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DEVELOPMENT AND OPTIMIZATION OF SUITABLE METHOD OF PREPARATION FOR A STABLE DUAL DRUG LOADED NLC OF 5-FU AND RSV FOR THE TREATMENT OF SKIN CANCER VIA DERMAL DELIVERY

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Abstract

Hot melt encapsulation was used to load SLNs with 5-FU, and a gelling agent was then used to transform the SLNs into a gel (Carbopol 940). Particle size ranged from 76.821.48 to 3274.46 nm for the 5-FU-loaded SLNs, while zeta potential was between -11.32.11 and -28.42.40 mV and entrapment effectiveness (%) was between 63.461.13 and 76.082.42. The incorporation of 5-FU into nanoparticles was confirmed by powdered X-ray diffraction analysis. The quick initial release was followed by a persistent release over 48 hours in an in vitro release investigation of 5-FU-loaded SLNs. After 48 hours, the cytotoxic impact of 5-FU-loaded SLNs on cutaneous melanoma and squamous cell carcinoma was larger than that of free 5-FU medication solution.

Key words: Resveratrol; 5-FU-loaded SLNs ; drug delivery systems; lipid nanoparticles

INTRODUCTION

Non-melanoma skin cancer (NMSC) is more frequent than melanoma among both the general public and the Caucasian population; more than 3 million instances of NMSC are recorded annually throughout the globe, and this number is predicted to treble in more than 30 years. Sunlight's ultraviolet (UV) rays are a known carcinogen and a leading cause of skin cancer. This is because UV radiation breaks down DNA bases, which disrupts signaling pathways. Chemotherapy, targeted treatment, and immunotherapy are all being used to treat patients with skin cancer [2, 4]. Combination medications, which mitigate the problems caused by synthetic pharmaceuticals by mixing them with those of natural origin, have been the subject of much study in recent years. Because of the many benefits it offers over monotherapy, combination cancer treatment has recently received a lot of attention. 5-fluorouracil (5-FU) has been shown to have a significant antitumor effect in the treatment of skin cancer and has been given FDA approval. Cancer cells treated with 5-FU undergo accelerated apoptosis and decreased cell proliferation as a result of the drug's ability to inhibit thymidylate synthase activity. The combination of RSV and 5-fluorouracil (FLU) increases the susceptibility of cancer cells to apoptosis.

5-fluorouracil (5-FU) is one of the most widely used chemotherapeutic medicines, and it is effective against a wide variety of malignancies, including pancreatic, breast, colorectal, and skin cancers, as well as a number of topical disorders [9]. To exert its lethal impact on cancer cells, 5-FU integrates its metabolites into RNA following intracellular activation. This prevents the synthesis of new RNA and causes DNA replication to fail. Nevertheless, 5-FU's hydrophilic nature and unfavorable hydrophilic/lipophilic balance present problems with its distribution to the tumour site owing to limited skin permeability. While treating skin cancer, standard drug delivery techniques have been shown to provide suboptimal 5-FU distribution, necessitating the employment of alternative strategies.

Low solubility and bioavailability limit the pharmaceutical potential of 5-fluorouracil (5-FU) and reovirus (RSV), two very efficient anticancer medicines. Since RSV is a BCS class II medication and 5-FU is a BCS class III medicament, using them together for dermal application to treat skin cancer would

be problematic due to their distinct physiochemical features. Free 5-FU has a hard time reaching the stratum corneum due to its low partition coefficient (0.88). While RSV's lipophilicity is unrivaled, its larger molecule size, instability in the presence of UV radiation, and fundamental behavior all act to prevent it from reaching its destination. Nanostructured lipid carriers (NLCs) were designed, improved, and validated for dermal application as a means of overcoming the drawbacks of both medications. NLC has recently emerged as a potential innovative lipid-based carrier for dermal targeting due to its capacity to increase drug absorption over the skin's barrier. Nanoscale lipidic coatings (NLC) adhere to the skin as a thin occlusive layer, alter the organization of corneocytes and increase intercellular gaps to increase medication deposition into the living skin. Moreover, this lipid-nanosystem as carriers has benefits including increased drug loading, a tunable release profile [18], and storage stability. Because of their increased occlusiveness and large surface area (a result of their nanosize), these carriers are swiftly absorbed into the malignant cells, where they may do their work. In addition, NLC is useful in the prevention and treatment of UV-induced skin cancer because it contains lipids that function as a UV screen [14].

