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# PREPARATION AND *IN-VITRO* EVALUATION OF 0.05% 5-FLUOROURACIL SOLID LIPID NANOPARTICLE GEL IN THE TREATMENT OF ACTINIC KERATOSIS

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**Keywords:**

5- flurouracil, Solid lipid nanoparticle, Actinic keratosis, Cell viability.

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**ABSTRACT:** The present study focused on the formulation and in-vitro evaluation of 0.05% 5-fluorouracil solid lipid nanoparticle gel in treating actinic keratosis. The *in-vitro* release profile of solid lipid nanoparticle gel is higher than the marketed cream formulation; thus, we can reduce the dose of formulation by this method. This is prepared using tween 80, tween 20, glycerol monostearate, glycerol, and lecithin. Pre-formulation studies for the pure drug were conducted, and formulations were evaluated based on viscosity, encapsulation efficiency and *in-vitro* drug release study using franz diffusion cell. FTIR study of pure drug-polymer and formulation proves there is no chemical interaction between them. Formulation SLN2 had appropriate viscosity of 1738.34, the highest encapsulation efficiency of 94.45%, drug loading 31.97%. SLN2 shows 95% cell viability. The *in-vitro* release study was found to have a zero-order release profile. Compared to all other formulations, this indicates the predominant mechanism of drug release is diffusion.

**INTRODUCTION:** The major concern in the novel drug delivery is the controlled delivery of the pharmacological agent to its site of action at a therapeutically optimal rate of dose regimen. The site-specific or targeted drug delivery will not only for improving efficiency but also for reducing toxicity. Colloidal drug delivery includes the drug carriers like liposomes, niosomes, nanoparticles and microemulsion [1, 2, 3].

**Nanoparticles:** They are made of artificial or natural polymer. In addition to the properties of the carrier material, *i.e.*, the polymer and auxiliary substance, bio acceptability is also influenced by the particle size. A reduction of particle size enables an intravenous injection. The final choice of the appropriate polymer, particle size, and manufacturing method will primarily depend on the bio acceptability of the polymer, secondarily on the drug's physicochemical properties and the therapeutic goal to be reached. One unique class named solid lipid nanoparticle provides some advantage in site-specific drug delivery applications among all these colloidal carriers.

General ingredients used in the preparations include solid lipid (s), emulsifier (s), and water. The term lipid is used here in a broader sense and includes triglycerides (*e.g.*, tristearin, tricaprin, tripalmitin, partial glycerides (*e.g.*, Imwitor, glycerol behenate), fatty acids (*e.g.*, stearic acid), steroids (*e.g.*, cholesterol), and waxes (*e.g.*, cetyl palmitate).

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All classes of emulsifiers (with respect to charge and molecular weight) have been used to stabilize the lipid dispersion (*e.g.,* poloxamers, Tween 80, soya lecithin, and sodium dodecyl sulphate). The choice of the emulsifiers and their concentration greatly impact the quality of the SLN dispersion. It has been found that the combination of emulsifiers might. High concentrations of the emulsifier reduce the surface tension [2, 3, 4, 7].

**Actinic Keratosis:** Actinic keratosis or solar keratosis is a pre-cancerous disease induced by UV light. These growths are common in fair-skinned people. The skin appears as a white, scaly plaque of variable thickness and redness. The lesions were produced to show evidence of solar damage. The lesions are asymptomatic, but it is tenderly bleed and produce a burning sensation. Based on the clinical presentation, it is graded into 3 grade-1, grade-2 and grade-3. The most important cause of actinic keratosis is solar radiation. The various mechanisms involved in the same include the mutation of the p53 tumor suppressor gene and dysregulation of the p53 pathway. The topical medication for the treatment is fluorouracil cream, imiquimod cream, genolmebutate gel, retinoids, photodynamic therapy, cryotherapy, surgical technique [16, 17, 18]. Fluorouracil [19, 20] is a drug that is a pyrimidine analog that is used in the treatment of cancer. It is a suicide inhibitor and works through irreversible inhibition of thymidylate synthase. It belongs to the family of drugs called anti-metabolites.

**Uses:**

- The chemotherapy agent 5-FU, Which is used as a thymidylate synthase inhibitor. Used in colorectal cancer and pancreatic cancer.
- It is sometimes used in the treatment of inflammatory breast cancer, an especially aggressive form of breast cancer.
- 5-FU is used in ophthalmic surgery, specifically to augment trabeculectomy.
- Fluorouracil can be used topically (as a cream) for treating actinic (solar) keratoses and some types of basal cell carcinomas of the skin.

**Mechanism of Action:** As a pyrimidine analogue, it is transformed inside the cell into different cytotoxic metabolites, which are then incorporated into DNA and RNA, finally inducing cell cycle arrest and apoptosis by inhibiting the cell's ability to synthesize DNA. It is an S-phase specific drug and is only active during certain cell cycles. In addition to being incorporated in DNA and RNA, the drug has been shown to inhibit the activity of the exosome complex, an exoribonuclease complex of which the activity is essential for cell survival

**Adverse Effects**

- Side effects include myelosuppression, mucositis, dermatitis, and diarrhea.5-FU injection and topical even in small doses affect acute central nervous system 5-FU-induced damage to the oligodendrocytes that produce the insulating myelin sheaths.
- One study showed that 79 percent of the patients who switched from 5-FU (with leucovorin) to Xeloda (capecitabine) had a serious side effects.
- DPD deficiency, a pharmacogenetic syndrome leading to partial or total loss of ability to detoxify 5-FU in the liver, is strongly associated with increased risk of severe/lethal toxicities with 5-FU or oral 5-FU.

**Dosage and Administration:** Fluorouracil cream can apply twice daily.

**Common Problems with 5% Fluorouracil:** Burning sensation, increased sensitivity to sunlight, itching, oozing, darkening of skin compelling the patient's ton switched from the 5-fluorouracil.

