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Research paper

Overcoming instability and low solubility of new cytostatic compounds: A comparison of two approaches

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ABSTRACT

The pharmaceutical use of some 3-hydroxyquinolinone derivatives with high cytotoxic and cytostatic activities (under *in vitro* conditions) as well as potential immunosuppressive properties is seriously limited by their low solubility in water accompanied by instability in oxidative environment, like physiological fluids. In an attempt to improve the bioavailability and the stability of four of these derivatives, we propose here two different approaches: complexation with β-cyclodextrin derivatives and incorporation of these substances inside antioxidant micelles. The comparison of the two different methods is the focus of this work, as well as the investigation of some physicochemical properties of the micellar aqueous dispersions. Antioxidant micellar dispersions appear to be suitable for increasing the apparent solubility and stability for all the compounds studied, most probably because of the antioxidant activity of the specific surfactant used, combined with the low amount of water present in the center of the micelles. On this regard, ^{1}H NMR and UV–*vis* spectroscopy result as efficient tools to verify that the drug molecules are indeed placed in the core of the micelles. Moreover, freeze-drying provides a very easy and powerful technique to obtain solid formulations starting from micellar dispersions. On the contrary, cyclodextrins could potentially be used as solubilizing agents, but the drawback connected to degradation in aqueous media could not be overcome with this type of solubilizer.

1. Introduction

It has been well known for considerable time that solubility and permeability through biological membranes are two of the main factors that influence the pharmaceutical activity of substances [1]. Nevertheless, in the last decade, the majority of new chemical entities that has been found pharmaceutically relevant unfortunately suffered of poor solubility in water and physiological fluids. It has been estimated that nearly 40% of drugs present on the market are poorly water-soluble substances, and because of that, development of innovative strategies to solubilize these types of substances has become a necessity [2]. The technologies that are commonly employed to overcome poor drug solubility can be generally divided into physical modifications (e.g. changing in physical state of the drugs), chemical modifications (e.g. salt formation, co-crystallization), and use of agent that can increase the apparent solubility of the drug mostly by stoichiometric complexing of the drug molecules (e.g. cyclodextrins) or forming nanosystems or microsystems to carry the drug in homogeneous dispersions (e.g. micelles, liposomes, nanoparticles, nanoemulsion) [2–4]. One class of poorly soluble compounds with promising properties in terms of very high cytotoxicity and cytostatic activity in *in vitro* conditions are 3-hydroxyquinolinone derivatives (flavones analogue) [5]. Due to their low solubility in any aqueous media, all the activity studies have been carried out using DMSO as a solvent, hampering a proper assessment of their real intrinsic cytotoxic activity. In an attempt to prepare these compounds for better tests by avoiding the use of organic solvents, we have previously shown that liposomes can be good vehicles for increasing solubility in aqueous media for these substances by a factor from 100 to 500, depending on the compound structures [6]. Unfortunately, the liposome dispersions were not able to stabilize these compounds over a sufficiently long period of time. Furthermore, for cancer therapy, poor solubility of drugs is not the only problem that has to be overcome for induction of a relevant pharmaceutical effect: the majority of anticancer drugs suffer from a very high metabolic clearance by the liver. Cyclodextrins, since their introduction in the 1950s, have been considered good vehicles to enhance apparent solubility and stability of poorly soluble drugs

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[7,8], especially for oral administration purposes. Unfortunately, in the majority of the cases, insufficient accumulation of the drug in the tumor tissues was observed, resulting in inefficiency of the therapy and in the development of collateral effects. In the last years, several nanotechnologies have been developed to improve targeting efficiency in anticancer therapy such as liposomes, solid nanoparticles, and micelles [3,9]. A major breakthrough in this field has been reached when it was discovered that big hydrophilic polymers, like polyethylene glycol (PEG), bonded on the nanocarrier surfaces, could significantly increase carrier circulation time in the blood stream [10] (the so-called stealth effect). Most recently, it has been shown that some of these hydrophilic polymers are also able to inhibit some drug efflux pumps (e.g. P-glycoprotein), resulting in a better drug accumulation in tumor cells [11]. As a consequence of all these improvements in nanoparticle drug delivery technology, a number of auto assembling systems containing hydrophilic polymers have been successfully developed as possible carriers for pharmaceutical substances [12]. In this work, the suitability of d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) as a solubilizing agent, as well as some cyclodextrin derivatives, has been investigated. Moreover, solid mixtures composed of solubilizer and compounds obtained by freeze-drying were studied and developed as potential final formulations for *in vivo* and *in vitro* studies.

