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(54) **Title:** PHOTOACTIVATABLE NANOPARTICLES FOR PHOTODYNAMIC APPLICATIONS, METHOD OF PREPARATION THEREOF, PHARMACEUTICAL COMPOSITIONS CONTAINING THEM, AND USE THEREOF

(57) **Abstract:** The present invention relates to photoactivatable nanoparticles for photodynamic applications containing a photo-sensitizer, a (C₈ to C₂₂) fatty alcohol and a polymeric surfactant, preferably poly(ethylene oxide) monomethyl ether-*block*-poly(ϵ -caprolactone), whereas the size of the nanoparticle is in the range of from 1 to 1000 nm, and the core of the nanoparticle is solid at the temperature of 4 °C, and liquid at the temperature around 39 °C. The present invention further relates to a method of preparation of the photoactivatable nanoparticle, a pharmaceutical composition containing it, and use thereof.



Photoactivatable nanoparticles for photodynamic applications, method of preparation thereof, pharmaceutical compositions containing them, and use thereof

Field of Art

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The present invention relates to photoactivatable nanoparticles for photodynamic applications, method of preparation thereof, pharmaceutical compositions containing them, and the use thereof as medicaments in photodynamic therapy.

10 Background Art

Photodynamic therapy (PDT) is a relatively new method of treating various diseases, with a particular focus on the treatment of tumors, for example squamous cell carcinoma of the head and neck. PDT consists in combination of photosensitizer (PS), molecule of oxygen, and subsequent
15 laser irradiation of tumor lesions. After laser irradiation of the tumor, the molecules of the photosensitizer absorb the photons of light and subsequently excite oxygen while releasing singlet oxygen, which is highly destructive causing damages of the cellular structures, such as DNA or membranes in close surrounding. Efficiency of PDT largely depends on the photochemical, photobiological, and phototherapeutic properties of PS. Critical factor is the formulation of PS,
20 which ensures, that the effect is selectively focused on the target tumorous tissue. PDT is a preferred method of treatment not only in tumorous, but also in many non-tumorous applications, for example in cosmetics, where the photosensitizer is combined with a biological filling material (comprising collagen, hyaluronic acid, and other synthetic or natural products) and the formulation is subsequently injected to the treated location (US20110021973 A1). PDT is used also as a method
25 of treatment of unwanted cellulitis (US8414880 B2) or in the prevention and treatment of vascular or inflammatory diseases (US20090306032 A1), where the authors describe solid lipidic nanoparticles obtained by micro-emulgation in heat, containing cholesteryl propionate and/or cholesteryl butyrate.

30 Nanomedicine is a fast developing discipline of medical research, which is focused especially on the development of nanoparticles (NPs) for prophylactic, diagnostic, and therapeutic applications. In the area of pharmacy, the size of nanoparticles is generally usually defined in the range of 1 nm thru 1000 nm. Nanomedicine functions on the same principle as many biological processes and cellular mechanisms. Photosensitizers incorporated inside the nanoparticles provide a very
35 promising approach in solving the problem of both bioavailability and targeted delivery to the tumorous tissue. Methods and procedures for the synthesis, functionalization and the use of NPs are

today widely studied and they represent new strategies for molecular targeting, individual therapy and minimally invasive diagnostic techniques.

In general, there may be various ways of administration of PSs to the patient, for example topical (US20090081281 A1), peroral (US20100273803 A1) or intravenous, and each of them has its advantages and disadvantages. Especially intravenous administration is problematic, as many PSs are hydrophobic or amphiphilic substances usually insoluble in water. In some cases, PSs (especially temoporfin) are administered in alcoholic solution (ethanol, propylene glycol). For PDT, mainly porphyrins and chlorines were tested. Temoporfin (Biolitec Pharma, Scotland, United Kingdom) was approved in EU for palliative tumors of head and neck, tumors prostate and pancreas (US20060040912 A1).

Solid lipid nanoparticles (SLNPs) with stabilized surface with non-covalently bound medicaments represent a new type of medical formulation. In the bloodstream, these SLNPs, after adhesion to apolipoproteins, imitate low-density-lipoprotein (LDL) transporting the lipids to the tissues. LDL receptors are overexpressed on the surface of many tumorous cells (Kretzer, Iara F., Durvanei A. Maria, and Raul C. Maranhão. 2012. *Cellular Oncology* 35 (6): 451–60; Huntosova, Veronika, Diana Buzova, Dana Petrovajova, Peter Kasak, Zuzana Nadova, Daniel Jancura, Franck Sureau, and Pavol Miskovsky. 2012. *International Journal of Pharmaceutics* 436 (1-2): 463–71), which leads to targeted delivery of the medicament to the tumor. For the specific delivery of PS as close as possible to the tumorous tissue, it is necessary to suppress the mechanisms restricting the effectivity of PDT. Due to accumulation of PS leading to unwanted photosensitizing of the entire surface of the body of patients, the skin is the decisive organ (Moore, Caroline M., Mark Emberton, and Stephen G. Bown. 2011. *Lasers in Surgery and Medicine*. 3:768–75). SLNPs are then an effective system of administration of medicaments improving biological availability also for the substances poorly soluble in water. Formulations on the basis of SLNP provide generally higher bioavailability and a higher therapeutic effect *in vitro* and *in vivo* in comparison with commercial formulations, especially for use in intravenous drug application form.

US5250236 describes solid lipid spherical particles that have the diameter smaller than one micrometer. Lipid particles were obtained by adding heated lipids (above their melting point) to the mixture composed of water, surfactant, and co-surfactant.

Patent application WO 1993005768 A1 describes a system of drug delivery based on solid particles of lipidic materials or mixtures thereof, with the diameter from 10 nm to 10 μ m. They provide longer time of controlled release of active substance and enable incorporation of hydrophilic drugs to the solid core of the particle.

US20080090803 A1 describes covalent conjugates of fatty alcohols and pharmaceutical carriers for the treatment of tumorous and viral diseases and psychiatric disorders.

US20060127471 A1 describes the preparation of liposomal formulations containing hydrophobic photosensitizer (temoporfin) and their use in therapy, particularly using intravenous application.

5 Phospholipids are modified by PEGylation [containing poly(ethylene oxide) as an integral part]. The resulting PEGylated liposomes include a hydrophobic photosensitizer within the membrane. This mechanism delivers the photosensitizer to the cellular membrane of the system directly to the site of action.

10 US7354599 B2 describes pharmaceutical liposomal formulations for PDT that contain a hydrophobic photosensitizer of porphyrin type (temoporfin), monosaccharide (fructose and glucose) and one or more synthetic phospholipids. Thanks to the lyophilization process, the resulting formulations are stable on storage and suitable for intravenous administration.

15 US8709449 B2 describes the formulation increasing the efficiency of PDT and reducing phototoxic reactions of skin caused by PDT. The lipid emulsion contains soybean oil, triglycerides with medium long chains and phospholipids from eggs as hydrophobic components. PS is selected from the group of porphyrins, phthalocyanines, metallo-graphite derivatives, dyes and synthetic photosensitizers.

20 US20080311214 A1 describes modified lipidic nano- /micro-particles that contain on their surface a molecule or a ligand targeting to a specific binding site, and their use for peroral administration of medicaments and antigens.

Patent application WO 2006069985 A2 describes methods of preparation of lipid-based nanoparticles by homogenization for the purpose of incorporating a compound into the already pre-prepared nanoparticles using a dual asymmetric centrifuge.

25 Patent application WO 2010112749A1 describes the method of preparation and composition of suspensions of solid lipidic nanoparticles in aqueous phase, containing the minoxidil in a lipid matrix (mono-, di- and triglycerides non-containing stearic acid). SLNPs contain at least one amphiphilic compound selected from phospholipids (soy lecithin, composed mainly of phosphatidylcholines). SLNPs contain mainly phosphatidylcholine, lecithin and one or more surfactants (selected from esters of fatty acids with sorbitans) and water phase (comprising water, 30 propylene glycol and eventually ethanol). The authors focus on the cosmetic and pharmaceutical use.

35 Patent application WO 2011116963 A2 describes a system of delivery of medicaments using SLNP that - as lipidic carriers - comprise at least one active substance and are coated with polymers. The active substance is selected from cosmetic, pharmaceutical and/or alimentary active substances and/or adjuvans. Polymers (prepared by simple or complex coacervation) are selected from the

group of proteins, polysaccharides, polyesters, polyacrylates, polycyanoacrylates, and/or mixtures thereof.

US5785976 A describes the preparation of colloidal suspensions of solid lipidic particles of anisometric shapes with a lipid matrix which is in a stable polymorphic modification. They further
5 comprise a suspension of micro- and submicron particles of bioactive compounds. These suspensions were prepared especially for parenteral administration, which is very convenient for bioactive substances poorly soluble in water, especially medicaments, and their use in cosmetic, food and agricultural industries.

US4880634 A describes lipidic nano-pellets containing pharmacologically active substances, the
10 size of which is in the range of from 50 to 1000 nm, and may be used as adjuvants in the system of transport of drugs for peroral administration.