LITERATURE REVIEW

Iqubal MK, et al. (2019), This research details the creation, optimization, and use of a resveratrol and 5-fluorouracil-containing nanostructured lipid carrier gel for the treatment of skin cancer. From the beginning, stability experiments were conducted to fine-tune the formulation process, and extensive testing led to the creation of a unique, modified emulsiosonication approach. This served two purposes at once: first, it displayed a thorough report card on formulation durability and second, it gave the best-optimized approach for building a combinatorial lipid-nanosystem. Drug release from the modified lipid-nanosystem was shown to be much enhanced, sluggish, and extended in an in vitro research (***p 0.05) and to follow non-Fickian Higuchi kinetics. Combinatorial linogel was also more effective than the standard formulation in killing the A431 cell line (**p 0.01).

Caldas, A.R.; et al. (2019), Researchers in the dermocosmetic and pharmaceutical industries are increasingly interested in the bioactives resveratrol (RSV) and omega 3 (3) due to their biologically beneficial properties; However, many bioactives have technical barriers that prevent them from being delivered to the desired skin layer. This research presents a hybrid approach using two different lipid nanosystems, each of which contains 3 fatty acids, as a means of overcoming the difficulties associated with the stability and epidermal penetration of free bioactives. Moreover, only liposomes that provide an appropriate amphiphilic environment are capable of encapsulating RSV. Each formulation's physical and chemical characteristics are described in detail. However, many bioactives have technical barriers that prevent them from being delivered to the desired skin layer. This research presents a hybrid approach using two different lipid nanosystems, each of which contains 3 fatty acids, as a means of overcoming the difficulties associated with the stability and epidermal penetration of free bioactives, and liposomes' increased flexibility, provided by edge activator components, facilitates this process. Incorporating both lipid nanosystems into a common semisolid basis represents an exciting new approach to treating autoimmune, inflammatory, and malignant skin conditions.

Souto, E.B., de Souza, A.L.R., dos Santos, F.K., Sanchez-Lopez, E., Cano, A., Zieli 'nska, A., Staszewski, R., Karczewski, J., Gremio, M.P.D., and Chorilli, M. (2019). Depending on the severity and location of the wounds, hyperproliferative skin disorders (HSD) may range from skin cancer to precancerous lesions to illnesses of unclear cause. Some medications are notoriously insoluble in water, which limits their ability to penetrate the skin. Hence, it has been suggested to include them in lipid nanocarriers with the primary aim of increasing the efficacy of topical therapy while decreasing adverse effects. This paper attempts to outline the benefits of loading a wide variety of chemically distinct medications into lipid nanoparticles and liposomes for the purpose of treating HSD.

Intagliata, Sebastiano; Modica, Maria N. (2019), Due to their wide range of biological effects, natural chemicals have garnered considerable interest in recent years. Several clinical illnesses, including neurological and cardiovascular diseases, cancer, diabetes mellitus, and inflammation, are linked to oxidative stress, and polyphenols, a class of plant compounds, have been extensively studied for their potential to prevent and cure these diseases. Due of its excellent antioxidant and free radical scavenging properties, resveratrol (RSV) has garnered a lot of attention as one of these polyphenols. Unfortunately, RSV's limited bioavailability, caused by its poor water solubility and fast metabolism, reduces its therapeutic usefulness. This review will first go over the primary biochemical pathways at play in RSV's biological activities, then go on to the methods used to enhance RSV's efficacy through systemic and topical delivery. In particular, methods of incorporating RSV into various delivery systems will be outlined, with examples including liposomes, polymeric and lipid nanoparticles, microemulsions, and cyclodextrins. Results from in vitro and in vivo research will be presented with details about chemical alterations made to this antioxidant in an effort to improve its physicochemical qualities.

Materials and methods

Materials

Gattefosse was nice enough to send over a free sample of their Precirol1 ATO 5 (Glyceryl palmitostearate 100%). (NJ, USA). Merck's Poloxamer 188 was acquired (Germany). Sigma-Aldrich Co. was where I got my supplies of Tween 80, ethanol (95%), and 5-FU (>99%). Spectrum Laboratories dialysis bags were bought because of their low MWCO (10K Da) (Rancho Dominguez, Canada). Merck KGaA was contracted to provide the ethanol (Germany). The ATCC in the United States was where we got our skin melanoma (B16F10) and squamous cell carcinoma (A-431) cell lines.