2. Materials and methods

2.1. Materials

The quinolinone derivatives (Fig. 1) have been synthesized in the Department of Organic Chemistry (Faculty of Science) of Palacky University (Czech Republic) as described in [5]. All compounds were of high degree of purity, between 98.5% and 99.5%. Methyl-β-cyclodextrin (M-β-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD) with molar degrees of substitution of 0.57 and 0.62, respectively, (kind gifts from Roquette Freres, Lestrem, France) were used for the complexation experiments. D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) (kind gift from BASF SE, Ludwigshafen, Germany) was used for the micelle formulations. For the preparation of the physiological buffer (pH 7.4 and 286 mOsm), sodium-di-hydrogen-phosphate, di-sodium-hydrogen-phosphate, and sodium chloride (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) were dissolved in microfiltrated distilled water. Ethanol (Kemetyl A/S, Køge, Denmark) and *tert*-butanol (Sigma–Aldrich Chemie GmbH) were used for the preparation of the organic mixtures.

2.2. Complexation with cyclodextrins

For the complexation studies, the compound Q1 was chosen as an example to study the suitability of CD as a solubilizing agent. The compound Q1 was dissolved in *tert*-butanol resulting in a stock solution of a concentration of 1.5 mM. For both types of CD (M-β-CD and HP-β-CD), stock solutions were prepared (concentrations of 50 mM) in the same solvent. A 1 mL of the corresponding CD solution was mixed with 2 mL of the Q1 solution and the obtained mixture shock-frozen using liquid nitrogen. The solvent was then sublimated by freeze-drying (24 h, 1.054 mbar, shelf temperature 25 °C) using a Gamma 1–16 LSC (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany). The obtained powder was resuspended in 2 mL of phosphate buffer and the UV spectra of the solution recorded using a Genesys UV-spectrophotometer, model 10 UV Scanning (Thermo Electron Corporation, Cambridge, UK).

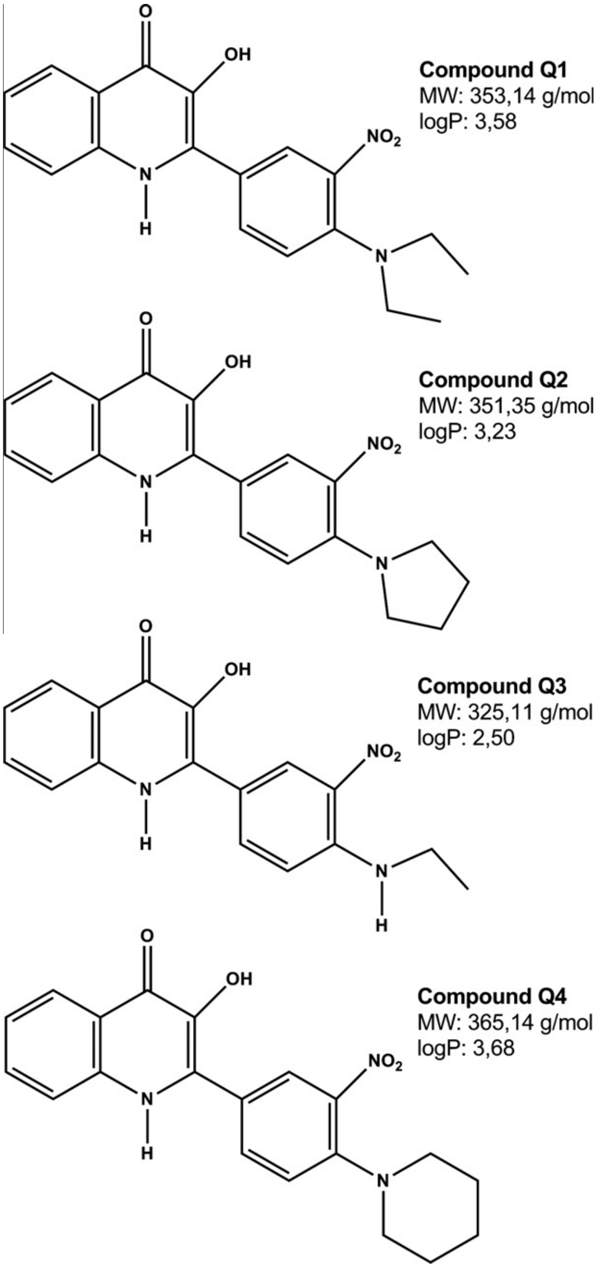

Fig. 1. Chemical structures of the quinolinone derivatives.