PCT/EP2012/055222 (WO 2012/127037) describes a pharmaceutical composition, which comprises a corticosteroid and/or vitamin D derivative incorporated as a solid solution or dispersion in lipid nanoparticles, said lipid nanoparticles being solid at ambient temperature and
15 comprising a first lipid with a melting point above body temperature, and a pharmaceutically acceptable surfactant.

US 2015/0273084 A1 relates to a methylene blue nanoparticle for bioimaging and photodynamic therapy, and a use thereof as a cancer therapeutic agent and a contrast agent. The nanoparticle contains a methylene blue – fatty acid (or salt thereof) complex and an amphiphilic polymer
20 (polyoxyethylene-polyoxypropylene block copolymer), wherein the methylene blue – fatty acid complex is enclosed in an amphiphilic polymer micelle (Pluronic F-68).

Xingwang Zhang, Huan Wang, Tianpeng Zhang, Xiaotong Zhou, Baojian Wu, *European Journal of Pharmaceutical Sciences* 62 (2014) 301–308 describes the preparation, solubilization, stabilization and bioavailability of polymeric mixed micelles with stiripentol as model drug. The
25 micelles were prepared by rapid dispersion of an ethanol solution containing stiripentol, monomethoxy poly(ethylene glycol)-b-poly(ϵ -caprolactone) and sodium oleate into water.

Jingxin Gou, Shuangshuang Feng, Helin Xu, Guihua Fang, Yanhui Chao, Yu Zhang, Hui Xu and Xing Tang, *Biomacromolecules* 16.9 (2015): 2920-2929 relates to a preparation of well-controlled particle size micelles, containing medium chain triglyceride (MCT) and a drug incorporated inside
30 the micelles. It also related to the influence of the MCT presence on the drug content. The coat of the micelles is composed of the block copolymer mPEG-b-PCL.

Taratula O., et al., *Chemistry of Materials* 27.17 (2015):6155-6165 describes a combined therapeutic/diagnostic agent based on silicon naphthalocyanine encapsulated in a hydrophobic core of PEG-PCL polymeric nanoparticles. These nanoparticles, upon their accumulation in the target
35 tumor tissue, may be detected using NIR fluorescence imaging. Upon NIR laser irradiation of the

nanoparticles, the absorbed energy generates reactive oxygen species (ROS) and heat, which are emitted into the surrounding tumor tissue.

To select a suitable type of nanoparticles as a transport medium allowing targeted application of the respective active pharmaceutical ingredients, it is necessary to consider also all the drawbacks and limitations of the system concerned. In case of liposomes, the use of high-energy ultrasound during the preparation often causes oxidation and degradation of phospholipids (T. P. Chelvi and R. Ralhan, *International Journal of Hyperthermia*, vol. 11, No. 5, pp. 685–695, 1995; S. Batzri and E. D. Korn, *Biochimica et Biophysica Acta-Biomembranes*, vol. 298, No. 4, pp. 1015–1019, 1973; J. J. Escobar-Chávez, *Skin*, Vol. 19, p. 22, 2012; M. R. Mozafari, *Cellular and Molecular Biology Letters*, Vol. 10, No. 4, pp. 711–719, 2005). In micelles and liposomes, there is further a risk of for example decomposition of the transporting system immediately after the administration because of its insufficient stability (M. Malmsten, *Soft Matter*, Vol. 2, No. 9, pp. 760–769, 2006). For the systems using encapsulation into cyclodextrins, there was demonstrated irritability and therefore security concerns and limitations of their use for parenteral administration (R. A. Rajewski and V. J. Stella, *Journal of Pharmaceutical Sciences*, vol. 85, no. 11, pp. 1142–1169, 1996; A. F. Soares, R. D. A. Carvalho, and F. Veiga, *Nanomedicine*, Vol. 2, No. 2, pp. 183–202, 2007). In liquid crystals, there are problems, due to their high viscosity, with the preparation and administration, and they further manifest toxicity because of high content of surfactants (M. Malmsten, *Soft Matter*, Vol. 2, No. 9, pp. 760–769, 2006; P. S. Prestes, M. Chorilli, L. A. Chiavacci, M. V. Scarpa, and G. R. Leonardi, *Journal of Dispersion Science and Technology*, Vol. 31, No. 1, pp. 117–123, 2009; M. Rückert and G. Otting, *Journal of the American Chemical Society*, Vol. 122, No. 32, pp. 7793–7797, 2000). For various types of nanoparticles, there may be fundamental also other characteristics, for example the method of administration of a medicament, colloidal stability and interaction with blood proteins, biodegradability of the carrier, low incorporation and adsorption of the curative substance, complex development of methodologies for the control of quality of the transporting system, problems with stability during storage, toxicological tests of some types of nanoparticles are not fully completed and there is often need to increase the efficiency of the production (W. Mehnert and K. Mäder, *Advanced Drug Delivery Reviews*, Vol. 47, No. 2-3, pp. 165–196, 2001; E. B. Souto, P. Severino, M. H. A. Santana, and S. C. Pinho, *Química Nova*, Vol. 34, No. 10, pp. 1762–1769, 2011).

Disclosure of Invention

The present invention provides fully biodegradable formulation of photoactivatable nanoparticles for photodynamic applications with minimum side effects. It is based on solid lipid nanoparticles (SLNPs) of the photosensitizer and fatty alcohols, whereas the surface of these solid lipid

nanoparticles is stabilized with a non-toxic surface active polymer, such as a copolymer poly(ethylene oxide) monomethyl ether - *block* - poly(ϵ -caprolactone), which prevents entrapping of SNLPs to reticuloendothelial system. Fatty alcohols are then fully degradable during metabolism by β -oxidation, while the surface active copolymer itself is also biodegradable to the 6-hydroxyhexanoic acid and poly(ethylene oxide), and based on the relatively low molecule weight, both types of fragments are eliminable by kidneys. SLNPs are at the storage temperature of 4 °C solid, stable, and do not aggregate. Melting temperature of the core is 39 °C, which causes releasing of the photosensitizer in the tumor in a controllable way as a result of intensive hyperthermic metabolism.

The subject of the present invention is a photoactivatable nanoparticle for photodynamic applications, containing a core and a shell, the core of which contains from 1 to 50 weight % of a photosensitizer, referred to the total dry weight of the nanoparticle, preferably from 1 to 4 weight % of the photosensitizer, referred to the total dry weight of the nanoparticle, and the said photoactivatable nanoparticle further contains from 1 to 80 weight % of (C₈ to C₂₂) fatty alcohols, referred to the total dry weight of the nanoparticle, preferably from 12 to 72 weight % of (C₈ to C₂₂) fatty alcohols, referred to the total dry weight of the nanoparticle; and the shell contains from 1 to 80 weight % of polymeric surfactant, preferably of poly(ethylene oxide) monomethyl ether - *block* - poly(ϵ -caprolactone), referred to the total dry weight of the nanoparticle, preferably the shell contains from 14 to 72 weight % of the said polymeric surfactant, referred to the total dry weight of the nanoparticle; wherein the size of the nanoparticle is in the range of from 1 to 1000 nm, preferably in the range of from 11 to 760 nm, most preferably in the range of from 11 to 30 nm, and wherein the core of the nanoparticle is solid at the temperature of 4 °C, and liquid at the temperature of 39 °C, determined by differential scanning calorimetry with flushing with nitrogen gas with the volume of 50 cm³/min while using indium as a standard in temperature cycles: heating – cooling - heating, at 5 °C/min, and the temperature reading was performed from the first curve of heating of the differential scanning calorimetry, where the result was a bimodal thermogram with two endotherms, one for the eutectic mixture of the photosensitizer and the fatty alcohol, and the second one for the fatty alcohol.

Inclusion of temoporfin to the lipidic matrix leads to the melting temperature around 26 °C, when the eutectic mixture of the photosensitizer and of the (C₈ to C₂₂) fatty alcohol melts, and between 35 and 39 °C, preferably between 37 and 39 °C, most preferably at around 39 °C, when the fatty alcohol-based core matrix itself melts. It means, that two phases exist in the lipid core, containing the photosensitizer and the (C₈ to C₂₂) fatty alcohol. This composition enables the stability of solid

lipid nanoparticles during their storage at the temperature of 4 °C, and, at the same time, the melting temperature of the core of 39 °C.

In a preferred embodiment, the photosensitizer is selected from derivatives of chlorine and/or porphyrin and/or anthraquinone, more preferably the photosensitizer is selected from the group comprising temoporfin, verteporfin, lutetium texaphyrin, hypericin and porphycene.

In one embodiment of the invention, the core of the photoactivatable nanoparticle contains one type of (C₈ to C₂₂) fatty alcohol; preferably the fatty alcohol is the (C₁₄) fatty alcohol.

In another embodiment of the invention, the core of the photoactivatable nanoparticle contains a combination of (C₈ to C₂₂) fatty alcohols.

In one embodiment of the invention, the polymeric surfactant is the poly(ethylene oxide) monomethyl ether-*block*-poly(ε-caprolactone), preferably with the degree of polymerization of the poly(ethylene oxide) block in the range of from 10 to 100, and with the degree of polymerization of the poly(ε-caprolactone) block in the range of from 3 to 60, more preferably the degree of polymerization of the poly(ethylene oxide) block is in the range of from 20 to 50, and the degree of polymerization of the poly(ε-caprolactone) block is in the range of from 3 to 20; most preferably the degree of polymerization of the poly(ethylene oxide) block is 45, and the degree of polymerization of the poly(ε-caprolactone) block is 7.

