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Nano delivery Systems of Resveratrol to Enhance its Oral Bioavailability

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ABSTRACT

Resveratrol is a polyphenol found in grapes and red wines. Interest in this polyphenol has increased due to its pharmacological cardio- and neuroprotective, chemopreventive, and antiaging effects, among others. Nevertheless, its pharmacokinetic properties are less favorable, since the compound has poor bioavailability, low water solubility, and is chemically unstable. To overcome these problems, we developed two novel resveratrol nanodelivery systems based on lipid nanoparticles to enhance resveratrol's oral bioavailability for further use in medicines, supplements, and nutraceuticals. Solid lipid nanoparticles (SLNs) and nano structured lipid carriers (NLCs) loaded with resveratrol were successfully produced by homogenization and ultrasonication technique. These were completely characterized for particle size, zeta potential, DSC and TEM, to evaluate the quality of the developed resveratrol-loaded nanoparticles. The *in vitro* release studies showed that both the nanosystems are highly stable system allowing sustained and controlled release after uptake.

Keywords: Nanosystems, solid lipid nanoparticles, ultrasonication, homogenization.

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INTRODUCTION

Nanoparticles offer several advantages in drug delivery owing to their small particle size, large surface area and the capability of changing their surface properties. In general, nanoparticles can be used to target the delivery of drugs, to sustain its effect, to improve bioavailability, to solubilize it for intravascular delivery and to improve its stability against enzymatic degradation[1]. Based on the type of the inactive ingredient used, there are four main classes of nanoparticles: Lipid based nanoparticles[2], polymeric nanoparticles[3], metal based nanoparticles[4] and biological nanoparticles[5]. SLNs were developed in the early 1990s as an alternative carrier system to other colloidal formulations such as emulsions, liposomes, and polymeric nanoparticles. SLNs are produced by replacing the liquid lipid (oil) component of an oil/water emulsion with lipids that are solid at both room and body temperature[6]. In the second generation of lipid nanoparticle technology, the particles are produced using blends of solid lipids and oils – so called nanostructured lipid carriers (NLCs)[7]. Resveratrol (RSV) (trans 3, 4, 5 trihydroxystilbene), a phytoalexin found in grapes, nuts, fruits, and red wine, is a potent antioxidant with strong anti-inflammatory and antiproliferative properties[8]. RSV induces promyelocytic leukemia, cell differentiation, and exhibits anticancer properties by mediating apoptosis, arresting cell cycle progression, antiproliferation and inhibiting ribonucleotide reductase, ornithine decarboxylase, and cyclooxygenase through modulation of prostaglandin production[9]. The interest in resveratrol has increased due to several other pharmacological effects and properties, which include neuroprotection, anti-inflammatory effects, chemopreventive and antiaging properties, and its potential role in diabetes and obesity prevention[10]. Despite the beneficial therapeutic effects of resveratrol, its pharmacokinetic properties are less favorable, since the compound has poor bioavailability, low water solubility, and is chemical unstable, being rapidly and extensively metabolized and excreted[11–13]. However, in most studies resveratrol has been used in its free form, which is not suitable for drug delivery. NLC have rarely been considered[14–19], thus presenting a challenge still to be pursued. The development of site-specific drug delivery systems that protect resveratrol during its transit inside the organism is extremely important to preserve its pharmacological properties, while enhancing its bioavailability after oral administration. The main goal of this work was to develop novel resveratrol nanodelivery systems based on lipid nanoparticles to enable resveratrol's further use in medicines, supplements, and nutraceuticals.

MATERIALS AND METHOD

Resveratrol was kindly gifted by Zenith Nutritions Pvt Ltd, Bangaluru, cetyl palmitate was provided by Gattefosse, Tween60 and miglyol-812 from Sigma Aldrich. All other ingredients were of analytical grade.