2.3. Preparation of the micellar formulations

2.3.1. Preparation of the micellar dispersions

A modification of the method previously described in Mi et al. [13] was employed for preparing the micelle dispersions: Each compound was dissolved in ethanol (concentration of the corresponding stock solutions approximately 100 μg/mL) and mixed with an ethanolic solution of TPGS (200 mg/mL) in a round-bottom flask. The solvent was removed by rotary vacuum evaporation using a rotary evaporator system model Laborota 4000 Efficient (Heidolph Instruments GmbH, Schwabach, Germany) equipped with a vacuum pump model PC 3001 Vario (Vacuumbrand GmbH, Wertheim, Germany) under the following conditions: water bath temperature = 37 °C, time = 1.5 h, and pressure = 45 mBar. The obtained film was resuspended in isotonic and isohydric phosphate buffer (PBS) and the resulting micellar dispersion purified by centrifugation (21,000*g*, 35 min, 15 °C) using a centrifuge model

5804-R (Eppendorf AG, Hamburg, Germany). Once the saturation of the micelles with the drug was reached, precipitation of the free drug was observed after incubation for 18 h at room temperature.

2.3.2. Freeze-drying of micellar dispersions

After 18 h of incubation at room temperature, the amount of drug not incorporated in the micelles was separated from the micellar dispersion by a second centrifugation step (21,000g, 35 min, 15 °C). One mL of supernatant was placed in a 2-mL Eppendorf vial, frozen overnight at −20 °C, and freeze-dried (18 h, Vacuum 0.9 mbar, shelf temperature +15 °C). No cryoprotectants were used.

2.4. Characterization of the TPGS micelle formulations

2.4.1. Drug incorporation efficiency

The method described in di Cagno et al. [6] was used to identify the maximum incorporation efficiency for each compound in the TPGS micelles. While the amount of TPGS was kept constant, the amount of each compound was systematically increased until precipitation of the free drug was observed after incubation. The supernatant (0.2 mL; previously separated from the precipitation of free drug by a second centrifugation step) was diluted to 10 mL with ethanol and the solution sonicated for 15 min in an ultrasonic bath to ensure complete destruction of the micelles. The solution was filtered through a prewashed 0.22-μm pore-size filter and the concentration of the compound detected by UV spectroscopy. Extinction coefficients for each compound are reported in Table 1.

2.4.2. Size analysis

Size of the micelles was detected by Photon Correlation Spectroscopy (PCS) technique using a Brookhaven apparatus, model TurboCorr (Brookhaven Instruments Corporation, Holtsville, USA). In brief, micelle dispersions were diluted adequately (by a factor of 10) with filtered phosphate buffer until reaching a counting rate in the range of 30–50 kcounts/s. Run time of 2 min was found to be suitable for the routine size analysis, and each analysis was repeated three times.

2.4.3. Stability of the compound in micelle formulations

2.4.3.1. UV analysis. To evaluate the stability of the drug in the TPGS micelles, formulations were prepared with a molar ratio drug to micelles 10 times smaller than the maximum incorporation efficiency to avoid precipitation of drug outside the micelles. The supernatant was diluted immediately after resuspension of the film in phosphate buffer and the UV spectra of the solution registered for 18 h (recording frequency of 15 min) following two specific wavelengths respectively of the benzyl chromophore (close to 250 nm for all compounds) and the quinolinone ring chromophore (which is for all compounds between 366 and 376 nm).

2.4.3.2. Nuclear Magnetic Resonance (NMR). NMR spectroscopy was used in order to identify eventual changes in molecular conformation in the micelles. Spectra were recorded on a Varian INOVA spectrometer (Varian Inc., Palo Alto, USA), operating at proton frequency of 500 MHz. Temperature was stabilized at 25 °C during all experiments. The residual proton impurity of the deuterated solvent (D_2O) was used as an internal reference and set at 4.640 ppm. Samples (0.6 mL) of a freshly prepared micelle dispersion were placed in an NMR tube, and the ^1H NMR spectrum was recorded (time = 0) immediately. Subsequently, spectra were recorded every 15 min for 18 h (recording time 5 min each).

Table 1
UV–*vis* spectroscopic parameters of the investigated compounds detected in ethanol.