The subject of the present invention is also a method of preparation of the photoactivatable nanoparticle according to the present invention, wherein:

- the photosensitizer is dissolved in a solvent, preferably in dichloromethane, and it is added to the (C₈ to C₂₂) fatty alcohol heated to 45 °C while forming a first phase;
- the polymeric surfactant is dissolved in an organic solvent, preferably in dichloromethane, then water is added, and the mixture is heated to 45 °C while forming a second phase;
- both heated phases are mixed together and homogenized, preferably for the period of 2 minutes at 8000 rotations per minute (RPM);
- the resulting emulsion is subjected to sonication by ultrasound;
- the resulting emulsion is cooled down to the laboratory temperature.

Ultrasound sonication may be performed, for example, at the laboratory temperature and the ultrasound amplitude between 50 % and 75 %, preferably at the ultrasound amplitude of 50 % for

the time period of 35 min and then at the ultrasound amplitude of 70 % for the time period of 5 min.

The subject of the present invention is also a pharmaceutical composition which comprises the photoactivatable nanoparticle according to the present invention, and the said pharmaceutical composition further comprises pharmaceutically acceptable additives, selected from the group comprising anti-adhesive agents, binders, coating agents, coloring agents, swelling agents, flavoring agents, lubricants, preservatives, sweeteners, cryoprotectives, absorbents.

The subject of the present invention is also the photoactivatable nanoparticle according to the present invention and/or the pharmaceutical composition according to the present invention for the use as a medicament.

The subject of the present invention is also the photoactivatable nanoparticle according to the present invention and/or the pharmaceutical composition according to the present invention for the use as a medicament for intravenous applications. An intravenous application is understood as injection of a liquid form of the medicament directly into a vein or a artery of a patient.

The subject of the present invention is also the photoactivatable nanoparticle according to the present invention and/or the pharmaceutical composition according to the present invention for the use in the photodynamic therapy and/or photodynamic diagnostics.

The present invention of the system of solid lipid nanoparticles provides a solution to overcome all of the aforementioned limitations of the background art, such as for example simple preparation, stability during the period of storage, the core of the nanoparticle at the storage temperature (4 °C) is solid, stable, and it does not aggregate at the physiologic pH (pH ~ 7,4), while the core melting temperature of 39 °C allows for release of the photosensitizer in the tumor in a controlled manner as a result of intensive hyperthermic metabolism. The nanosystem of this unique composition is fully biodegradable and it provides an increase of bioavailability and of the therapeutic effect *in vitro* and *in vivo* (in comparison with commercial formulations). The use of the nanoformulation is suitable both for the peroral as well for the intravenous medicament application forms.

Brief Description of the Drawings

Figure 1: ^1H -NMR spectrum of the copolymer poly(ethylene oxide)-*block*-poly(ϵ -caprolactone), (PEO-*b*-PCL). Integral intensities of proton signals marked as: **a** ($\delta = 3.63$ ppm, corresponds to the PEO block) and **c** ($\delta = 1.64$ ppm, corresponds to the PCL block) in the ^1H -NMR spectrum.

Figure 2: Thermograms of the DSC analysis of the solutions without a content of temoporfin (marked as SLNP with the corresponding PEO_xPCL_y), with incorporated temoporfin (prepared in accordance with the Examples 1, 2, and 3), and the pure temoporfin (10 to 20 mg).

Figure 3: Transmission electron micrographs of samples of solid lipid nanoparticles with incorporated temoporfin, prepared in accordance with the Examples 1, 2, and 3.

Figure 4: Time profile of *in vitro* release of temoporfin (%) from solid lipid nanoparticles prepared in accordance with the Examples 1, 2, and 3, at pH 7.4. The data are expressed as the average $\pm$ SD ($n = 3$).

Figure 5: The influence of "one-shot" PDT on the growth of a human breast carcinoma line MDA-MB-231. A formulation of solid lipid nanoparticles with incorporated temoporfin prepared in accordance with the Examples 1, 2, and 3 (the dose of temoporfin 0.8 mg/kg mouse, i.v.) was intravenously applied to a group of Nu/Nu mice ($n = 5$), and compared with a non-treated group of mice (in graphs as a control group) and with a commercial formulation (Originator, containing 4 mg of temoporfin, 376 mg of ethanol, and 560 mg of propylene glycol per gram). Three hours after the application, the tumorous areas were irradiated (100 J/cm^2) and subsequently dimensions of the tumors were measured regularly.

Figure 6: The tumor growth inhibition curve - TGI % as a function of time after intravenous application (i.v.) of tested formulations of solid lipid nanoparticles with incorporated temoporfin prepared in accordance with the Examples 1, 2, and 3 (the dose of temoporfin 0.8 mg/kg mouse) in comparison with the commercial formulation (Originator, containing 4 mg of temoporfin, 376 mg of ethanol, and 560 mg of propylene glycol per gram). The graph shows the growth inhibition of a human breast carcinoma line MDA-MB-231 in a group of Nu/Nu mice ($n = 5$).

Examples

Example 1

The synthesis of the copolymer poly(ethylene oxide)-*block*-poly(ϵ -caprolactone) (PEO-*b*-PCL) is based on the ring-opening polymerization of ϵ -caprolactone without the presence of a catalyst, initiated using the poly(ethylene oxide) monomethyl ether (MPEO), weight-average molecular weight 2 kDa. Three grams (1.49 mmol) MPEO were placed into a 50 ml graduated flask with magnetic stirrer. The system was degassed in 5 vacuum - argon cycles. Three point four g (30 mmol) ϵ -caprolactone were added under argon atmosphere. Subsequently, the system was cooled

down with dry ice/ethanol, evacuated, and heated to 185 °C. Ring opening polymerization took 33 h. After polymerization, the system was filled with argon and it was cooled down to the laboratory temperature. The solids in the flask were dissolved in 10 ml of dichloromethane and precipitated into 350 ml of diethyl ether. Once dried, the polymer was suspended in water and dialyzed against water in the dialyzation membrane with molecular weight cut-off (MWCO) 2 kDa. Example of the ¹H-NMR spectrum of the PEO-*b*-PCL copolymer is depicted in Figure 1. The ratio of monomer units of the individual products was calculated using the intensities of proton signals marked as **a** (δ = 3.63 ppm, corresponding to the PEO block) and **c** (δ = 1.64 ppm, corresponding to the PCL block) in ¹H-NMR spectrum, following equation (1):

$$DP(PCL) = DP(PEO) * \frac{c}{a} \quad (1)$$

wherein DP(PCL) is a degree of polymerization of the poly(ε-caprolactone) block, DP(PEO) is a degree of polymerization of the poly(ethylene oxide) block, and *c* and *a* are integral intensities of signals of the corresponding protons (see Figure 1).

The length of the PEO block of the macro initiator in the copolymer with the degree of polymerization was DP = 45 monomeric units. The length of the PCL block with the degree of polymerization DP = 7 monomeric units. The resulting copolymer (polymeric surfactant) has therefore average formula: PEO₄₅PCL₇.

Solid lipid nanoparticles with incorporated photosensitizer temoporfin were prepared using a high-power homogenization and ultrasound method. Lipid phase composed of 1-tetradecanol (10 mg/ml) was put in a glass vial and warmed to 45 °C. Temoporfin (10 mg/ml) was dissolved in 1 ml of dichloromethane and added to the lipid phase to obtain the lipid-active pharmaceutical ingredient mixture after evaporation of the dichloromethane. Water phase was obtained by dissolving PEO₄₅PCL₇ copolymer (50 mg/ml) in 1 ml of dichloromethane, subsequent addition of 5 ml of H₂O and warming up to 45 °C. The hot lipid phase was added to the hot water phase and subsequently the mixture was homogenized during the period of 2 min at 8000 RPM. The resulting emulsion was subjected to ultrasound using the standard ultrasound tip (T25 digital Ultra-Turrax, IKA, Schoeller Instruments, s.r.o., Czech Republic). The amplitude of the ultrasound was set to 50 % for the period of time of 35 min and to 70 % for the period of 5 min. The resulting emulsion was immediately cooled to room temperature.

For the measurement of particle size (hydrodynamic radius, *R_H*), index of polydispersity (PDI), and the zeta potential (ZP) of the solid lipid nanoformulations with incorporated temoporfin under test, the Zetasizer (Zen 3600, Malvern Instruments Ltd.) was used using dynamic light scattering (DLS). To achieve the appropriate concentration prior to the actual measurement, the samples were diluted with re-distilled water (pH ~ 5 of the the resulting solution). All measurements were made at the

temperature of 25 °C. For the representation of the NPs size distribution data, the intensity-weighted distribution was used. The results are shown in Table 1.

