are facing challenges in developing drug delivery system which is safe as well as effective. Lipospheres are made of solid hydrophobic triglycerides containing active moiety either dissolved (Lipophilic) or dispersed (Hydrophobic) & having a monolayer of phospholipids embedded on the surface of the particle. Lipospheres derived their name from lipid microspheres .

Composition of liposphere

Lipospheres are composed of solid lipid core surrounded by a single unit phospholipid layer that may entrap the drug or enrich its coat with the drug. The emulsifier or stabilizer is used to form uniform coat around the core material and to facilitate partitioning of the drug between the lipid and aqueous phases. Low molecular polyethylene glycols (PEGs), as plasticizers could impart tensile strength to the external lipid coat. The strong affinity between progesterone, a lipophilic drug and lipid was observed and evidenced with highentrapment efficiency (EE) of 70% which resulted in sustained release. Sodium cromoglycate release was found to be dependent on the principle of the stabilizer. Different stabilizers such as gelatin and poloxamer 407 used in hydrophilic drug containing liposphere formulation have exhibited sigmoid release and biphasic release sequentially. Morphology and characteristics of lipospheres are affected of excipient. [1-3]

Materials and Methods

Formulation and development of Liposphere of Verapamil[4-12]

Lipospheres containing drug were developed using the melt dispersion technique as outlined by Bhosale et al. in 2016. The formulation details for various batches are provided in Table 7.1. In a nutshell, the lipid core was melted using a water bath maintained at 70-72 ^{0}C. The finely powdered drug was dispersed into the molten lipidic phase. Simultaneously, the aqueous phase was prepared by heating a combination of water and surfactant to 70-72 ^{0}C along with a stabilizer.

The molten lipidic phase was gradually introduced into the hot aqueous phase, resulting in an oil-in-water (o/w) emulsion. Emulsification was facilitated by stirring the content on a sonicator continuously. The resulting milky dispersion was swiftly cooled to 20 ^{0}C by immersing the formulation in an ice bath, all while maintaining agitation to ensure a consistent dispersion of lipospheres. Subsequently, the obtained lipospheres underwent washing with water and were isolated through filtration.

Table 7.1: Preparation of Liposphere of Verapamil

F. Code	Drug (mg)	Lipid core (mg)		Tween 80 as Surfactant (ml)	Gelatin or pectin as Stabilizer (mg)	Water (ml)
		Stearic acid (mg)	Cetyl alcohol (mg)			
	120	50	50	I .5ml	2	98
F2	120	100	100	I .5ml	2	98
	120	150	150	1.5m1	2	98
	120	50	100	1.5m1	2	98
	120	100	200	1.5m1	2	98
	120	150	300	I .5ml	2	98

7.2 Characterization of Verapamil encapsulated lipospheres

7.2.1 Percentage yield of Lipospheres

Yield of Lipospheres percent w/w was calculated according to the following formula:

$$\% \text{ Yield} = \frac{\text{Weight of lipospheres}}{\text{Wt. of drug + Wt. of excipients}} X100$$

7.2.2 Drug loading and Entrapment efficiency

The amount of Verapamil present in lipospheres was determined by taking the known amount of lipospheres in which I Omg of drug should be present theoretically. Then the lipospheres were crushed and the powdered microspheres was taken and dissolved in 10 ml of methanol and stirred for 15 minutes with an interval of 5 minutes and allowed to keep for 24 hours (Cherniakov et al., 2012). Then the solution was filtered through whatmann filter paper. Then the absorbance

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after appropriate dilution was measured spectrophotometrically at 278nm by UV-visible spectrophotometer.

$$\text{Drug entrapment efficiency(\%)} = \frac{\text{Experimental Drug Content}}{\text{Initial Drug Content in the formulation}} \times 100$$

7.2.3 Microscopic Evaluation

An optical microscope (Cippon-Japan) with a camera attachment (Minolta) was used to observe the shape of the prepared microspheres for each drug: lipid ratio (Brown et al., 2013).

