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Novel PEGylated derivatives of α-tocopherol for nanocarrier formulations; synthesis, characterization and *in vitro* cytotoxicity against MCF-7 breast cancer cells

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ABSTRACT

Despite numerous beneficial therapeutic effects namely antioxidant and anti-inflammatory activity, Vitamin E has limited clinical applications due to its low water solubility. Throughout the present work, α-tocopherol's new PEGylated derivatives alongside with polyethylene glycol 300 (α-[1]TPGT^{300}), 400 (α-TPGT^{400}), and 1000 (α-TPGT^{1000}) were synthesized. A 1,2,3-triazole ring was utilized as a linker for the attachment of alpha tocopherol to the PEGs through a click reaction. The purified derivatives were characterized by the means of ^{1}H NMR, ^{13}C NMR, mass spectroscopy, UV–vis and FT-IR methods. Synthesized derivatives' capacity to produce self-assembly nanoparticles was evaluated employing the critical micelle concentration (CMC) values. The stability of the micelles was studied by size analysis. *In vitro* cytotoxicity of the products was investigated using MTT assay against MCF-7 breast cancer cells. The IC_{50} value for TPGT^{1000} after 24 h treatment was 15.0 ± 1.8 μM, whereas no significant cytotoxicity effect was observed following the treatment of MCF-7 cells by TPGT$^{300,\ 400}$. The present study showed that polymeric micelle TPGT^{1000} possessed better physicochemical and biological properties including relatively lower CMC value, higher stability in FBS environment in addition to higher cytotoxicity against MCF-7 breast cancer cells compared to the lower molecular weight PEGylated derivatives. These results confirmed that increasing PEG chain length left a positive effect on the polymeric micelle properties and also improved the cytotoxicity effect of new PEGylated vitamin E derivatives.

Vitamin E belongs to a family of eight-membered lipid-soluble compounds divided into two main subgroups known as tocopherols (T) and tocotrienols (T3). Vitamin E plays a vital role in quenching free radicals when used together with other antioxidant compounds such as vitamin C and beta–carotene. It has been proven that vitamin E is essential for the function of nerve and muscle cells. The anti-inflammatory effect of vitamin E by inhibition of lipoxygenase enzyme leads to the inhibition of inflammatory intermediates like leukotrienes.[1,2] Vitamin E leaves a therapeutic effect on a variety of illnesses such as cancer,[3] heart diseases, and the last but not the least the Alzheimer's disease.[4] However, vitamin E's low aqueous solubility has confined its clinical applications. To overcome the mentioned drawback, a polyethylene glycol (PEG) chain attached to the α-tocopherol succinate and characterized as D-alpha tocopherol polyethylene glycol 1000 succinate (vitamin E TPGS) has been synthesized and marketed extensively. TPGS has an effective role in enhancing the solubility and absorption of many poor water-soluble drugs. The tocol ester TPGS^{1000} is a water-soluble form of vitamin E with an amphiphilic structure that can self-assemble to form micelles.[5]

Micelles are well known nanocarriers extensively used in pharmaceutical industries for drug delivery and targeting purposes. Regarding the high loading capacity and the possibility of targeting the surface of this nanocarrier, micelles can be considered as effective carriers for drug delivery.[6] Polyethylene glycol (PEG) is one of the most universal polymers hired for the fabrication of nanocarriers based on its unique properties such as biocompatibility, biodegradability, and non-toxicity.

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[1] α-Tocopherol polyethylene glycol triazole