Preparation of SLNs and NLCs

The best method for preparation of nanoparticles was combination of homogenization and ultrasonication[20]. Homogenization reduces the particles to micro-range whereas ultrasonication reduces the particle size to nano-range. For the preparation of Solid-Lipid Nanoparticles (SLNs) (S1-S5, Blank S) cetyl palmitate and Tween 60 were used while preparation of Nanostructured lipid carriers (NLCs) were obtained with (N1-N5, Blank N) cetyl palmitate, tween 60 and miglyol 812. (Table 1). The lipid phase containing the formulations as shown in table I was melted at 70°C and the melted formulation was dispersed in distilled water at same temperature by high speed stirring in homogenizer followed by ultrasonication. SLNs were stirred for 30 sec at 12,000 rpm followed by 5 min of sonication while NLCs were homogenized for 2 min and then sonicated for 15 min.

Table 1: Formulation parameters of SLNs and NLCs

Contents Code	Cetyl palmitate (mg)	Tween 60 (mg)	Miglyol-812 (mg)	Distilled Water (mL)	Resveratrol (mg)
S1	480	100	-	5	20
S2	485	100	-	5	15
S3	490	100	-	5	10
S4	495	100	-	5	5
S5	498	100	-	5	2
Blank S	500	100	-	5	-
N1	330	100	150	5	20
N2	335	100	150	5	15
N3	340	100	150	5	10
N4	345	100	150	5	5
N5	348	100	150	5	2
Blank N	350	100	150	5	-

Particle Size Analysis

Particle size and Polydispersity index were measured using Photon Correlation Spectroscopy after dilution of formulation (1:360) with bidistilled water to yield a suitable scattering intensity. Each sampling was done in triplicate.

Zeta Potential Measurement

The zeta potential of prepared SLNs and NLCs were measured using zeta potential analyzer.

Samples were diluted (1:360) using bidistilled water and were analyzed at 25°C. The measurement was conducted in triplicate.

Entrapment Efficiency

The entrapment efficiency was determined by calculating the difference between the total amount of drug used to prepare the formulation and the amount of free drug still present in the aqueous phase. Samples were diluted with distilled water (1:200) and centrifuged at 3300 rpm for 5 min. Supernatant was withdrawn and analyzed using UV-Spectrophotometer at 200-600 nm. Entrapment efficiency (EE) was calculated using formula

EE = Total amount of resveratrol- Untrapped resveratrol / Total amount of resveratrol x 100

Transmission Electron Microscopy (TEM)

Morphological examination of the prepared SLNs and NLCs was performed with Transmission electron microscopy. The samples were stained with 2% (w/v) phosphotungstic acid and placed on copper grid for viewing.

Differential Scanning Calorimetry (DSC)

The study of degree of crystallinity and the lipid polymorphism of SLNs and NLCs was performed by DSC analysis with the samples sealed in aluminum pans under nitrogen air atmosphere at a flow rate of 20 mL/min and evaluated in 30-300°C temperature ranges.

***In Vitro* Release studies**

The drug release was performed by dialysis bag method. The dialysis bag retains nanoparticles and allows the free drug into the dissolution media with a cut-off of 14 KDa. The bag was soaked in double-distilled water for 12 h before use. SLN dispersion was poured into the bag while the two ends were fixed by clamps. The bags were placed in a conical flask filled with 50 mL phosphate buffer (pH 7.4), which was placed into a thermostatic shaker at 37°C at the rate of 140 movements per min. Suitable aliquots were withdrawn at selected time intervals and volume was replaced with fresh medium. Aliquots were filtered through 0.22 µm membrane filter and analyzed by UV-spectrophotometer.

Stability Studies

Stability testing on the lipid formulations provides indication about variation in quality of an active substance or pharmaceutical product under the influence of environmental conditions. Stability was analyzed for selected formulations by keeping them at 40°C ± 2°C and 75% relative humidity (RH) in stability chamber for 180 days and then analyzed for physical parameters.

RESULTS AND DISCUSSION

Particle Size Analysis

The mean particle size of SLNs and NLCs are presented in table 2. Both drug loaded and blank formulation showed homogenous size distribution with a mean diameter of 155-265 nm and no statistical difference was observed indicating that the drug incorporation doesn't affect the nanoparticle size. While the mean diameters for SLNs and NLCs are compared, it was observed that there was no significant changes in the hydrodynamic mean diameter when a liquid lipid was added to NLCs. The liquid lipid must have been entrapped inside the core of nanoparticles, instead of making a layer on the surface, thereby having no significant effect on the size of the particles. The mean diameters conformed that both the lipid nanoparticles are submicron colloidal carriers, suitable for oral administration and GIT absorption. Meanwhile, PDI obtained was around 0.2 (table 2) indicating that the nanoparticles are distributed uniformly with no aggregation of the particles.