Compound n^r	λ_{MAX} (nm)	ε (mL/μg cm)	R^2
Q1	370	0.0554	0.999
Q2	373	0.065	0.999
Q3	366	0.062	1.000
Q4	369	0.050	1.000

3. Results and discussion

3.1. Complexation with cyclodextrins

Complexation with cyclodextrins is a well-known and easy-to-use technique to enhance apparent solubility and stability of poorly soluble drugs [8]. Fig. 2 shows the recorded UV spectra for the compound Q1 in ethanol, phosphate buffer and in the presence of HP-β-CD and M-β-CD. It is obvious that both types of CD have a positive effect on the apparent solubility of compound Q1, justified by the increased absorbance that is observed at 250 nm (transition $\pi \rightarrow \pi^*$, benzyl chromophore) compared to the saturated solution of Q1 in phosphate buffer. It should be noticed that the apparent solubility of the compound might increase not only due to complexation with cyclodextrins, but also due to amorphous state possibly produced by lyophilization. Moreover, all water (buffer)-based cyclodextrin-containing systems show a drastic change in the shape of the spectra and a reduction of the intensity of the peak at 370 nm (region of absorption for the quinolinone ring, $n \rightarrow \pi^*$ transition [14]) compared to the respective spectra recorded in organic solvents. This is a clear hint to degradation of the compound in the aqueous environment. Instability of quinolinone derivatives in oxidative environment was first described by Czaun and Speier [15]. They proposed the mechanism of the chemical reaction to be an oxygen-mediated quinolinone ring opening. Mass spectroscopy results suggest that this is the case also for the present compounds (data not shown). Considering these results, cyclodextrins are regarded not suitable as solubilizing agents for this kind of substances.

3.2. Drug incorporation efficiency in TPGS micelles

We have previously shown that liposomes may be good vehicles for increasing apparent solubility for all the studied compounds [6] which improve the solubility by a factor ranging from 100 to 500 depending on the compound. Using TPGS as a solubilizer, for all

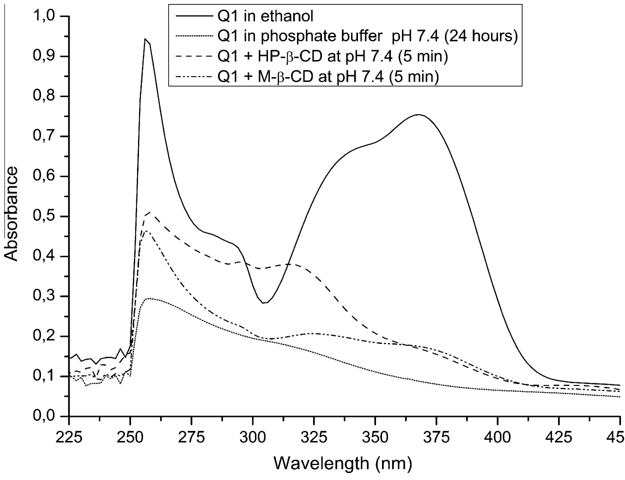

Fig. 2. UV–*vis* spectra of compound Q1 recorded in ethanol, in phosphate buffer pH 7.4, and in the presence of different types of CDs.

of these quinolinone derivatives, beside compound Q3, even slightly higher apparent solubilities compared to the liposome formulations were gained (Fig. 3). Incorporation efficiencies were between 150 μg (compound Q3) and 624 μg (compound Q4) per 100 mg of TPGS, respectively. Compounds Q1 and Q2 show an intermediate incorporation (356 μg and 466 μg per 100 mg of TPGS, respectively). It is also of importance to notice that the rank order of incorporation is the same that was observed for the liposome-based formulations. TPGS forms micelles in water, above a concentration of 0.02 mM (CMC value reported in Sadoqi et al. [16]). It is hypothesized that the quinolinone molecules are incorporated into these micelles. It has been described by Mall et al. [17] that the incorporation of several structurally related drug compounds, in that case sulfonamides, in micelles (in that case sodium dodecyl sulfate) is directly proportional to the logP of the drug. The present results show that the general behavior of the investigated compounds and surfactant is in good agreement with those findings. However, the calculated logP for compounds Q1 and Q2 is quite similar; therefore, the deviation of amount of drug incorporated and logP in this case may be assumed to be related to the higher rigidity of the aza-cycle-pentane ring (substituent of compound Q2) compared to the di-ethylene-amino group (substituent of compound Q1). Furthermore, the flexibility of the molecules themselves may also have an effect on the interaction of drug with lipophilic environment [18]. The present results clearly indicate that TPGS is a promising vehicle for the solubilization of all four quinolinone derivatives studied.