**AIM:** To formulate and evaluate gel containing solid lipid nanoparticle of 5-fluorouracil: Fluorouracil is a drug used commonly against colon, stomach, breast, and pancreatic cancers, vaginal cancer, basal cell carcinoma. It is a fluorinated analog of pyrimidine base uracil, which is metabolized intracellularly to its active form, fluorodeoxy uridine monophosphate. The active form inhibits DNA synthesis by inhibiting the thymidine. However, the efficacy of this drug is limited (41%) due to the extensive first-pass metabolism following oral administration.

5-FU is an S-phase-active anticancer agent, and it has no activity when cells are in G0 or G1. It is sparingly soluble in water. On intravenous administration, it causes severe toxic effects of gastrointestinal, haematological, neural, cardiac, and dermatological origin. The bioavailability of 5-FU is greatly limited by rapid catabolism in the blood, liver, and other organs. After IV injection in humans, the drug has a half-life in blood of only 8 to 20 min.

Therefore5-FU requires an effective delivery system for appropriate therapy. SLNs combines the advantages of polymeric nanoparticles, fat emulsions, and liposomes. In addition, they also avoid some of their disadvantages. Solid matrices in SLNs provide controlled release of drugs, thereby avoiding the burst release generally associated with fat emulsions. 5-FU-loaded SLNs is prepared by hot homogenization method. This route is particularly beneficial for the drugs which need to be administered in chronic diseases like cancer.

**Objective:**

- To formulate and evaluate solid lipid nanoparticle-containing 5-fluorouracil.
- Optimise the best formulation.
- Incorporating the solid lipid nanoparticle into gel base.

**Methodology:**

**Formulation and Evaluation of Solid Lipid Nanoparticle:**

**Pre-formulation of 5-Fluorouracil[15, 16]:**

**Organoleptic Property:** The drug was visually inspected to find the organoleptic property like colour odour and appearance.

**Determination of Solubility of 5-Fluorouracil:** Excess drug (50 mg) was added to 15 ml of each fluid taken in a 25 ml stoppered conical flask, and the mixtures were shaken for 24 h at room temperature (28 ± 1 ºC) on Rotary Flask Shaker.

After 24 h of shaking, 2 ml samples were withdrawn at 2 h interval and filtered immediately using a 0.45 µ disk filter. The filtered samples were diluted suitably and assayed for 5-fluorouracil by measuring absorbance at 266 nm. Shaking was continued until two consecutive estimations were the same. The solubility experiments were replicated four times each (n=4).

**Melting Point Determination:** The melting point of 5-fluorouracil was determined by using the melting point apparatus.

**Determination of Partition Coefficient:** 30 ml of water and 30 ml of n-octanol solution were taken to the separating funnel. 100 mg of the drug (5-fluorouracil) was added to it and shake for 1 h. 1 ml of the aqueous layer was removed and transferred into a 100 ml standard flask and made up to the mark with water. The absorbance was measured at 266 nm by using water as a blank.

**UV spectroscopy [18]:**

**Determination of $\lambda_{max}$:** 5-fluorouracil was accurately weighed and dissolved in distilled water to make 1 mg/ml. This solution was then suitably diluted to 100ml using distilled water to get a final solution of concentration 100 µg/ml. UV spectrum was recorded in the wavelength range 200- 400 nm.

**Preparation of Calibration Curve For 5-Fluorouracil:** A stock solution of 5-fluorouracil (500 µg/mL) was prepared in water. The solution of 5-fluorouracil was transferred into a series of 10 mL volumetric flask up to the mark with methanol to get the concentration in the range 10-50 µg/mL. The absorbance of all the resulting solutions was measured at 266 nm.

**FTIR Studies [16, 18]:** The FTIR spectra of the sample were determined using the potassium bromide (KBr) disc technique. Samples equivalent to 2 mg of 5 - FU were mixed with potassium bromide (100 mg) in a clean glass pestle and mortar and were compressed to obtain a pellet.

The baseline was corrected, and the samples were scanned against a blank KBr pellet background at a wave number ranging from 4000-400 $cm^{-1}$ with a resolution of 1.0 $cm^{-1}$.

**Preparation of Solid Lipid Nanoparticle[15, 18]:** The drugs were dispersed in melted lipid (60-700), and then the mixture was dispersed in a hot aqueous solution with a surfactant concentration of 5 % w/w and 1 % w/w lecithin as co-surfactant at the same temperature, by high-speed stirring, using

an Ultra-Turraxhomogenizer at 12000 rpm for 10 minutes as mentioned in **Table 1.** The resulting dispersion was then cooled, and each sample was diluted with water. SLNs were prepared by the same technique using 50% w/w glycerol as viscosity enhancer [10].

**TABLE 1: FORMULATION TABLE OF FLUOROURACIL SOLID LIPID NANOPARTICLE**

| S. no. Code | Drug (mg) | Tween 80 (ml) | Tween 20 (ml) | Glycerol Monostearate (gm) | Glycerol (ml) | Lecithin (mg) | Water (ml) |
|---|---|---|---|---|---|---|---|
| SLN-1 | 50 | 5 | - | 5 | 50 | 1 | 50 |
| SLN-2 | 50 | 5 | - | 5 | - | 1 | 100 |
| SLN-3 | 50 | - | 5 | 5 | 50 | 1 | 50 |
| SLN-4 | 50 | - | 5 | 5 | - | 1 | 100 |

**Evaluation of Solid Lipid Nanoparticle [15, 16, 18]:**

**Measurement of Particle Size:** The mean particle size distribution was determined using a laser diffraction particle size analyzer (Nano ZS - Malvern instuments orsay, France).

Prepared nanoparticulate dispersions were appropriately diluted with distilled water thrice and stirred at 5000 rpm for 15 min in order to reduce aggregation. From the diluted sample, 1 ml was pipetted and analyzed for particle size distribution.

**Zeta Potential:** The zeta potential of nanoparticles was recorded using zeta sizer. The samples which have good *in-vitro* release were subjected to zeta potential analysis.

