Study on β-cyclodextrin-complexed nanogels with improved thermal response for anticancer drug delivery

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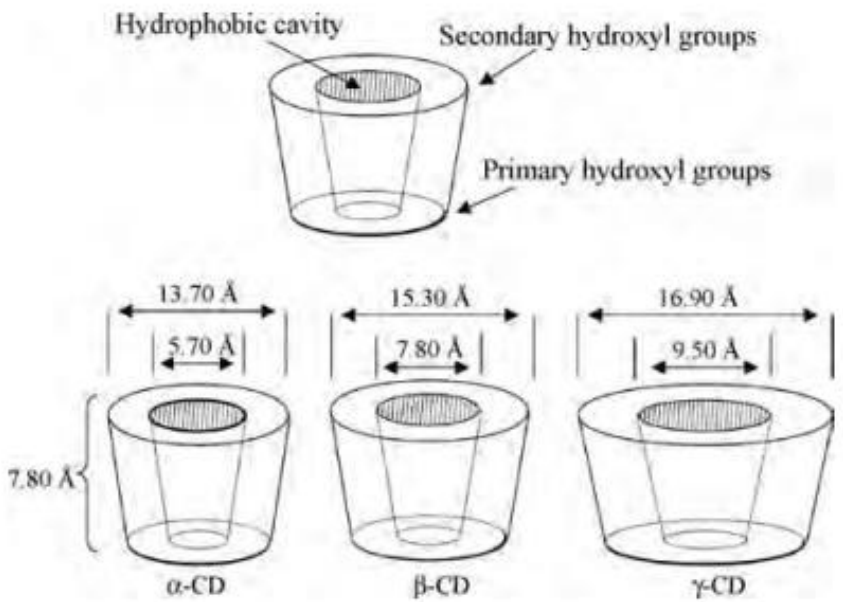
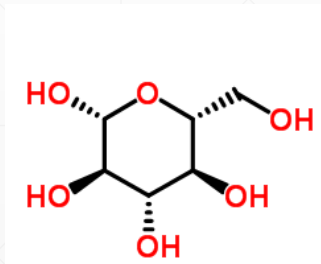
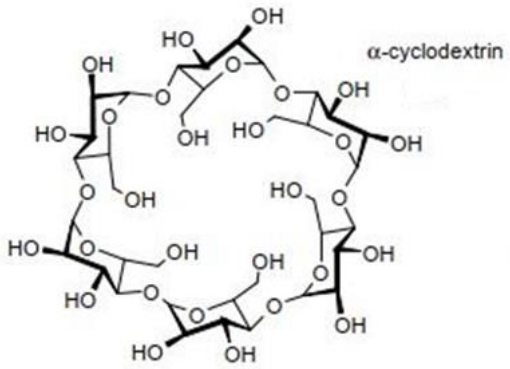
Introduction

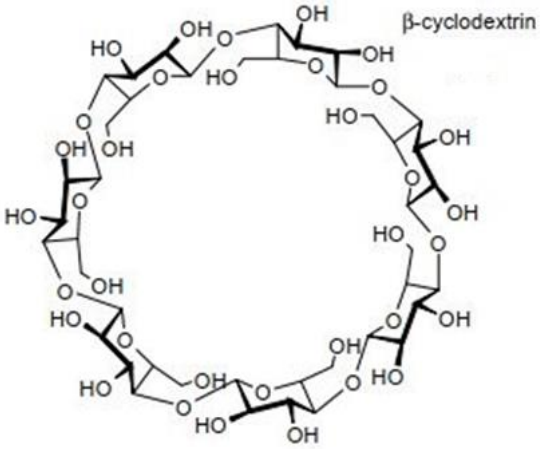
- **Cancer** is one of the most serious diseases, which causes death of large population around the world each year.
- **thermosensitive nanocarriers** have been receiving more and more attention due to their remote manipulation ability for controllable release.
- **Poly(N-isopropylacrylamide)** (**PNIPAM**) is a popular polymer candidate for fabrication of thermosensitive nanocarriers.
- there were several reports on employment of PNIPAM-based nanogels for delivery of various types of therapeutic reagents, such as **doxorubicin** (**DOX**), protein, as well as antibacterial drug.

