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FORMULATION OF NOVEL ANTI-CANCER SOLID LIPID NANO-PARTICLES

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Abstract- A human prostate cancer cell line known as LNCaP was used in this study to investigate the anti-cancer effects of CRC-encapsulated SLN formulations, both loaded and unloaded. CRC encapsulated SLN (CRC-SLN) demonstrated anti-tumor action, whereas blank SLN (BL-SLN) did not show any evidence of anti-tumor activity. At a concentration of 100 g/ml of CRC, both lipid-based and non-lipid-based CRC-loaded SLNs decreased the viability of LNCaP cells to practically 0%. Studies that investigated cellular uptake provided evidence that CRC-SLN was taken inside the cell, where it was discovered to be positioned in the cytoplasm close to the nucleus. Studies using flow cytometry provided evidence that CRC-SLNs had the capacity to induce both early and late stages of the apoptotic process. An increased level of anticancer activity was observed in retinoic acid (RTA) loaded SLN that had been adjusted by adjusting the process parameters (pressure and temperature) and making use of a variety of lipid grades to form nano-dispersions. High-pressure homogenization was used to form the RTA-SLN dispersions, which were then studied in terms of particle size, zeta potential, drug entrapment efficiency, transmission electron microscopy (TEM), atomic force microscopy (AFM), X-ray diffraction (XRD), and in vitro drug release. The effectiveness of the anticancer treatment was determined by incubating RTA-SLN with LNCaP cells.

Keywords— human prostate cancer cell, anti-cancer effects, CRC-encapsulated SLN formulations, LNCaP cells, RTA-SLN dispersions, particle size, zeta potential, drug entrapment efficiency, transmission electron microscopy (TEM).

INTRODUCTION

Solid lipid nanoparticles (SLNs) have emerged as a viable alternative to various colloidal drug delivery systems like oil-in-water emulsions, liposomes, microparticles, and polymeric nanoparticles. Comprising of nanometer-sized spherical lipid particles, SLNs have revolutionized the controlled and targeted delivery of drugs, accommodating both hydrophilic and lipophilic drugs with ease. The composition of SLNs includes solid lipids, along with emulsifiers and/or coemulsifiers, combined with water. Notably, the solid lipids used in these delivery systems possess melting points higher than the body's temperature (37°C). A diverse array of lipids has been explored for their suitability in SLNs, encompassing fatty acids, steroids, waxes, triglycerides, acylglycerols, and various combinations thereof. Thestability of lipid dispersion is effectively ensured by employing a wide range of emulsifiers, either independently or in combination. Among the emulsifiers studied are lecithin, bile salts like sodium taurocholate, nonionic emulsifiers like ethylene oxide/propylene oxide copolymers, sorbitan esters, fatty acid ethoxylates, and their synergistic combinations. As for the

dispersion medium, deionized water is the preferred choice. The versatile and dynamic nature ofsolid lipid nanoparticles heralds a new era of drug delivery, promising precise therapeutic outcomes and advancing pharmaceutical possibilities to new heights. Because the smallest blood capillaries in the body are roughly 5-6 m in diameter, particlesin the blood stream should be smaller than 5 m in diameter without forming aggregates to reduce embolism. As a result, SLNs are more suited for I.V. administration. The size of the microparticles limits their ability to traverse the intestinal lumen into the lymphatic system after oral administration of vaccines, peptides, and other biomacromolecules. While microparticles persist in Peyer's patches, SLNs are dispersedsystematically.

(i) Production processes for solid lipid nanoparticles - Delay in drug release has long been achieved by using solid lipids in the form of pellets. In the early 1980s, Speiser and colleagues developed spray-dried, congealed micropellets and nanopellets of lipids for oral delivery. Microparticle concentrations were usually high in Speiser's nanopellets. Using high shear mixing or ultrasonication, Domb created lipospheres. However, Speiser and Domb's lipospheres and nanopellets were also tainted by microparticles. Over the past ten years, a numberof scientists have realized the promise of SLNs technology, and their study has led to improvements in the production of solid lipid nanoparticles.