3.3. Size of the micelles

The size of micelles is a fundamental parameter to evaluate the general possibility to inject this type of formulations. Dispersions containing empty micelles, drug-loaded micelles, and reconstituted micelles made from the solid mixture obtained after freeze-drying respectively (same concentration of surfactant in each sample) were analyzed by a light scattering technique. In Fig. 4, a typical example of the size distributions obtained for drug-containing TPGS micelle dispersions is reported. For all the three replicates, average sizes are between 11.9 and 12.4 nm, with a very narrow distribution (polydispersity index value 0.05). In the same way, the freeze-drying process seemed not to affect the average size of the micelles that was found to be close to 12 nm after reconstitution of the solid mixture in buffer. Therefore, it can be concluded that the micellar dispersion is formed spontaneously upon reconstitution after freeze-drying.

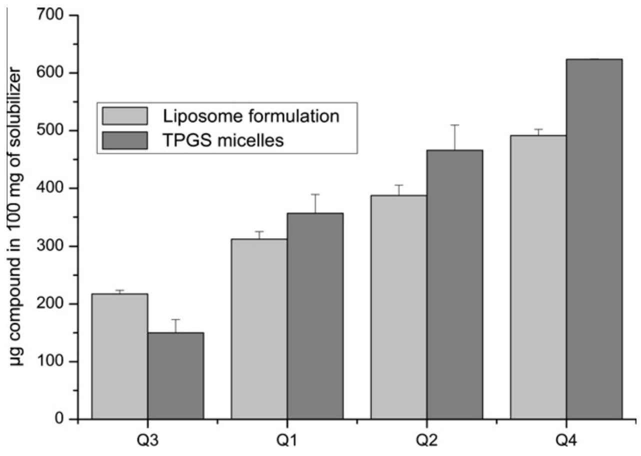

Fig. 3. Maximum incorporation of the four quinolinone derivatives in micelle dispersions compared to liposome formulations.

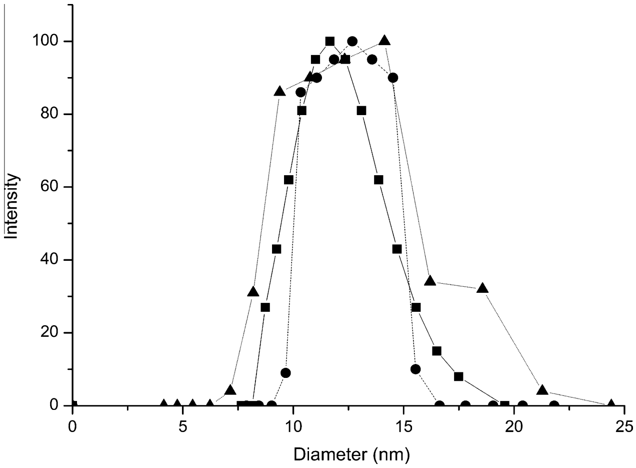

Fig. 4. Example of size analysis of TPGS micellar dispersion containing compound Q2. Each curve represents one typical single analysis.

3.4. Stability of the compounds in the micelles

3.4.1. UV spectroscopy

UV–*vis* spectroscopy is a well-established technique that has been employed to study the interactions between drugs and micelles [19–21]. As a typical example, we report the spectra of the compound Q4 in ethanol compared to the same compound in the TPGS micelles in Fig. 5. For all compounds, the UV spectra in ethanol (thereby destructing the micelles) and in phosphate buffer (micellar dispersion) are widely similar: The specific wavelengths of absorption for each compound are reported in Table 2. As can be seen from Fig. 5 and Table 2, both characteristic peaks of absorption, around 250 nm (λ^{I}) and 370 nm (λ^{II}), are maintained even if a small batochromic shift induced by the solvent is observed. Moreover, in the case of drug encapsulated in the micelles, the absolute intensities of both peaks are reduced in comparison with the spectra of the compound in ethanol. This effect is probably due to the lower dielectric constant in the micelles in which drug molecules are incorporated compared to the free solvent [22]. Following the intensities at two wavelengths recorded for the micelle formulations in PBS, it was found that, for all compounds, no big changes in the intensity of peak λ^{I} were detected after 18 h. In contrast to this, for all formulations tested, a decrease

