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Development of PEG-free Excipients for Drug Delivery

Enabling Technology

The Challenge

Polyethylene glycol (PEG) is a well-known polymer whose unique physicochemical properties increased its extensive application in the pharmaceutical and cosmetic industry. Notably, PEG-lipid conjugates, where PEG is covalently linked to a lipid-based component, are commonly applied in the formulation of some of the most successful nucleic acid delivery systems. These lipid nanoparticles (LNPs), act as shielding components to reduce immunogenicity and enhance blood circulation times, maximizing therapeutic output of the delivery system. PEG-shielded LNPs have recently been positioned as key non-viral vectors for gene delivery applications with BioNTech/Pfizer and Moderna/NIAID mRNA COVID-19 vaccines leading the way, as well as many others in clinical trials for cancer, infectious, and neurodegenerative diseases.

One particular example of this is the PEG-containing adjuvant PEG-vitamin E conjugate amphiphile (also known as tocofersolan or TPGS), designated as a safe excipient by the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA), that can be found in a variety of drug delivery systems, some of which are in clinical evaluation.

While the physicochemical properties of TPGS can enhance the solubility and biocompatibility of Active Pharmaceutical Ingredients (APIs), the use of PEG components, in the pharmaceutical and cosmetic industry, has raised concerns related to immunogenicity and loss of efficiency of PEG-containing medicines. The production of anti-PEG antibodies has the potential to clear PEGylated excipients from the bloodstream by a phenomenon called Accelerated Blood Clearance (ABC).

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These concerns are driving demand for PEG-free excipients being particularly evident in the case of PEG-shielded LNP formulations. In fact, the tremendous exposure of the population to PEG, by the worldwide vaccination campaign against COVID, has accelerated the interest in PEG-free alternatives by pharma industries.

The Solution

To cover this need, Curapath has developed a portfolio of PEG-free polymer-vitamin E novel functional excipients for use in nanomedicine applications. One conjugate combines the vitamin E derivative alpha-tocopherol and the PEG analogue polysarcosine, a biodegradable, biocompatible, and non-immunogenic polymer (***Figure 1***).

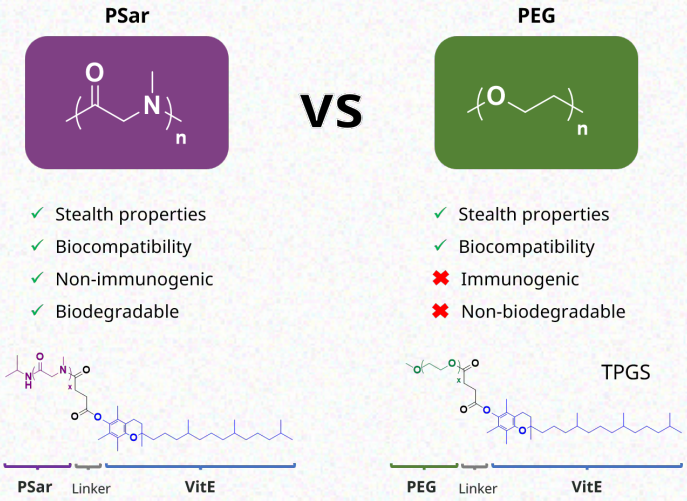

Figure 1. Properties and structure of PSar and PEG polymers summarizing the advantages of PSar over PEG.

A variety of these polysarcosine-vitamin E (PSar-vitE) excipients have been produced and scaled up in technical batches with demonstrated batch-to-batch reproducibility and QC compliance. Proof-of-concept of their use as LNP components was centered around a side-to-side comparison with Benchmark LNPs formulated using chaotic mixing with microfluidic techniques. Furthermore, the ability of PSar-vitE to form micelles and encapsulate cargo are comparable to those formed with TPGS.

Results

We have shown that different PSar-vitE prototype excipients with varying chain lengths can be produced and transferred to relevant scales with reproducibility and controlled QC protocols.