7.2.4 Measurement of mean particle size

The mean size of the liposphères was determined by Photo Correlation Spectroscopy (PCS) on a submicron particle size analyzer (Malvern Instruments) at a scattering angle of 90^0 . A sample (0.5mg) of the liposphères suspended in 5 ml of distilled water was used for the measurement (Nasr et al., 2008).

7.2.5 Determination of zeta potential

The zeta potential of the drug-loaded liposphères was measured on a zeta sizer (Malvern zetasizer Instruments) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water.

7.2.6 Surface morphology (Scanning electron microscopy)

Morphology and surface topography of the liposphères were examined by scanning electron microscopy. The liposphères from the optimized batch were mounted on the SEM sample stab using a double-sided sticking tape and coated with gold (— 200 nm) under reduced pressure (O. 133 Pa) for 5 min using an Ion sputtering device. The gold coated liposphères were observed under the scanning electron microscope and photomicrographs of suitable magnifications were obtained.

7.2.7 Flow property determination of the Liposphères

Bulk density: Both loose bulk density (LBD) and tapped bulk density (TBD) were determined (Newman, 1995). Accurately weighed amount of granules taken in a 50 ml capacity measuring cylinder was tapped for 100 times on a plane hard wooden surface and estimated the LBD and TBD, calculated by using following formulas.

$$\text{LBD (Loose bulk density)} = \frac{\text{Mass of powder}}{\text{Volume of Packing}}$$

$$\text{TBD (Tapped bulk density)} = \frac{}{\text{Tapped volume of packing}}$$

Compressibility index: Percent compressibility of powder mix was determined by Carr's compressibility index, calculated by using following formula (Newman, 1995):-

$$\text{Carr's Index} = \frac{\text{TBD --LBD}}{\text{TBD}} \times 100$$

7.2.8 In-vitro drug release studies

The dissolution of Verapamil from the prepared liposphères was monitored using USP XXV paddle Il apparatus. The Amount of the liposphères equivalent to I ()mg of Verapamil was dispersed into the dissolution medium. The dissolution media was 900 ml of pH 1.2 buffers maintained at 37 +0.5 ^{0}C and rotating at 50 +1 rpm. The 5mI aliquots were withdrawn at pre-determined time intervals and the withdrawn samples were replaced with fresh dissolution medium. The samples were then analyzed spectrophotometrically at 278 nm for Verapamil content.

Results and Discussions

Characterization of Verapamil encapsulated liposphères

8.1.1 Percentage yield of liposphères

The percentage yields of liposphères for various formulations (Fl to F6) were determined and are summarized in the provided table. Formulation F3 stands out with the highest percentage yield of 81.12%, indicating a highly efficient production process and successful generation of the intended liposphères. Formulation F 2 also demonstrates a substantial yield of 76.65%, suggesting good reproducibility and consistency in the manufacturing method. Formulations F I, F5, and F6 exhibit percentage yields ranging between 72.25% and 74.45%, indicating satisfactory outcomes with minor variations. However, Formulation F4 shows a slightly lower yield of 69.98%, prompting further investigation into potential factors influencing this deviation. The standard deviations accompanying the mean values provide insights into result precision. Overall, the variations in percentage yields among formulations highlight the importance of process optimization for enhanced reproducibility and yield consistency in liposphere production. Further studies and refinement of the manufacturing parameters may contribute to optimizing the overall efficiency of the process.

Table 8.1: Percentage yields of liposphères

S.No	Formulation Code	% Yield*
1	F1	73.25±0.15
2	F2	76.65±0.32
3	F3	81.12±0.22
4	F4	69.98±0.14
5	F5	72.25±0.32
6	F6	74.45±0.15