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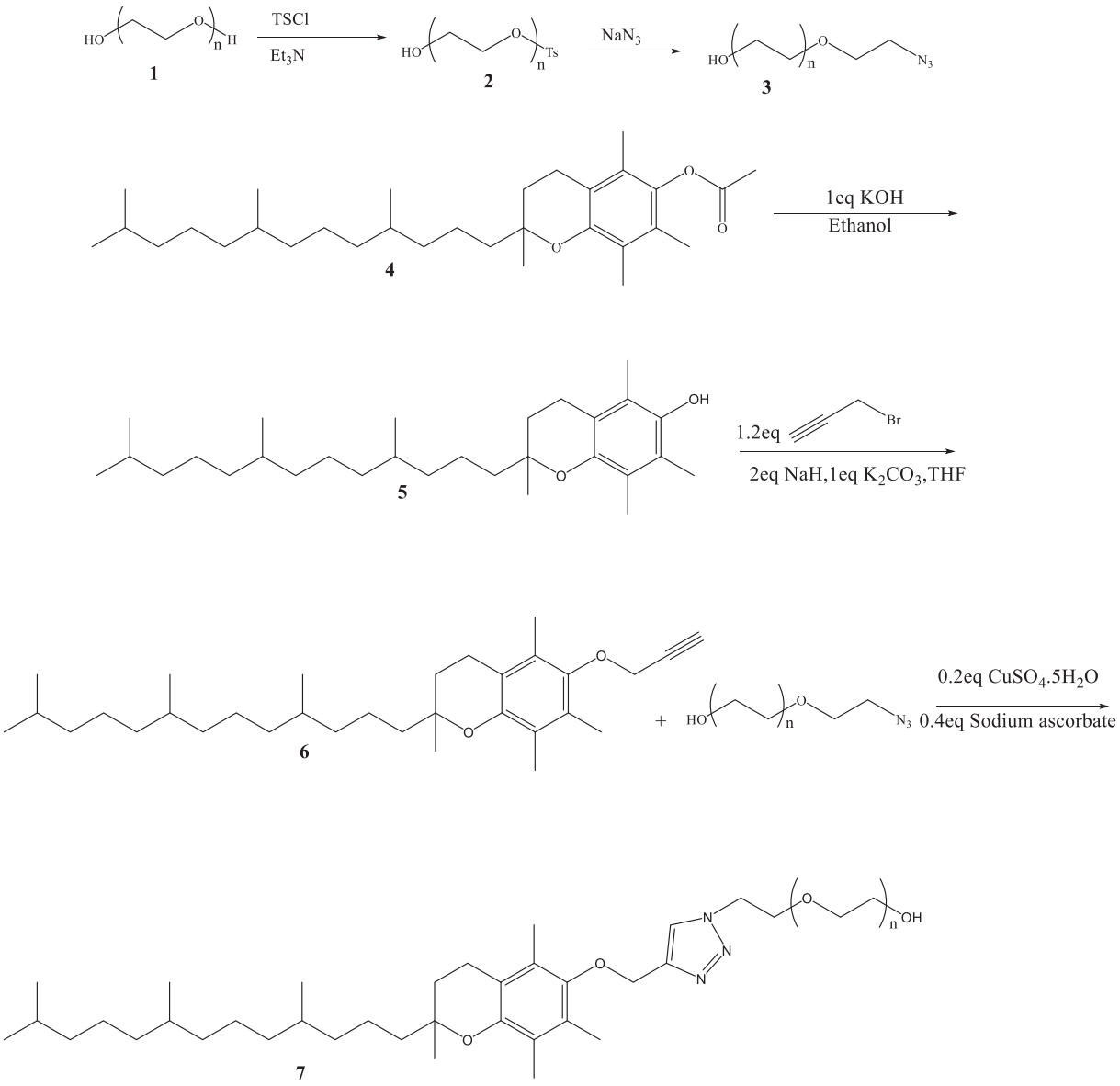

Scheme 1. Synthesis of PEGylated derivatives of vitamin E.

The surface PEGylation of nanoparticles also leads to the enhancement of the nanoparticles half- life *in vivo*, aggregation reduction, facilitation of water solubility, plus reduction of opsonization by the immune system cells.[7,8,9]

Click chemistry, introduced by K. Barry Sharpless in 1998, was fully describes in 2001 for the first time. Click chemistry has several advantages such as very fast reaction, simple to use, easy purification of the products, and finally high yield of the products due to selectivity of the reactions.[10]

The Huisgen 1,3-dipolar cycloaddition reaction is a recognized click reaction which is also termed as the [3+2] azide-alkyne cycloaddition, whose responsibility is to fuse two unsaturated reactants including azides and alkynes to afford five-membered heterocycles.[11] 1,2,3-Triazole rings are nitrogen-containing heterocycles able to form hydrogen bonds and leading to improved solubility and ability to interact with the biomolecules. They are highly stable against metabolic degradation