**Incorporation of Solid Lipid Nanoparticle into Gel[14]:** Gel is prepared using 4 polymers include carbopol934 (1%), xantham gum (1%), hydroxyl propyl methyl cellulose (2%), and chitosan (1%) Hydrogel was prepared by dispersing the gel-forming polymer in double distilled water containing glycerol (10%). SLN and hydrogel was mixed in a high-speed stirrer at approximately 1000 rpm for 5 min to yield gel containing SLN.

**Evaluation of Solid Lipid Nanoparticle Containing Gel [14 , 16, 10]:**

**Evaluation of SLN Gel pH and Viscosity:** The pH of the gel is measured using pH meter before and viscosity by brook field viscometer.

**Determination of % Entrapment Efficiency:** The % drug entrapment efficiency (%EE) of 5- FU in SLN s formulations was determined by centrifugation of the colloidal samples at 14000 rpm at 25 ºC for 30 min. The free fluorouracil in the supernatant is estimated by UV spectroscopy at 266 nm after centrifugation.

$$\%EE = W_{total\ 5\text{-}FU} - W_{free\text{-}5\text{-}FU}/W_{total5\text{-}FU} \times 100$$

$$\%DL = W_{total\ 5\text{-}FU} - W_{free\text{-}5\text{-}FU}/\ W_{total5\text{-}FU} + W_{total\ lipid} - W_{free5\text{-}FU}$$

***In-vitro* release study**

***In-vitro* Release Studies:** of SLN was performed in Franz diffusion cell that has receptor compartment with an effective volume of approximately 70 ml and an effective surface area of permeation of 3.14 sq cms. The cellophane membrane was mounted between the donor and receptor compartment. A weighed amount of SLN gel is placed on one side of the membrane; the receptor medium was phosphate buffer pH 7.4. A water jacket surrounds the receptor compartment to maintain the temperature at 37 ± 0.5 °C. Heat is provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirred by a Teflon coated magnetic bead fitted to a magnetic stirrer. At each sampling interval, the sample is withdrawn and is replaced by an equal volume of fresh receptor fluid on each occasion. Samples withdrawn were analyzed spectrophotometrically at 266 nm.

**Stability Studies[11]:** A physical stability test was carried out to investigate the leaching of drugs from SLN. The SLN samples were sealed in 20-ml glass vials and stored at refrigeration temperature (4-8 ºC) and at 25 + 2 ºC for three months. The EE of all the samples was determined after three months.

**Optimization of Prepared SLN:** Optimization of SLN was done based on the evaluated parameters such as particle size, % EE and drug release profile, and stability study. One of the criteria in optimizing SLN is drug release, *i.e.*, the SLN with high cumulative drug release will be selected as the best batch.

***In-vitro* Cytotoxicity Study;** The optimized formulation is subjected to an *in-vitro* cytotoxicity test**.**

The cell viability assay did the evaluation of the viability and proliferation of cells. The main reagent 3-(4, 5-dimethyl thialzol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) was prepared in distilled water. Before assay human normal cells L929, cell lines were subcultured from the stock culture and seeded in a multi-well plate. Cells were incubated at 37 ºC under 5% $CO_2$ atmosphere. Samples and positive controls were added in duplicates and incubated for 24 h at 37 ºC in a 5% $CO_2$ atmosphere. After 24 h of incubation, the samples were removed, and an MTT reagent (0.2 mg/ml) was added to each well and incubated.

**Release Studies**

***In-vitro* Diffusion Studies[7, 11]:** *In-vitro* drug diffusion study was carried out by using Franz-diffusion cell. In this method, a pre-hydrated cellophane membrane (pore size 0.45µ) is used as the model membrane. The membrane was placed between the donor compartment and the reservoir compartment (phosphate buffer pH 7.4). The patch was placed on the membrane, and the compartments clamped together. The receptor compartment (70 ml capacity) was filled with phosphate buffer pH 7.4, and hydrodynamics in the receptor compartment was maintained by stirring with a magnetic bead at 100 rpm. 5 ml of sample is withdrawn and replaced with receptor medium. The Amount of drug present is assayed spectrophotometrically at 266 nm, and amount of drug release at various time intervals was calculated

**Optimization of Best Formulation:** Optimization of the best gel was done based on the drug release and all other evaluation techniques.

**Curve Fitting Analysis:** To explain the exact mechanism of drug release, the optimized formulation was undergone a curve fitting analysis.

**Zero Order Kinetics:**

$$A_t = A_0 - K_{0t}.$$

Where, At is drug release at time‘t’. Ao is initial drug concentration. K0 is Zero-order rate constant (hr-1) when the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys zero-order equal to K

**First Order Kinetics:** Predicted by the equation;

$$\text{Log } C = \log Co - Kt/2.30.$$

Where, C is the amount of drug remaining at time‘t’, Co is initial amount of drug, and K is the First order rate constant (hr-1). When the data is plotted as log cumulative percent drug remaining versus time yields a straight line indicated that the release follow first order kinetics. The constant ‘k’ can be obtained by multiplying 2.303 with the slope values.

**Higuchi Model:** Drug release from the matrix device by diffusion has been described by diffusion equation;

$$Q = [D€ /\tau (2A-€Cs) Cst] 1/2$$

Where, Q is amount of drug released at time ‘t’, D is diffusion coefficient of the drug in the matrix, A is total amount of in unit volume of matrix, Cs is the solubility of drug in the matrix, € is porosity of the matrix, τ is Tortuosity, t is time (hrs) at which ‘q’ amount of drug is released. Above equation may be simplified if one assumes that ‘D’, ‘Cs’ and ‘A’ are constant. Then equation becomes Q=Kt1/2 When the data is plotted according to the equation i.e. cumulative drug release versus square root of time yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to ‘K’.

**Korsemeyer peppa’s Equation Model:** To study the mechanism of drug release from the formulation, the released data were fitted to exponential equation, which is often used to describe the drug release behavior from the polymeric system.

$$Mt /M_\alpha = K_{tn}$$

Where, Mt/Mαis the fraction of drug released at a time ‘t’, K is the constant term incorporating the structural and geometrical characteristics of the drug/ polymer system, n is diffusion exponent related to the mechanism of the release. The above equation can be simplified by applying log on both sides;

$$\text{Log } Mt /M_\alpha = \text{Log } K + n \text{ Log } t.$$

When the data is plotted as a log of drug released versus log time, yield a straight line with a slope equal to ‘n,’ and the ‘K’ can be obtained from the y-intercept.