*Average of three determinations

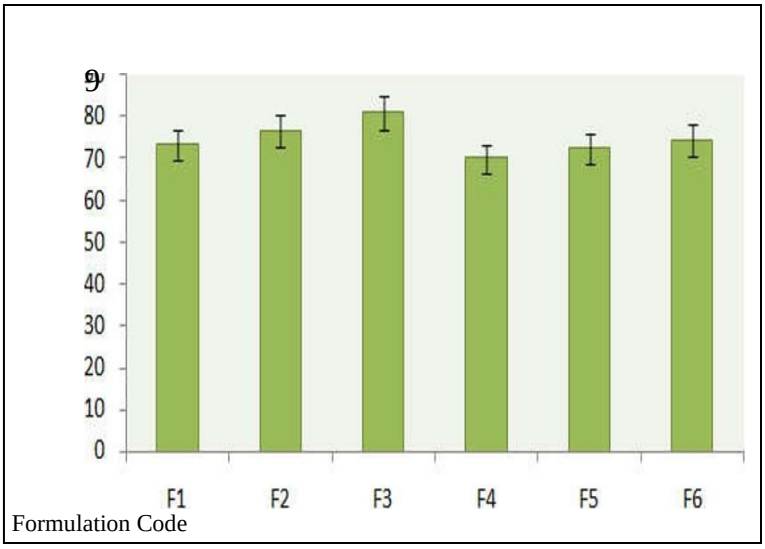


Figure 8.1: Percentage yields of liposphères

8.1.2 Drug loading and Entrapment efficiency

Table 8.2 presents the percentage drug entrapment efficiency for the prepared Verapamil lipospheres formulations, with each formulation identified by a specific code (Fl to F6). The entrapment efficiency reflects the proportion of the drug successfully encapsulated within the lipospheres during the manufacturing process.

Formulation F3 exhibits the highest drug entrapment efficiency at 80.22%, suggesting an effective encapsulation of Verapamil within the lipospheres. This formulation appears to be particularly successful in retaining a significant amount of the drug, which is crucial for ensuring the therapeutic efficacy of the lipospheres.

Formulations F2, F5, and F6 also demonstrate favorable drug entrapment efficiencies ranging from 71.15% to 75.65%. These values indicate reliable drug encapsulation, showcasing the robustness of the manufacturing process across different formulations.

Formulation Fl shows a drug entrapment efficiency of 70.15%, indicating a satisfactory encapsulation level. However, attention should be paid to the standard deviation (0.25) to assess the precision of the results and the potential variability in drug entrapment.

Formulation F4, while exhibiting a slightly lower drug entrapment efficiency of 65.58%, may warrant further investigation into optimizing the formulation or manufacturmg parameters to enhance drug encapsulation.

Table 8.2: % Drug entrapment efficiency of prepared Verapamil lipospheres formulation

S.No	Formulation Code	% Drug entrapment efficiency
1	F1	70.15±0.25
2	F2	75.65±0.32
3	F3	80.22±0.14
4	F4	65.58±0.16
5	F5	71.15±0.22
6	F6	73.65±0.18

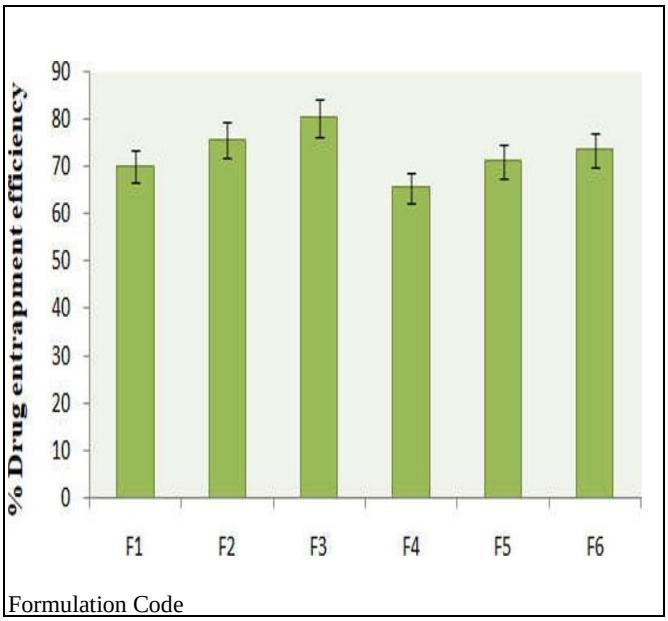
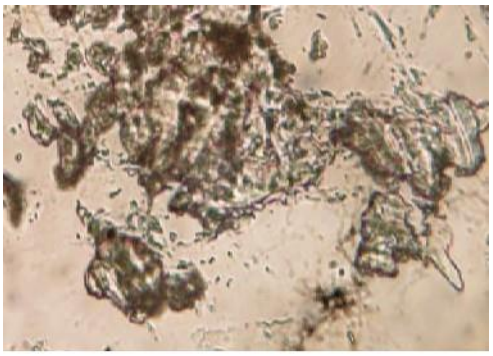