The analysis using the differential scanning calorimetry (DSC), the calorimeter (Q2000, V24.11 Build 124, TA Instruments) with gaseous nitrogen flushing (50 cm³/min) was used. The instrument was calibrated for measuring the temperature and the heat flow using indium as standard. The samples analyzed were both without the content of temoporfin (marked as SLNP with the corresponding PEO_xPCL_y), and with incorporated temoporfin (prepared in accordance with the Examples 1, 2, 3), and the temoporfin alone (solid substance, 10 to 20 mg), (see Figure 2). Nanoformulations were placed to T-zero hermetic aluminum pans and the analysis was done in temperature cycles: heating - cooling - heating, from 10 °C to 50 °C, at 5 °C/min. Melting temperatures of these samples were read from the 1st DSC-curve of heating. Inclusion of temoporfin into the lipid matrix results in bimodal thermogram with two endotherms: one at around 26 °C (eutectic mixture of temoporfin and 1-tetradecanol) and another at around 39 °C (1-tetradecanol). It means that there exist two phases in the lipid core, containing temoporfin and 1-tetradecanol. The composition according to the present invention enables the stability of solid lipid nanoparticles at storage temperature of 4 °C, and, equally, the core melting temperature at 39 °C.

The tested nanoformulations were stored in glass vials in the refrigerator at temperature of 4 to 8 °C for the period of 1 month. On day 1, 14, and 30, DLS was used to measure the size of particles (R_H, nm), PDI, and ZP (mV), at pH ~ 5 and 7.4. Based on the one-way statistic analysis of variance (one-way ANOVA), no significant difference was found during the storage (day 1, 14, 30). The value of $\alpha = 0.05$ was used as a threshold value of statistical significance.

The content of temoporfin in the lipid matrix was determined using UV-VIS spectrometry (Evolution 220, Thermo Scientific™) in the following way: Samples of nanoformulations (1 µl) were dissolved in methanol (0.999 ml) and analyzed at 652 nm. The concentration of temoporfin was calculated using Lambert-Beer law, $A_{\max} = 652 \text{ nm}$, $\epsilon_{652} = 22\,400 \text{ M}^{-1}\text{cm}^{-1}$ (2). The efficiency of temoporfin encapsulation (EE %) and the content of temoporfin in the system of nanoparticles (DL %) were calculated following equations (3) and (4), and the results are listed in the Table 2.

$$A = \epsilon_{\lambda} \cdot c \cdot l \quad (2)$$

$$EE (\%) = \frac{W_{\text{drug in SLNP}}}{W_{\text{total}}} \times 100 \% \quad (3)$$

$$DL (\%) = \frac{W_{\text{drug in SLNP}}}{W_{\text{drug in SLNP}} + W_{\text{lipid}} + W_{\text{surfactant}}} \times 100 \% \quad (4)$$

(1) A = absorbance; ϵ_{λ} = molar absorption coefficient at the wavelength λ (652 nm); c = molar concentration of the absorption component in mol/L; l = length of the absorption layer. (2) EE (%)

= encapsulation efficiency; $w_{\text{drug in SLNP}}$ = weight of temoporfin in solid lipid nanoparticles; w_{total} = total weight of temoporfin used in the preparation of tested nanoformulations; (3) DL (%) = content of temoporfin in the nanoparticle system (drug loading); w_{lipid} = weight of 1-tetradecanol; $w_{\text{surfactant}}$ = weight of PEO₄₅PCL₇.

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Tested samples were analyzed and visualized using transmission electron microscope (TEM) (Tecnai G2 Spirit Twin 12, FEI). A sample (2 μ l) was dropped on the perforated, carbon-coated grid and the excess of the solution was sucked by a narrow strip of filtration paper (1 min). Nanoparticles were left to dry completely at the laboratory temperature, and subsequently they were observed using the TEM microscope (bright field view, accelerating voltage 120 kV). The particles are shown as black spots on a gray background (positive contrast), they have a spherical shape and the size correlating with the hydrodynamic radius, measured using DLS, as shown in the Figure 3.

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15 In order to study the release of temoporfin *in vitro* from the tested formulations, a method of dialysis through a dialyzation membrane was used (see Figure 4). The dialyzation membrane (3.5 kDa) was immersed for 20 min to distilled water. Aliquot volume of the formulation (2 ml) was first diluted with 2 ml of 2-time more concentrated PBS. This mixture was displaced into dialyzation membranes and subsequently put in the preheated (37 °C) solution of PBS with pH ~ 7,4 (200 ml) and left stirred on the magnetic stirrer (200 RPM). Aliquot parts (0,1 ml) of samples were taken in the time intervals of 0,5; 1; 2; 4; 6; 8; 24; and 72 h from the internal space of the dialyzation membrane and analyzed using UV-VIS spectrometry (after suitable dilution with methanol). Subsequently, the percentage of released temoporfin was calculated based on the difference of its concentration used for the preparation of the solution of SLNP (10 mg/ml) and the determined concentration using UV-VIS spectrometry in the sample of the solution taken from the inside of the dialyzation membrane.

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The tumor cell lines 4T1 (murine mammary carcinoma) and MDA-MB-231 (human breast adenocarcinoma) were maintained in the exponential phase of growth under sterile conditions in the medium RPMI 1640 enriched with 10 % of fetal calf serum (FCS), penicillin (100 units/ml), streptomycin (0,1 mg/ml), and L-glutamine (2 mmol). They were incubated at 37 °C in the atmosphere of 95 % air and 5 % CO₂. Cell lines were passaged every second or third day.

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For the analysis of *in vitro* cytotoxicity of the tested formulations, the cells 4T1 (2×10^5) were placed in Petri dishes (35 mm) and incubated overnight at 37 °C. Then the tested formulations were added to the cells so that the final concentrations of temoporfin were within the range of from 0.25 to 2 μ M, and subsequently they were further incubated for 16 h. The concentration of temoporfin in

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SLNP was chosen in order not to cause more than 10 % cell mortality on its own. Testing dishes were flushed with medium without phenol red (PR), and after addition of cultivation medium without PR they were irradiated for 30 min with halogen lamp (75 W) equipped with the filter for wavelengths of 620 to 680 nm (Andover, Salem, NH). The maximum excitation of temoporfin corresponds to $\lambda_{ex} = 652$ nm. The intensity of irradiation of cells was 3.7 mW/cm^2 with the overall irradiation dose of 6.6 J/cm^2 . The ratio of live and dead cells (% , cells/ml) was determined in Bürker chamber 24 h after irradiation using the absorption of trypan blue, thus obtaining the dose - effect curve, from which the values of IC_{50} were numerically evaluated. The value of IC_{50} represents the concentration of temoporfin, which is necessary to kill 50 % of cells, and it was calculated for each curve (Table 3). In control experiments, their toxic effect without irradiation was evaluated (the so-called "dark toxicity", toxicity without the photodynamic effect) and also the toxicity of SLNP formulations without the content of temoporfin ("empty", i.e. 1-tetradecanol and PEO_xPCL_y) at the same concentration conditions. A very low toxicity was found without irradiation as well as in case of SPLP without temoporfin (below 10 % in the entire concentration range).

Subcellular localization of the photosensitizer of temoporfin was studied using fluorescence microscopy in comparison with the formulation of the originator (commercial, containing 4 mg of temoporfin, 376 mg of ethanol, and 560 mg of propylene glycol per gram). Tumor cells 4T1 inoculated to cover glasses, placed in Petri dishes (diameter 35 mm) were incubated with the formulations of temoporfin with the final concentration of $2 \mu\text{M}$ (1 h or 4 h incubation) or $1 \mu\text{M}$ (16 h of incubation) in the cultivation medium at 37°C . After incubation, the dishes with the cells were washed three times with PBS, poured with colorless medium without PR, and investigated under the microscope (DM IRB, Leica) equipped with the camera (DFC 480) while using the object-lens with 63-times oil immersion and filtration cube (N2.1, Leica), excitation: BP 515-560 nm, emission: LP 590 nm). Fluorescence of the formulations of the photosensitizer of temoporfin in the tumor 4T1 cells was measured 1, 4, and 16 h after the addition of the formulation into the cultivation medium. The tested nanoformulation, in comparison with the formulation of the originator, has shown significantly faster kinetics of fluorescence growth (after 4).

To monitor subcellular co-localization, the cells were incubated with tested nanoformulation of temoporfin, and 100 nM of MitoTracker Green (to label mitochondria) or 250 nM of ER-Tracker Blue to label endoplasmic reticulum (ER) was gradually added to the cells for 30 minutes. In order to label lysosomes, 500 nM of LysoTracker Green were added to the cultivation medium (1 h). Filtration cube Type A (Leica, excitation: BP 340-380 nm and emission: LP 400 nm) was used for the analysis of ER Tracker Blue; filtration cube I3 (Leica, excitation: BP

450-490 and emission: LP 515) was used for MitoTracker Green and LysoTracker Green. The tested nanomulation was localized mainly in ER.