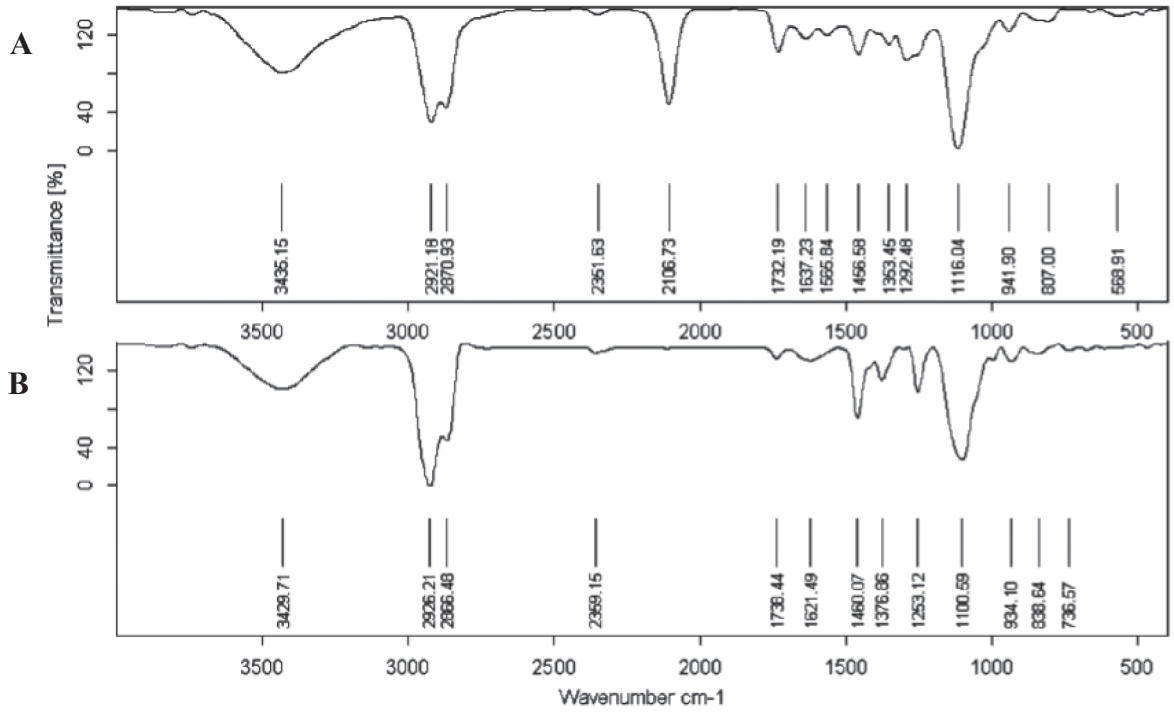

Fig. 1. FT-IR spectrum of A: PEG-300-azide, B: $TPGT^{300}$.

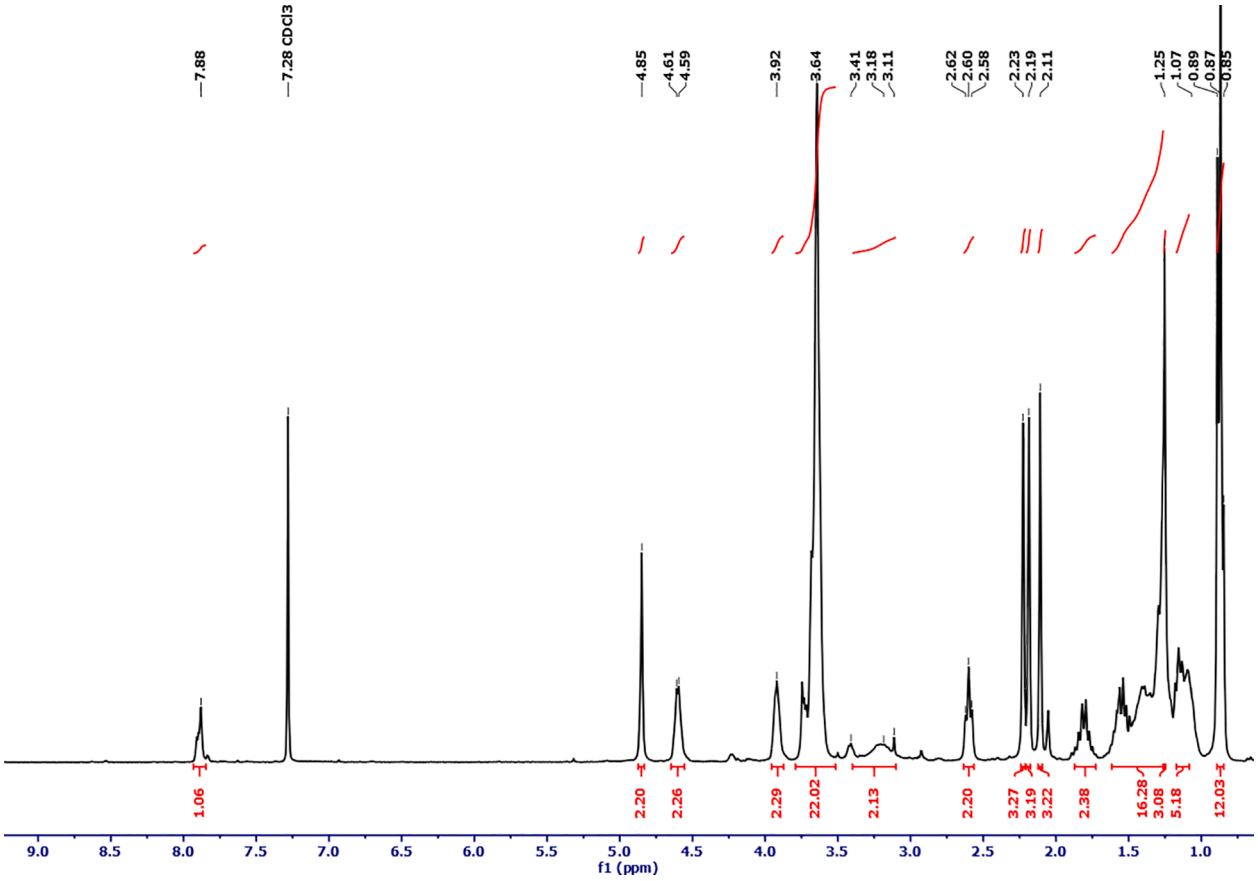

Fig. 2. ^{1}H NMR spectrum of $TPGT^{300}$.