Table 2: Characterization of drug loaded and blank SLNs and NLCs

Characterization Code	Particle Size (nm)	Zeta Potential (mV)	PDI	EE (%)
S1	196.94±9.3	-33.9±1.7	0.219±0.021	77.0±2.3
S2	191.02±3.6	-25.6±6.9	0.190±0.011	79.3±6.4
S3	182±5.1	-28.8±2.2	0.211±0.035	84.8±1.2
S4	169.8±7.5	-37.1±4.1	0.197±0.035	86.2±2.1
S5	161.3±4.8	-32.54±7.2	0.237±0.031	91.6±4.2
Blank S	155.7±5.3	-31.2±6.7	0.234±0.029	-
N1	238±6.2	-31.4±6.3	0.190±0.033	59.7±3.6
N2	265±4.7	-29.5±6.9	0.194±0.037	61.6±4.7
N3	261±1.7	-21.1±3.7	0.198±0.030	63.1±3.4
N4	210.8±2.2	-19.5±5.2	0.204±0.027	68.5±3.7
N5	171.8±3.5	-25.1±6.7	0.217±0.029	79.2±4.1
Blank N	168.1±4.2	-23.6±7.3	0.199±0.036	-

Zeta Potential Measurement

The zeta potential indicates the degree of charge present on suspended particles in dispersion and evaluates the stability of colloidal dispersion. A suitably high value of zeta potential (positive or negative) confers stability because particles resist aggregation. Particles are considered stably dispersed when the zeta potential value is above 20mV due to electric repulsions between the particles, while the particles between 5-15mV results in limited flocculation and below 5mV results in maximum flocculation . As shown in table 2, most of the formulations exhibited zeta potential values above 20mV (except for N4 but S2, N3, N5 and Blank N may present lower zeta, as can be seen by their s.d), indicating that the lipid nanoparticles are considered physically

stable due to electrostatic repulsions conferred by the chemical nature of lipid matrix, polysorbate surfactant used and the adsorption of negatively charged ions onto the surface of lipid nanoparticles.

Entrapment Efficiency

The entrapment efficiency of SLNs and NLCs formulation with different concentration is shown in Table 2. The percent encapsulation efficiency was found to be satisfactorily high, with an average EE of 70%. Statistical Analysis showed that resveratrol concentration used in the preparation of formulation had no significant effect on the percentage of entrapment obtained.

Transmission Electron Microscopy (TEM)

The shape and surface morphology of SLN (S5) and NLC (N5) loaded dispersion were studied using TEM (figure 1). In the TEM study, the size of the lipid nanoparticles was found to be in agreement with the particle size observed in table 2. All the particles were found to be roughly spherical in shape with a well-defined periphery. The lipid nanoparticles appear to be less dense in the core with a well-defined shell. No obvious aggregation of the lipid nanoparticles was observed in the TEM images.