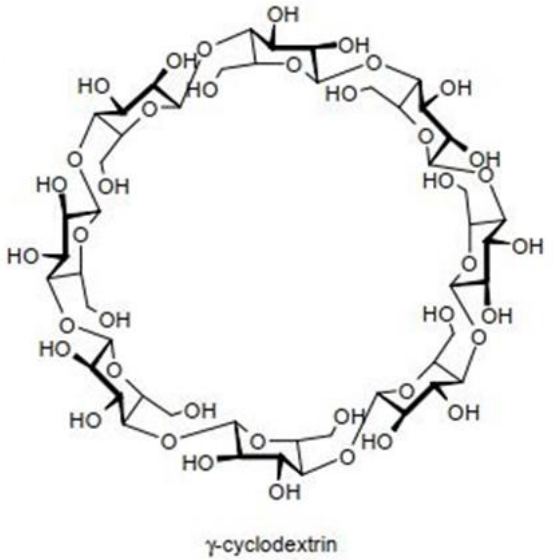
Introduction

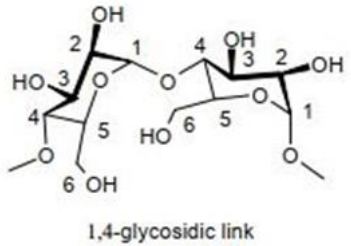












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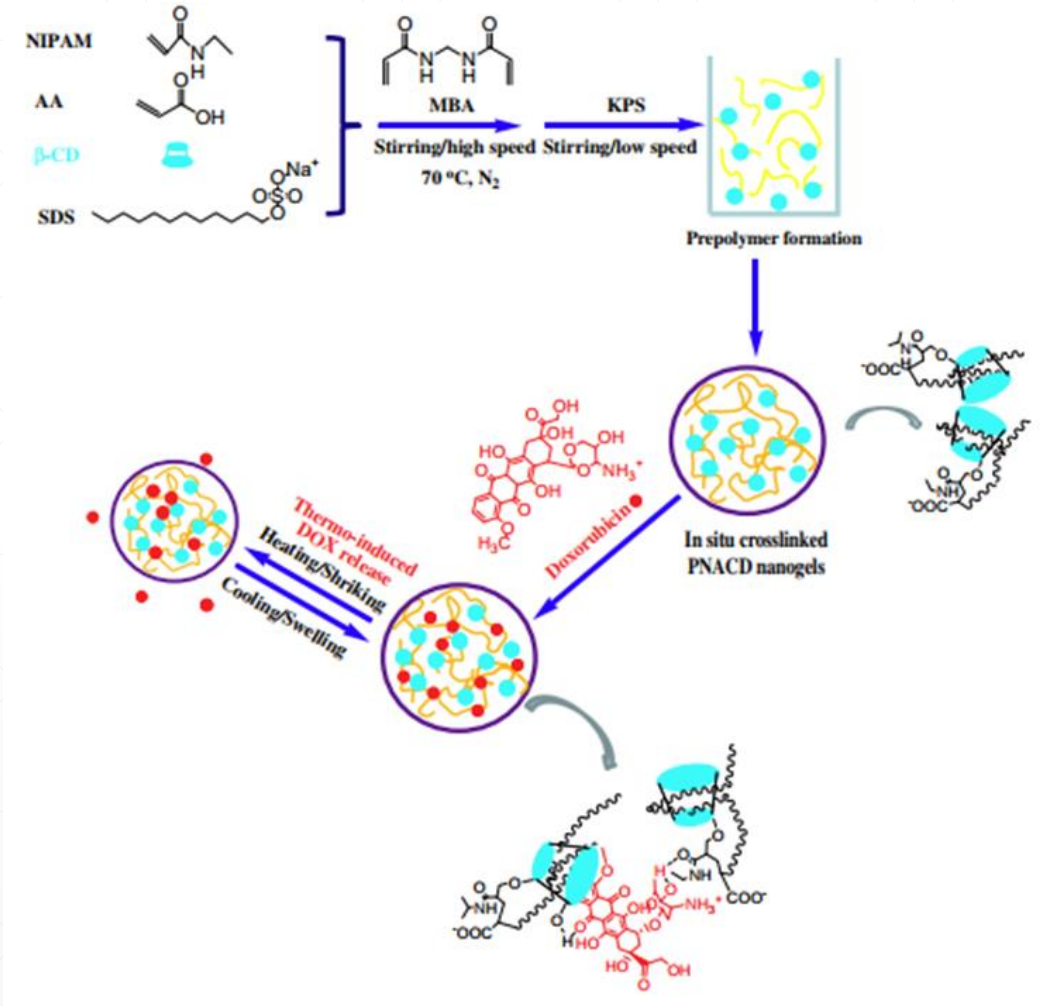