(ii) High pressure homogenization- The high pressure homogenization (HPH) approach was initially used to create solid lipid nanoparticles by Muller and Lucks. Since a few years ago, homogenizers have been employed commercially to create nanoemulsions for parenteral nutrition, such Intralipid® and Lipofundin®. Scaling up is therefore less problematic and more economical than other ways. Naturally, numerous research teams have used this technique extensively to make better solid lipid nanoparticles. To boost the aqueous dispersion's physical stability, a homogeneous dispersion with a restricted size distribution is preferred. This method involves forcing a liquid at high pressure (100–2000 bar) through a microscopic opening.

(iii) Method of hot homogenization- The active component is initially dissolved in the lipid melt in this approach. By swirling a hot surfactant solution that has been heated to a temperature over the melting point of the lipid while adding the melted lipid, a coarse pre-emulsion is created. After that, a high pressure homogenizer is used to pass the pre-emulsion through three to five times while exerting pressurebetween 500 and 1500 bar. Then applied to the created nano-emulsion cooled to or below room temperature. As they cool, the lipid nano-droplets solidify, forming an aqueous dispersion of solid lipid nanoparticles. Because this raises production costs, increases the risk of metal contamination, and in some cases leads to an increase in particle size due to aggregation because of the high surface free energy of the particles, homogenization pressure and the number of cyclesshouldn't be higher than necessary to achieve the desired effects

LIPIDS AND SURFACTANTS' EFFECT

The physical stability of the formulation, the drug's rate of release, and the fate of the particles in vivo are all greatly impacted by the particle size of SLNs. The characteristics of the lipid and surfactant, production methods, and processing circumstances (such as time, temperature, pressure, and cycle count) all have an impact on particle size. With an increase in the melting point of lipids, the average particle size of the SLN dispersion increases for

both high pressure homogenization and high shear homogenization procedures. Larger particle sizes have been attributed to an increase in the viscosity of the dispersed phase with an increase in the melting point of the lipids. Each type of lipid has different requirements in terms of other factors including crystallization rate, lipid structure, and size. The lipid's composition has a significant impact on the SLN dispersion's quality. Additionally, because the majority of the used lipids are blends of several chemical compounds, their composition differs across suppliers and batches. Due to the increased viscosity of the liquid SLN dispersion, which affects the homogenization efficiency and increases the rate of particle agglomeration, lipid content exceeding 5–10% causes an increase in particle size and polydispersity index. The size and effectiveness of the SLNs as a drug delivery mechanism are influenced by the properties and concentration of the surfactant. The SLN surface area grows as particle sizes get smaller. Phase separation from the Ostwald ripening phenomena occurs from this increased surface area's thermodynamic instability. Following SLN preparation, there should be enough surfactant in the concentration to cover all newly created surfaces. Surfactants reduce the interfacial tension between the lipid and aqueous phases, which prevents phase separation from occurring. The formulation may contain excessive surfactant in the form of monomer, micelles, or liposomes. Research teams have identified a few trends between surfactant concentration and SLN quality, and it is important to identify the surfactant's ideal concentration in each formulation. Additionally, it has been found that SLNs stabilized with a surfactant/cosurfactant combination had lower particle size and improved stability compared to those made with a surfactant alone. Tyloxapol 10%w/v was necessary to stabilize 10%w/v tripalmitin dispersion, according to Siekmann et al. When compared to an SLN dispersion stabilized by a nonionic surfactant (200 5 nm), an ionic surfactant-stabilized SLN dispersion displayed smaller particle size (70 2 nm), according to research by Cavalli et al. Depending on the kind of surfactant used in the SLN, a certain formulation's homogenization parameter will need to be adjusted. Poloxamer 188 stabilized systems homogenized best with 500 bar pressure and three cycles, whereas lecithin stabilized systems required 1500 bar pressure.

RESULTS AND DISCUSSION

(i) Analysis of Particle Size- Nanoparticles of solid lipids are spherical lipid particles that fall within the nanometer size range. Therefore, measurement of the particle size both during and after the creation of solid lipid nanoparticles is an essential step in the process. Table 1 displays the particle size data of SLN dispersions that were generated by microemulsion and magnetic stirring processes, as well as the influence of a number of different surfactants and surfactant- cosurfactant combinations on the particle size. When the hot microemulsion was dispersed in the cool aqueous medium, it was sometimes noted that a few particles with a bigger particle size were present; as a result, no further research were carried out utilising the microemulsion technique.