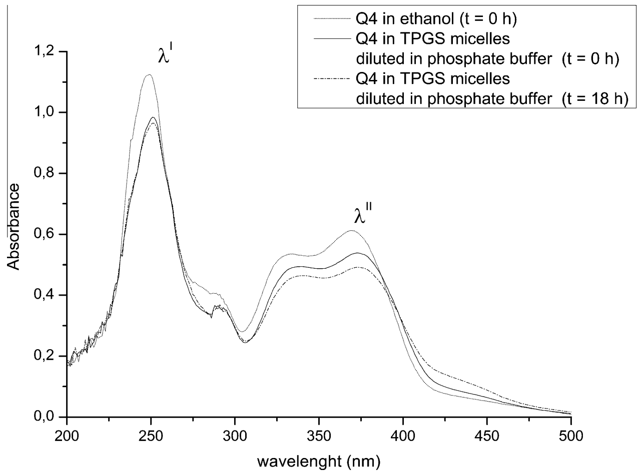

Fig. 5. Example of UV–*vis* spectra recorded for a micellar formulation of compound Q4 in phosphate buffer after immediate resuspension of the solid mixture ($t = 0$), and 18 h after resuspension ($t = 18$ h), and after dissolution in ethanol.

Table 2
Specific wavelengths of absorption for the investigated compounds in ethanol, and in micelle dispersions in phosphate buffer respectively.

Compound	λ^{I} ethanol (nm)	λ^{I} buffer (nm)	λ^{II} ethanol (nm)	λ^{II} buffer (nm)
Q1	250	251	370	375
Q2	247	250	373	375
Q3	242	246	366	370
Q4	249	251	369	373

in intensity at λ^{II} was observed, followed by reaching a plateau after approximately 2 h. Moreover, a negative exponential curve can be obtained if the ratios of the absorbances (λ^{II}/λ^{I}) are plotted versus time ($R^2 = 0.9880$) (Fig. 6). This phenomenon may typically be interpreted as to indicate degradation of the compounds also in this type of formulations. To verify this hypothesis, the concentration of compound in the supernatant after 18-h incubation was detected using ethanol as solvent. Surprisingly, for all the samples tested, no change in concentration was detected in the ethanolic solutions, and it was concluded that after incubation at room temperature for 18 h, the compounds show no sign of degradation. This fact suggests that the spectroscopic phenomenon observed is not related to degradation, but most probably to a structural rearrangement of the micelles in the first hours after formation.

3.4.2. NMR investigation

^{1}H NMR spectroscopy has been employed to indentify the exact location of some drug molecules in some micelles [23,24]. For the present formulations, ^{1}H NMR spectra were recorded every 15 min for 18 h to follow any changes with time. The spectra were compared using the water signal (impurity of D_2O) as a reference. It was observed that the peaks generated by the polyethylene glycol motif of the molecule (lying at 3.5 ppm) did not shift during time. Similarly, there was no change for the peaks related to the α-tocopherol motif of the TPGS. However, the peaks associated with the methyl groups bound to the aliphatic chain (lying around 0.7 ppm) shifted with time, which is regarded a clear sign of a change in the electrical density in the core of the micelles. Fig. 6 depicts the position of the peaks generated by the change of the methyl group environment with time. It shows a logarithmic dependence versus time ($R^2 = 0.9958$) until reaching a plateau after approximately 2 h. These results are interesting because they indicate that all drug molecules interact with the motif of the TPGS located in the core of the micelles and not with the portion in contact with the water (i.e. the polyethylene glycol motif). Moreover, Puvvada and Blankschtein [25], using a molecular thermodynamic approach, have found that for similar surfactants, the cores of non-ionic micelles are regarded to be water-free. This may explain why the chemical degradation of the quinolinone compounds is not observed in phosphate buffer when the compounds are incorporated in the micelles, in addition to the antioxidative effect of the α-tocopheryl segment of the TPGS. Fig. 7 shows that the reduction in the UV absorption and the change in the electrical density of the core of the micelles recorded via NMR, both recorded during the first hours of rehydration of the freeze-dried product, are linearly correlated (R^2 of 0.9987). It is the authorś hypothesis that this phenomenon is due to a rearrangement of the micellar systems that takes place after their formation. However, further studies should be performed to clarify this observation exactly.

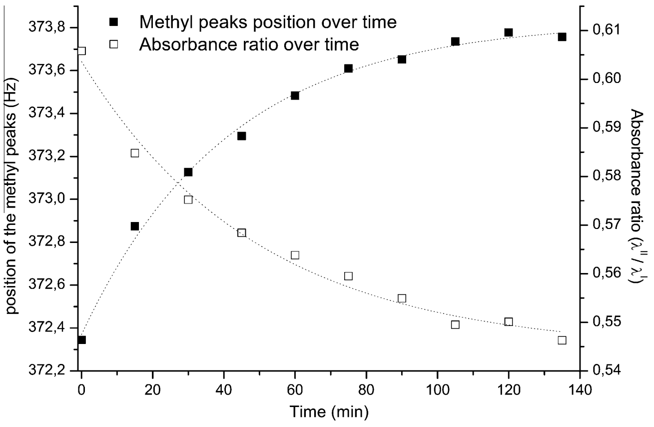

Fig. 6. Example of change in UV absorbance and position of the methyl peak signals recorded by NMR, registered after redispersion of the solid mixture of compound Q3 obtained by freeze-drying.