Figure 8.2: Drug entrapment efficiency of lipospheres

8.13 Microscopic Evaluation

An optical microscope (MSW) with a camera attachment (olympus) was used to observe the shape of the prepared microspheres for each drug: lipid ratio. Microscopic examination can provide information about the size and size distribution of the lipospheres. It can reveal whether the lipospheres are uniform in size or if there is a wide range of particle sizes. A narrow size distribution is often desirable for pharmaceutical formulations, as it can lead to more consistent drug release and distribution in the body. The microscopy images can show the shape and morphology of the lipospheres. This information is crucial as it can impact drug release kinetics and stability. Ideally, the lipospheres should have a spherical or nearly spherical shape, as this shape is associated with better flow properties and ease of administration. The presence of agglomerates or clusters of lipospheres can be observed under the microscope. Agglomeration can affect the dispersibility of the formulation and may lead to inconsistent drug release. It's important to assess whether agglomeration is present and, if so, consider strategies to minimize it during formulation. Microscopy can reveal surface characteristics such as smoothness or roughness. A smooth surface is often preferred for drug delivery systems, as it can reduce interactions with biological components and improve stability



F3

Figure 8.3: Microscopic observation of liposphere formulation F3

8.1.4 Particle size

The mean size of the lipospheres was determined by photo correlation spectroscopy (PCS) on a submicron particle size analyzer (Horiba Instruments) at a scattering angle of 90^0 . A sample (0.5mg) of the lipospheres suspended in 5 ml of distilled water was used for the measurement. The results of measurement of mean particle size of optimized formulation F3 lipospheres were found 215.45 nm.

Figure 8.4: Particle size data of optimized lipospheres formulation F3

The mean particle size of the lipospheres was determined using photo correlation spectroscopy (PCS) with a submicron particle size analyzer (Horiba Instruments) at a scattering angle of 90^0. For the measurement, a sample containing 0.5 mg of lipospheres suspended in 5 ml of distilled water was utilized. The results indicate that the mean particle size of the optimized formulation, F3 lipospheres, was found to be 215.45 nm.

The particle size is a crucial parameter in drug delivery systems, as it directly influences various aspects of their performance, including bioavailability, stability, and cellular uptake. The obtained particle size of 215.45 nm for formulation F3 suggests

the successful generation of submicron-sized lipospheres. Submicron sizes are often desirable in pharmaceutical formulations as they can enhance drug delivery properties, such as improved drug solubility, increased surface area for drug release, and potential for enhanced cellular uptake.

8.1.5 Zeta Potential

The zeta potential of the drug-loaded lipospheres was measured on a zeta sizer (Malvern Instruments) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water Results of zeta potential of optimized formulation F3 lipospheres was found -29.4 Mv

Figure 8.5: Zeta potential data of lipospheres formulation F3

Zeta potential is a measure of the electrostatic charge at the surface of nanoparticles, including lipospheres. The negative zeta potential value of -29.4 mV indicates that the lipospheres in this formulation have a predominantly negative charge. This can be beneficial for their stability in aqueous dispersion because particles with high absolute zeta potential values (either positive or negative) tend to repel each other, reducing the likelihood of aggregation or flocculation. Since the measurements were conducted in water, the negative zeta potential suggests that the lipospheres are well-dispersed in an aqueous medium. This is important for pharmaceutical formulations, as it ensures that the lipospheres remain in a dispersed state, preventing them from clumping or settling, which can negatively impact drug delivery.

