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Drug release and sperm motility protection studies of vitamin E encapsulated in liposome, cyclodextrin or polyethylene glycol

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ABSTRACT

Nanoencapsulation may be a good way to preserve hydrophobic bioactive components from unfavorable conditions while also increasing their solubility and bioavailability. Vitamin E has major hurdles because of these chemicals' limited solubility and bioavailability. In this study, the drug release and sperm motility effects of several nanoencapsulation delivery strategies for alphatocopherol, such as inclusion complexes through cyclodextrins, liposomes, and PEG, are investigated. The alpha tocopherol release test was performed on the dialysis bags, and the motility parameters were analyzed using Casa. Because of the rigidity of the liposomal barrier, which was enhanced by adding cholesterol, liposomes did not release the vitamin E immediately, but instead kept it for 24 h. After that, within 48 h, all of the vitamin E molecules had been released. Due to both agitation and fence hydration, release was most likely by Fickian diffusion, which involved the formation of mini-ducts. Furthermore, compared to all other therapies, semen motility improved significantly when vitamin E liposome formulations were employed. Liposomes developed in this study modified the release of vitamin E, making them a promising option for sperm preservation during cryopreservation.

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1. Introduction

Recently, to increase the stability, safety, efficacy, and therapeutic profile of a drug, a range of drug delivery methods have been created based on drug release mechanisms such as rapid release, delayed release, sustained release, and controlled release [1]. The actions of releasing encapsulated chemicals from carrier and displaying specified concentration–time curves at the target site are referred to as a release profile. The nature of the release mechanisms has a big impact on the release profiles. When compared to traditional solution or emulsion-based systems, pharmaceutical scientists and formulators are interested in NC vehicles because of the potential advantages in managing biodistribution and release profiles for parenteral delivery. Polymer nanoparticles [2], dendrimers [3], liposomes [4], micelles [5], hydrogels [6], proteins [7], and inorganic materials [8–11] are some of the materials that have been studied. Due to their unique structure, characteristics, and ability to effectively entrap drug molecules, cyclodextrins (CDs) are particularly promising options for the controlled release of pharmaceuticals among drug delivery carriers [12,13]. CDs are natural cyclic oligosaccharides having six, seven, or eight glucose units connected by α-1, 4 glucoside linkages, and are designated by the letters α-, β- and γ-CD [14]. All CDs have a cone-like shape, with a hydrophilic external surface and a hydrophobic inside hollow. CDs can form inclusion complexes with a wide range of poorly water-soluble and size-matched guest molecules thanks to their hydrophobic cavity. Drug solubility [15], stability, permeability, effectiveness, biosafety, and bioavailability [16–19] are all improved by them. Liposomes can encapsulate a variety of water-soluble medicines, however with little control over the rate of release and often low drug mass loadings [20]. Liposome (remote) loading methods that vary drug solubility inside the liposome have boosted drug loading in liposomes, however they do not give independent release control [21,22]. PEG or its derivatives have been conjugated to bioactive molecules or grafted on to deliv-

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ery systems, mitigating the interactions between bioactive-loaded systems and physiological environments, known as the stealth effect [23]. Polyethylene glycol (PEG), liposomes and cyclodextrins are widely used in delivery systems to protect sperm against drawback of cryopreservation, such as oxidative stress and cold choc. As a result, supplementing extenders with antioxidants such as vitamin E has been suggested as a method of mitigating the harm caused by the freezing-thawing mechanism [24–26]. Vitamin E (Vit E) is a fat-soluble antioxidant with the principal biological function of interrupting lipid peroxidation chain events in cell membranes [27]. Vit E is one of the most potent lipid-soluble antioxidants in humans, scavenging lipid peroxyl radicals by donating hydrogen from their phenolic group [28]. The major goal of this research is to find an encapsulating technology that optimizes alpha tocopherol release and sperm protection during cryopreservation (see Fig. 1.).