Therapeutic efficiency for the targeted transport of drugs with PDT application was tested on the tumor model Nu/Nu mice *in vivo* and it was compared with the original formulation (commercial, marked as originator). The human breast carcinoma cells line MDA-MB-231 (1×10^7 cells) were suspended in 0.1 ml of PBS and 0.1 ml of Matrigel and subsequently subcutaneously (s.c.) injected to hind flank of mice. When the weight of the tumor reached the volume of 200 to 300 mm³ (7 to 10 days after transplantation), the tested formulation was injected in the volume of 0.1 ml per 20 g of mice intravenously (i.v.) to the tail vein in the dose of temoporfin of 0.8 mg/kg of the mouse. After three hours, the area of the tumor (2 cm²) was irradiated using a xenon lamp, (500-700 nm, λ_{ex} 652 nm), with the total maximum dose of light 100 J/cm² and the intensity of irradiation of 200 mW/cm². The control group of mice was irradiated under the same conditions but without the action of the medicament (see Figure 5). Each experimental group consisted of 5 mice. Forty-two days after administration of the tested nanoformulations, the mice were sacrificed by overdose of ketamine and the tumors were evaluated histologically. All aspects of animal experiments and rearing were conducted in accordance with national and European legislation and were approved by the constitutional committee (Bříza, Tomáš, Jarmila Králová, Petr Cigler, Zdeněk Kejík, Pavla Poučková, Petr Vašek, Irena Moserová, Pavel Martásek, and Vladimír Král. 2012. *Bioorganic & Medicinal Chemistry Letters* 22 (1): 82–84; Králová, Jarmila, Tomáš Bříza, Irena Moserová, Bohumil Dolenský, Petr Vasek, Pavla Poucková, Zdenek Kejík, et al. 2008. *Journal of Medicinal Chemistry* 51 (19): 5964–73). The data obtained for each tested group are expressed as an arithmetic average ($n = 5$), the error of measurement was calculated as the standard deviation ($\pm$ SD) and plot as a function of time. Reduction of the volume of the tumor was in comparison with the non-treated control group statistically significant at the level of significance $\alpha = 0.01$ (Table 4).

The tumor growth inhibition (% TGI) as a measure of efficiency of the therapy was compared with the commercial formulation (containing 4 mg of temoporfin, 376 mg ethanol, and 560 mg of propylene glycol per gram), (refer to Figure 6). It was calculated in accordance with the equation (5), wherein V is the mean volume of the tumor of the specific group under test at the given time, V_0 is the mean volume of the tumor of the specific group under test at the beginning of the experiment, V_k is the mean volume of the tumor of the control group (with the application of physiological solution) at the given time, and V_{k0} is the mean volume of the tumor of the control group at the beginning of the experiment:

$$TGI (\%) = 100 - \left[\frac{V/V_0}{V_k/V_{k0}} \times 100 \right] \quad (5)$$

Example 2

The formulation was analogical to Example 1, the modification consisted in other composition (degree of polymerization of the poly (ϵ -caprolactone) block) of the copolymer used (PEO₄₅PCL₁₇). The results from the DLS analysis (R_H , PDI, ZP) are shown in Table 1. The results from DSC are shown in Figure 2. The results for the effectivity of encapsulation of temoporfin (%) and the contents of temoporfin (%) in SLNP are shown in Table 2. Transmission electron micrographs of the tested samples are shown in Figure 3. The time profile of *in vitro* releasing of temoporfin (%) from the nanoformulations is shown in Figure 4. The analysis of the *in vitro* cytotoxicity was done in accordance with Example 1, the modification consisted in the use of the final concentrations of temoporfin incorporated in the SLNPs under test, which were in the range of from 1 to 20 μ M. The results from the mortality of the cells (%), IC₅₀ (μ M) are listed in Table 3. The procedure of subcellular localization of the photosensitizer of temoporfin using the fluorescence microscopy was performed in accordance with Example 1. The procedure of determination of the subcellular co-localization of the nanoformulations using fluorescence microscopy was performed in accordance with Example 1, the modification consisted in its localization - mainly in lysosomes. The results of therapeutic efficiency for the targeted transport of temoporfin with the application of PDT *in vivo* are shown in Figure 5 and their statistical evaluation is shown in Table 4. The graph showing the tumor growth inhibition is shown in Figure 6.

Example 3

The formulation in accordance with Example 1, the modification consisted in other composition (degree of polymerization of the poly (ϵ -caprolactone) block) of the copolymer used (PEO₄₅PCL₃₄). The results from the DLS analysis (R_H , PDI, ZP) are shown in Table 1. The results from DSC are shown in the Figure 2. The results for the effectivity of encapsulation of temoporfin (%) and the contents of temoporfin (%) in SLNP are shown in Table 2. Transmission electron micrographs of the samples under test are shown in the Figure 3. The time profile of *in vitro* releasing of temoporfin (%) from the nanoformulations is shown in the Figure 4. The analysis of the *in vitro* cytotoxicity in accordance with the Example 1, the modification consisted in the use of the final concentrations of temoporfin incorporated in the SLNPs under test, which were in the range 1 - 20 μ M. The results from the mortality of the cells (%), IC₅₀ (μ M) are shown in Table 3. The procedure of subcellular localization of the photosensitizer of temoporfin using the fluorescence microscopy in accordance with Example 1. The results of therapeutic efficiency for the targeted transport of temoporfin with the PDT application *in vivo* are shown in Figure 5, the modification consisted in the statistic analysis of data, where the reduction of volume of the tumor was in comparison with the non-treated group control group statistically insignificant on the level of significance $\alpha = 0.01$, as it is shown in Table 4. The graph showing the tumor growth inhibition is shown in Figure 6.

Example 4

Formulation in accordance with Example 1, its modification lies in weight composition of the lipidic phase, wherein the weight of 1-tetradecanol was 50 mg/ml, and in the weight composition of the aqueous phase, where the weight of PEO₄₅PCL₇ was 10 mg/ml. The results from the DLS analysis (R_H , PDI, ZP) are shown in Table 1. The results for the effectivity of encapsulation of temoporfin (%) and the contents of temoporfin (%) in SLNP are shown in Table 2.

Example 5

Formulation according to Example 1, modification lies in weight composition of the lipid phase, where the weight of 1-tetradecanol was 50 mg/ml, and in the weight composition of the aqueous phase, where the weight of PEO₄₅PCL₁₇ was 10 mg/ml. The modification further lies in other composition (degree of polymerization of the poly (ϵ -caprolactone) block) of the copolymer used (PEO₄₅PCL₁₇). The results from the DLS analysis (R_H , PDI, ZP) are shown in Table 1. The results for the effectivity of encapsulation of temoporfin (%) and the contents of temoporfin (%) in SLNP are shown in Table 2.

Example 6

Formulation according to Example 1, modification lies in weight composition of the lipid phase, where the amount of the 1-tetradecanol was 50 mg/ml, and in the weight composition of the aqueous phase, where the amount of PEO₄₅PCL₃₄ was 10 mg/ml. The modification further consisted in other composition (degree of polymerization of the poly (ϵ -caprolactone) block) of the copolymer used (PEO₄₅PCL₃₄). The results from the DLS analysis (R_H , PDI, ZP) are shown in Table 1. The results for the effectivity of encapsulation of temoporfin (%) and the contents of temoporfin (%) in SLNP are shown in Table 2.

Example 7

Formulation according to Example 1, the modification lies in the amount of temoporfin used in the preparation of the nanoformulation under test, which was 20 mg/ml. The results from the DLS analysis (R_H , PDI, ZP) are shown in Table 1. The results for the effectivity of encapsulation of temoporfin (%) and the contents of temoporfin (%) in SLNP are shown in Table 2.

Example 8

Formulation according to Example 1, the modification lies in the amount of temoporfin used in the preparation of the nanoformulation under test, which was 20 mg/ml. Further modification consisted in another composition (degree of polymerization of poly(ϵ -caprolactone) block) of the copolymer used (PEO₄₅PCL₁₇). The results from the DLS analysis (R_H , PDI, ZP) are shown in Table 1. The

results for the effectivity of encapsulation of temoporfin (%) and the contents of temoporfin (%) in SLNP are shown in Table 2.

Example 9

- 5 Formulation according to Example 1, the modification was in the amount of temoporfin used in the preparation of the nanoformulation under test, which was 20 mg/ml. Further modification consisted in different composition (degree of polymerization of poly(ϵ -caprolactone) block) of the copolymer used (PEO₄₅PCL₃₄). The results from the DLS analysis (R_H , PDI, ZP) are shown in Table 1. The results for the effectivity of encapsulation of temoporfin (%) and the contents of temoporfin (%)
- 10 in SLNP are shown in Table 2.

Table 1: Size of particles (R_H , nm), polydispersity index (PDI), zeta potencial (ZP, mV) of samples of solid lipid nanoformulations with incorporated temoporfin (mean $\pm$ SD, $n = 3$), at pH~5.

Formulation	Concentration (mg / ml)			Weight % (contents in dry weight of the system)		R _H (nm) ± SD	PDI ± SD	ZP (mV) ± SD
	1-tetradecanol	PEO _N PCL _Y	temoporfin	1-tetradecanol	PEO _N PCL _Y			
Example 1						13.3 ± 0.8	0.7 ± 0.3	-8.2 ± 1.7
Example 2	10	50	10	14.3	71.4	7.8 ± 2.3	0.4 ± 0.2	-17.8 ± 3.2
Example 3						8.9 ± 1.1	0.9 ± 0.2	-13.1 ± 0.5
Example 4						316.2 ± 22.5	0.9 ± 0.01	-9.2 ± 1.6
Example 5	50	10	10	71.4	14.3	349.2 ± 15.1	0.6 ± 0.01	-7.5 ± 1.3
Example 6						353.2 ± 25.3	0.9 ± 0.01	-6.5 ± 1.3
Example 7						6.3 ± 0.4	0.8 ± 0.02	-9.2 ± 1.3
Example 8	10	50	20	12.5	62.5	10.4 ± 1.6	0.9 ± 0.003	-4.5 ± 1.6
Example 9						20.6 ± 1.4	0.5 ± 0.01	-14.6 ± 1.2

Table 2: Effectivity of encapsulation of temoporfin (EE %) and the contents of temoporfin (DL %) in tested SLNP with incorporated temoporfin (mean $\pm$ SD, n = 3).

