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Preparation, characterization, and evaluation of solid lipid nanoparticles containing Herbal extracts

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Abstract:

Objective: In this work, Solid lipid nanoparticles (SLNs) loaded with Herbal extracts (Kalonji, Gorakhmundi, Kamrakh, Lotus) were synthesized via the microemulsion method, and their physicochemical properties were studied.

Methods: Herbal extracts loaded SLNs were prepared using the microemulsion method. The particle size of SLNs was determined by a particle size analyser. The morphology and size of SLNs were evaluated by scanning electron microscopy (SEM). Compatibility study between pure extracts & SLNs loaded extracts was done by FTIR.

Results: The spherical structure of SLNs was confirmed by SEM images. The mean particle size of the obtained SLNs ranged from 48-68 nm for different extracts. The study shows a compatibility study of pure drug extracts with prepared SLNs by FTIR, which does not show any major interactions. Based on SEM analysis, SLNs had a spherical shape & good & suitable stability. Thus, it can be concluded that SLNs can be successfully prepared by the microemulsion technique.

Conclusions: According to the above results, SLNs loaded with Herbal extracts showed acceptable particle size and shape.

Keywords: Solid Lipid Nanoparticles, Herbal Extracts, Microemulsion, Stearic acid, Tween 80, Soy lecithin.

1. Introduction[1, 2]:

The use of herbal supplements has increased unbelievably over the past 50 years due to fewer side effects. Solid lipid nanoparticles (SLNs) are important in cosmetics because they can improve the efficacy & functionality of cosmetic products. Ingredients: SLNs provide a protective environment for encapsulated ingredients, which reduces degradation & improves shelf life. It enhances skin penetration: SLNs' nano size promotes better skin hydration & the targeted delivery of bioactive compounds. SLNs can protect skin against harmful radiation. SLNs are used to increase the bioavailability of drugs & other substances. Compared to colloidal carriers, SLNs offer several advantages such as controlled drug release, drug targeting, ability to combine hydrophobic & hydrophilic drugs, lack of biotoxicity, no use of organic solvents, helping the stability of the drug & the absence of large-scale manufacturing issues. Here, SLNs were produced using the microemulsion technique. Microemulsion technique offers some advantages over other techniques, such as reproducibility, simplicity, does not require organic solvents, requires low mechanical energy output, ease of preparation, has a high-water content & resulting in low cost & improved formulation flexibility. Here, SLNs were constituted mainly of physiological or physiologically related lipids such as stearic acid. Stearic acid is used as a lipid matrix in solid lipid nanoparticles. Stearic acid is a good choice for SLNs because it has a lower heat of fusion than other long-chain fatty acids, & it's a single compound, so the quality of the SLNs doesn't change based on lipid composition. Soy lecithin was used as a surfactant & stabilizer, which stabilizes the shells of the SLNs, which are made up of various surfactants &

emulsifiers. Soy lecithin stabilizes the particles in SLNs. Tween 80 was used as a co-emulsifying agent in solid lipid nanoparticles. Tween 80 is a hydrophilic non-ionic surfactant, used to reduce the size of globules in lipid emulsions. This study aimed to prepare and evaluate Herbal extracts (Kalonji, Karakh, Lotus, Gorakhmundi)-loaded SLNs. The effects of the type and concentration of surfactants on the characteristics of SLNs, for example, particle size, FT-IR, Zeta potential, and morphology, etc. were observed.

2. Materials & methods[1, 2, 7, 12,]:

2.1. Materials:

Stearic acid, Tween 80 & Soya lecithin were used as Lipid, Surfactant & Co-surfactant, respectively. All reagents and solvents used in the study were of analytical grade. All plant extracts were obtained from Bhagvati Herbals & Healthcare Private Limited, Vapi.

2.2. Preparation of SLNs:

SLNs were prepared by the microemulsion method. Stearic acid (500 mg) was added into a conical flask & the flask was put into a porcelain dish filled with water & it was put on a magnetic stirrer at 70˚C. After that drug extract (1 gm) (Kalonji, Gorakhmundi, Kamrakh, Lotus), Tween 80 (3.4 ml) & Soy lecithin (1.1 ml) & water (2.1 ml) were added & stirred at 1000 RPM until it was well mixed. The prepared emulsion was diluted with ice-cold water by adding it dropwise into ice-cold water & it was stirred by using a mechanical stirrer in a cold-water bath at 3000-3100 RPM for 2-3 hours. The prepared suspension was sonicated for half an hour. Sonicated suspension was centrifuged at 14500 RPM at 8˚C for 5 minutes to separate probable nanoparticles & nanoparticles were collected by using methanol & kept drying until it was completely dry.