Preparation of 5-FU-loaded SLNs

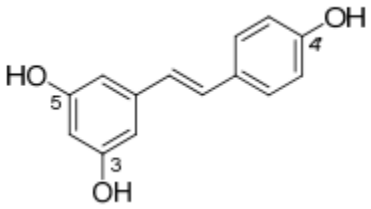
Hot melt encapsulation (HME) was adapted from its original form in the literature to create the SLNs. The drug and co-surfactant concentrations were remained constant, while the solid lipid and surfactant concentrations were changed in 5-FU-loaded SLNs (Table 1). The lipid phase and the water used to create the aqueous phase were both heated to the same temperature. Poloxamer 188 and Tween 80 were then added to the water. Three minutes later, at 12,000 rpm, the mixture was homogenized in a homogenizer. Following 5 minutes, we disabled the hot plate stirrer's heating element and allowed the resultant hot melt emulsion to cool to room temperature while being constantly swirled and sonicated. Deionized water was used to wash the SLNs before they were centrifuged at room temperature for 20 minutes at 14000 rpm. At the end, SLNs were lyophilized for 24 hours at -45 degrees Celsius and low pressure.

Table 1. Physicochemical characteristics of 5-FU-loaded SLNs

Code	Precirol[1] ATO 5 (mg)	Poloxamer 188: Tween 80 (%w/v)	Size (nm)	PDI	Zeta Potential (mV)	%EE
SLN1	100	3%: 0.5%	327± 4.5	0.442±0.009	-9.66± 1.2	70.60%± 1.2
SLN2	100	2%: 0.5%	220.3± 3.3	0.430±0.007	-11.3± 2.1	67.72%± 2.1
SLN3	100	1.5%: 0.5%	125.3± 4.8	0.354±0.013	-20.4± 1.5	63.46%± 1.1
SLN4	100	1%: 0.5%	100.3± 2.9	0.257±0.006	-28.4± 2.6	76.08%± 2.4
SLN5	75	1%: 0.5%	84.09±1.01	0.345±0.004	-21.3± 1.2	73.40%± 3.2
SLN6	50	1%: 0.5%	76.82± 1.5	0.356±0.008	-19.4± 2.4	71.16%± 2.9

Preparation of trans-resveratrol (RSV)

5-[(E)-2-(4-hydroxyphenyl)ethenyl] Figure 1 shows the phenolic stilbenoid compound trans-resveratrol (IUPAC name: benzene-1,3-diol). Stilbene molecules, in particular, include a modified diarylethene skeleton with hydroxyls, methyl, methoxy groups, or glycosides attached. Wine, Itadori tea, soy, and red fruits are all good food sources, but RSV may also be found in peanuts, Japanese knotweed, and other plants. There is an ethylene moiety in the middle of the RSV molecule, thus it may exist as either the cis or trans stereoisomer. Natural RSV is mostly the trans-isomer, but it may be photochemically transformed to the cis-isomer when exposed to UV light. RSV functions as a phytoalexin in plants, protecting them from UV radiation, damage, and fungal diseases. Many consequences on human health have been attributed to RSV, which has been studied extensively. These benefits include prevention of cardiovascular disease and metabolic syndrome in addition to antioxidant, anticancer, and anti-inflammatory actions.



When a dispersion of 5-FU-loaded SLNs containing 2 mg of medicine was added to a solution of 1% w/v Carbopol-940 and agitated at 1000 rpm for 2 hours, a gel was formed. Gel consistency was improved and pH was balanced with the addition of triethanolamine (0.9%). For the purpose of contrasting the 5-FU loaded SLNs gel with the 5-FU plain gel, both were made according to the identical protocol.

Particle size, zeta potential, and polydispersity index (PDI) were measured at 25 degrees Celsius and a fixed angle of 90 degrees using dynamic light scattering (DLS) using a Zeta Sizer-ZS90 for synthesized SLNs. Three replicates of each sample were examined, and the average and standard deviation are shown below. An indirect technique was used to calculate the %EE of 5-FU-loaded SLNs. Centrifuged

at 12,000 rpm, the SLNs filled with 5-FU were ready for use. The quantity of medication that was not enclosed by the nanoparticles was determined by analyzing the supernatant.