Formulation	DL (%) $\pm$ SD	EE (%) $\pm$ SD
Example 1	3.92 $\pm$ 0.06	22.22 $\pm$ 0.3
Example 2	3.93 $\pm$ 0.09	22.65 $\pm$ 0.1
Example 3	3.96 $\pm$ 0.08	23.06 $\pm$ 0.4
Example 4	2.98 $\pm$ 0.11	14.83 $\pm$ 0.6
Example 5	3.08 $\pm$ 0.05	17.02 $\pm$ 1.1
Example 6	3.12 $\pm$ 0.08	18.13 $\pm$ 0.8
Example 7	1.70 $\pm$ 0.34	5.26 $\pm$ 0.3
Example 8	1.82 $\pm$ 0.12	7.15 $\pm$ 0.4
Example 9	1.90 $\pm$ 0.02	9.12 $\pm$ 0.3

Table 3: Cell mortality (%) and IC₅₀ (μ M) after administration of SLNP with incorporated temoporfin, prepared according to Examples 1, 2 and 3 (mean $\pm$ SD, n = 5).

Formulation	Concentration (μ M) (temoporfin)	Cell mortality (%) (mean $\pm$ SD)	IC ₅₀ (μ M) (temoporfin)
Example 1	0.25	24 $\pm$ 6.8	0.39
	0.5	67 $\pm$ 24.7	
	1	88 $\pm$ 0.9	
	2	93 $\pm$ 2.4	
Example 2	1	47 $\pm$ 12.9	2.41
	2.5	44 $\pm$ 4.2	
	10	66 $\pm$ 7.7	
	20	61 $\pm$ 11.5	
Example 3	1	21 $\pm$ 1.6	>20
	2.5	22 $\pm$ 7.6	
	10	26 $\pm$ 15.7	
	20	29 $\pm$ 9.7	

Table 4: Statistical analysis of *in vivo* data („one-shot“ PDT) using one-way statistical analysis of variance (one-way ANOVA) from Origin 9 (OriginLab software), at threshold values of statistical significance $\alpha = 0.05$ and 0.01 .

	Control	Originator	Example 1	Example 2	Example 3
Control		++	++	++	+
Originator	++		0	0	0
Example 1	++	0		0	++
Example 2	++	0	0		+
Example 3	+	0	++	+	

0 No statistically significant variations among the groups, $\alpha = 0.05$.

5 + Statistically significant variations among the groups, $\alpha = 0.05$.

++ Statistically significant variations among the groups, $\alpha = 0.01$.

Example 10

10 Formulation according to Example 1 wherein verteporfin was used as photosensitizer.

Example 11

Formulation according to Example 1 wherein lutecium texafyrin was used as photosensitizer.

15 Example 12

Formulation according to Example 1 wherein hypericin was used as photosensitizer.

Example 13

Formulation according to Example 1 wherein porficen was used as photosensitizer.

CLAIMS

1. A photoactivatable nanoparticle for photodynamic applications, containing a core and a shell, **characterized in that** the core of which contains from 1 to 50 weight % of a photosensitizer, referred to the total dry weight of the nanoparticle, preferably from 1 to 4 weight % of the photosensitizer, referred to the total dry weight of the nanoparticle, and the said photoactivatable nanoparticle further contains from 1 to 80 weight % of (C₈ to C₂₂) fatty alcohols, referred to the total dry weight of the nanoparticle, preferably from 12 to 72 weight % of (C₈ to C₂₂) fatty alcohols, referred to the total dry weight of the nanoparticle; and the shell contains from 1 to 80 weight % of polymeric surfactant, preferably of poly(ethylene oxide) monomethyl ether-*block*-poly(ϵ -caprolactone), referred to the total dry weight of the nanoparticle, preferably the shell contains from 14 to 72 weight % of the said polymeric surfactant, referred to the total dry weight of the nanoparticle; wherein the size of the nanoparticle is in the range of from 1 to 1000 nm, preferably in the range of from 11 to 760 nm, most preferably in the range of from 11 to 30 nm, and wherein the core of the nanoparticle is solid at the temperature of 4 °C, and liquid at the temperature of 39 °C, determined by differential scanning calorimetry with flushing with nitrogen gas with the volume of 50 cm³/min while using indium as a standard in temperature cycles: heating – cooling - heating, at 5 °C/min, and the temperature reading was performed from the first curve of heating of the differential scanning calorimetry, where the result was a bimodal thermogram with two endotherms, one for the eutectic mixture of the photosensitizer and the fatty alcohol, and the second one for the fatty alcohol.
2. The photoactivatable nanoparticle according to claim 1, **characterized in that** the photosensitizer is selected from derivatives of chlorine and/or porphyrin and/or anthraquinone, more preferably the photosensitizer is selected from the group comprising temoporfin, verteporfin, lutetium texaphyrin, hypericin and porphycene.
3. The photoactivatable nanoparticle according to claim 1 or 2, **characterized in that** the core of the photoactivatable nanoparticle contains one type of (C₈ to C₂₂) fatty alcohol; preferably the fatty alcohol is the (C₁₄) fatty alcohol.
4. The photoactivatable nanoparticle according to claim 1 or 2, **characterized in that** the core of the photoactivatable nanoparticle contains a combination of (C₈ to C₂₂) fatty alcohols.

5. The photoactivatable nanoparticle according to any one of the claims 1 to 4, **characterized in that** the polymeric surfactant is the poly(ethylene oxide) monomethyl ether-*block*-poly(ϵ -caprolactone), preferably with the degree of polymerization of the poly(ethylene oxide) block in the range of from 10 to 100, and with the degree of polymerization of the poly(ϵ -caprolactone) block in the range of from 3 to 60; more preferably the degree of polymerization of the poly(ethylene oxide) block is in the range of from 20 to 50, and the degree of polymerization of the poly(ϵ -caprolactone) block is in the range of from 3 to 20; most preferably the degree of polymerization of the poly(ethylene oxide) block is 45, and the degree of polymerization of the poly(ϵ -caprolactone) block is 7.

6. A method of preparation of the photoactivatable nanoparticle according to any one of the preceding claims, **characterized in that**:

- the photosensitizer is dissolved in a solvent, preferably in dichloromethane, and it is added to the (C_8 to C_{22}) fatty alcohol heated to 45 °C while forming a first phase;
- the polymeric surfactant is dissolved in an organic solvent, preferably in dichloromethane, then water is added, and the mixture is heated to 45 °C while forming a second phase;
- both heated phases are mixed together and homogenized, preferably for the period of 2 minutes at 8000 rotations per minute (RPM);
- the resulting emulsion is subjected to sonication by ultrasound;
- the resulting emulsion is cooled down to the laboratory temperature.

7. A pharmaceutical composition, **characterized in that** it comprises the photoactivatable nanoparticle according to any one of the preceding claims, and the said pharmaceutical composition further comprises pharmaceutically acceptable additives, selected from the group comprising anti-adhesive agents, binders, coating agents, coloring agents, swelling agents, flavoring agents, lubricants, preservatives, sweeteners, absorbents.

8. The photoactivatable nanoparticle according to any one of the claims 1 to 5 and/or the pharmaceutical composition according to claim 7 for the use as a medicament.

9. The photoactivatable nanoparticle according to any one of the claims 1 to 5 and/or the pharmaceutical composition according to claim 7 for the use as a medicament for intravenous applications.

10. The photoactivatable nanoparticle according to any one of the claims 1 to 5 and/or the pharmaceutical composition according to claim 7 for the use in the photodynamic therapy and/or photodynamic diagnostics.