FIG 1 - Steps of preparation of SLNs

2.2.1. Freeze-drying of SLN:

The SLNs were lyophilized to prolong the shelf-life of the Herbal extract-loaded SLNs. Lyophilization of particles was done using 5% (w/v) mannitol as cryoprotectant to limit the risk of particle aggregation. SLN's emulsion was frozen at -40˚C for 24 hours, and then lyophilization was done. Particle size, zeta potential, and polydispersity index were evaluated after freeze-drying again to ensure no significant size enlargement was happening.

2.2.2. Characterization of SLNs[20, 21, 22]:

2.2.2.1. Particle Size & Zeta Potential:

The mean particle size and polydispersity index of the SLN formulations were assessed by the dynamic light scattering (DLS) (Zeta Sizer Nano-ZS; Malvern Instruments) method. Zeta potential was measured by Horiba Scientific SZ-100.

2.2.2.2. Scanning Electron Microscopy (SEM):

The morphological examination of SLNs was performed by SEM (JEOL JSM 7600F FE-SEM). Before analysis, 100 μl of SLN dispersion was dried overnight under vacuum on an aluminium stub. This was then sputter-coated using a thin platinum layer under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. These coated samples were then subjected to scanning, and photomicrographs were taken at an acceleration voltage of 15 kV.

2.2.2.3. FTIR Characterization of SLNs:

FTIR is used for identifying functional groups, studying chemical reactions. FTIR was done by FTIR 7800A- A model from Shimadzu.

3. Results & Discussion[15, 16, 18, 19]:

3.1. Particle size analysis & PDI Determination:

In the present study, the analysis of prepared SLNs is summarized in the table given on slide 107. PS ranging from 48-68 nm & PDI ranging from 0.17-0.37. Formation of all SLNs showed that about 82%, 95%, 98% & 100 % volume of the particles are in the narrow range in lotus, Kamrak, kalonji & gorakhmundi extracts, respectively. PDI values of less than 0.5 were obtained for all SLNs. The low value of PDI indicated that, using optimal conditions, we could manufacture stable SLNs with a relatively narrow particle size distribution. Surfactant plays an important role in an emulsion. It helps the stabilization of the system & controls the particle size. It was found that PS decreased as the concentration of surfactant was increased. This could be due to the significant reduction in the interfacial tension between the organic phase, which leads to a more homogenized lipid in the aqueous phase & the reduction of the particle size of SLNs. Also, at high surfactant concentration, surfactant molecules sufficiently cover the lipid matrix, leading to more stable particles. On the other hand, there is a report that a relatively high concentration of surfactant is needed to prevent particle aggregation. The particle size of SLNs was increased with the incorporation of drug extracts. Highly positive or negative values of zeta potential, an indicator of surface charge, stabilize SLNs & prevent aggregation. The surface charge of SLN dispersion is considered a significant characteristic of the particles, as this often controls the stability behavior when particles approach each other.

Table 1: Particle size & PDI Values

SLNs	PDI	Particle size Average (nm)
Gorakhmundi	0.172	56.90
Kalonji	0.251	48.77
Kamrakh	0.285	50.18
Lotus	0.374	68.75

3.2. Zeta Potential:

Highly positive or negative values of zeta potential, an indicator of surface charge, stabilize SLNs & prevent aggregation. The surface charge of SLN dispersion is considered a significant characteristic of the particles, as this often controls the stability behavior when particles approach each other. Zeta potential indicates a repulsive force between NPs to prevent the aggregation of NPs in the process of emulsion stabilization. The measured potential is the most appropriate potential, which is known as the zeta potential. Zeta potential of the prepared SLNs ranges from -5.0 mV to -19.1 mV. All SLNs showed a negative zeta potential since tween 80 & stearic acid have a negative charge on their surface. All SLNs were found to be negatively charged due to fatty acid residues of stearic acid. Furthermore, surfactant concentration revealed a significant impact on zeta potential as SLNs were perfectly covered by a non-ionic surfactant like Tween 80. SLNs tend to remain stable despite having a lower zeta