## RESULTS:

### Formulation and Evaluation of Solid Lipid Nanoparticle:

### Pre-formulation Studies of Fluorouracil:

### Organoleptic Property:

**Colour:** white to off-white
**Odour:** odourless
**Taste:** bitter
**Appearance:** powder
**Nature:** hygroscopic and crystalline

**Determination of Solubility of Fluorouracil:** The solubility of the received sample of fluorouracil was examined in various solvents (aqueous and organic). The results observed were tabulated in **Table 2.**

**TABLE 2: SOLUBILITY OF FLUOROURACIL IN DIFFERENT SOLVENTS**

| S. no. | Solvent | Solubility |
|---|---|---|
| 1 | Water | Slightly soluble |
| 2 | Methanol | Freely soluble |
| 3 | Ethyl ether | Insoluble |
| 4 | Methylene chloride | Sparingly soluble |

**Melting Point Determination:** The melting point of pure fluorouracil was determined by the Melting point determining apparatus, and the melting point was found out to be 282-283 °C.

**Determination of Partition Coefficient:** The partition coefficient of fluorouracil was determined by using water and n-octanol as solvents. It was found to be 3.5.

**IR Spectrum Interpretation:** The infrared spectrum of the pure fluorouracil sample was recorded, and spectral analysis was done. From the scanning of the drug, it was concluded that the drug had, an IR spectrum was concordant with the reference spectrum of fluorouracil.

**Determination of $\lambda_{max}$:** The $\lambda_{max}$ of fluorouracil was determined by using UV spectrophotometer. The $\lambda_{max}$ was found out to be 266 nm.

**Calibration Curve of Fluorouracil:** Calibration curve of fluorouracil in **Table 3** and **Fig. 1**.

**TABLE 3: CALIBRATION CURVE OF FLUOROURACIL**

| Functional group | Characteristic peak | Observed peak |
|---|---|---|
| N-H(Stretching) Free | 3500-3100 | 31749 |
| C=O(Stretch) | 1649-1643 (amide ) | 1649 |
| C-N(stretch) | 1350-1000 | 1246 |
| C-H (in plane) | 1300-1000 | 813 |
| C-O | 1300-1246 | 1246 |

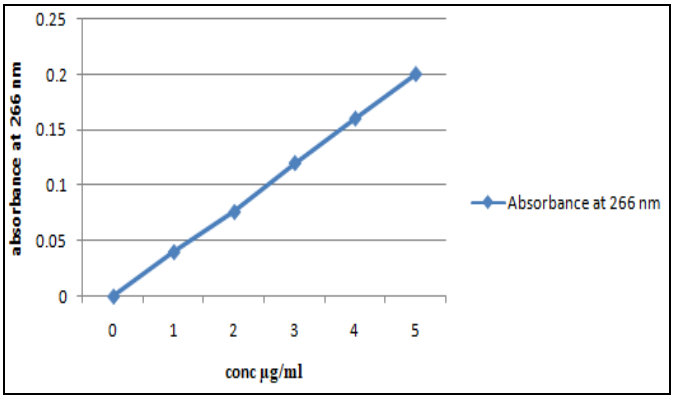


**FIG. 1: CALIBRATION CURVE OF FLUOROURACIL**

**FTIR Studies:** Different peaks obtained from the graph of sample drug **Fig. 2** is tabulated in **Table 4.**

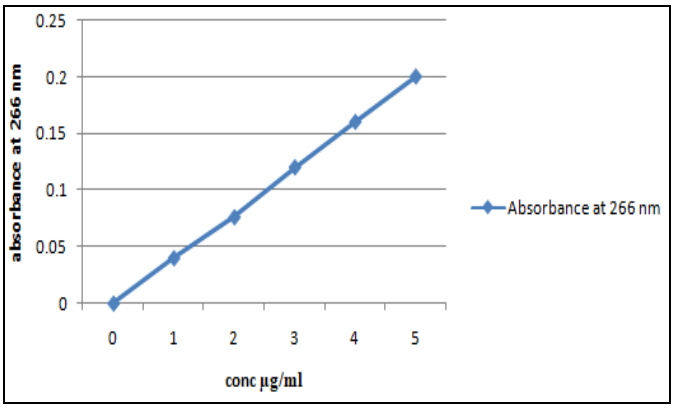


**FIG. 2: FTIR SPECTRA OF FLUOROURACIL**

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| C-O | 1300-1246 | 1246 |

The drug- lipid, drug-polymer, physical mixture, and final formulation FTIR was done **Fig. 3 and 4**, N-H stretching was in the range of 3500-3100 and found to be 3282 in drug- excipient and 3283.57 in the final formulation.

The carbonyl groups amide stretching range from 1649-1643 and found to be 1649, 1646.89 in drug - excipients and final formulation.

The C-N in-plane stretching ranging at 1350-1000 and found to be 1330 and 1211.85. Medium to strong absorption in the region of 1600 – 1550 $cm^{-1}$ often imply an aromatic ring. The absorption at 1416.20 implies that no degradation in aromatic structure of drug.