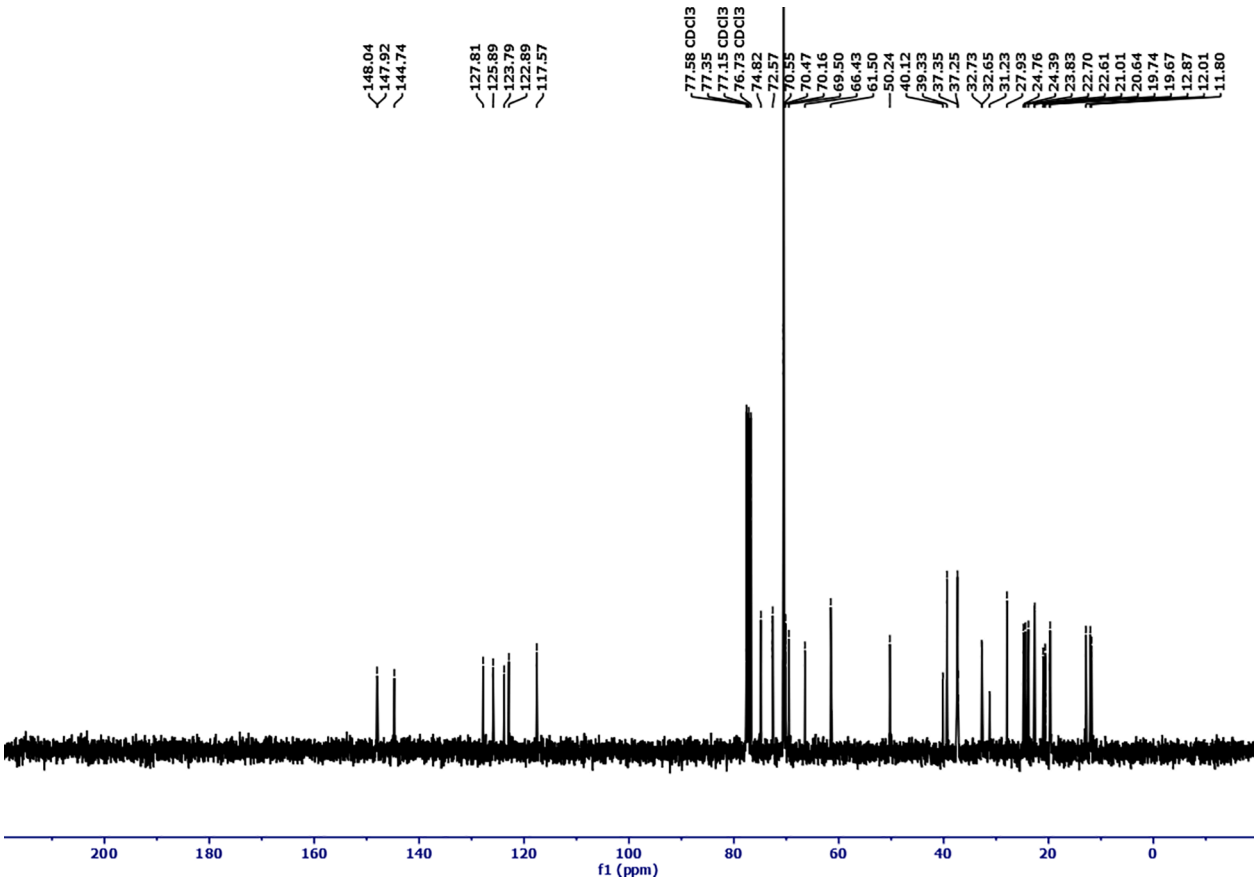

Fig. 3. ^{13}C NMR spectra of TPGT^{300}.

compared to the other compounds.[12] A wide assortment of therapeutic applications have been reported for triazoles due to their diverse biological activities including antimicrobial, antiviral, anti-inflammatory, analgesic, anticancer, antifungal, and anticonvulsant activities. In this regard, these particular scaffolds attract considerable attention in medicinal chemistry.[13]

The objectives of the current study were to (a) synthesize new PEGylated α-tocopherol derivatives utilizing polyethylene glycol 300 (α-TPGT^{300}), 400 (α-TPGT^{400}), and 1000 (α-TPGT^{1000}) by a click reaction; (b) fabricate nano-structured micelles from PEGylated derivatives, and characterize the designed micelles by DLS and evaluate the micelles' stability in different media; (c) assess the biological activity of the PEGylated α-tocopherol derivatives by *in vitro* cytotoxicity assay against human breast cancer MCF-7 cell line.