2. Materials and methods

Alpha-tocopherol (vitamin E) was purchased from SIGMA-ALDRICH, T3634, lot#SLBJ1634,PCODE: 1002263195, CAS: 59–02-9.

Cholesterol was purchased from SIGMA-ALDRICH, C8503, lot #SLBR0583V. PCODE: 1002334996.CAS: 57–88-5.

Saturated phospholipids were purchased from RHONE POULENC (Phospholipon 90H lot: 90060).

Fructose was purchased from SIGMA-ALDRICH, F0127, lot#SLBM7710V, PCODE: 101675782, CAS: 57–48-7.

TRIS (hydroxymethyl) aminomethane, SIGMA ALDRICH, T6066, lot #SLBS9646, PCODE: 101860987, CAS: 77–86-1.

Ethanol and methanol HPLC grade was purchased from Biochem, PCode: 205042500, 64–17-5.

Polyethylene glycol 6000 was purchased from Biochem.

Permethyl beta Cyclodextrin, SIGMA ALDRICH, Germany

2.1. Method

Buffer Tris preparation : 2.5 g acetic acid, 1.8 g fructose, 4.35 g TRIS, and 0.2 g penicillin were dissolved in 180 ml of distillated water to make a buffer TRIS solution.

Nano systems preparation

Cyclodextrin/ Vitamin E: In 75 ml of ethanol, cyclodextrin containing cholesterol or vitamin E was dissolved. The resulting mixture was kept at room temperature for 24 h, stirring constantly and protected from light. The solvent was then evaporated under vacuum at 40 °C by rotary evaporation and the residue was kept in a desiccator until used.

PEG 6000/ Vitamin E dispersion:Polyethylene glycol 6000 and vitamin E were dissolved in ethanol by agitation, the solvent was then evaporated by rotary evaporation under vacuum at 40 °C, and the residue was stored in a desiccator until required.

Liposome/ Vitamin E: Ethanol injection was used to form liposomes. In 15 ml of ethanol, 10.9 mg/ml Phospholipid 90H, 1.6 mg/ml cholesterol, and 2 mg/ml vitamin E were dissolved. Under magnetic fields, the resultant organic phase was injected into 40 ml of aqueous phase. The ethanol was then removed via rotary evaporation at lower pressure, resulting in the formation of spontaneous liposomes.

2.1.1. In vitro release studies

The dialysis method was used to conduct in vitro release testing of liposome VitE CD (VE –CHL) PEG (VE –CHL). 1 ml liposome solution, cyclodextrin, and peg complexes in the amount of vitamin E corresponding to 2 mg/ml were placed in a dialysis bag (Spectra/Por 4, molecular cut-off 12–14 kD, Californina, CA, US8A) that had been cleaned and soaked for 30 min in this study. A dialysis clip was used to seal both boundaries. After that, the gadget was immersed in 50 ml of dialysis fluid. In a phosphate buffer pH 7.4, this solution contained 25% ethanol and 0.5 percent Tween80 (v/v). The dialysis bags had been agitated. 500 L was taken at specified intervals of 0 h, 1 h, 2 h, 24 h, and 48 h. A HPLC at 208 nm was used to quantify the samples.

2.1.2. Kinetic analysis of drug release data

Drug release data were analyzed using four model-dependent approaches were used to compare the Vit E dissolution profiles. The model-dependent approaches included the zero-order, the first-order, the Higuchi, and the Korsmeyer-Peppas models.

2.1.3. Motility analysis

The sperm motility characteristics were assessed using a Computer Assisted Sperm Analyzer (CASA) (Sperm class analyzer, SCA Microptic, S.L., Version 3.2.0, Barcelona, Spain). To promote image acquisition easier and avoid spermatozoa cell overlapping, the sperm were diluted to a concentration of less than 40 106Spz/ml.