Experimental

- Preparation of the PNA/DOX and PNACD/DOX nanogels

- **NIPAM**, **β-CD**, **AA** and **SDS** were added to **MBA** in 25 mL **ultrapure** (**UP**) **water** under magnetic stirring. And **KPS** was dropped in after the mixture heated to 70 °C and degassed by N_2 for 1 h. Under nitrogen atmosphere, polymerization was performed at 70 °C for 7 h. The product was dialyzed against UP water for 3 days (500 mL × 9 times). The **β-CD-free PNA nanogels** were prepared through a similar method in the absence of β-CD.

- The drug loading experiment was realized by 1 mL **DOX solution** (2 mg/mL) added to 5 mL water containing 50 mg nanogels. The mixture was stirred at room temperature overnight and purified by dialysis to remove the free drug. An ultraviolet-visible (UV–Vis) spectrometer was used to detect the absorption of the media removed from the dialysis membrane for indirect determination of the DOX **encapsulation efficiency** (**EE**). The **PNA/DOX** and **PNACD/DOX nanogels** were obtained after the purified samples lyophilized, and then were kept at 4 °C for further study.

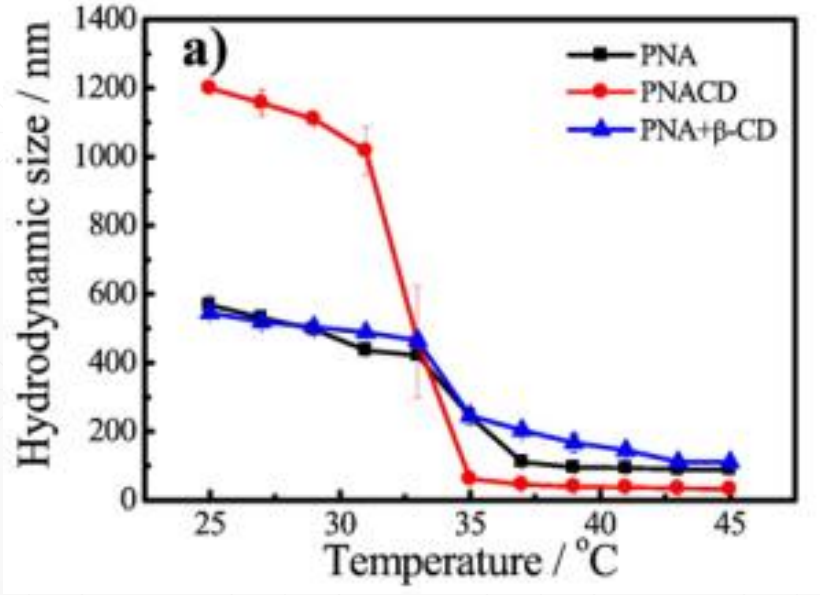
Results and discussion

- Preparation and physical characterization of DOX-free or –loaded nanogels (PNACD/DOX)
- PNACD nanogels were fabricated via in situ polymerization of NIPAM and AA monomers in the presence of β-CD as a host for complexation and SDS as surfactant.
- The formation of prepolymer of NIPAM (PNIPAM) at the temperature (70 °C) would increase its hydrophobicity, which may create a driving force to make its chain through the hydrophobic cavity of β-CD to form guest-host complexes.
- At the same time, the complexed structure can be fixed through the in situ crosslinking by MBA as a crosslinker.
- Upon cooling/heating switch, the variation of hydrophilicity of PNIPAM block may allow for their shuttle out/in the cavity of β-CD, probably resulting in an enhanced thermosensitivity.