The zeta potential is influenced by the surface properties of the lipospheres, including the composition of the lipids and any surface-active agents or stabilizers used in the formulation. The negative charge may be attributed to the presence of negatively charged functional groups or surfactants on the liposphere surface.

The negative zeta potential can also affect the interaction of lipospheres with biological systems. In some cases, lipospheres with a negative charge may have reduced interactions with negatively charged biological components, such as cell membranes, which can be advantageous for drug delivery applications.

8.1.6 Surface morphology (SEM)

Morphology and surface topography of the lipospheres were examined by scanning electron microscopy.

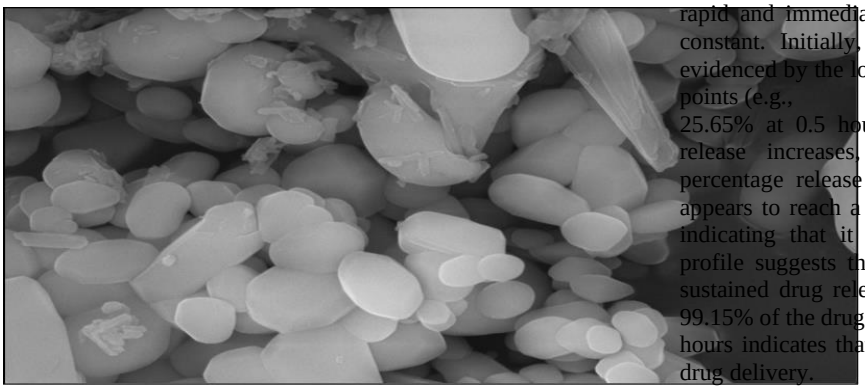


Figure 8.6: SEM Image of Optimized Formulation

8.1.7 Flow property determination of the Liposphere s

Table 8.3: Result of flow properties of different liposphere formulation

The flow properties of different liposphere formulations are assessed using parameters like loose bulk density, tapped bulk density, Carr's index, and Hausner's ratio. These properties are essential in pharmaceutical formulations

Bulk Density (Loose and Tapped):

Loose bulk density represents the density of the lipospheres when they are loosely packed or have minimal compaction. It ranges from 0.632 gm/ml (F3) to 0.785 gm/ml (Fl). Tapped bulk density represents the density of the lipospheres when they are subjected to tapping or compaction. It ranges from 0.853 gm/ml (F3) to 0.995 gm/ml (F I). The difference between loose and tapped bulk densities provides insight into the compressibility and cohesion of the lipospheres. Higher differences between loose and tapped bulk densities (as seen in FI and F4) suggest better flowability, as the lipospheres can undergo more compaction and still flow freely.

Carr's Index:

Carr's index is a measure of flowability and compressibility. It is calculated using the formula: (Tapped bulk density - Loose bulk density) / Tapped bulk density * 100%.

Carr's index is inversely related to flowability; lower values indicate better flowability.

The Carr's index values for these formulations range from 20.966% (F6) to 25.909% (F3). F6 has the lowest Carr's index, suggesting that it has the best flowability among the formulations, while F3 has the highest Carr's index, indicating relatively poorer flowability.

Hausner's Ratio:

Hausner's ratio is another indicator of flowability and is calculated as Tapped bulk density / Loose bulk density.

A Hausner's ratio less than 1.25 typically indicates good flow properties, while values above I .25 suggest poorer flow.

All formulations have Hausner's ratios less than I .25, which is generally favorable for flow.

F3 has the highest Hausner's ratio (1.350), indicating less favorable flow properties, while

F6 has the lowest ratio (I. 265), suggesting better flowability.