%EE = (Weight of 5-FU added-Free amount of 5-FU) / (Weight of 5-FU added) x 100

Transmission electron microscopy was used to examine the morphology of 5-FU-loaded SLNs after they had been manufactured. To examine the morphology of the formed SLNs, Five-fluorouracil (5-FU)-treated SLNs were placed on the microscope's copper grid, and images were taken at different magnifications. Phosphate buffered saline (PBS) at pH 7.4 was used for in-vitro drug release tests using lyophilized 5-FUloaded SLNs. The dialysis bag was reconstituted with 3 mL of pH 7.4 phosphate-buffered saline (PBS) and 5 mL of lyophilized SLNs containing 5 mg of 5-FU. Clamping the dialysis bags shut, 200 mL of dissolving medium solution, 37 0.5 C, and 50 rpm were added to a vessel of USP type II dissolve apparatus. Phosphate-buffered saline (PBS) was used to dilute the two-milliliter (mL) samples that were obtained at regular intervals. Lastly, a UV-visible spectrophotometer was used to measure the amount of 5-FU released into the dissolving media at a wavelength of 266 nm.

Results

As no hazardous organic solvents were used in the preparation of the SLNs for this investigation, the HME approach may be considered the gold standard. In this work, Because of its wide range of fatty acids and its looser structure, Precirol1 ATO 5 was selected as the lipid core for the production of 5-FU-loaded SLNs. To prevent the formulation from becoming unstable due to particle aggregation, surfactants Poloxamer 188 and Tween 80 were added.

Because of their increased drug concentration in the tumor microenvironment and decreased lymphatic outflow in tissue, nanoparticles approximately 200 nm in size have a greater therapeutic impact. Increased permeability and retention (EPR) is the term used to describe this phenomena. Pharmacokinetic characteristics, cytotoxicity, and drug release rate may all be affected by the particle size of a drug delivery system. The content and quantity of lipids and surfactants, among others, may significantly alter the average particle size of lipid nanoparticles.

Particle size is also considerably affected by the concentration of poloxamer 188, as seen in Table 1. All three formulations maintained the same lipid ratio, whereas poloxamer 188 was employed at 3%, 2%, and 1.5% w/v in each. As larger concentrations of poloxamer 188 enable it to be absorbed on the surface of nanoparticles, reducing the concentration causes the particle size to drop, from 3274.46 nm to 1254.75 nm (Table 1).

Entrapment efficiency for all 5-FU-containing SLN formulations is shown in Table 1. Because of its surfactant properties, poloxamer 188 facilitates drug solubilization and stability when it is confined inside a lipid matrix or on the surface of nanoparticles, decreasing the quantity of poloxamer 188 in SLN1 to SLN3 resulted in a drop in EE from 70.60%1.16 to 63.46%1.13. When t-tested, this impact was shown to be statistically significant (p0.05). Poloxamer 188 has been shown to have the similar effect on EE.

Morphology by TEM analysis. Transmission electron microscopy (Jeol, USA) analysis confirmed that the morphology of SLN4 was optimal. TEM examination, as seen in Fig. 1, revealed nanoparticles with a spherical form. TEM examination of SLNs confirmed the size established by the DLS method. Conformity with previously published works on SLN morphology was observed.

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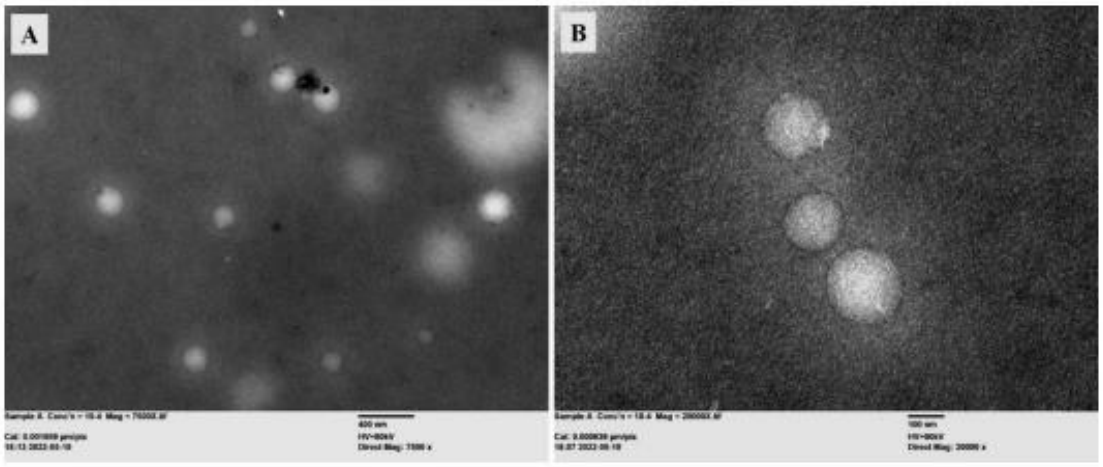