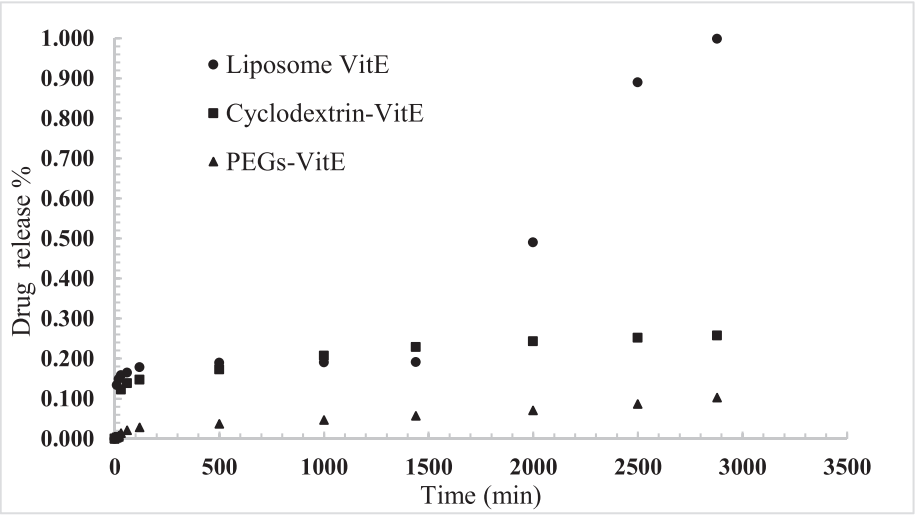

Fig. 1. Drug release profils (liposome-VitE, Cyclodextrin-VitE, PEG-VitE).

A phase-contrast microscope (Nikon E200®-LED microscope, Japan) was used to analyze five (5) l of each sperm sample. At a magnification of x10, images were acquired using a video camera (Caméra Digital Basler A312fc, Germany) (negative phase contrast). Total motility (percent), percentage of spermatozoa (STR percent), and straight linear velocity (VSL) were the sperm motility characteristics evaluated by CASA.

3. Results and discussions

3.1. In vitro release studies

Due to its insolubility in aqueous environments, vitamin E has limited dissolution [28]. VitE dissolution in PEG was significantly improved to percent after 48 h. This is likely owing to VitE dispersion in PEG during the preparation procedure, which is due to VitE solubility augmentation by changing its state from crystalline to amorphous, according to literature [29]. The encapsulation of VitE in the hydrophobic compartment of cyclodextrin increased VitE dissolution in CD significantly to percent after 48 h [30]. This is likely due to VitE encapsulation in the hydrophobic compartment of cyclodextrin. The profile of vitamin E loaded in liposomes is biphasic:

- A quick release of vitamin E was obtained in the first part: 0 to 24 h, stabilizing at roughly percent over time (during 24 h). This could be related to the burst effect, which occurs when only free drugs and drugs that were adsorbed on the surface of the liposome (due to weak binding force) are released [31]. In their study of the preparation of a liposomal delivery system and its in vitro release of rapamycin, Miao et al found that the release of rapamycin encapsulated in liposomes is divided into two phases, one due to the burst effect (presence of active ingredient on the liposome surface) and the other due to the burst effect (presence of active ingredient on the liposome surface) [31].
- The dissolution of Vit E improved significantly in the second part: 24–48 h, reaching 99 percent at 48 h. This is likely due to Vit E being trapped in the liposomal bilayer and being released via diffusion first to the exterior of the liposome, then to the outside of the dialysis bag. Indeed, multiple studies in the literature mention drug controlled release by liposome entrapment [32,33]. This behavior has also been documented by Xing et al. in their paper Recent Developments of Liposomes as Nanocarriers for Theranostic Applications [34]. Furthermore, the stiffness of the membrane caused by cholesterol contributed to the delaying of Vit E release [35].