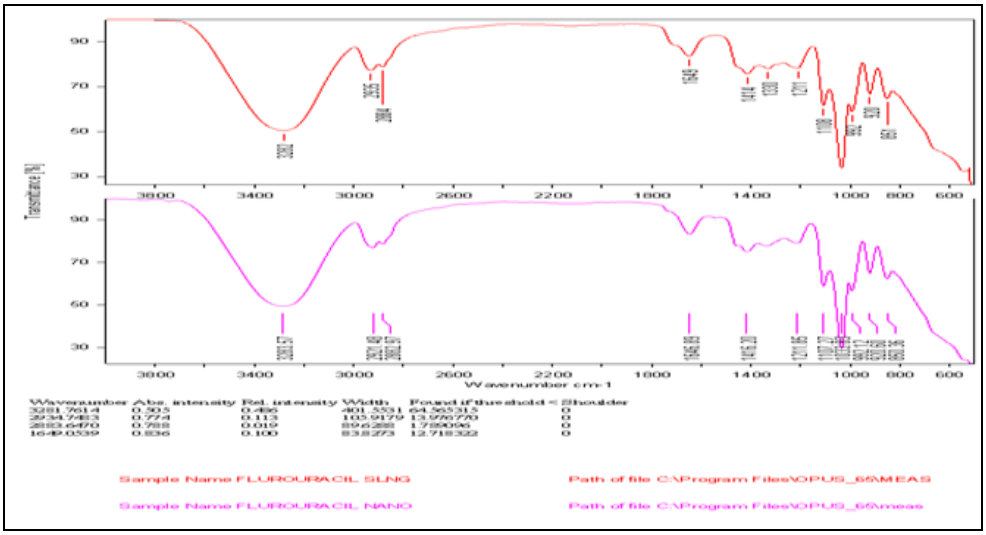


**FIG. 3: FTIR SPECTRA OF PHYSICAL MIXTURE AND FINAL FORMULATION**

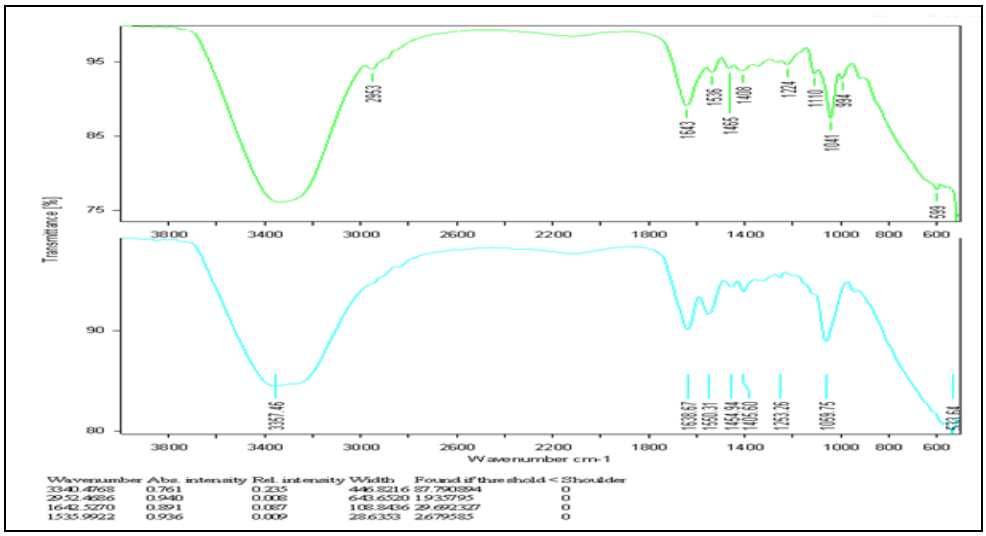


**FIG. 4: FTIR SPECTRA OF DRUG- LIPID AND DRUG-POLYMER MIXTURE**

**Evaluation of Solid Lipid Nanoparticle:**

**Measurement of Particle Size:** Different sizes and zeta potential of nanoparticle obtained by measuring particle size **Fig. 5** are mentioned in **Table 5.**

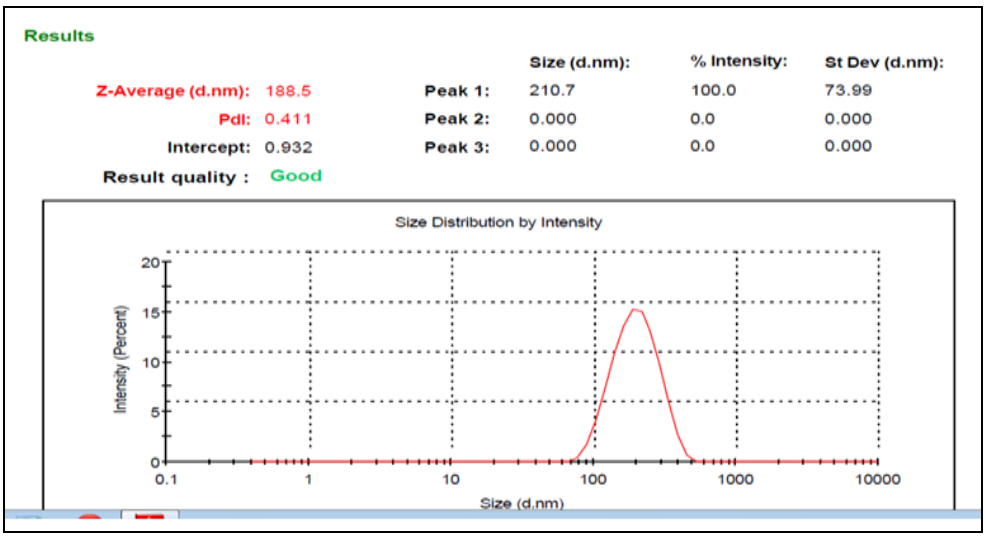


**FIG. 5: MEASUREMENT OF PARTICLE SIZE**

**TABLE 5: PARTICLE SIZES AND ZETA POTENTIAL OF VARIOUS FORMULATIONS**

| Formulation | Particle size (nm) | Zeta potential (mv) |
|---|---|---|
| SLN1 | 356.4 | 40.8 |
| SLN2 | 210.7 | 32.6 |
| SLN3 | 332.5 | 38.2 |
| SLN4 | 240.0 | 34.5 |

**Evaluation of Solid Lipid Nanoparticle Containing Gel:**

**Colour and Appearance:** The formulation shows white in colour with semisolid consistency.

It was observed that gel formulation shows good spread ability and viscosity. a) *in-vitro* release study**.** The *in-vitro* fluorouracil release from SLN was investigated by using Franz- diffusion cell **Table 6** and **Fig. 6.** Formulations that have glycerol (SLN-1 and SLN-2) are seen to have less drug release over a period of 24 h. Hence, glycerol seems to be a consequent reduction in permeability.