To overcome poor water solubility and limited absorption plus vitamin E's low bioavailability, PEGylation of α-tocopherol was performed by click reaction utilizing the triazole function as a linker. The novel derivatives of vitamin E were synthesized by using monoazide PEGs with different molecular weights (i.e. 300, 400, and 1000 g/mol) and propargylated vitamin E through 1,2,3-triazole linker in the presence of copper (II) sulfate as a catalyst and sodium ascorbate as a reducing agent. The synthetic pathway is exhibited in Scheme 1. The products were characterized by ^{1}HNMR and FT-IR spectra. FT-IR spectrum of PEGylated derivative (300 g/mol) is presented in (Fig. 1). According to this spectrum, the azide group signal disappeared in 1206 cm^{-1}. The presence of the signals in 1100, 1253, 1621, and 1738 cm^{-1} are correlated to the C—O bond, C—N bond, C═C double bond, and C═N double bond stretching, respectively, that confirmed the formation of triazole ring. ^{1}HNMR of PEGylated 300 g/mol derivative (Fig. 2) demonstrated a unique signal in 7.88 ppm that is correlated to the

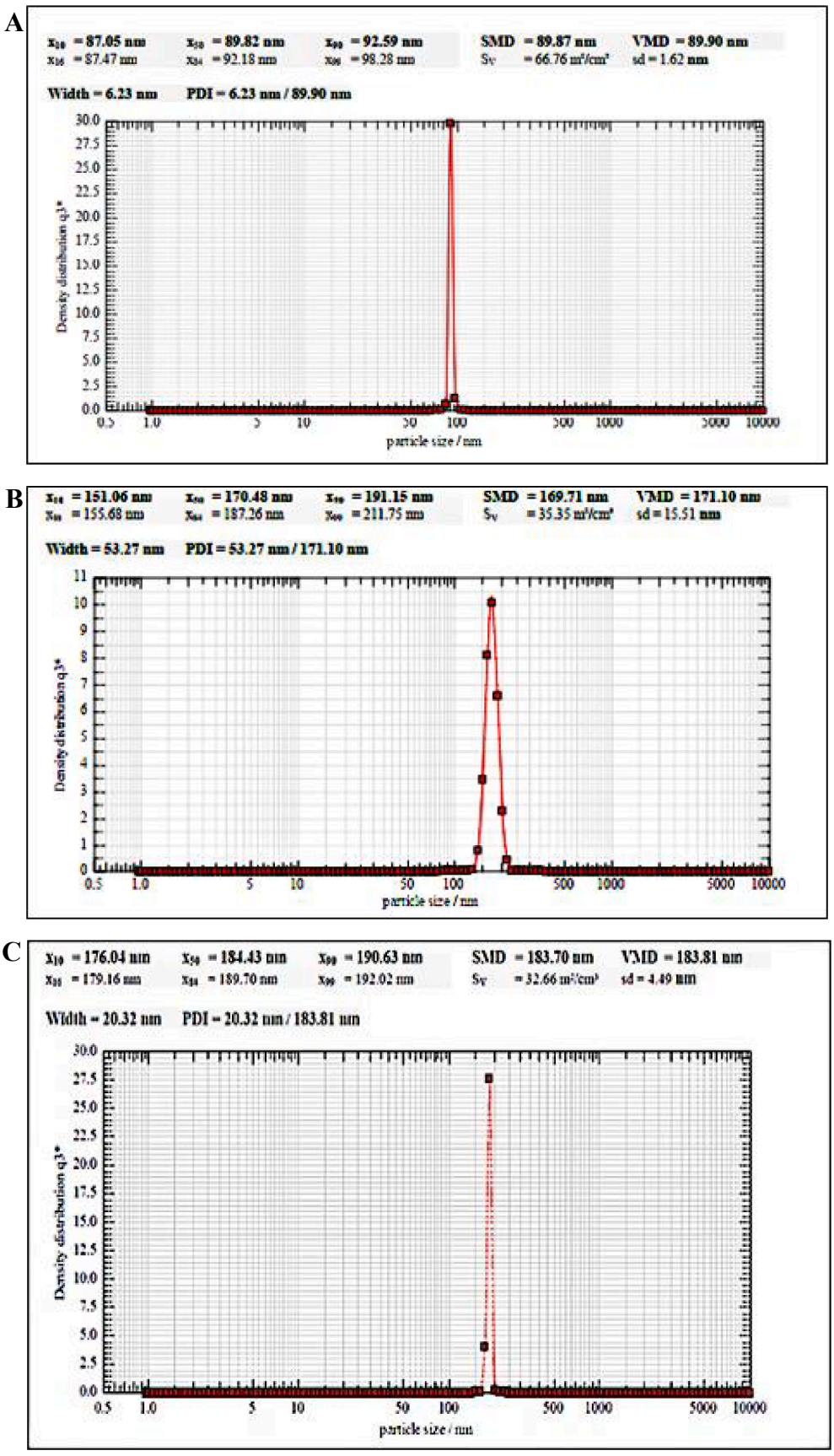

Fig. 4. Particle size distribution diagram of micelles, A: TPGT^{1000}, B: TPGT^{400}, C: TPGT^{300}.