3.2. Kinetic analysis of drug release data

The drug release kinetic was studied. Many different models exist for describing drug release, some of them can be applied to nano formulations, such as Korsmeyer-Peppas and Higuchi, etc [36,37]. In our case, four different fitting models were performed: The Korsmeyer-Peppas model, Higuchi model, first-order kinetic model, and zero order. From the R2 values presented in Table 1, it can be deduced that the Korsmeyer-Peppas model was the most accurate one. This model was already described for nanoparticles [38–40]. In addition, kinetic parameters corresponding to this model were extracted, specifically the release rate constant (K) and the release exponent (n). The n values that were obtained corresponded to a Fickian diffusion behavior. This result confirms that the release of Vit E through the bilayer and the dialysis bag follows release by diffusion, commended by the concentration gradient.

Table 1
Drug release kinetic data obtained from fitting drug release experimental data by various mathematical models (liposome-VitE, Cyclodextrin-VitE, PEG-VitE).

Parameters	Liposome-VitE	Cyclodextrin-VitE	PEG-Vit E
Zero order			
K_0	0,0020	0,0001	0,0080
R^2	0,7820	0,2295	0,8672
First order			
K_1	0,0020	0,0001	0,0080
R^2	0,7180	0,3216	0,8801
Higuchi order			
K_H	0,0140	0,0057	0,0017
R^2	0,5980	0,7033	0,9636
Krosmeyer pepass			
K_{KP}	0,0290	0,0263	0,0063
n	0,4000	0,3000	0,3000
R^2	0,7910	0,9363	0,9702

3.3. Motility analysis

Sperm resistance to cryodamage is determined by the cell membrane's structure and antioxidative power. Freezing extenders have been augmented with several treatments to improve post-thawed sperm quality, including the inclusion of cholesterol and -tocopherol with various encapsulation techniques (Cyclodextrin, polyethylene glycol (PEG 6000), or liposome). The sperm motility metrics following cryopreservation are shown in Fig. 2. As can be shown, the different treatments enhanced the STR, VSL, and ALH considerably when compared to the control group (P 0.05). After 48 h of chilling, liposome-loaded vitamin E followed by PEG (Vit E-CHL) had the most significant effect (P0.05) on all motility measures examined. Individually, supplementing with vitamin E or cholesterol improves motility metrics when compared to the control group. Their combination, on the other hand, produced a more efficient result, which is consistent with prior research that found cholesterol and α-tocopherol to have similar beneficial effects in post thawing motility [41,25].

In this study, vitamin E loaded in liposome effect on spermatozoa survival after chilling has been investigated. The results show that the best sperm protection effect is obtained when using liposome vitamin E during 48 h of chilling, it is due to the simultaneous effect of vitamin E and cholesterol (antioxidants protection) and also to the interactions between liposomes and cells likely facilitate lipid and cholesterol transfer causing rearrangement of cell membrane components affecting cryostability of the cells. In addition, it was demonstrated that liposomes can be loaded with lipid-related content (such as lecithin or cholesterol) [42] to improve the plasma membrane regeneration efficacy during the freeze–thaw process of ram spermatozoa. Also, Liposomes were used as cryoprotectant additives in several animal species including equine [43,44], buffalo [45], ovine [46–48], porcine [49], and bovine [50] with reported improvement in fertility after artificial insemination [51]. Finally, it has been reported that liposomes with their contents of phospholipids saturated and unsaturated fatty acids can fuse with the sperm plasma membrane and abate the damage to spermatozoa caused by the freeze–thaw process [52,53].

4. Conclusion

Nanoencapsulation of hydrophobic bioactive chemicals with various carriers has the potential to improve their solubility, bioavailability, and stability, as well as their application in food and pharmaceutics. This research looked at various nanocarriers for vitamin E, including inclusion complexes with cyclodextrin, liposomes, and PEG. The release of nanoencapsulated systems as

