Communications

A New Paclitaxel Formulation with a Cationic Cyclotriphosphazene

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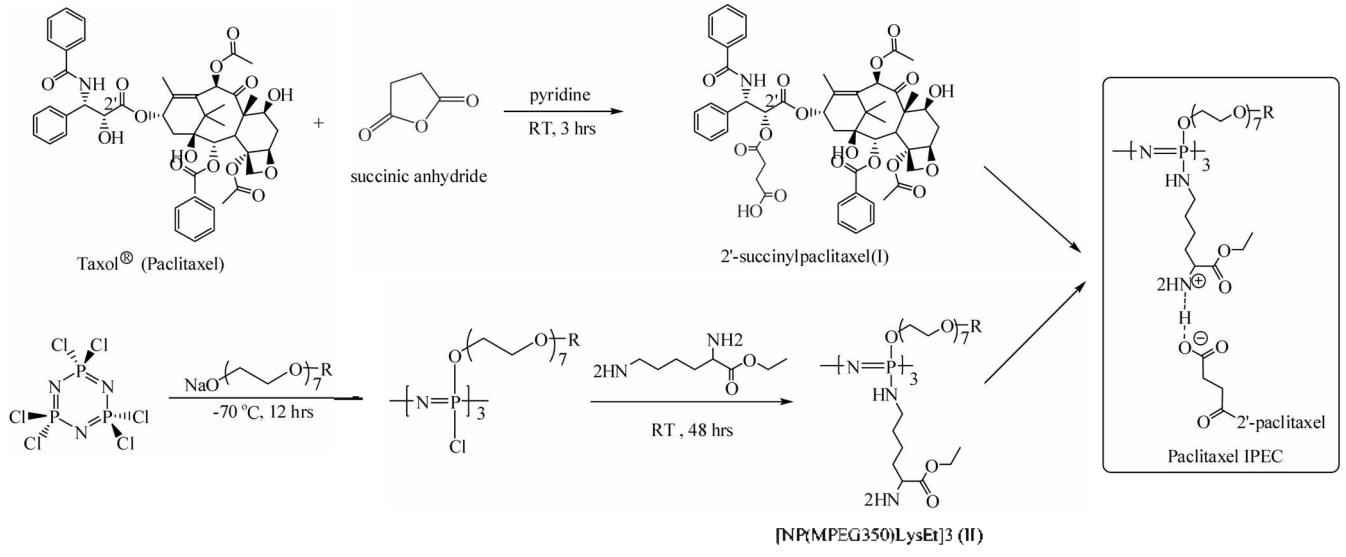
Recently the inter-polyelectrolyte complexes (IPECs) have received considerable attention.[1] IPECs represent a special class of chemical compounds formed as a result of cooperative electrostatic interactions between cations and anions. The properties of IPECs depend strongly on the feeding ratio of the oppositely charged polyions. First studies on the utilization of IPECs were reported on gene delivery in the late 1980s.[2]

Paclitaxel is one of the most important antitumor agents currently in clinical use, since it exhibits effective antitumor activity against various cancers.[3] However, its clinical applications are limited due to its extremely low water solubility (< 1 μg/mL)[4] as well as its serious side effects. Therefore, a great deal of efforts has been made to overcome such problems. There are numerous studies on solubilization of paclitaxel by chemical conjugation to hydrophilic polymers or micellar encapsulation using amphiphilic polymers.[5] However, to our knowledge, there is no report on the formulation of paclitaxel based on IPEC. In this paper we report a novel formulation method for paclitaxel by incorporation of 2'-succinylpaclitaxel (2'-SPTX) into a cationic cyclotriphosphazene resulting in chemically stable micelles.