Fig 1. TEM analysis of 5-FU-loaded SLNs at (A) 7500X magnification (scale bar 400 nm) and (B) 20000X magnification (scale bar 100 nm).

pXRD analysis. The crystalline character of 5-FU was shown by the pXRD pattern, which revealed a prominent peak at 28.7 and a series of weaker peaks between 16.5 and 33.5. Crystalline poloxamer, represented by peak positions of 18.9 and 23 angstroms in poloxamer188, was also observed. Characteristic peaks of 5-fluorouracil (5-FU), pyrrolo-1-ato-5 (Precirol1 ATO-5), and poloxamer 188 are shown in the pXRD In the pXRD pattern of the optimal formulation (SLN4), the typical peak of 5-FU had vanished, showing that the medication had been successfully encapsulated in the lipid matrix and converted from a crystalline state. The medication may have been encapsulated inside the lipid crystal lattice, which resulted in a minor reduction in the crystallinity of the lipid, nevertheless the regular peaks of solid lipid didn't disappear or move entirely.

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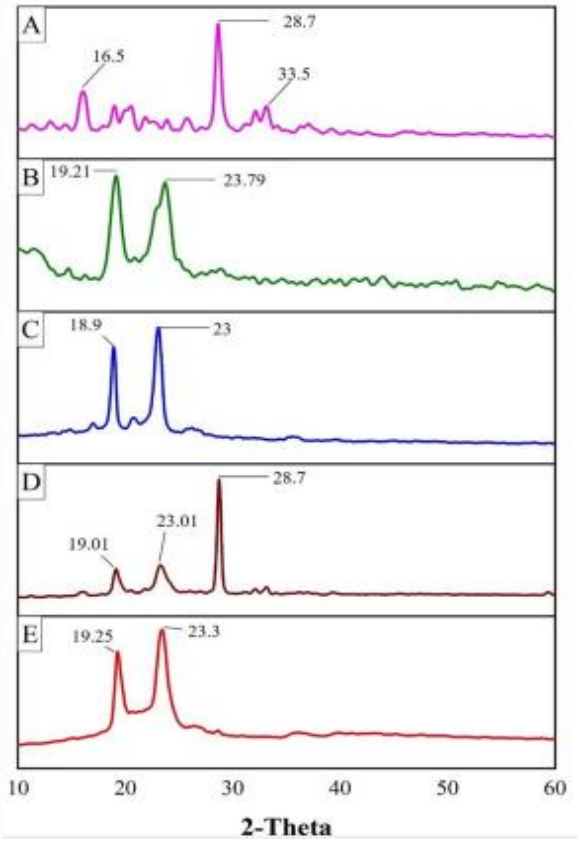

Fig 2. pXRD analysis of 5-FU (A), Precirol1 ATO5 (B), Poloxamer 188 (C), Physical mixture (D), and optimized formulation (E).

In-vitro release studies. Five-fluorouracil (5-FU) drug solution and SLNs loaded with the drug were subjected to in-vitro release testing (Fig 3). By 6 hours, almost all of the free 5-FU had leaked out of the dialysis bag. Under constant sink circumstances, the release profile of 5-FU may be utilized to distinguish between the release patterns of 5-FU and SLNs. Rapid release of 40-45% of 5-fluorouracil (5-FU) from SLNs occurred during the first 3 hours, followed by a steady release over the following 48 hours. It has been hypothesized that SLN surface absorption of the medication is responsible for the quick onset of release [75, 76]. Since 5-FU is a hydrophilic medication, it tends to move into the aqueous phase during SLN production, where it becomes concentrated on or near the particle surfaces that undergo the first burst of release. Sustained release of 5-fluorouracil (FU) from SLNs was observed, with values ranging from 88-98% for all formulations over 48 hours. This was likely the result of diffusion of the entrapped medication via the lipid matrix.