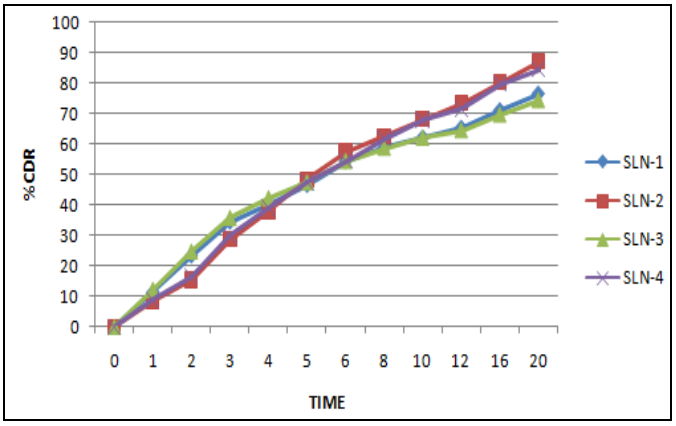


**FIG. 6: *IN-VITRO* DRUG RELEASE OF FLUOROURACIL FROM SLN GEL**

**TABLE 6: THE FORMULATION ARE SUBJECTED TO VARIOUS EVALUATION TESTS AND RESULTS ARE TABULATED AS BELOW**

| S. no | Formulation Code | PH | Viscosity (CPS) | Encapsulation Efficiency | Drug loading |
|---|---|---|---|---|---|
| 1 | SLN-1 | 8.12 | 1354.67 | 88.46 ± 0.16 | 30.55% |
| 2 | SLN-2 | 6.52 | 1738.34 | 94.45 ± 0.4 | 31.97% |
| 3 | SLN-3 | 8.06 | 1452.78 | 87.82 ± 0.56 | 30.05% |
| 4 | SLN-4 | 6.82 | 1682.25 | 92.34 ± 0.24 | 31.50% |

**Stability Studies:** Physical stability of SLN gel was studied for a period of three months. The EE were determined for all formulations stored at 4-8 ºC and 25 ± 2 ºC, which indicate a decrease in EE when stored in both temperature **Table 7** and **Fig. 7**; but approximately 90% of the drug was retained after three months. All formulations seemed to exhibit better stability.

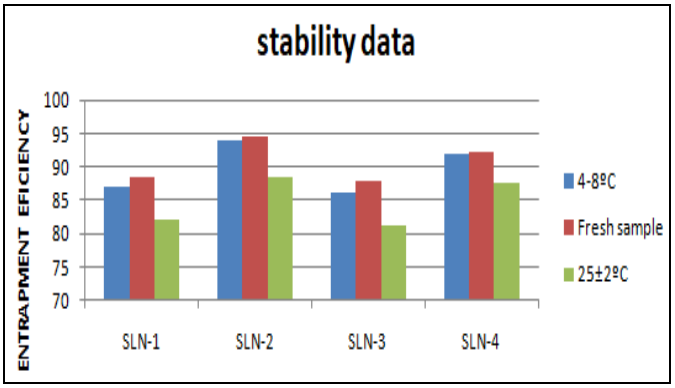


**FIG. 7: STABILITY DATA OF SLN GEL STORED AT 4-8 ºC AND 25 ± 2 ºC**

**TABLE 7: % CDR OF FLUOROURACIL FROM SLN-1 TO SLN-4**

| S. no. | Time | SLN-1 | SLN-2 | SLN-3 | SLN-4 |
|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 |
| 2 | 1 | 11.43 | 8.234 | 12.22 | 8.859 |
| 3 | 2 | 23.456 | 15.494 | 24.6712 | 16.59 |
| 4 | 3 | 34.567 | 28.716 | 35.747 | 29.82 |
| 5 | 4 | 40.123 | 37.99122 | 42.1562 | 39.02 |
| 6 | 5 | 46.567 | 48.39387 | 47.45466 | 47.45 |
| 7 | 6 | 54.46 | 57.63161 | 54.35973 | 54.36 |
| 8 | 8 | 58.894 | 62.61976 | 58.73817 | 61.74 |
| 9 | 10 | 62.187 | 68.20847 | 61.95483 | 67.95 |

| | | | | | |
|---|---|---|---|---|---|
| 10 | 12 | 65.386 | 73.64712 | 64.45126 | 71.45 |
| 11 | 16 | 71.1234 | 80.2323 | 69.5454 | 79.55 |
| 12 | 20 | 76.5645 | 87.1134 | 74.3287 | 84.32 |
| 13 | 24 | 82.2045 | 92.1338 | 80.93 | 90.93 |

***In-vitro* Cytotoxicity Study:** *In-vitro* cytotoxicity data collected from **Fig. 8.** Whose % cell viability is tabulated in **Table 8**.

**TABLE 8: STABILITY STUDY DATA**

| S. no | Formulation | 4-8 ºC | Fresh sample | 25 ± 2 ºC |
|---|---|---|---|---|
| 1 | SLN-1 | 87.12 | 88.46 | 82.12 |
| 2 | SLN-2 | 94.01 | 94.45 | 88.56 |
| 3 | SLN-3 | 86.26 | 87.82 | 81.34 |
| 4 | SLN-4 | 91.91 | 92.34 | 87.66 |

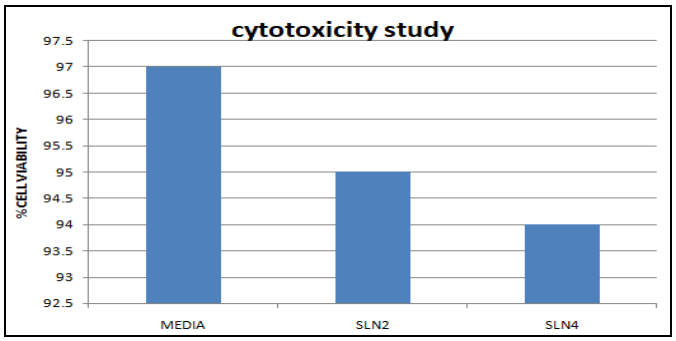


**FIG. 8: *IN-VITRO* CYTOTOXICITY STUDY OF SLN2 AND SLN4**

**Optimization of Best Gel [12, 16]:** Out of four formulations SLN2 **Table 9** shows good release pattern, proper viscosity, highest encapsulation efficiency, and drug loading ability. It also exhibited good cell viability. So SLN2 had been optimized as the best formulation. To describe the exact mechanism and order of drug release, curve fitting analysis was done with SLN2.