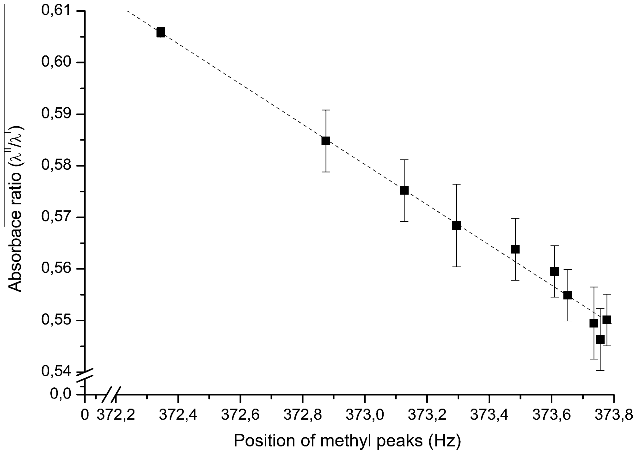

Fig. 7. Correlation between the change in UV absorbance and the methyl peak signal positions recorded by NMR for compound Q3.

3.5. Formulation aspects

Freeze-drying is a powerful technique to obtain solid formulations especially when substances show instability in aqueous media. For all the formulations tested, freeze-drying of the micellar dispersions resulted in a high-volume solid mixture. Resuspension of the obtained solid mixture required short time of manual agitation directly inside the vial (1–2 min). As has been shown above, even without using cryoprotectants, no change in drug concentration or size of micelles was seen after freeze-drying and resuspension of the solid mixture, compared to the freshly prepared micellar dispersions. Moreover, the presence of the salt in the solid mixture gives the possibility to obtain isotonic and isohydric micellar dispersions, thereby being suitable for immediate intravenous administration. Further studies will investigate the physical characteristics of freeze-dried formulations.

4. Conclusions

In this work, TPGS micelles have proven to be excellent vehicles to solubilize and stabilize new potential cytotoxic and cytostatic quinolinone drugs. For the chemical stabilization, the antioxidant effect of the α-tocopherol motif in the surfactant molecules plays an important role as well as the absence of water in the core of the micelles, where the compounds seem to be placed. Freeze-drying was found to be an efficient technique to obtain formulations in the solid state, obtaining a better stability over time in comparison with liposome dispersions. On the contrary, cyclodextrins appear not suitable as solubilizers in this case due to the fact that they are not able to stabilize these substances in aqueous media against oxidation and/or hydrolysation. Interestingly, NMR and UV–*vis* spectroscopy results show that the stabilization of the micelles in dispersion is not immediate but seems to take place during a period of approximately 2 h after reconstitution of the solid mixture in water/buffer. Further studies will be performed to study this phenomenon closely.