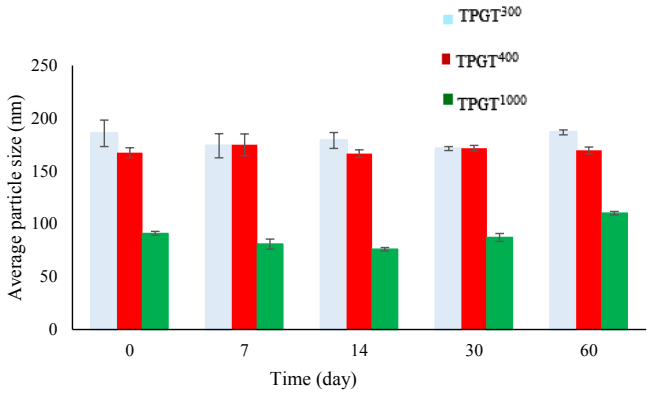

Fig. 5. Polymeric micelle size stability after 2 months storage at 4 °C.

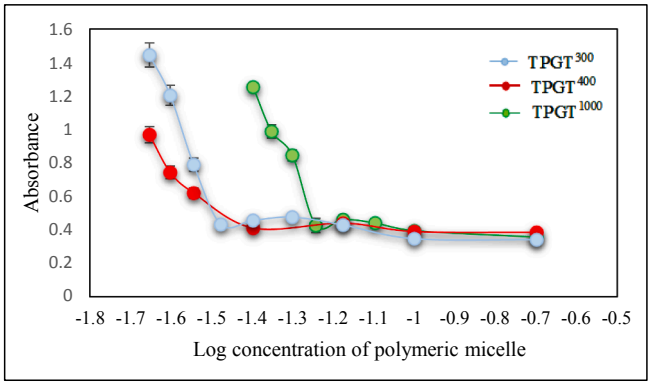

Fig. 6. CMC values for polymeric micelle systems in water.

hydrogen of the triazole ring. Singlet signal in 4.85 ppm represents methylene protons of the propargyl group. Methylene protons attached to the triazole ring appeared in the triplet form with an integral of 2 in 4.60 ppm. In Fig. 3, the ^{13}C spectrum of $TPGT^{300}$ displayed six carbon signals in 117.57, 122.98, 126.07, 127.96, 147.87, and 148.24 ppm that contributed to phenyl group of α-tocopherol and also showed two carbon signals in 123.79 and 144.74 ppm that respectively contributed to methine and quaternary carbon of triazole ring. These indicative peaks confirmed formation of the product. Full proton and carbon assignment from $TPGT^{300,\ 400,\ 1000}$ was mentioned within the supplementary data.

The size and size distribution of PEGylated derivatives were together measured employing dynamic light scattering (DLS) and the relative diagrams are presented in (Figs. 4 and 5). As can be observed, the mean particle size of the prepared micelles ranges from 100 nm to 200 nm. No significant changes in the micelle size or size distribution were recorded subsequent to storing the micelles at 4 °C for a period of two months, which confirms the high stability of the polymeric micelles.

The CMC values of the polymeric micelles have an essential effect on their stability, both *in vitro* and *in vivo*.[14] Within this study, iodine had a task as a hydrophobic probe to evaluate the CMC values of the polymeric micelles TPGT with different PEG weights of (PEG 300, 400, and 1000 g/mol). Solubilized I_2 distinction to participate in the hydrophobic section of TPGT, leads to the transformation of I_3 to I_2 from the surplus potassium iodide (KI) in the solution. To determine the CMC values of polymeric micelles, the ultraviolet absorbance of KI/I_2 at 366 nm profile were plotted versus the concentration of polymeric micelles. According to Fig. 6, the results showed that increasing the molecular weight of PEG in polymeric micelles produced a profound reduction in the CMC values. The relatively lowness of CMC of the polymeric micelles $TPGT^{1000}$, is able to improve the micelles' stability as well as boosting resistance against degradation upon dilution by blood circulation in the body, which is significant for effectively delivering to diverse types of tumors.[15,16] The hydrophilic sections of the amphiphilic molecules and the size of PEG have an effective part in micelles' function. Longer PEG chain in $TPGT^{1000}$ compared to $TPGT^{300,\ 400}$ provides higher HLB [2]value that leads to a relatively low CMC in $TPGT^{1000}$.[17,18]

The TEM images showed that the polymeric micelles of $TPGT^{1000}$ were spherical (Fig. 7) within the size range of 100–200 nm which was in conformity with the DLS result. In PEGylated derivatives of 300 and 400 g/mol, an aggregation of the micelles was observed in TEM micrographs. For DLS analysis, sample solutions of the synthesized compounds were diluted with deionized water prior to particle size measurement in order to retain the particle count in the range of 20–200 kcps. However, for TEM analysis, sample solutions of particles were dropped on a carbon-coated copper grid and dried subsequently. Once the water in preparation medium was removed, a close contact of nanoparticles in TEM analysis was observed (probably through formation of loose aggregates or flocculates). The mean particle size of individual particles within the flocculates is in harmony with the results of DLS analysis.