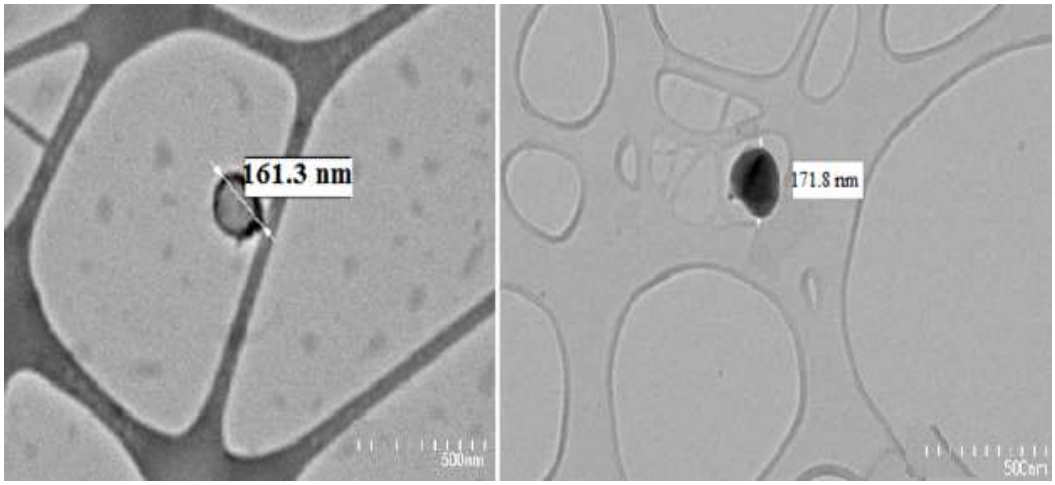

Figure 1: Transmission Electron Microscopy of a) S5 b) N5

Differential Scanning Calorimetry (DSC)

Formulation S5 and N5 were subjected to DSC studies along with blank samples because of better particle size, PDI, and zeta potential. DSC thermogram of drug loaded SLN and NLC showed an endothermic peak at 51.50°C and 49.15°C respectively (figure 2, table 3). While there was slight shift of melting point in case of blank formulations for SLNs and NLCs. The melting enthalpy values was calculated and it was observed that the decrease in the enthalpy was due to the presence of surfactant. Recrystallization Index (RI) set at 100% crystallinity (used as

reference) showed that the RI of SLNs and NLCs was decreased by 11 and 38 respectively, indicating that the lipid nanoparticles have a lower crystal organization.

Table 3: DSC parameters of SLNs and NLCs

Formulation	Melting Point (°C)	Enthalpy (J/g)	RI (%)
S5	51.50	21.4	89
Blank S	50.12	23.7	94
N5	49.15	9.1	62
Blank N	48.78	8.7	77

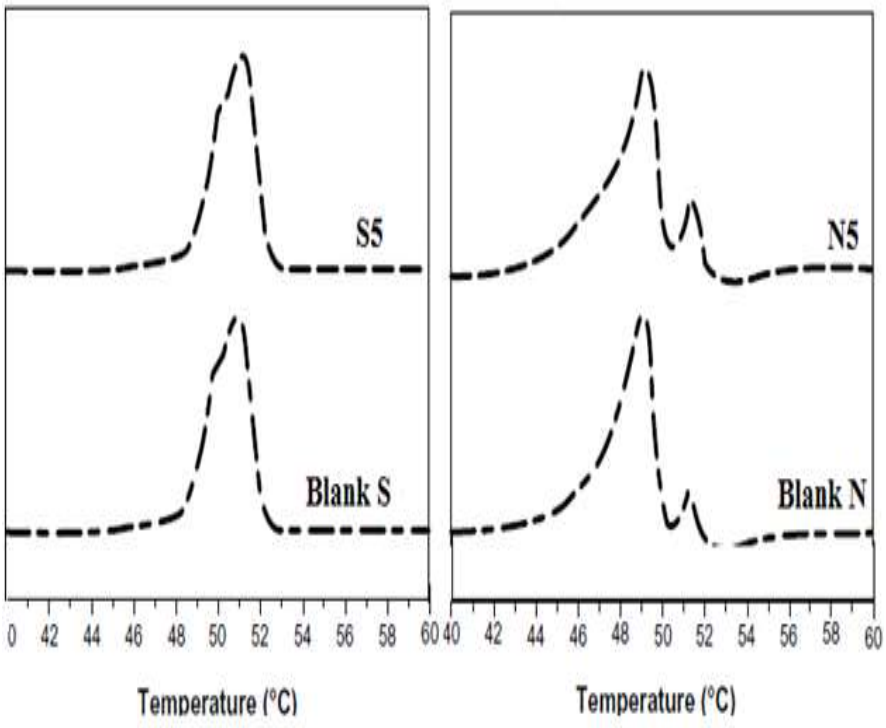

Figure 2: Differential Scanning Calorimetry of a) SLNs b) NLCs

***In Vitro* Release studies**

In vitro release studies were done in triplicate using Phosphate Buffer pH 7.4. For S5 formulation, after 60 min of time interval 33.98±0.22% drug release was observed whereas it was only 14.73±0.18% for N5 formulation. Nearly 50% of drug release was observed in S5 formulation after 4 hr of time interval whereas for N5 formulation it was observed after 6 hr. Thus, a sustained release formulation was observed in this case. The difference in drug release (Figure 3) for NLC (N5) was due to less ordered lipid matrix conferred by liquid lipid in NLC that promotes controlled release. Thus, both nanodelivery system can be considered suitable for oral administration conferring protection to the incorporated resveratrol and allowing controlled release after uptake.