A cationic and water soluble cyclotriphosphazene drug carrier bearing a hydrophilic poly(ethylene glycol) (PEG) with a molecular weight of 350 and lysine ethyl ester as cationic moiety was prepared according to the same procedure as described in our previous work[6] with modification. Instead of an oligopeptide, lysine ethyl ester (LysEt) was employed as a cationic moiety for electrostatic interaction with anionic 2'-SPTX. The synthetic schemes for the anionic 2'-SPTX (I) and the cationic cyclotriphosphazene carrier (II) are shown along with their final ion paired IPEC in Scheme 1. The final paclitaxel IPEC could easily be prepared by a direct feeding method as follows. The 2'-SPTX (1 mg) solution in ethanol was added to the cyclotriphosphazene carrier (20 mg) in ethanol. The mixed solution was evaporated to dryness and then the residue was dissolved in distilled water. After sonication for 10 min, the resulting solution was filtered with 0.45 μm syringe filter to obtain a clear solution of the paclitaxel-loaded IPEC, which was fully characterized by multinuclear (^{1}H, ^{31}P) NMR spectroscopy along with its dynamic light scattering (DLS) and critical micelle concentration (CMC) measurements.

The ^{1}H-NMR spectra of the paclitaxel IPEC in $CDCl_3$ displayed in Figure 1(a) clearly show all the proton resonances both of the hydrophilic methoxy poly(ethylene glycol) (MPEG) and the LysEt group forming an ionic pair with 2'SPTX with good resolution, but in D_2O most proton



Scheme 1. Reaction routes to 2'-succinylpaclitaxel (I) and a cationic cyclotriphosphazene (II) for paclitaxel IPEC.

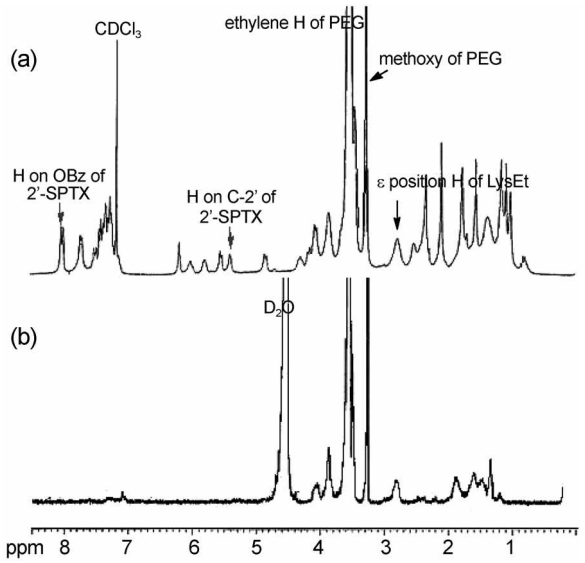


Figure 1. ^{1}H-NMR spectra of paclitaxel IPEC in $CDCl_3$ (a) and D_2O (b).

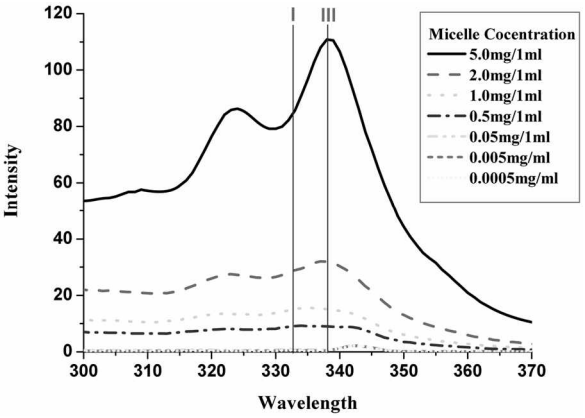


Figure 2. Fluorescence spectra of pyrene for CMC determination.

resonances of LysEt and 2'SPTX disappeared or broadened while the MPEG protons remained with almost the same intensity as shown in Figure 1(b). Such an observation strongly indicates that the paclitaxel IPEC itself forms micelles by self-assembly with orientation of the Lysine groups including the paclitaxel molecule into the core and the MPEG groups on the outer shell of the micelles.

Therefore, we have performed DLS measurements for the paclitaxel IPEC displaying relatively large sized micelles with a mean diameter of about 115 nm. It was confirmed that the free cyclotriphosphazene carrier (II) did not form micelles in aqueous solution probably because of low hydrophobic and cationic properties of lysine, but electrostatic interaction between phosphazene trimer and highly hydrophobic 2'-SPTX seems to drive micelle formation. The paclitaxel drug, 2'-SPTX could be loaded up to approximately 20% of the trimeric carrier.