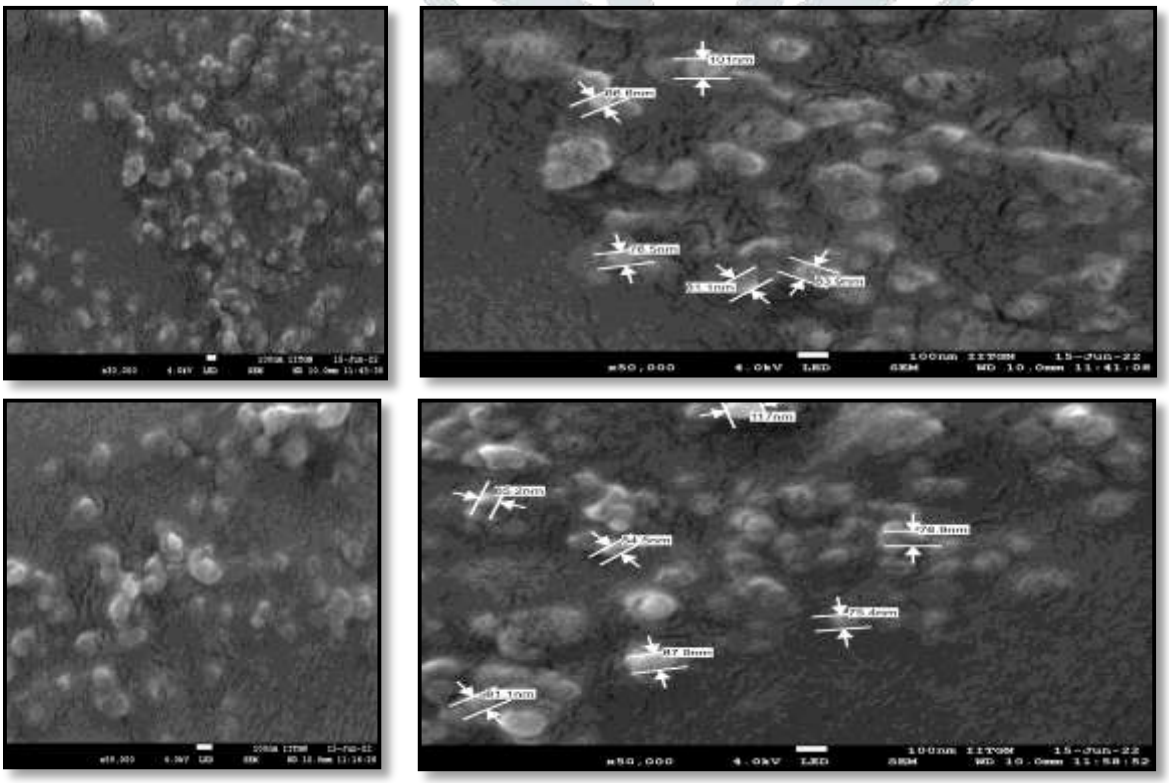
potential. This could be attributed to greater stabilization. All SLNs had lower zeta potential values due to the existence of oleic acid traces in Tween 80. In addition, the surface coverage of the SLNs reduces the electrophoretic mobility of the particles & thus lowers the zeta potential. On the other hand, a higher concentration of surfactant showed a negative effect on the zeta potential & this is due to the non-ionic nature of Tween 80 that broadens the electric double layer surrounding the SLNs & thus decreases zeta potential. Thus, the size distribution exhibited good unimodal behavior as deduced in the figures & showed polarity of the prepared SLNs with zeta potential in the range of -5.0 mV to -19.1 mV.

Table 2: Zeta Potential Value

SLNs	Zeta potential (mV)
Gorakhmundi	-5.0
Kalonji	-17.8
Kamrak	-19.1
Lotus	-9.0

3.3 Scanning Electron Microscopy:

The scanning electron microscope (SEM) is one of the most widely used techniques in the characterization of nanomaterials and nanostructures. The signals that derive from electron-sample interactions reveal information about the sample, including surface morphology (texture). From surface morphology study by SEM, it was observed that formulated SLNs had near-spherical shapes with smooth surfaces & uniformly distributed. The particle size of all SLNs was below 100 nm. The SEM results revealed spherical NPs morphology for SLNs, which confirmed the size results with no evident sign of aggregation. SEM images confirmed the stability of SLNs.