The effect of FBS on the particle size alternates the polymeric micelles at 37 °C within a period of 0–72 h and was monitored by DLS (Fig. 8). The results displayed that polymeric micelles of PEGlayted derivatives 300 and 400 g/mL were stable for 24 h and then an enhancement in the size of the polymeric micelle was observed. However, the polymeric micelle $TPGT^{1000}$ was stable for 48 h. It can be proposed that the increase of the PEG chain in $TPGT^{1000}$ positively influences the stability of the polymeric micelle structure in a simulated biological environment of the body. Probably increasing the PEG chain length reduces the protein adsorption on the surface of the polymeric micelle via creating spatial hindrance and as a result reduces the micelle degradation and protein corona.[19]

The cytotoxicity effects of the polymeric micelles were investigated against MCF-7 cells. Fig. 9 shows the viability of MCF-7 cells after 24 h treatment with the polymeric micelles using various PEG chains (300, 400, 1000 g/mL). As shown in Fig. 9, the polymeric micelle of $TPGT^{1000}$ exhibited higher cytotoxicity and inhibited the cell viability about 65.09 ± 1.13% in 25 μM concentration with an IC_{50} value of 15 ± 1.8 μM. As reported in previously published studies, free α-tocopherol has no significant cytotoxicity effect against MCF-7 cells in 25 μM.[20] Another report mentions that the IC_{50} value of $TPGS^{1000}$ against MCF-7 breast cancer cells is 26.75 ± 1.07 μM.[21] According to these results, conjugation of PEG 1000 to α-tocopherol result in better cytotoxicity against MCF-7 cells compared to free α-tocopherol. The higher degree in cytotoxicity might be correlated to the ability of $TPGT^{1000}$ to generate [3]ROS, which consequently induces apoptotic cell death in more effective manner than that of α-tocopherol.[22] Polymeric micelles with PEGs 300 and 400 g/mL showed no significant cytotoxicity effect against MCF-7