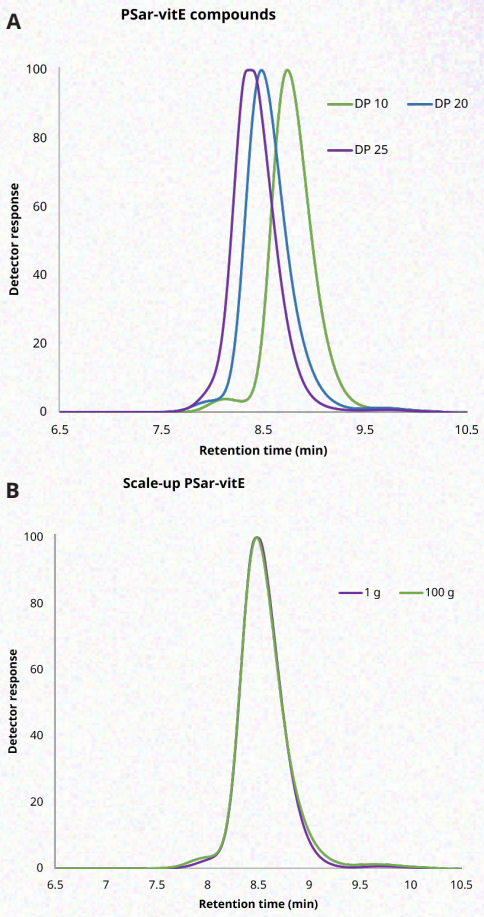

Figure 2. GPC traces of ***A***) PSar-vitE derivatives with different chain lengths or DP (degree of polymerization) showing good consistency with expected DPs; and ***B***) GPC traces of 1 and 100 g batches of PSar-vitE showing batch-to-batch reproducibility and scalability of the synthesis.

At Curapath, PSar-vitE excipients have been successfully incorporated in LNP formulations, in a scalable way, using qualified equipment (IJM NanoScaler, Knauer) (***Figure 3***); Development and optimization of LNP formulations using the NanoScaler, Curapath/Knauer Application Note, October 2022, www.curapath.com

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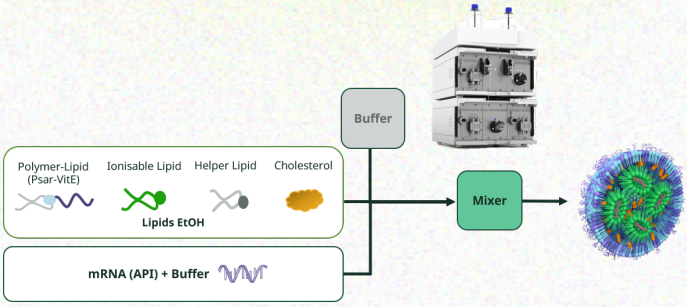

Figure 3. Workflow of the formation of LNP with PSar-vitE as polymer-lipid shielding component using Knauer's IJM NanoScaler formulation instrument.

We have formulated reproducible and stable prototype LNPs using up to 5% in molar ratio of VitE-lipid anchored PSar as a shielding excipient, and substitute of the Benchmark DMG-PEG. LNP Sizes obtained were comparable to those of Benchmark LNPs with suitable polydispersity index and high encapsulation efficiency in all cases (***Table 1***). Moreover, particle size could be tuned by using different PSar chain length in the conjugates highlighting the versatility of the excipients.

Hence, this Proof of Concept (POC) positions PSar-vitE as a very appealing alternative for LNP-based nucleic acid delivery applications.

Proof of the versatility of these new excipients for their use in other types of nanomedicines is that they can also form spherical micelles with size ranges analogous to those produced using TPGS (***Figure 4 and Table 2***).

These micelles easily encapsulate hydrophobic drugs such as carbamazepine (logP: 2.67) at comparable encapsulation efficiencies as TPGS.

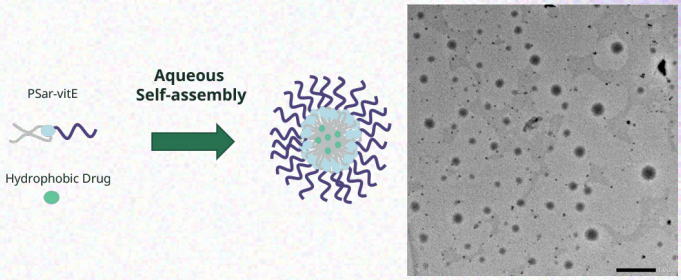

Figure 4. Aqueous micelle formulation of PSar-vitE encapsulating carbamazepine.