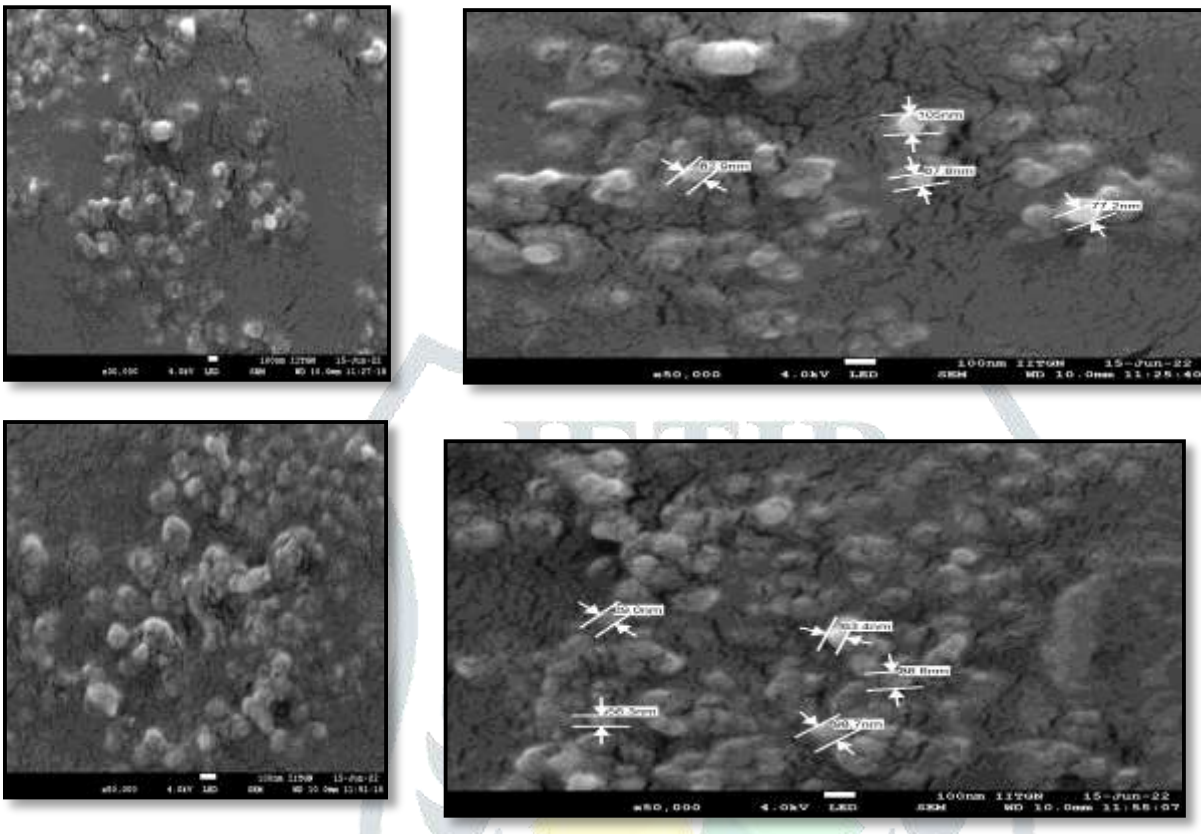
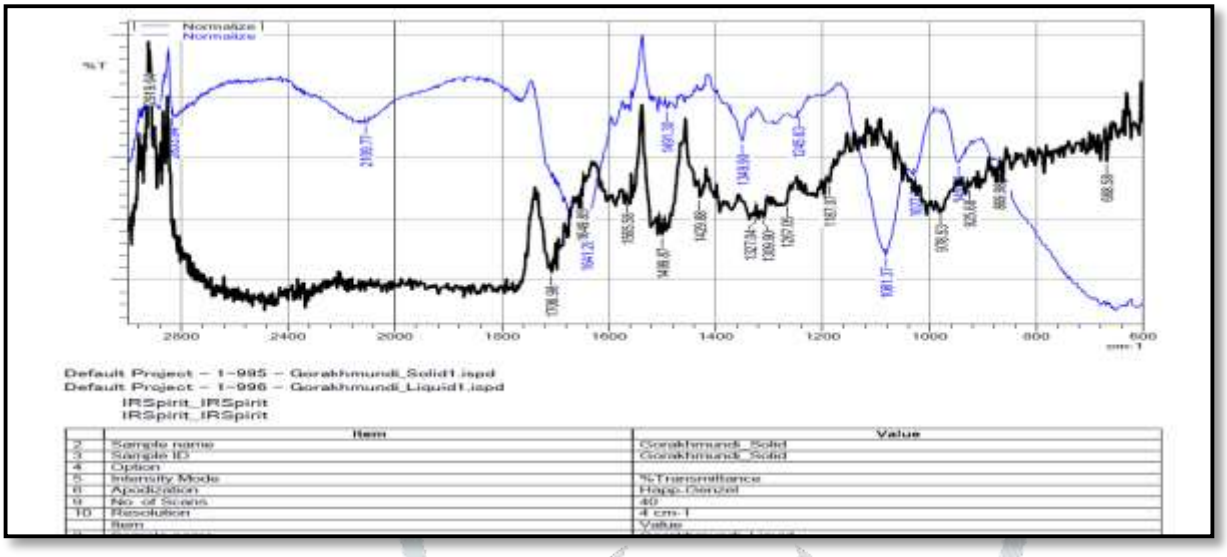