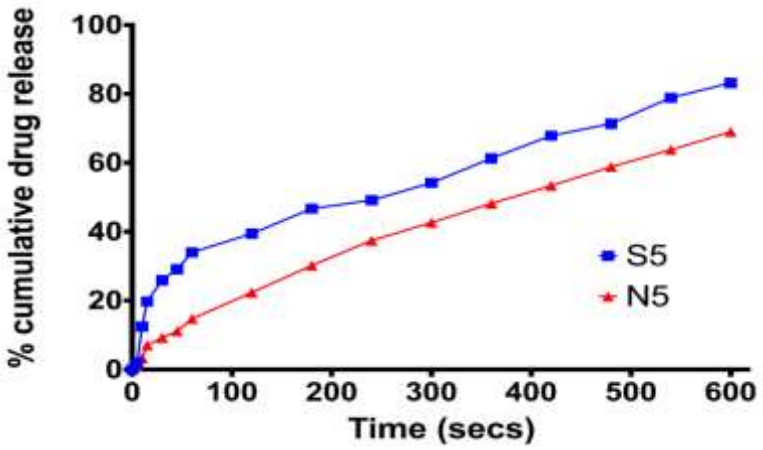

Figure 3: *In Vitro* Release Profile for SLN (S5) and NLC (N5) formulation

***error bars not visible due to low data variability**

Stability Studies

All the formulations were found to be stable after 180 days as shown in table 4. There was no significant differences observed for particle size, zeta potential, PDI and EE. Thus, it can be said that S5 and N5 formulations were the most stable formulations.

Table 4: Physical characterization of SLN (S5) and NLC (N5) after stability studies

Characterization Code	Particle Size (nm)	Zeta Potential (mV)	PDI	EE (%)
S5	161.1±4.8	-31.54±7.1	0.238±0.030	90.9±4.2
N5	170.9±3.5	-24.9±7.7	0.216±0.027	78.6±3.9

CONCLUSION

These challenging controlled-release systems are suitable for transporting and protecting this important bioactive compound against degradation, increasing its physical stability, and enhancing its oral bioavailability. Lipid nanoparticles are submicron colloidal carriers composed of biodegradable and biocompatible lipids that are generally recognized as safe and suitable for the incorporation of lipophilic and poorly water soluble active ingredients such as resveratrol, promoting its oral absorption[21, 22]. In fact, lipid nanoparticles have a superior ability to penetrate cell membranes, allowing the increased cellular uptake of compounds they are loaded with[23]. In the present study, SLN and NLC were successfully prepared by homogenization and ultrasonication method using cetyl palmitate, tween 60 and miglyol 812. Tween 60 was used as a surfactant to increase the stability and to avoid the aggregation of the particles. Morphological studies showed that the particles were spherical in shape with smooth surfaces. Particle size and Zeta Potential was found to be in range of 155.7±5.3 to 265±4.7, -19.5±5.2 to -37.1±4.1 respectively. PDI value around 0.2 indicated satisfactory homogeneity. DSC studies also

conformed the solid crystalline state for both the optimized formulations. NLCs having less ordered crystalline structure than SLNs which was confirmed by the inclusion of liquid lipid, since the values of melting enthalpy, and RI was less for NLCs. *In vitro* release studies showed that both the nanosystems are highly stable system allowing sustained and controlled release after uptake. Thus, the physical and chemical protection conferred to resveratrol by these lipid nanoparticles will enhance the therapeutic effects of resveratrol by controlling its release profile. Future work in this project involves drug incorporation in SLNs by various other process, *in vitro* toxicological studies and testing on animal models.