The stability of micelles in aqueous solution is a critical factor for injectable delivery. Therefore, CMC of the paclitaxel IPEC was measured by the fluorescence dye solubilizing technique with pyrene.[7] The CMC value of the paclitaxel IPEC was calculated to be very low (1.3 mg/L) from the intensity ratio of III_{338}/I_{333} bands shown in Figure 2.

Table 1. Cytotoxicity of paclitaxel in different forms

	IC_{50} Values (nM) (mean ± SD, n = 3)			
	MCF-7	SK-OV-3	MDA-MB-231	MES-SA
Paclitaxel[a]	34.77±16.31	21.1±4.79	33.45±15.16	63.57±44.943
Conjugate[b]	447.4±138.2	503.4±114.3	379.9±89.2	327.2±54.5
IPEC[c]	267.3±81.5	108.8±44.9	273.3±37.4	243.4±119.6

[a] Formulated with Cremophore EL. [b] Ref. 10. [c] The feeding ratio of 2'-SPTX was 5% of the trimer carrier (wt/wt).

In order to evaluate the antitumor activity of the paclitaxel IPEC, we have assayed its *in vitro* cytotoxicity against selected human tumor cell lines according to our previous method.[8] The IC_{50} values of the paclitaxel IPEC measured after 72 h incubation are listed along with those of free and conjugated paclitaxel[9] in Table 1. The present paclitaxel IPEC exhibited lower cytotoxicity than free paclitaxel but higher cytotoxicity compared with cyclotriphosphazene-paclitaxel conjugate. Paclitaxel seems to be controlled-released from its IPEC, and further *in vivo* assay is planned.

In summary, the cationic lysine-grafted cyclotriphosphazene carrier and the anionic 2'-succinylpaclitaxel formed a stable IPEC due to self-assembly to micelles with the hydrophobic 2'-succinylpaclitaxel-LysEt ion pair in the core and PEG on the outer shell of the micelles in aqueous solution. The IPEC micelles have shown a narrow size distribution with a mean diameter of 115 nm and high stability with a low CMC value of 1.3 mg/L.

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References

1. Burkhardt, M.; Ruppel, M.; Tea, S.; Drechsler, M.; Schweins, R.; Pergushov, D. V.; Gradzielski, M.; Zezin, A. B.; Müller, A. H. E. *Langmuir* **2008**, *24*, 1769.
2. Wu, G. Y.; Wu, C. H. *J. Biol. Chem.* **1987**, *262*, 4429.
3. (a) Rowinsky, E. K.; Donehower, R. C. *New Engl. J. Med.* **1995**, *332*, 1004. (b) Spencer, C. M.; Faulds, D. *Drugs* **1994**, 794.
4. Goldspiel, B. R. *Pharmacotherapy* **1997**, *17*, 110S.
5. (a) Sugahara, S.; Kajiki, M.; Kuriyama, H.; Kobayashi, T. *J. Control. Release* **2007**, *117*, 40. (b) Greenwald, R. B.; Choe, Y. H.; McGuire, J.; Conover, C. D. *Adv. Drug Delivery Reviews* **2003**, *55*, 217.
6. Jun, Y. J.; Toti, U. S.; Kim, H. Y.; Yu, J. Y.; Jeong, B.; Jun, M. J.; Sohn, Y. S. *Angew. Chem. Int. Ed.* **2006**, *45*, 6173.
7. Allcock, H. R.; Powell, E. S.; Chang, Y. *Macromolecules* **2004**, *37*, 7163.
8. Yu, J. Y.; Jun, Y. J.; Jang, S. H.; Lee, H. J.; Sohn, Y. S. *J. Inorg. Biochem.* **2007**, *101*, 1931.
9. Jun, Y. J.; Min, J. H.; Ji, D. E.; Yoo, J. H.; Kim, J. H.; Lee, H. J.; Jeong, B.; Sohn, Y. S. *Bioorg. & Med. Chem. Lett.* **2008**, *18*, 6410.