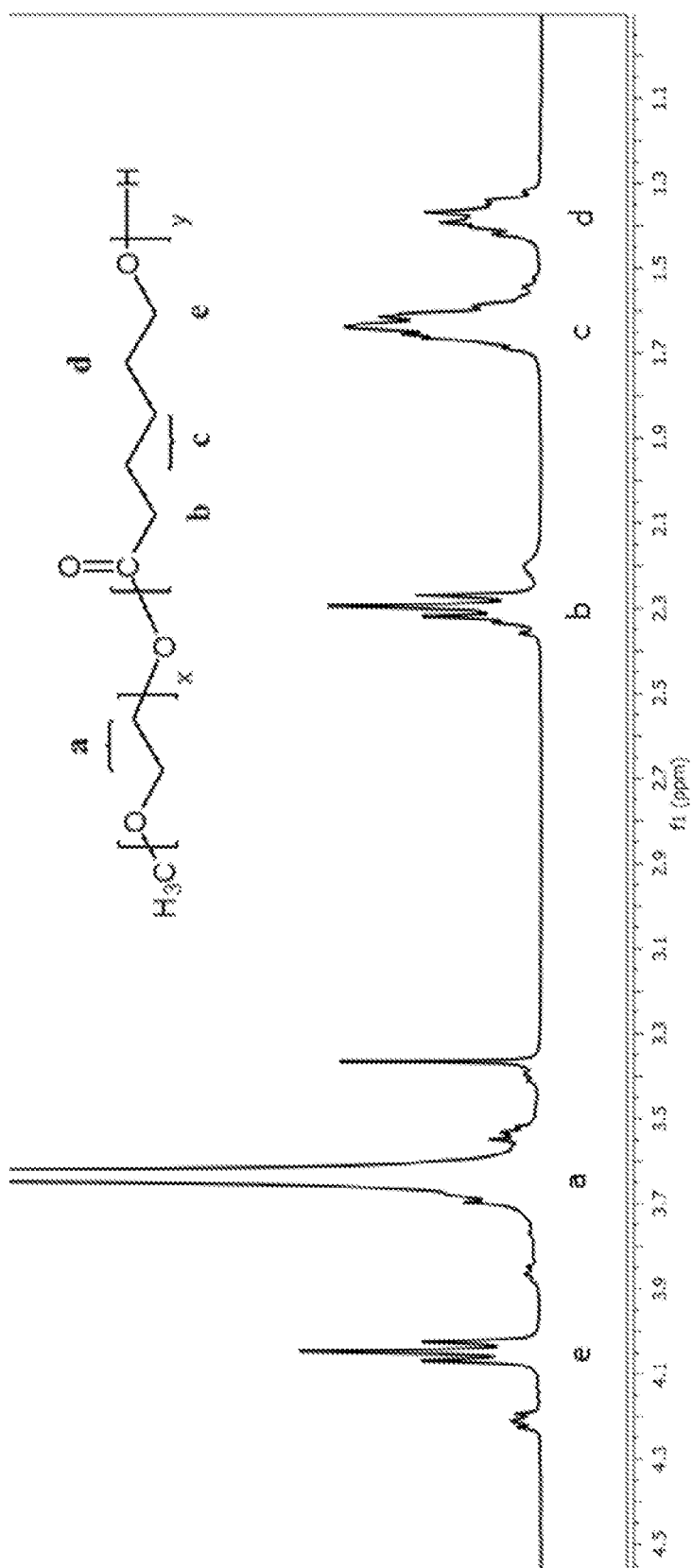


Figure 1

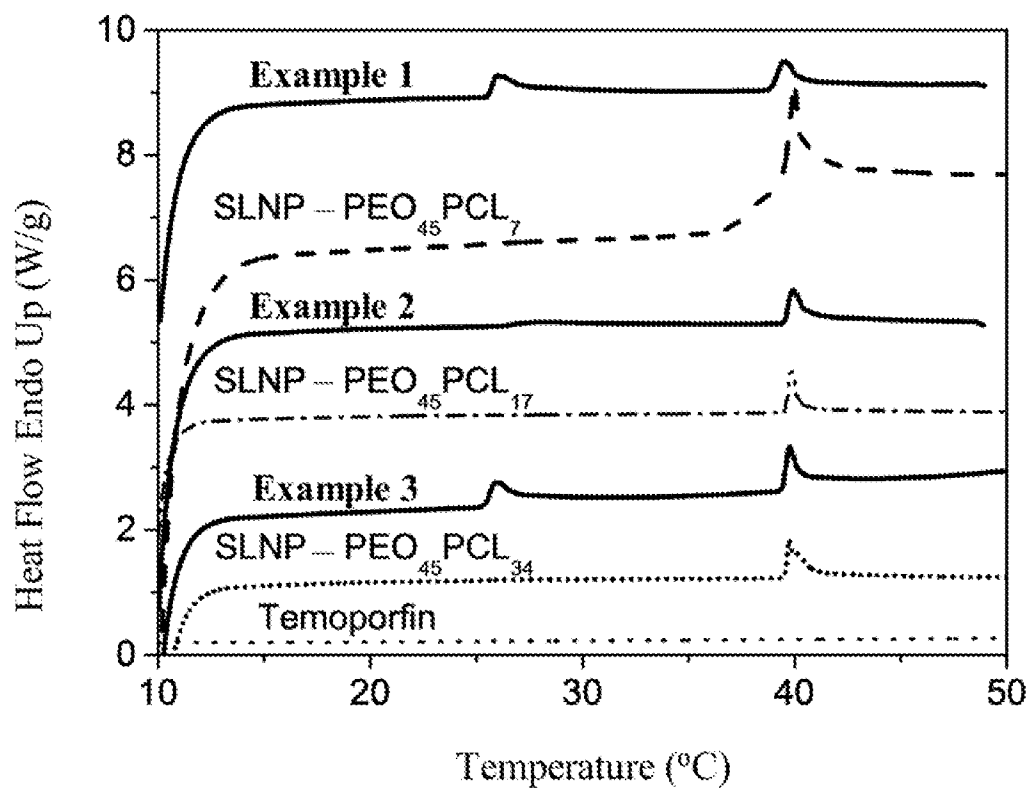


Figure 2

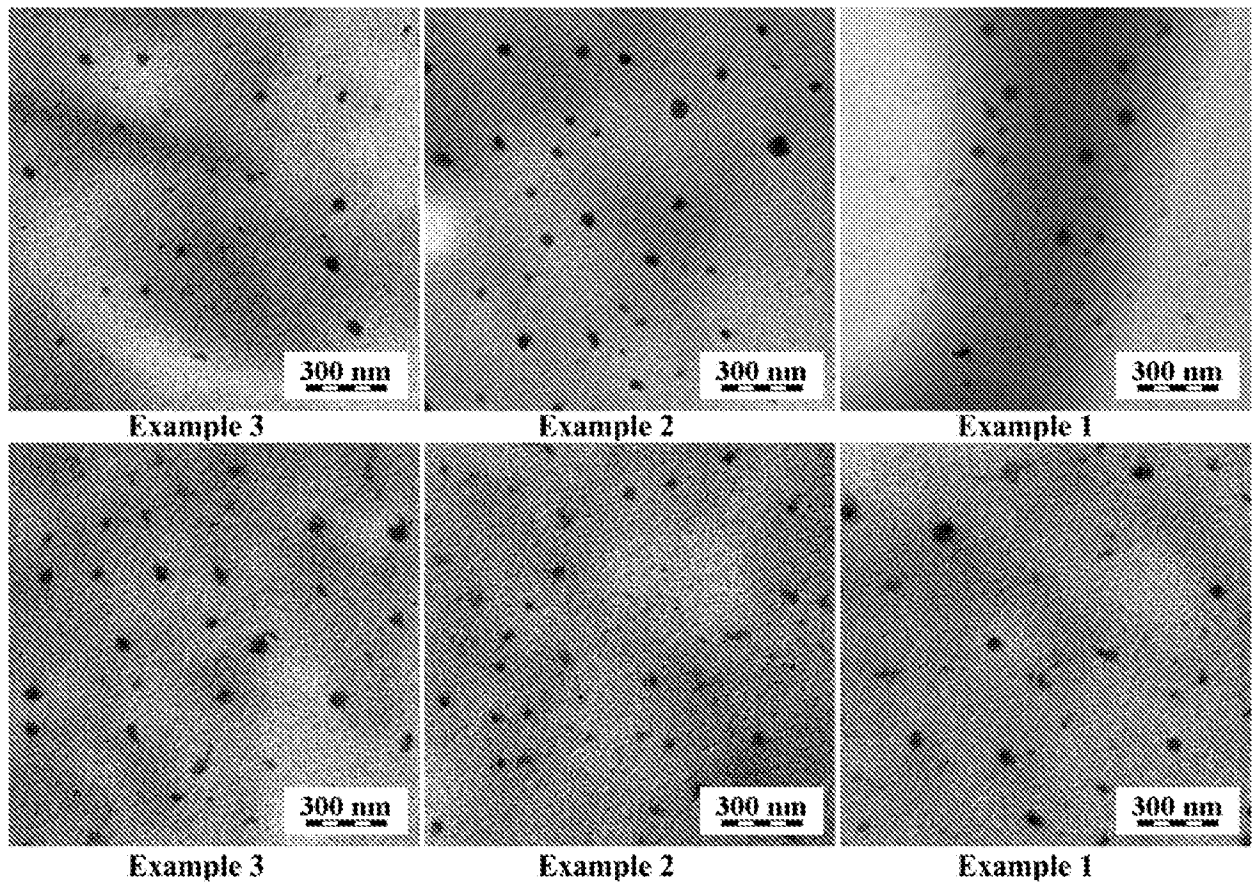


Figure 3

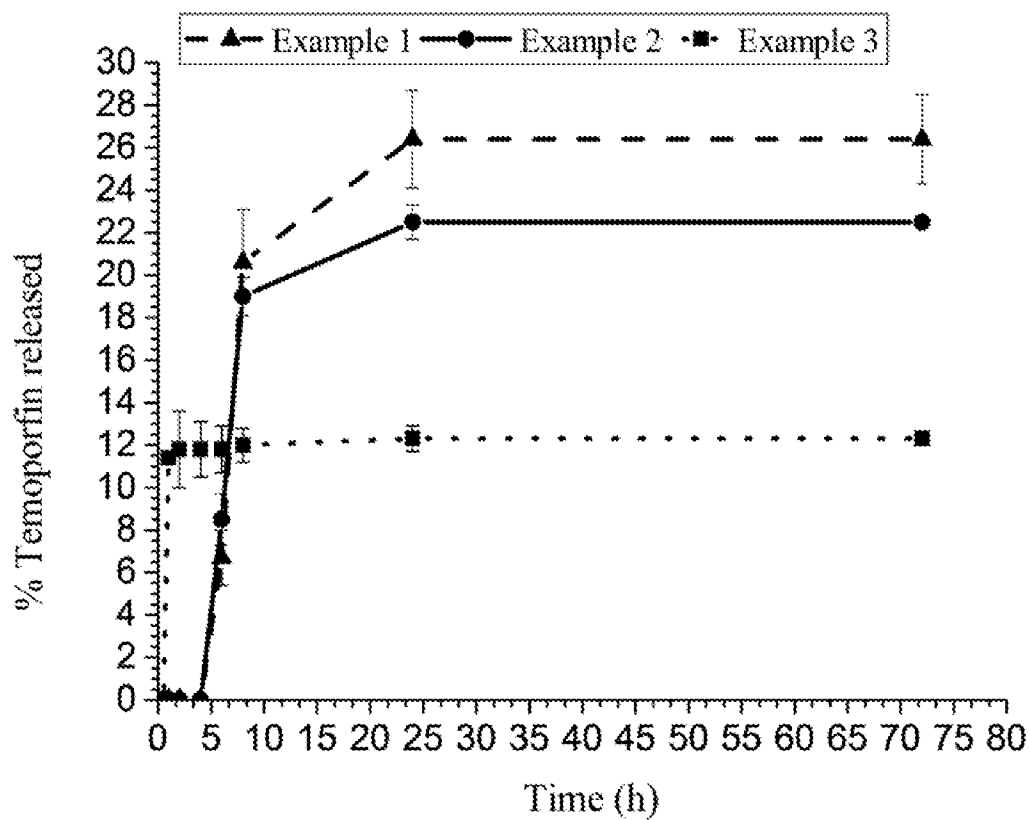


Figure 4

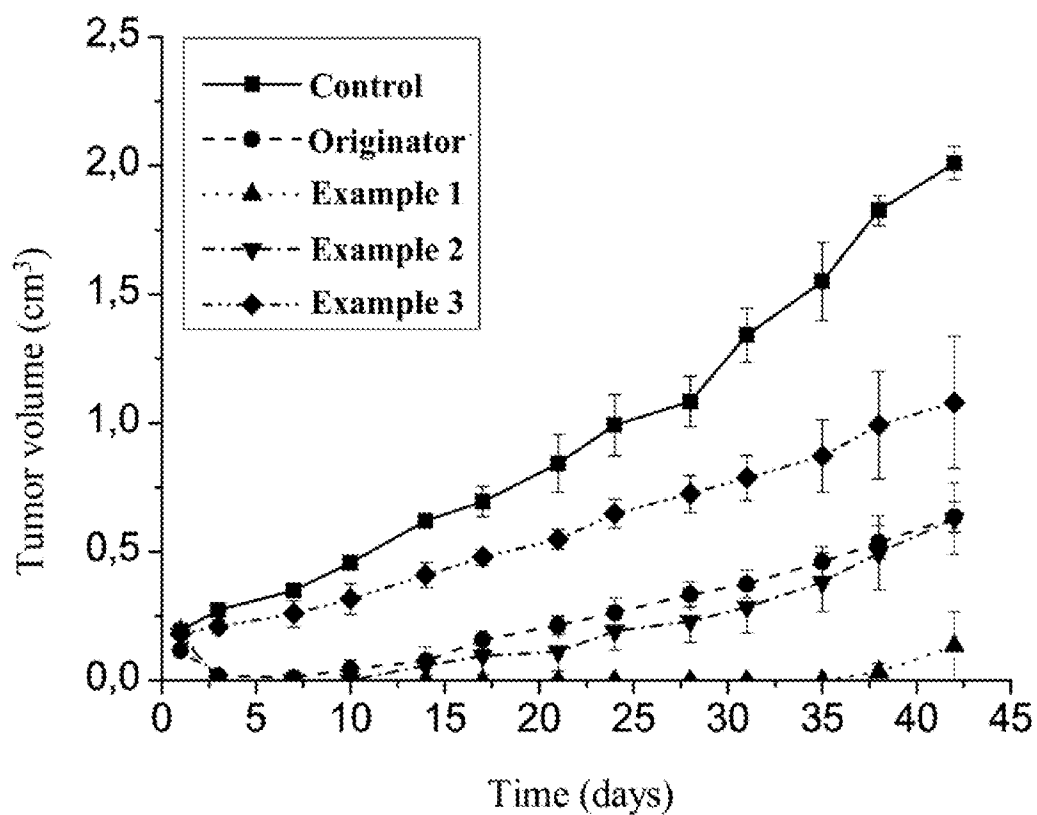


Figure 5

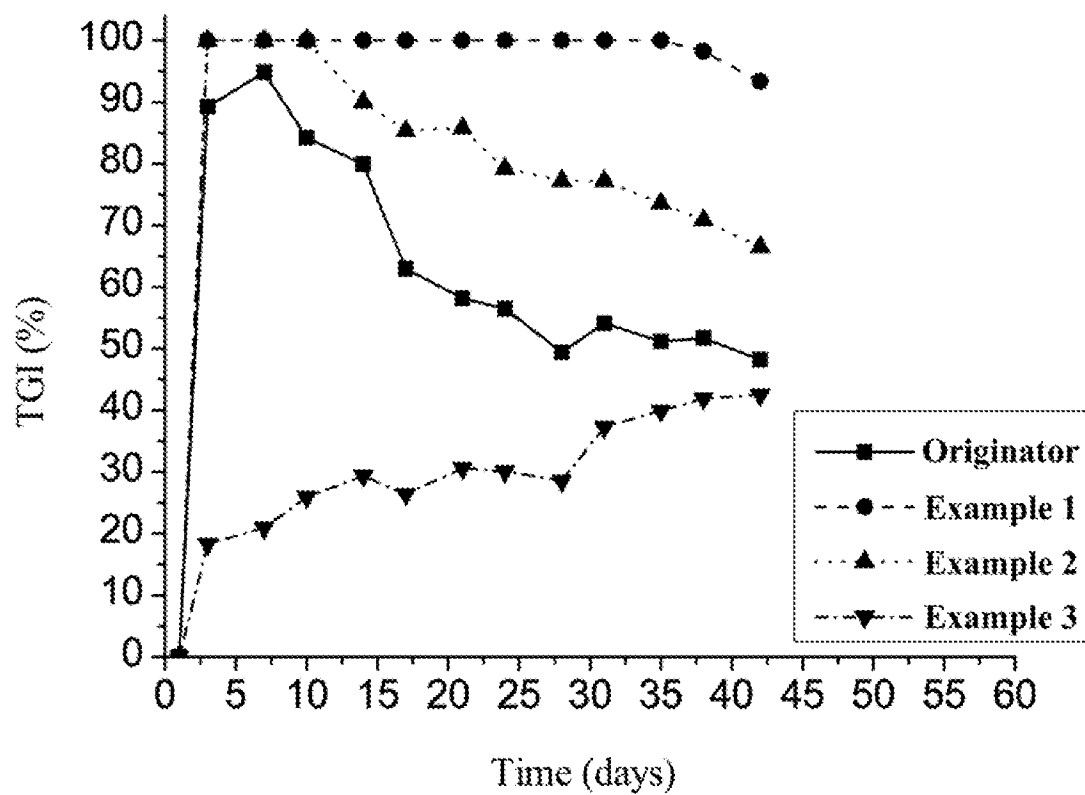


Figure 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/CZ2017/050008

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K41/00 A61K9/51 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, COMPENDEX, EMBASE, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NAVARRO FABRICE P ET AL: "Preparation and characterization of mTHPC-loaded solid lipid nanoparticles for photodynamic therapy", JOURNAL OF PHOTOCHEMISTRY AND PHOTOBIOLOGY B: BIOLOGY, vol. 130, 2014, pages 161-169, XP028616363, ISSN: 1011-1344, DOI: 10.1016/J.JPHOTOBIOL.2013.11.007	1-4,6-10
Y	abstract page 162, left-hand column, paragraph 2 pages 162-163, section 2.2. "Preparation of mTHPC-loaded LNP" page 166, right-hand column, paragraph 2 ----- -/-	5



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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INTERNATIONAL SEARCH REPORT

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DELMAS T ET AL: "Preparation and characterization of highly stable lipid nanoparticles with amorphous core of tuneable viscosity", JOURNAL OF COLLOID AND INTERFACE SCIENCE, ACADEMIC PRESS, INC, US, vol. 360, no. 2, 15 August 2011 (2011-08-15), pages 471-481, XP002686819, ISSN: 0021-9797, DOI: 10.1016/J.JCIS.2011.04.080 [retrieved on 2011-04-28] abstract page 472, section 2.2. "LNP fabrication process" figures 1,2 pages 475-477, section 3.3.2. "Core crystallinity" figure 4	1-10
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