Formulation process	Type of surfactant	Particle size (nm) ± σ
Microemulsion	Tween 80	97.25 ± 45.02
	Tween 80 + PEG 400	70.60 ± 12.34
Magnetic stirring	Tween 80	66.00 ± 6.23
	Tween 80 + PEG 400	56.9 ± 9.68 ,268.02 ± 128.64

Table 1- Particle size of SLN solution generated using the microemulsion with magnetic stirring method, as well as the influence of a variety of surfactants (denotes standard deviation, n = 3)

Oil : surfactant	Mean volume diameter (nm) ± σ
1:2	70.27 ± 2.81, 1074.2 ± 196.73
1:3	66.00 ± 6.23
1:4	55.75 ± 6.43
1:5	57.35 ± 6.57

Table 2- The effect of the surfactant concentration on the particle size of the SLN dispersion obtained by the use of the magnetic stirring process (denotes the standard deviation, n = 3)

Figure 1-1- A magnetic stirring procedure was used to prepare the SLN dispersion, and the oil to surfactant ratio was 1:2

Figure 1-2- SLN dispersion made using the temperature-modulated technique, with a ratioof oil to surfactant of 1:2.

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Figure 1-3- Lyophilized SLNs seen in this photograph

Comparable process characterization research was carried out on the temperature-modulated solidification process in order to investigate the impact that surfactant had on the particle size. On the basis of a comparison of visual clarity and the Tyndall effect (a bluish tinge when observed in light), which are shown in figures 1-1 and 1-2, an oil: surfactant ratio of 1:2 was found to be sufficient to prepare SLNs by the temperature modulated solidification process, whereas the magnetic stirring process produced large particles (figure 1-3). It is possible that the formulation contains an excessive amount of surfactant in one of several forms, such as monomer, micelles, or liposomes; also, the drug may be entrapped in micelles. Therefore, a ratio of oil to surfactant of 1:2 was employed in the optimised formulation that was prepared bythe temperature modulated procedure. Additionally, 150 ml of deionized water was used as the dispersion medium, and the nanoemulsion that was obtained was agitated for 45 minutes. All of the subsequent research was carried out on the same samples that had 5-fluorouracil already integrated into the SLNs before they were subjected to the temperature regulated solidification process.

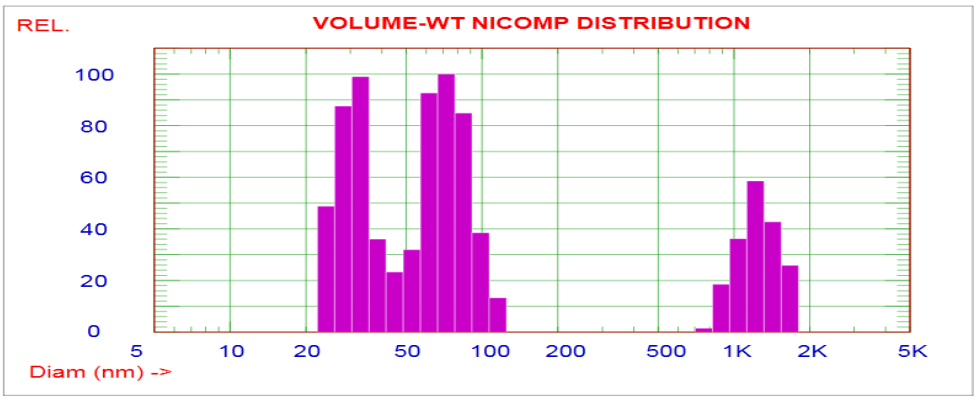
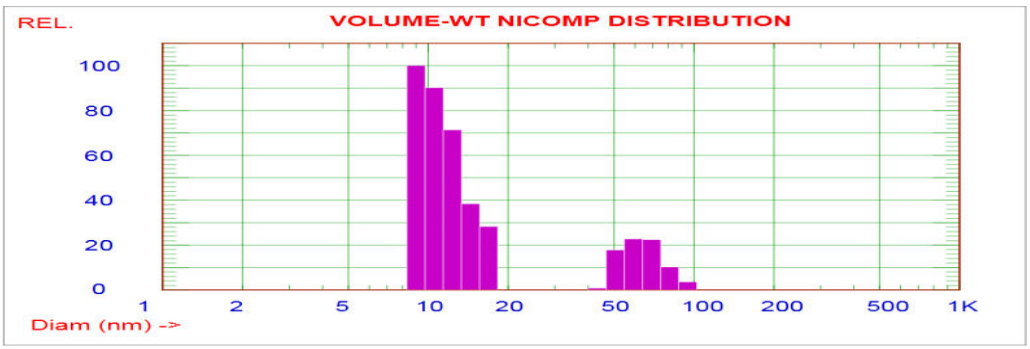