8,18 In vitro dug release study of optimized formulation F-3

Table 8.4: Release of Formulation F-3

The data indicates that there is progressive drug release over time. At each time point, more of the drug is released into the surrounding medium. This suggests that the formulation is designed for controlled or sustained drug release, as opposed to rapid and immediate release. The rate of drug release is not constant. Initially, there is relatively slow drug release, as evidenced by the low cumulative percentages at the earlier time points (e.g.,

25.65% at 0.5 hours). As time progresses, the rate of drug release increases, with a steeper incline in cumulative percentage release between 2 and 6 hours. The formulation appears to reach a plateau in drug release at later time points, indicating that it approaches complete release. The release profile suggests that the formulation provides controlled and sustained drug release over an extended period. The fact that 99.15% of the drug is released by 12

hours indicates that this formulation is designed for prolonged drug delivery.

The drug release kinetics is critical for achieving the desired therapeutic effect. In some cases, sustained release formulations are preferred to maintain a consistent drug concentration in the bloodstream over time, reducing the need for frequent dosing.lt's important to note that in vitro drug release data is a preliminary indicator of formulation performance. In vivo

studies are needed to confirm how the formulation behaves in the body and whether it achieves the desired therapeutic effect.

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	Size (r.nm)	% Number	Width (r.nmy:
Z-Average (r.nm): 215.45	Peak 1: 215.45	100.0	48.58
Pdl: 0.345	Peak 2: 0.00	0.0	0.000 Intercept: 0.615 peak 3: 0.000 0.0

0.000

Result quality : Good

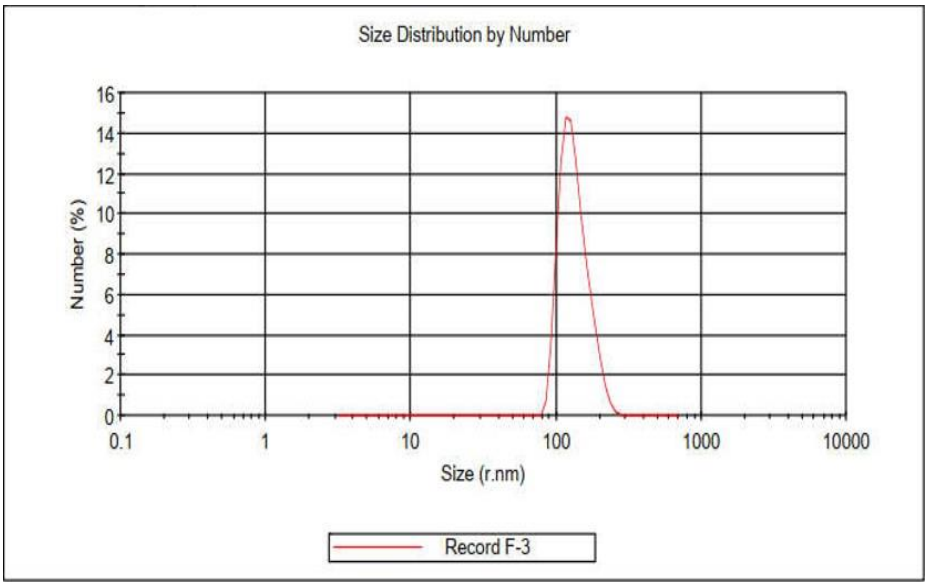

Figure 8.4: Particle size data of optimized lipospheres formulation F3

Results

	Mean (mV)	Area	Width	(mV)
zetapotenual (ITV): -29.4 peak 1: -29.4	100.0	322 zeta Deviation	322	peak 2:
0.00 0.0	0.00 Conductivity (mS/cm): 0.249	peak y. 0.00	0.0	0.00