FIG 2 - SEM Images of Kalonji, Kamarak, Lotus & Gorakhmundi SLNs

3.4. FTIR Characterization of SLNs:

Compatibility study between pure drug extract & SLNs was done by FTIR (SHIMADZU FTIR). The FTIR spectra of pure drug extract & the physical mixture of drug extract with stearic acid, soy lecithin & tween 80 are given in the diagram given above. The IR spectra of pure drug extract show principal peaks that are mostly near the principal peaks of the prepared SLNs. For Gorakhmundi, SLNs show major peaks at 2833.94, 2109.77, 1641.28, 1491.30, 1245.63, 1081.37, 1027.09, & 941.68 cm-1, which are mostly near the principal peaks of pure drug extract. For Kalonji, SLNs show major peaks at 2818.23, 1648.42, 1498.44, 1349.90, 1252.77, 1084.23, 1027.09 & 942.82 cm-1, which are mostly near the principal peaks of pure drug extract. For Kamrak, SLNs show major peaks at 2109.77, 1654.13, 1349.90, 1257.06, 1084.23, 1031.38 & 947.11 cm-1, which are mostly near the principal peaks of pure drug extract. For Lotus, SLNs show major peaks at 2923.93, 1655.56, 1475.69, 1349.90, 1296.62, 1252.77, 1099.94, 1024.24, 948.53 & 710.00 cm-1, which are mostly near the principal peaks of pure drug extract. Thus, it is concluded that pure drug extracts do not show any major interactions with the formulation components like lipid, surfactant & co-surfactant.



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Default Project = 1~996 = Gorakhmundi_Liquid1.ispd
IRSpirit_IRSpirit
IRSpirit_IRSpirit

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3	Sample ID	Gorakhmundi_Solid
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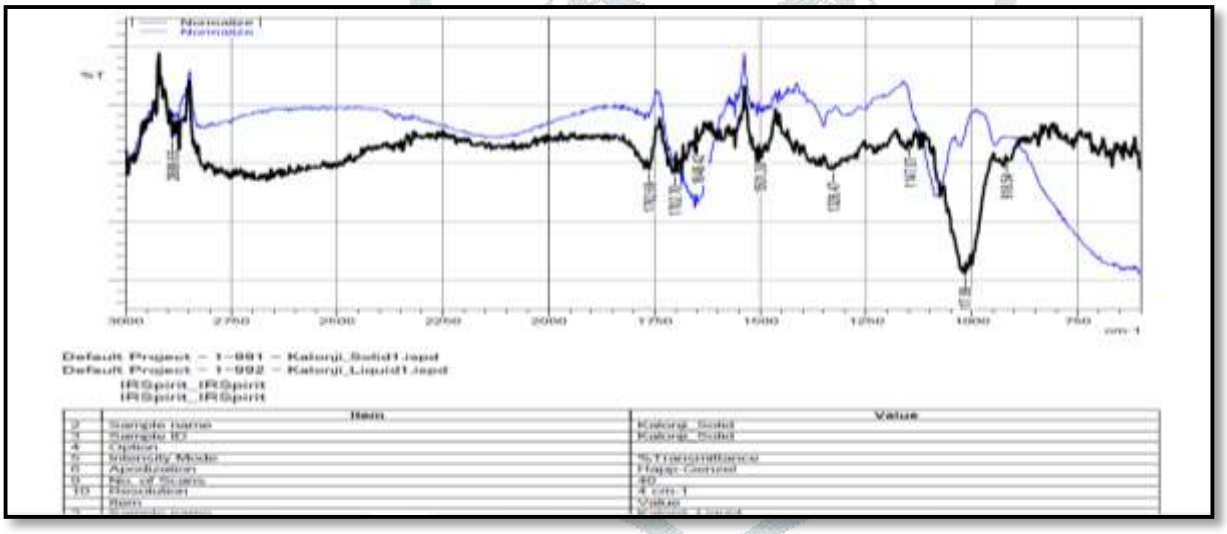
	Item	Value