Results and discussion

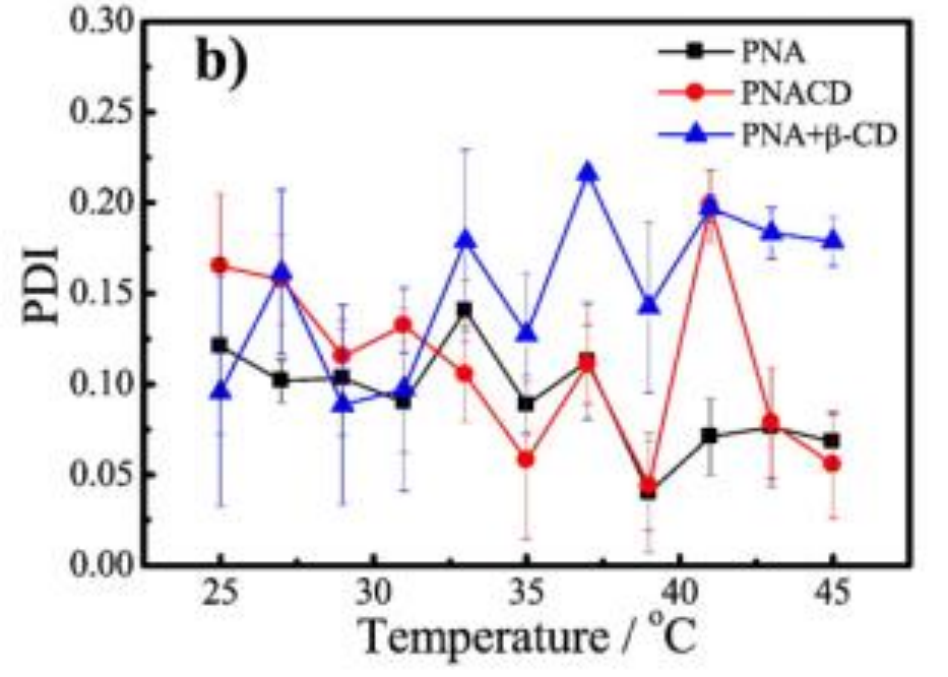
- the sizes of both the nanogels decreased with temperature increasing from 25 to 45 °C, which is reasonable because PNIPAM block becomes more hydrophobic at higher temperature to induce deswelling of the nanogels due to the repelling of water molecules from their hydrophobic parts.

- Hydrodynamic sizes of PNA, PNACD nanogels as well as PNA + β-CD blends (PNA nanogels + β-CD) at different temperatures.



Results and discussion

- all the nanogels (PNA, PNACD and PNA + β-CD) are all narrowly distributed with a small size distribution at different studied temperatures.
- the size distribution of PNA, PNACD nanogels and PNA + β-CD blends.



Results and discussion

- It can be seen from Table, both PNA and PNACD nanogels had high encapsulation efficiency, indicating that DOX drug can be effectively loaded into them.

Characterization of DOX-free and loaded nanogels.

Samples	Size, nm[a] 25 °C	Size, nm[a] 37 °C	Zeta, mV[a]	EE, %[b]	LC, %[c]
PNA	558 ± 13	111 ± 5	−11.7 ± 0.96		
PNA/DOX	573 ± 12	281 ± 11	−8.97 ± 0.45	62 ± 2	2.4 ± 0.1
PNACD	1200 ± 13	46 ± 1	−21.93 ± 1.05		
PNACD/DOX	1337 ± 34	299 ± 15	−16.63 ± 0.65	54 ± 5	2.1 ± 0.2

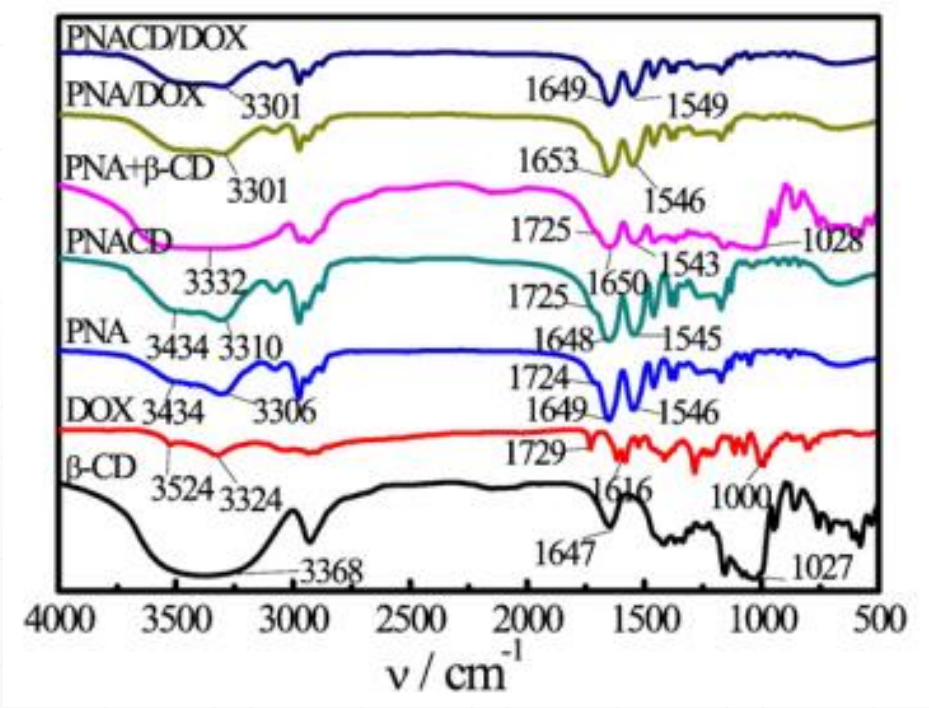
[a] Size and Zeta potential were measured in water.