Moreover, when applied topically in human skin for their use as permeation enhancers, PSar-vitE micelles have shown higher penetration and accumulation of a delivered agent in the epidermal layer, compared to the analogue TPGS (***Figure 5***). Both conjugates are accumulated mainly in the stratum corneum (SC), but the PSar-vitE micelle showed higher accumulation in the epidermis layer than the TPGS micelle.

#	% Ionizable Lípid*	% DSPC	%Chol	Shielding Lipid	% Shielding Lipid	N/P	PDI	Z-ave (nm)	EE%
1	46.3	9.4	42.7	DMG-PEG	1.6	6.2	0.16	74.7	> 90
2	46.3	9.4	39.3	PSar(10)-vitE	5	6.2	0.20	167.0	> 80
3	46.3	9.4	39.3	PSar(20)-vitE	5	6.2	0.17	82.6	> 90
4	46.3	9.4	39.3	PSar(20)-vitE	5	6.2	0.19	53.2	> 90

* Commercial ionizable lipid; DSPC= 1,2-distearoyl-sn-glycero-3-phosphocholine; DMG-PEG= 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000Q=total flow; Chol=Cholesterol; N/P= ratio nitrogen to phosphorus; PDI=polydispersity Index by DLS; Z-Ave= average Hydrodynamic diameter; EE%= Encapsulation efficiency by semiquantitative gel electrophoresis.

Table 1. Phys-chem characterization of LNPs formulated with PSar-vitE PEG alternatives encapsulating mRNA.

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#	Polymer	Mg Carb/ Polymer	EE (%)	Size Intensity (nm)	PDI
1	PSar-vitE	35.55	21.6	14.2	0.13
2	TPGS	35.63	21.8	14.1	0.03

Table 2. Phys-chem characterization of micellar formulations from PSar-vitE and TPGS.

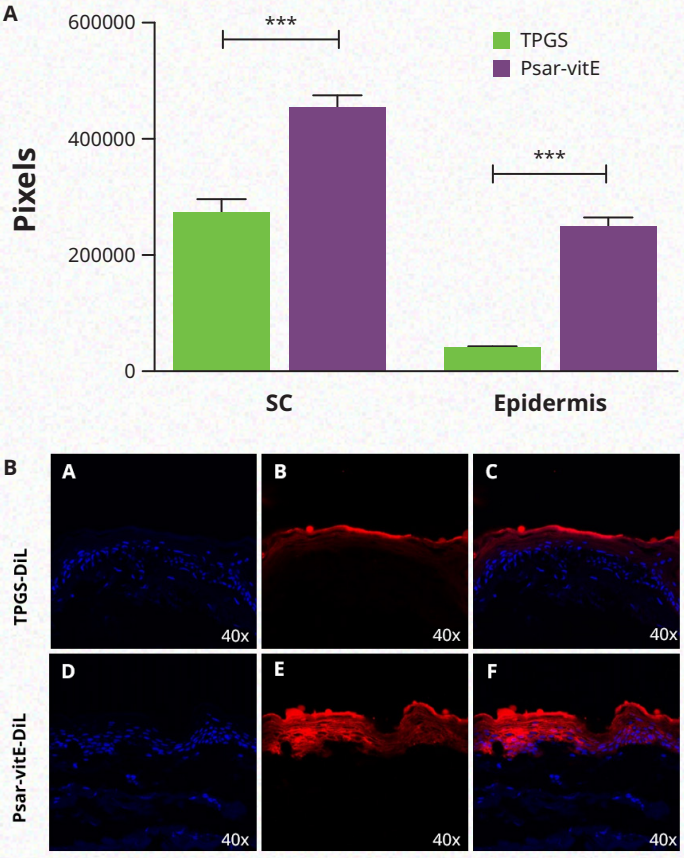

Figure 5. Skin permeation studies by Franz diffusion cells using human skin were performed employing TPGS-Dil and PSar-vitE-Dil. ***A***) Pixel quantification, ***B***) Confocal images.

Overall, the innovative PEG-free excipients can be used as solubilizer, absorption and permeation enhancer, emulsifier, and a surface stabilizer excipient. Envisaging what is ahead in the current nucleic acid delivery landscape, Curapath has engineered a library of different PEG replacement alternatives with the aim to not only overcome PEG drawbacks but to confer different surface features to help build the next generation LNPs.