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IRSpirit_IRSpirit
IRSpirit_IRSpirit

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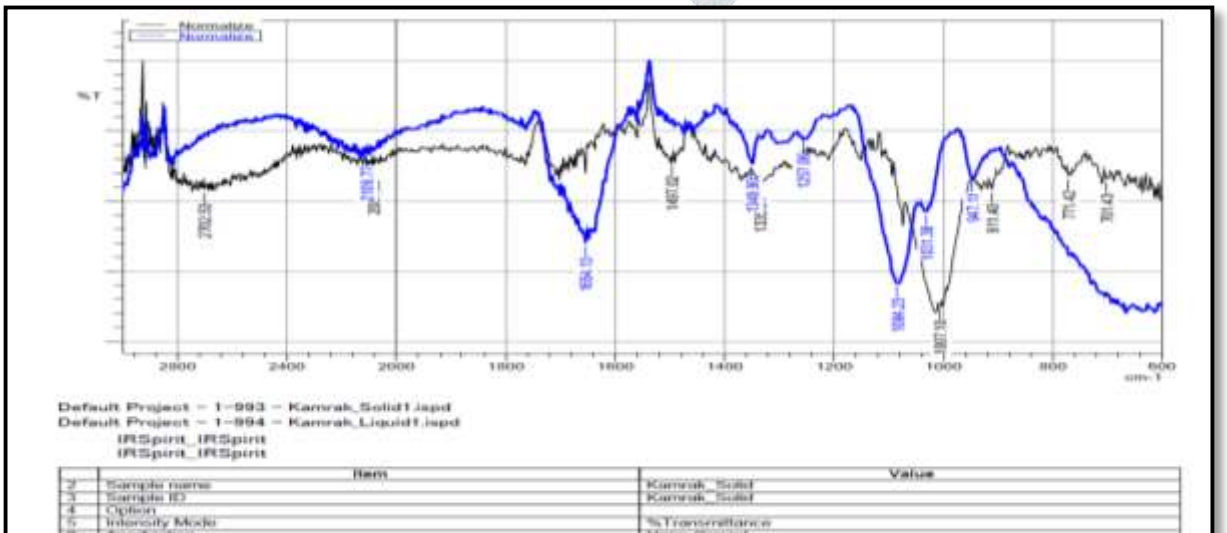