**TABLE 9: *IN-VITRO* CYTOTOXICITY STUDY OF SLN2 AND SLN4**

| Formulation | % Cell viability |
|---|---|
| Media | 97 |
| SLN2 | 95 |
| SLN4 | 94 |

## Curve Fitting Analysis:

### Zero Order Kinetics:

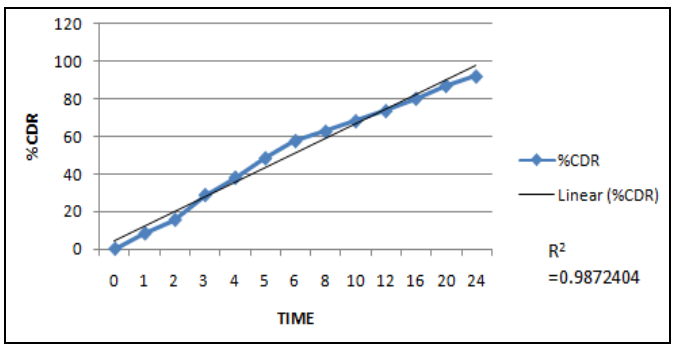


**FIG. 9: ZERO ORDER KINETICS OF SLN2**

**TABLE: 10: 1ZERO ORDER KINETICS OF SLN2**

| S. no. | TIME (HRS) | % CDR |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 1 | 8.234 |
| 3 | 2 | 15.494 |
| 4 | 3 | 28.716 |
| 5 | 4 | 37.99122 |
| 6 | 5 | 48.39387 |
| 7 | 6 | 57.63161 |
| 8 | 8 | 62.61976 |
| 9 | 10 | 68.20847 |
| 10 | 12 | 73.64712 |
| 11 | 16 | 80.2323 |
| 12 | 20 | 87.1134 |
| 13 | 24 | 92.1338 |

### First Order Kinetics:

**TABLE 11: FIRST-ORDER KINETICS OF SLN2**

| S. no. | TIME (HRS) | Log % CDR |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 1 | 0.9156 |
| 3 | 2 | 1.1901 |
| 4 | 3 | 1.4581 |
| 5 | 4 | 1.5796 |
| 6 | 5 | 1.6847 |
| 7 | 6 | 1.7606 |
| 8 | 8 | 1.7967 |
| 9 | 10 | 1.8338 |
| 10 | 12 | 1.8671 |
| 11 | 16 | 1.9043 |
| 12 | 20 | 1.9400 |
| 13 | 24 | 1.9644 |

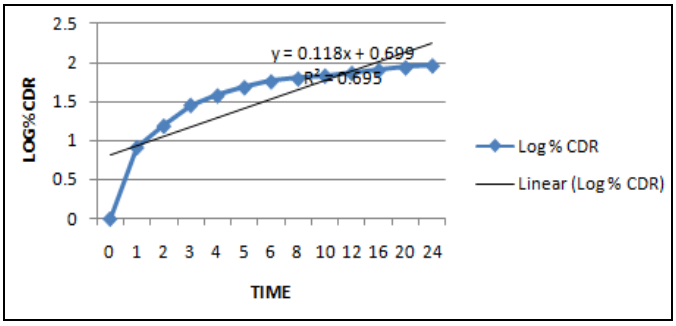


**FIG. 10: FIRST ORDER KINETICS OF SLN2**

## Higuchi Model

**TABLE 12: HIGUCHI MODEL SLN2**

| S. no. | Squre root time | %CDR |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 1 | 8.234 |
| 3 | 1.414214 | 15.494 |
| 4 | 1.732051 | 28.716 |
| 5 | 2 | 37.99122 |
| 6 | 2.236068 | 48.39387 |
| 7 | 2.44949 | 57.63161 |
| 8 | 2.828427 | 62.61976 |
| 9 | 3.162278 | 68.20847 |
| 10 | 3.464102 | 73.64712 |
| 11 | 4 | 80.2323 |
| 12 | 4.472136 | 87.1134 |
| 13 | 4.898979 | 92.1338 |

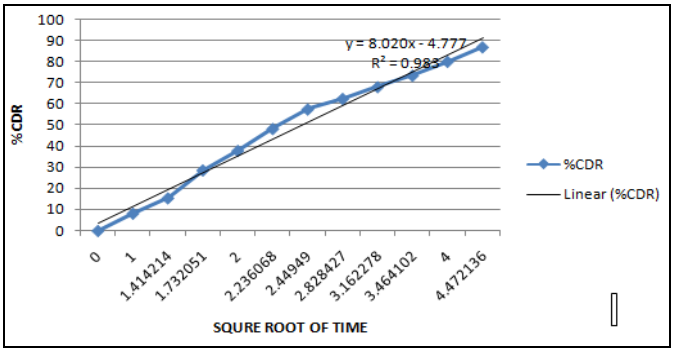


**FIG. 11: MODEL SLN2**

## Korsemeyer peppa's Equation Model:

**TABLE 13: KORSEMEYER PEPPA'S EQUATION MODEL OF SLN2**

| S. no. | LOG TIME | Log % CDR |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 0 | 0.9156 |
| 3 | 0.30103 | 1.1901 |
| 4 | 0.477121 | 1.4581 |
| 5 | 0.60206 | 1.5796 |
| 6 | 0.69897 | 1.6847 |
| 7 | 0.778151 | 1.7606 |
| 8 | 0.90309 | 1.7967 |
| 9 | 1 | 1.8338 |
| 10 | 1.079181 | 1.8671 |
| 11 | 1.20412 | 1.9043 |
| 12 | 1.30103 | 1.9400 |
| 13 | 1.380211 | 1.9644 |