REFERENCES

1. Basu, B., K. Garala, R. Bhalodia, B. Joshi, K. Mehta, Solid lipid nanoparticles: A promising tool for drug delivery system. J Pharm Res 2010; 3(1):84-92.
2. Muller, R.H., K. Mader, and S. Gohla, Solid lipid nanoparticles (SLN) for controlled drug delivery-a review of the state of the art. European J Pharma Biopharma 2000;50(1):161-177.
3. Kreuter J. Colloidal drug delivery systems. Vol. 66. 1994: CRC.
4. Bhattacharya, R. and P. Mukherjee, Biological properties of. Advanced drug delivery reviews, 2008; 60(11):1289-1306.
5. Manchester, M. and P. Singh, Virus-based nanoparticles (VNPs): platform technologies for diagnostic imaging. Advanced drug delivery reviews, 2006;58(14):1505-1522.
6. Mehnert W, Mader K. Solid lipid nanoparticles: production, characterization and applications. Adv Drug Deliv Rev. 2001; 47(2–3):165–196.
7. Muller RH, Mader K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art. Eur J Pharm Biopharm. 2000; 50(1):161–177.
8. Afaq F, Adhami VM, Ahmad N. Prevention of short term ultraviolet B radiation mediated damages byresveratrol in SKH1 hairless mice. Toxicol Appl Pharmacol. 2003; 186(1):28–37.
9. Van Ginkel PR, Sareen D, Subramanian L, et al. Resveratrol inhibits tumor growth of human neuroblastoma and mediates apoptosis by directly targeting mitochondria. Clin Cancer Res. 2007;13(17):5162–5169
10. Neves AR, Lucio M, Lima JL, Reis S. Resveratrol in medicinal chemistry: a critical review of its pharmacokinetics, drug-delivery, and membrane interactions. Curr Med Chem. 2012; 19(11):1663–1681.
11. Walle T, Hsieh F, DeLegge MH, Oatis JE Jr, Walle UK. High absorption but very low bioavailability of oral resveratrol in humans. Drug Metab Dispos. 2004; 32(12):1377–1382.

12. Vitaglione P, Sforza S, Galaverna G, et al. Bioavailability of trans-resveratrol from red wine in humans. Mol Nutr Food Res. 2005; 49(5): 495–504.
13. Lu X, Ji C, Xu H, et al. Resveratrol-loaded polymeric micelles protect cells from Abeta-induced oxidative stress. Int J Pharm. 2009; 375(1–2): 89–96.
14. Shi G, Rao L, Yu H, Xiang H, Yang H, Ji R. Stabilization and encapsulation of photosensitive resveratrol within yeast cell. Int J Pharm. 2008; 349(1–2):83–93.
15. Caddeo C, Teskac K, Sinico C, Kristl J. Effect of resveratrol incorporated in liposomes on proliferation and UV-B protection of cells. Int J Pharm. 2008; 363(1–2):183–191.
16. Das S, Ng KY. Resveratrol-loaded calcium-pectinate beads: effects of formulation parameters on drug release and bead characteristics. J Pharm Sci. 2010; 99(2):840–860.
17. Teskac K, Kristl J. The evidence for solid lipid nanoparticles mediated cell uptake of resveratrol. Int J Pharm. 2010; 390(1):61–69.
18. Shao J, Li X, Lu X, et al. Enhanced growth inhibition effect of resveratrol incorporated into biodegradable nanoparticles against glioma cells is mediated by the induction of intracellular reactive oxygen species levels. Colloids Surf B Biointerfaces. 2009; 72(1):40–47.
19. Hentschel A, Gramdorf S, Muller RH, Kurz T. Beta-carotene-loaded nanostructured lipid carriers. J Food Sci. 2008; 73(2):N1–N6.
20. Kotikalapudi LS, Adepu L, Ratna V, Diwan PV. Formulation and in-vitro characterization of domperidone loaded solid lipid nanoparticles. Int J Pharm Biomed Res. 2012; 3(1): 22-29.
21. Muchow M, Maincent P, Muller RH. Lipid nanoparticles with a solid matrix (SLN, NLC, LDC) for oral drug delivery. Drug Dev Ind Pharm. 2008; 34(12):1394–1405.
22. Acosta E. Bioavailability of nanoparticles in nutrient and nutraceutical delivery. Curr Opin Colloid Interface Sci. 2009; 14(1):3–15.
23. Neves RA, Lucio M, Martins S, Lima J, Reis S. Novel resveratrol nanodelivery system based on lipid nanoparticles to enhance its oral bioavailability. Int J Nanomed. 2013; 8: 177-187