Result quality : See result quality report

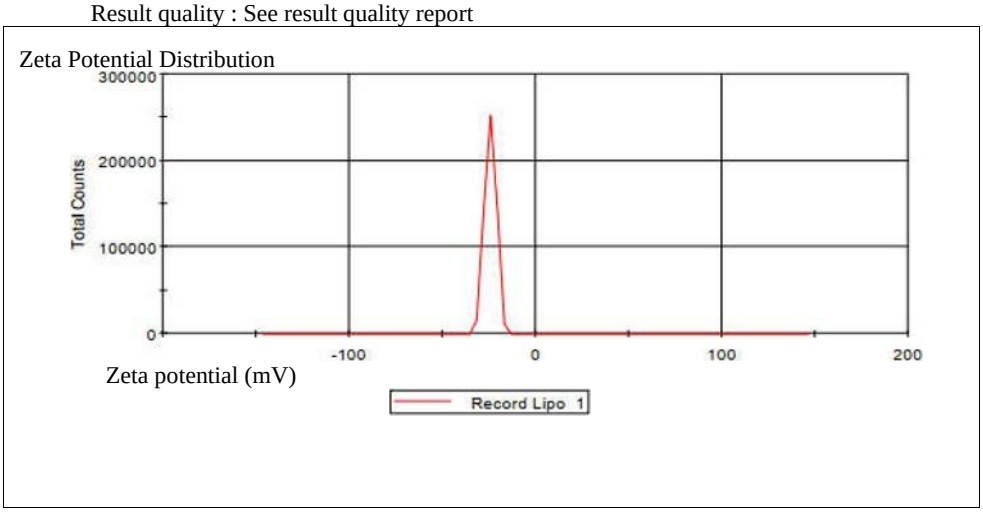

Figure 8.5: Zeta potential data of lipospheres formulation F3

Formulation code	Parameters			
	Loose Bulk density(gm/ml)	Tapped bulk density(gm/ml)	Carr's Index (%)	Hausner's Ratio
	0.785	0.995	21.106	1.268
	0.762	0.982	22.403	1.289
	0.632	0.853	25.909	1.350
	0.715	0.925	22.703	1.294
	0.712	0.926	23.110	1.301
	o. 769	0.973	20.966	1.265

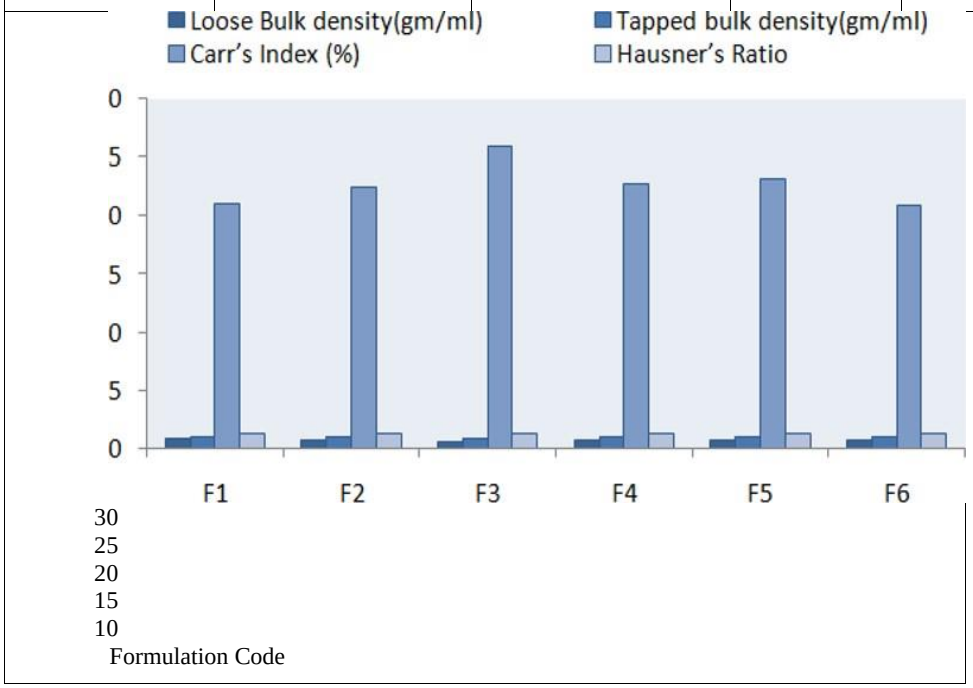

Figure 8.7: Graph of flow properties of different lisposphere formulation