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References

[1] G.L. Amidon, H. Lennernäs, V.P. Shah, J.R. Crison, A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability, Pharm. Res. 12 (1995) 413–420.
[2] A. Fahr, X. Liu, Drug delivery strategies for poorly water-soluble drugs, Expert Opin. Drug Deliv. 4 (2007) 403–416.
[3] L. Zhang, F.X. Gu, J.M. Chan, A.Z. Wang, R.S. Langer, O.C. Farokhzad, Nanoparticles in medicine: therapeutic applications and developments, Clin. Pharmacol. Ther. 83 (2008) 761–769.
[4] C.O. Rangel-Yagui, A. Pessoa-Jr, L. Costa Tavares, Micellar solubilization of drugs, J. Pharm. Pharmaceut. Sci. 8 (2005) 147–163.
[5] P. Hradil, J. Hlaváč, M. Soural, M. Hajdúch, M. Kolář, R. Večeřová, 3-Hydroxy-2-phenyl-4(1H)-quinolinones as promising biologically active compounds, Mini Rev. Med. Chem. 9 (2009) 696–702.
[6] M. di Cagno, J. Styskala, J. Hlaváč, M. Brandl, A. Bauer-Brandl, N. Skalko-Basnet, Liposomal solubilization of new 3-hydroxy-quinolinone derivatives with promising anticancer activity: a screening method to identify maximum incorporation capacity, J. Lipos. Res. 21 (2011) 272–278.
[7] T. Loftsson, M. Másson, M.E. Brewster, Self-association of cyclodextrins and cyclodextrin complexes, J Pharm. Sci. 93 (2004) 1091–1099.
[8] T. Loftsson, M.E. Brewster, Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization, J. Pharm. Sci. 85 (1996) 1017–1025.
[9] M.E. Davis, Z. Chen, D. M Shin, Nanoparticles therapeutics: an emerging treatment modality for cancer, Nat. Rev. Drug Discov. 7 (2008) 771–782.
[10] A.L. Klibanov, K. Maruyama, V.P. Torchilin, L. Huang, Amphipathic polyethyleneglycols effectively prolong the circulation time of liposomes, FEBS Lett. 1 (1990) 235–237.
[11] M. Werle, Natural and synthetic polymers as inhibitors of drug efflux pumps, Pharm. Res. 25 (2007) 500–511.
[12] G.S. Kwon, K. Kataoka, Block copolymers micelles as long-circulating drug vehicles, Adv. Drug Deliver. Rev. 16 (1995) 295–309.
[13] Y. Mi, Y. Liu, S.S. Feng, Formulation of Docetaxel by folic acid-conjugated d-α-tocopheryl polyethylene glycol succinate 2000 (vitamin E $TPGS_{2K}$) micelles for targeted and synergistic chemotherapy, Biomaterials 32 (2011) 4058–4066.
[14] P. Banerjee, S. Chatterjee, S. Pramanik, S.U. Hossain, S. Bhattacharya, S.C. Bhattacharya, Spectroscopic studies of 2-(2-bromo-ethyl)-6-nitro-benzo[de]isoquinolene-1,3-dione in water/alkanol mixed solvents and nonionic micelle of Igepal CO series, Spectrochim. Acta A 66 (2007) 1110–1114.
[15] M. Czaun, G. Speier, The base-catalyzed oxygenation of quinoline derivatives, Tetrahedron Lett. 43 (2002) 5961–5963.
[16] M. Sadoqi, C.A. Lau-Cam, S.H. Wu, Investigation of the micellar properties of the tocopheryl polyethylene glycol succinate surfactants TPGS 400 and TPGS 1000 by steady state fluorometry, J. Colloid Interf. Sci. 333 (2009) 585–589.
[17] S. Mall, G. Bckton, D.A. Rawlins, Dissolution behaviour of sulphonamides into sodium dodecyl sulfate micelles: a thermodynamic approach, J. Pharm. Sci. 85 (1996) 75–78.
[18] A.M. Navia, P.R. Chaturvedi, Design principles for orally bioavailable drugs, Drug Discov. Today 1 (1996) 179–189.
[19] T. Sobish, The use of methyl orange for the characterization of micelles in aqueous non-ionic surfactant solutions, Progr. Colloid Polym. Sci. 89 (1992) 44–48.
[20] M. Adams, G.S. Kwon, Spectroscopic investigation of the aggregation state of amphotericin B during loading, freeze-drying, and reconstitution of polymeric micelles, J. Pharm. Pharmaceut. Sci. 7 (2004) 1–6.
[21] Y. Kadam, U. Yerramilli, A. Bahadur, Solubilization of poorly water-soluble drug carbamezapine in Pluronic® micelles: effect of molecular characteristics, temperature and added salt on the solubilizing capacity, Colloid Surf. B 72 (2009) 141–147.
[22] L. Sepulveda, Absorbances of solutions of cationic micelles and organic anions, J. Colloid Interf. Sci. 46 (1974) 372–379.
[23] M. Verbrugghe, P. Sabatino, E. Cocquyt, P. Saveyn, D. Sinnaeve, P. Van der Meeren, J.C. Martins, Solubilization of flurbiprofen with non-ionic Tween 20 surfactant micelles: a diffusion^{1}H-NMR study, Colloid Surf. A 372 (2010) 28–34.
[24] G. Zheng, A.M. Torres, M. Ali, N. Manolios, W.S. Price, NMR study of the structure and self-association of core peptide in aqueous solution and DPC micelles, Peptide Sci. 92 (2010) 177–180.
[25] S. Puvvada, D. Blankschtein, Molecular-thermodynamic approach to predict micellization, phase behavior, and phase separation of micellar solutions. I. Application to nonionic surfactant, J. Chem. Phys. 92 (1990) 3710–3724.