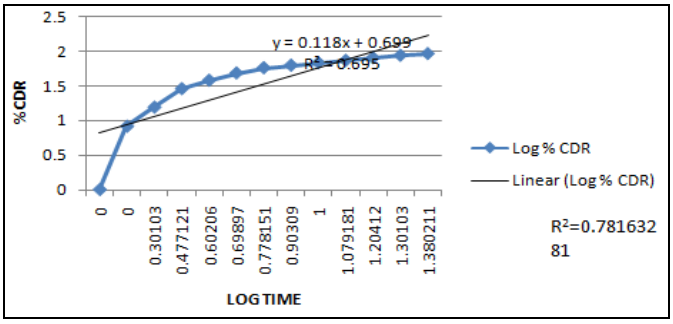


**FIG. 12: KORSEMEYER PEPPA'S EQUATION MODEL OF SLN2**

## DISCUSSION:

**Pre-formulation Studies:** Pre-formulation studies were carried out, and the drug was found to be white to off-white crystalline powder, which is hygroscopic in nature. The drug was found to be odourless and bitter in taste. Considering solubility, the drug is freely soluble in methanol, slightly soluble in water, sparingly soluble in methylene chloride, and insoluble in ethyl ether. Drug lipid and drug-polymer compatibility were studied using FTIR studies and found to have no interaction.

**Solid Lipid Nanoparticle:** Solid lipid nanoparticle is prepared using 4 different formulations and whose size and zeta potential is analyzed and found to be 356.4, 210.7, 332.5, 240.0 in size and zeta potential of 40.8, 32.6, 38.2, 34.5. From this, SLN2 and SLN4 were found to have optimized sizes. The gel was prepared from the formulation, and whose encapsulation efficiency and drug loading capacity were determined. The SLN2 has EE 99.45%and DL 31.97. SLN4 has EE 92.34% and 31.50 DL capacity.

For more optimizing these formulations the stability test and *in-vitro* release study were done. The SLN2 was found to be more stable in 4-8 degrees and 25 ± 2 degrees. SLN2 has the release of 92.13% in about 24 h. It is considered to be the optimized formulation. *The in-vitro* cytotoxic study was done, and the viability was found to have 95%. The chemical properties of the drug control the release of the drug from the gel. To examine the drug permeation kinetics and mechanism, the data of SLN 2 were fitted to models representing zero-order, first-order, Higuchi and Korsmeyer-Peppas. A higher drug release pattern was exhibited but SLN 2 at each time interval. This release is optimum and significant. This followed zero-order kinetics (By Zero-order plot).

The zero-order plot of SLN 2 was found to be fairly linear, as indicated by their high regression values. Therefore it was ascertained that the drug permeation from this formulation could follow zero-order kinetics. By plotting Higuchi's plot, it was found that the Release Obeys Diffusion Mechanism. Hence, To Confirm the Exact Mechanism of Drug Permeation from These Patches, the Data Were Fitted According to The Korsmeyer-Peppas model. Korsmeyer *et al.* Used a Simple Empirical Equation to Describe the General Solute Release Behaviour from Controlled Release Polymer Matrices

$$M_t/M_t = KT_n$$

Where mt/mt is the fraction of drug released, k is the kinetic constant, t is release time, and n is the diffusional exponent for drug release. In the present study, the coefficient of determination (r2) was found to be much closer to 1 (0.781) for the korsmeyer-peppas equation. Slope values ($n < 1.0$) suggest that the drug permeation from 0.05 fluorouracil gel followed the diffusion and non-fickian transport.

**CONCLUSION:** The pre-formulation studies were performed using the pure drug and were useful for the formulation. The lipid and polymer compatible studies with fluorouracil were done. FTIR was found to be not having any interaction. The nanoparticle of various formulations is prepared and selects one of them for further evaluation. This found that we can reduce the daily dose of 800mg drug daily dose to 50 mg dose form. The biological half-life can be increased. The formulation was prepared using the hot homogenization method. The formulation was subjected to evaluation procedures and found that the size of the particle is reduced to 210nm for the best formulation SLN2. This formulation had high drug-releasing ability of about 92% in 24 h. This is also having high drug loading ability and encapsulation efficiency. The formulation sln2 showed good consistency and optimum drug release pattern. The above formulation gave a maximum drug diffusion of 87.28% over a period of 24 h. A zero-order plot of optimized patch sln2 indicated that the release mechanism is concentration-independent. higuchi's plot for the formulation revealed that the predominant mechanism of drug release is diffusion. However, from peppa's plot the r2 value for SLN2 was found to be 0.75, thus indicating non-fickian diffusion.

**Summary:** In this study, an anticancer drug is to be used in the treatment of actinic keratosis, formulated as a solid lipid nanoparticle (SLN) and delivered topically as a gel form. The development of solid lipid nanoparticle and converting into gel form can overcome the problem existing with oral and intravenous administration and reducing the dose, poor bioavailability, frequent dosing and denaturation of the drug in the GIT pH. As the boi logical half-life of the drug is 10-15 min and the daily dose of the drug is not more than 800 mg for parental daily. By this method, we can able to maintain 0.05% of fluorouracil gel. The SLN is prepared by using an anticancer drug. The characterization of SLN, such as *in-vitro* drug loading ability, entrapment efficiency, particle size, zeta potential, surface morphology, and *in-vitro* drug release studies, are to be performed. These optimized formulations will be analyzed on the skin cancer cell lines to find the *in-vitro* cytotoxic effects. The outcome of the research will be to have an optimized drug delivery system that would deliver the drug directly to the skin; by reducing the dose of the drug, we can avoid all the adverse effects of the conventional dosage form.

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