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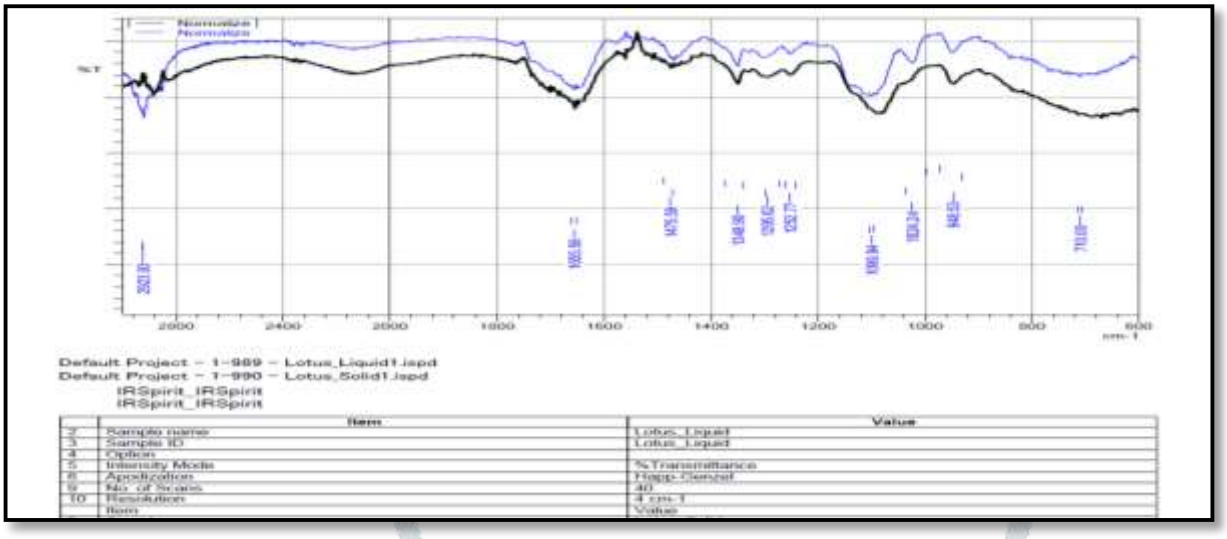

FIG 3 - FTIR Comparison of extracts & SLNs

3.5. Stability Parameters of SLNs[11]:

The changes that transpired over the 9-month test period have been summarized in the Table. Zeta-potential measurements of SLNs fluctuated over the 9 months, with the changes per formulation ranging from -3.72 mV to -7.82 mV. At the end of the 9-month testing period, all the average particle sizes were below 100 nm. PDI values ranged from 0.30 to 0.49 at the end of 9 months. SLNs stored at 4°C are said to have better stability as compared to SLNs stored at room temperature. Stability problems of nanoparticles are usually due to post-formulation expulsion of the active pharmaceutical ingredient (API) and particle aggregation. To increase the stability of nanoformulations, it may be necessary to increase surfactant content, as this increases the physical stability of the nanoparticles and also results in a high concentration of smaller nanoparticles. Initial physicochemical characterization was done on freshly prepared samples, while the stability experiments were done after freeze-drying.

Table 3: Zeta Potential stability parameters

SN	SLNs	ZP (mv) (3 months)	ZP (mv) (6 months)	ZP (mv) (9 months)
1	Gorakhmundi	-5.17	-4.03	-3.99
2	Kalonji	-7.14	-6.89	-6.71
3	Kamrak	-7.56	-7.48	-7.29
4	Lotus	-6.82	-6.31	-6.28

Table 4: Particle Size Stability Parameters

SN	SLNs	PS (nm) (3 months)	PS (nm) (6 months)	PS (nm) (9 months)
1	Gorakhmundi	84.07	83.59	83.76
2	Kalonji	77.64	71.40	71.20
3	Kamrak	72.20	71.83	71.40
4	Lotus	92.19	94.53	92.19

Table 5: PDI stability parameters

SN	SLNs	PDI (3 months)	PDI (6 months)	PDI (9 months)
1	Gorakhmundi	0.425	0.422	0.411
2	Kalonji	0.302	0.321	0.313
3	Kamrak	0.360	0.360	0.357
4	Lotus	0.493	0.474	0.486

4. Conclusion

In this research work, all herbal extracts loaded SLNs were prepared by the microemulsion technique by using stearic acid, Soy lecithin & tween 80. By the experimental study, it can be concluded that surfactant concentration affects the particle size of SLNs. All SLNs demonstrated a suitable particle size below 100 nm & PDI value obtained lower than 0.5, which is in an acceptable range in the pharmaceutical field of science. Thus, particle size confirmed the particle size in the nano range & low PDI indicated that we could manufacture stable SLNs with a relatively narrow particle size distribution. Zeta potential value indicated that prepared SLNs were attributed to greater stabilization & less electrostatic stabilization. The study shows a compatibility study of pure drug extracts with prepared SLNs by FTIR, which does not show any major interactions. Based on SEM analysis, SLNs had a spherical shape & good & suitable stability. Thus, it can be concluded that SLNs can be successfully prepared by the microemulsion technique.

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