Figure 1-4- The magnetic stirring technique yielded an accurate distribution of particle size ofthe blank SLN dispersion, which was created with an oil:surfactant ratio of 1:2

The results presented in Table 4.5 show that the integration of the medicine did not have a substantial impact on the particle size of the formulation. The particle size rose as a result of lyophilization of the SLN dispersion, although it continued to fall within the range of nanometers. The results of the instrumentation used in the DLS tests are depicted in figures 4-31 through 4-7. This result has been reported by other research groups as well, and it is



possible to see Tween 80 micelles in the particle size distribution that was produced by dynamic light scattering (size 6-10 nm).

Figure 1-5- A representative sample of the particle size distribution of the blank SLN dispersion that was generated using the temperature-modulated solidification process

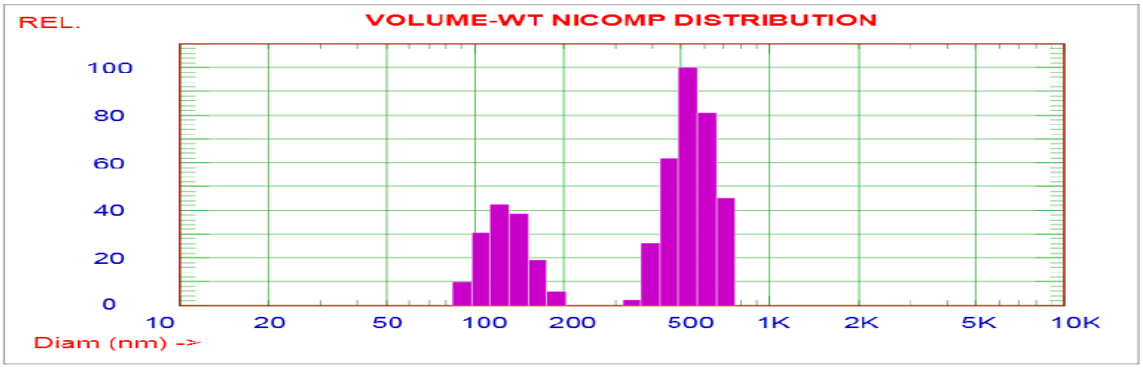


Figure 1-6- A temperature-modulated solidification technique was used to create blank redispersed lyophilized SLNs. The following chart shows the representative particle size distribution of the SLNs

CONCLUSION

It was discovered that the efficiency was roughly thirty percent, which may be because 5-FU is water soluble, leading to quick partitioning into the aqueous phase and, as a result, decreased encapsulation into the SLNs. This was discovered. According to the findings of in vitro drug release assays conducted with redispersed lyophilized SLNs, 17% of the encapsulated medication was released within two hours. An early burst release of 5-FU suggests that the compound has been adsorbed onto or very close to the surface of SLNs. It is possible that the structural integrity of the lipid matrix, which leads to the establishment of a barrier that prevents drug diffusion, is to blame for the low drug release from the SLNs. Future work in this research project will include exploring the influence of pH on in vitro drug release of 5-FU from SLNs, as well as investigating drug incorporation in the SLNs made via microemulsion process and magnetic stirring process, as well as conducting in vitro cell toxicity studies and formulation testing in animal models.

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