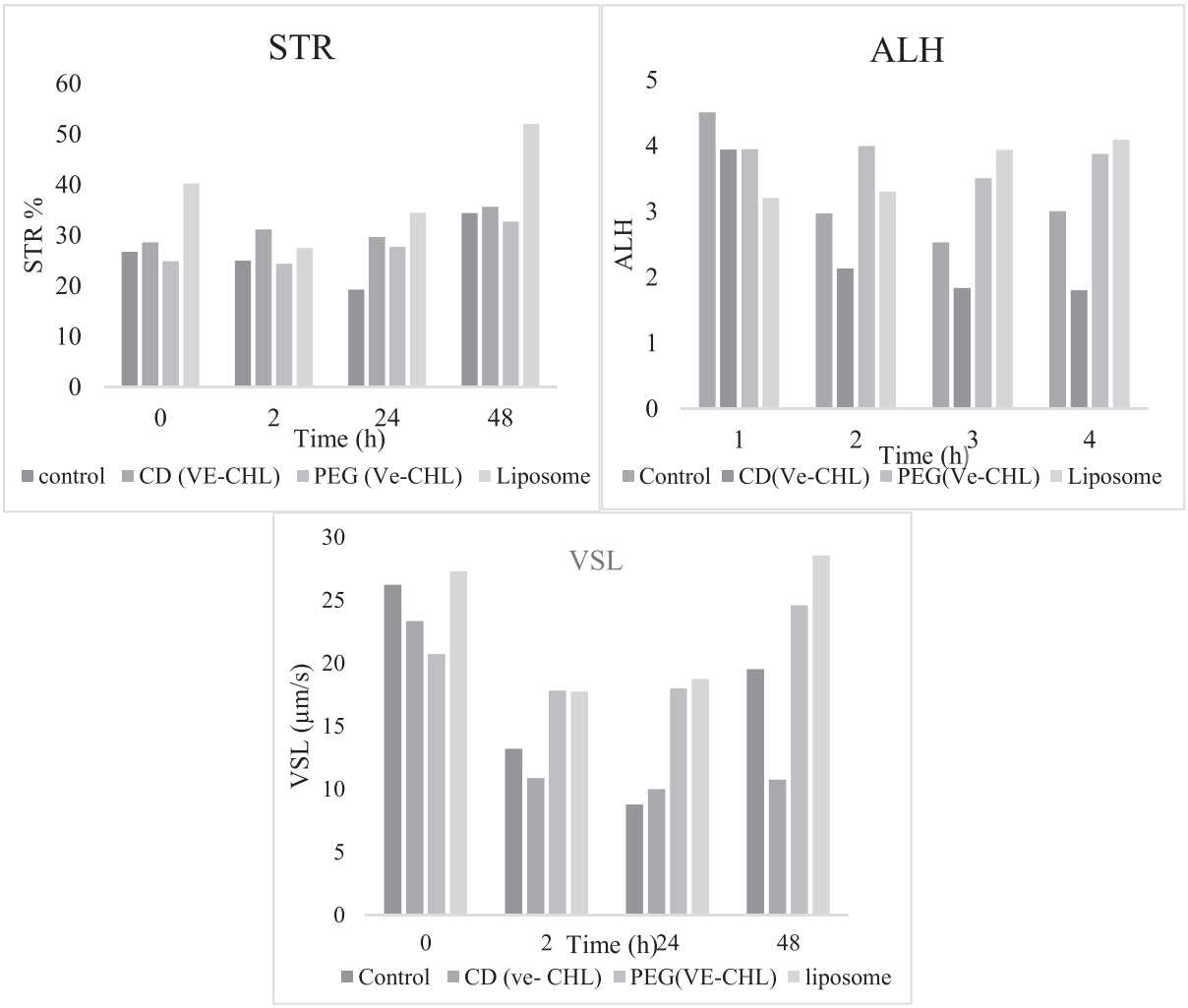

Fig. 2. Mean (±S.E.M) of curvilinear velocity (VCL), straight linear velocity (VSL), average path velocity (VAP), after semen cryopreservation in the control group (Cntrl), free VitE, CHL, (CD- Vit E- CHL), (PEG 6000-Vit E-CHL), and VitE loaded in liposome.

well as sperm motility metrics have also been studied. These findings showed that using liposomes to boost Vit E solubility and bioavailability in the cryopreservation medium while also improving cell penetration and sperm protection in the cryopreservation process is an interesting and promising strategy.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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