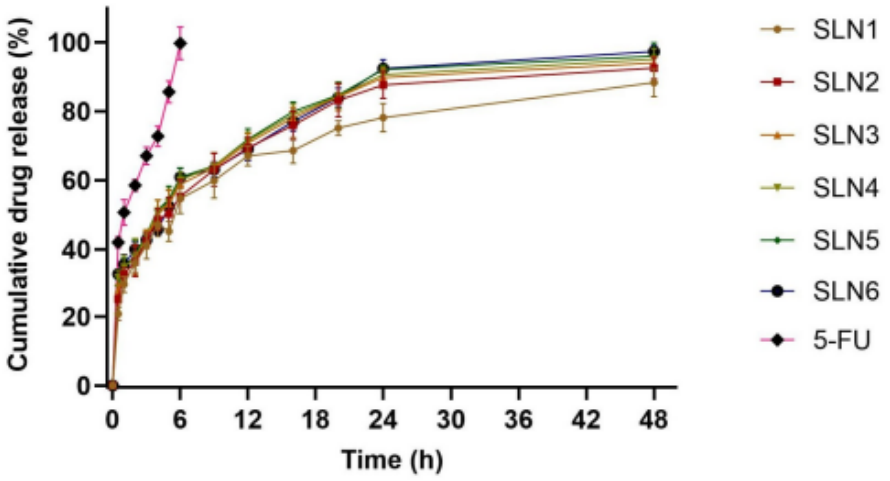

Fig 3. The in-vitro release profile of 5-FU and 5-FU-loaded SLNs in PBS pH 7.4.

Dermal Delivery of RSV

Many in vitro and in vivo investigations on the effects of RSV on the skin have been described, and its topical use in pharmaceuticals and cosmetics has been assessed. The intrinsic metabolic clearance of the entire skin is lower than that of the liver; hence, dermal use of RSV may be more successful than its systemic delivery, despite the fact that phase II biotransformations also occur in cutaneous tissues. The metabolism of the skin is still important; thus RSV prodrugs may be useful for treating skin illnesses or for cosmetic purposes. As a result, there has been a surge in enthusiasm for putting RSV prodrugs to use on the skin. (Table 2)

Table 2. RSV prodrugs for dermal delivery and their cosmetic application

Type of Prodrug	Compounds	Cosmetic Application
Triphosphate	**40**	not stated
Triacetate	**1**	anti-melanogenic agents/whitening effect
Triglycolate	**42**	anti-melanogenic agents/whitening effect
4^J-Acetate	**43**	skin antioxidant, skin anti-aging
4^J-, 3-, 3,5- Esters	**37, 38, 42–44**	skin antioxidant, skin anti-aging

To make topically administered RSV less susceptible to breakdown and release of the parent molecule by epidermal enzyme phosphatases, RSV triphosphate (40, Figure 4) was developed in 2007. Raman spectroscopy revealed that 40 was dephosphorylated in the stratum corneum, but since enzymes are heat-sensitive, the conversion to RSV did not take place when skin samples were subjected to steam. In addition, both RSV and 40 were tested for their mobility across space and through time. 40 was able to better penetrate the water-rich viable epidermis because it is more soluble in water than RSV. After

being delivered as a triphosphate prodrug, the RSV active component was more evenly distributed throughout the stratum corneum and the viable epidermis, since dephosphorylation was also detected in these layers.

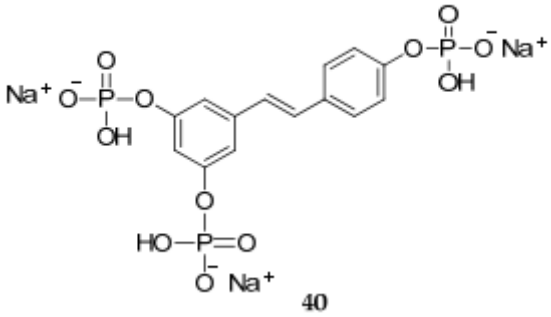

Figure 4. Chemical structures of RSV triphosphate (40)

CONCLUSION

In order to enhance permeability and retention at the tumour site, the current work effectively synthesized and evaluated 5-FU-loaded SLNs for physicochemical features. The therapeutic impact of 5-FU was increased while dose-related toxicity was reduced thanks to the SLNs loaded with the drug. There was a concentration- and time-dependent increase in cytotoxicity of the produced 5-FUloaded SLNs against cutaneous melanoma and squamous cell carcinoma cells. The acute toxicity research demonstrated that the engineered SLNs are reliable vehicles for transporting the chemotherapeutic drug.

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