[2] Hydrophilic–lipophilic balance

[3] Reactive oxygen species

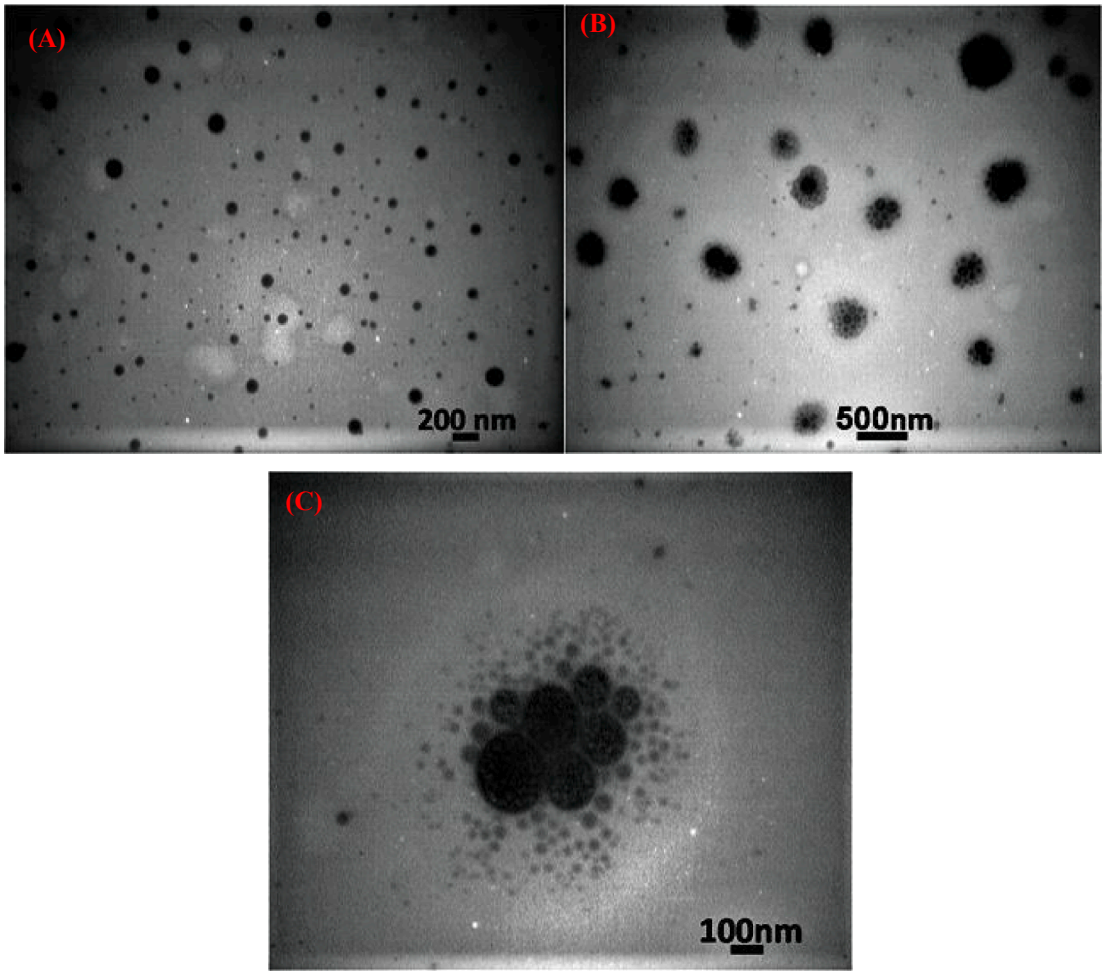

Fig. 7. TEM micrographs of polymeric micelles (A) $TPGT^{1000}$, (B) $TPGT^{300}$, (C) $TPGT^{400}$.

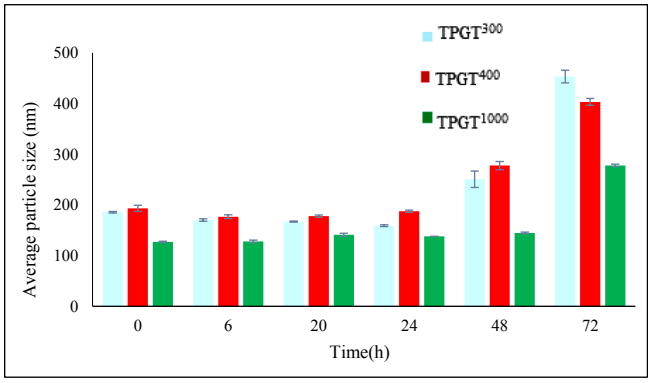

Fig. 8. Stability of polymeric micelles in 50% FBS.

cells below the concentration of 100 μM. It can be postulated that increasing the PEG chain length in polymeric micelle $TPGT^{1000}$ probably influences the interaction with the membrane protein receptors or protein channels and also facilitates the cell entrance via the endocytosis process and resultantly induced apoptosis.[23,24,25]

In this paper, we reported the synthesis and characterization of tree novel PEGylated derivatives of α-tocopherol and investigated the physicochemical and biological effect of these compounds. The results implied that $TPGT^{1000}$ put on display better physicochemical properties such as relatively lower CMC value and higher stability in FBS environment. What's more, $TPGT^{1000}$ showed better biological effect, as a result of its higher cytotoxicity against MCF-7 cells compared to that of

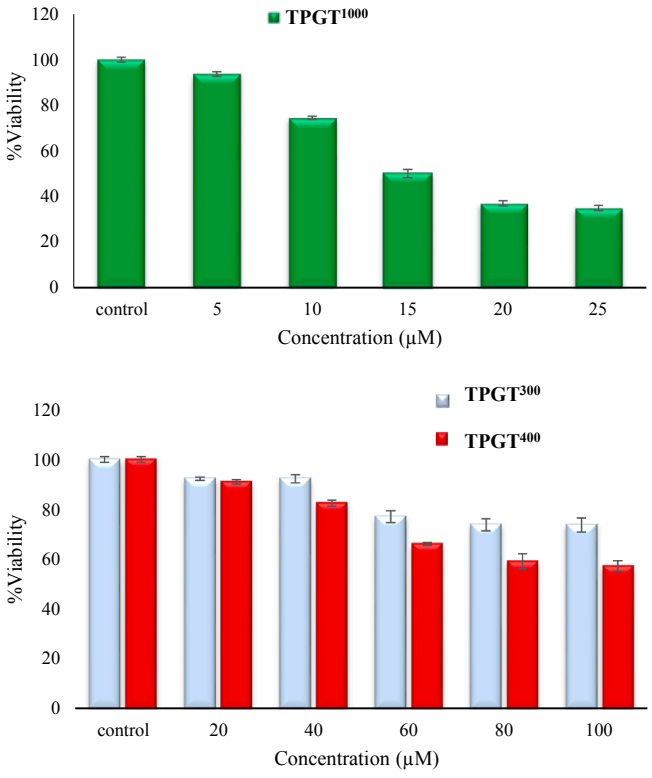

Fig. 9. Diagrams of MCF-7 cancer cell viability at various polymeric micelle concentrations after 24 h treatment.

$TPGT^{300, 400}$. From the results presented in this study, it can be proposed that increasing the PEG chain length in polymeric micelles can improve the stability, solubility and therapeutic effects of the synthesized vitamin E derivatives.

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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Disclosure statement

No potential conflict of interest was reported by the authors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.127907.

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