[b] Encapsulation efficiency (EE) = $100 * W_{te} / W_0$, W_0 and W_{te} are the total DOX weight used for encapsulation and the weight of encapsulated DOX, respectively.

[c] Loading capacity (LC) = $100 * W_{te} / W$, W_{te} and W are the weight of encapsulated DOX and the weight of DOX-loaded nanogels, respectively.

Results and discussion

- β-CD sample: 3368 (O-H bond) &1027 (C-O-C bond)
- PNA & PNACD: 1649 ((C-O stretching) &1546 (N-H bending) & 1724 (-COOH)



Results and discussion

- PNA and PNACD nanogels presented average sizes of 177 ± 6 and 759 ± 38 nm, respectively, which were smaller than those measured by DLS method.
- PNACD nanogels presented a more monodispersed state than PNA nanogels, which is important for biomedical applications.

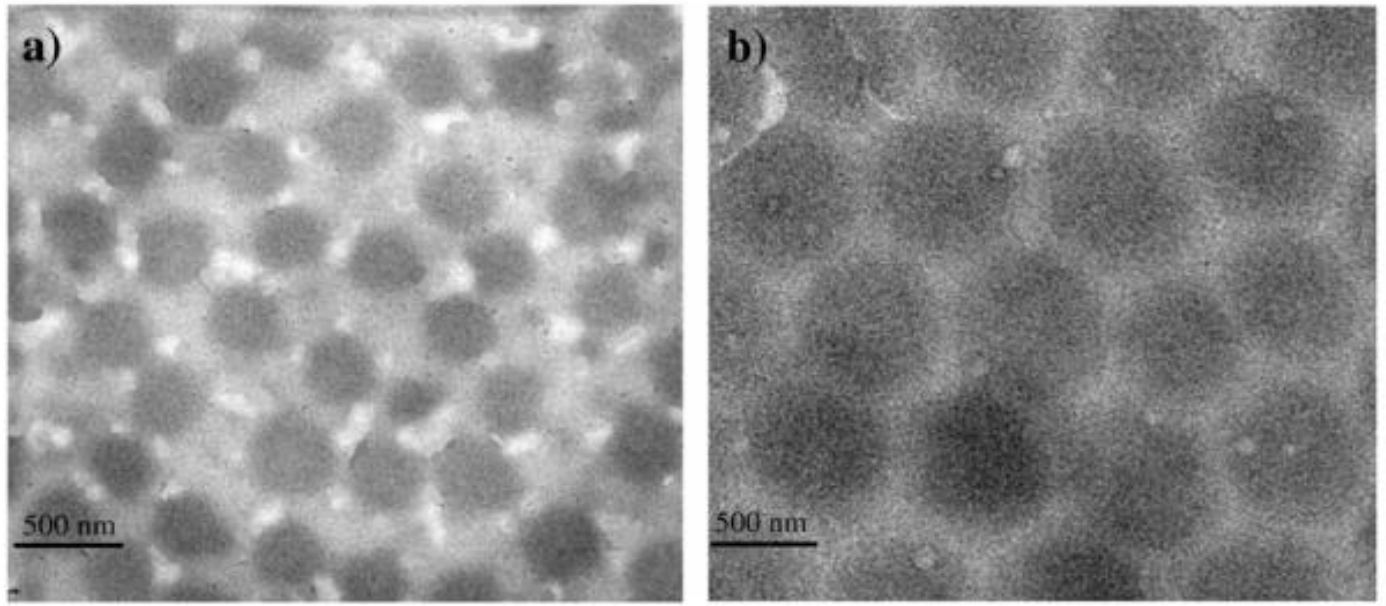
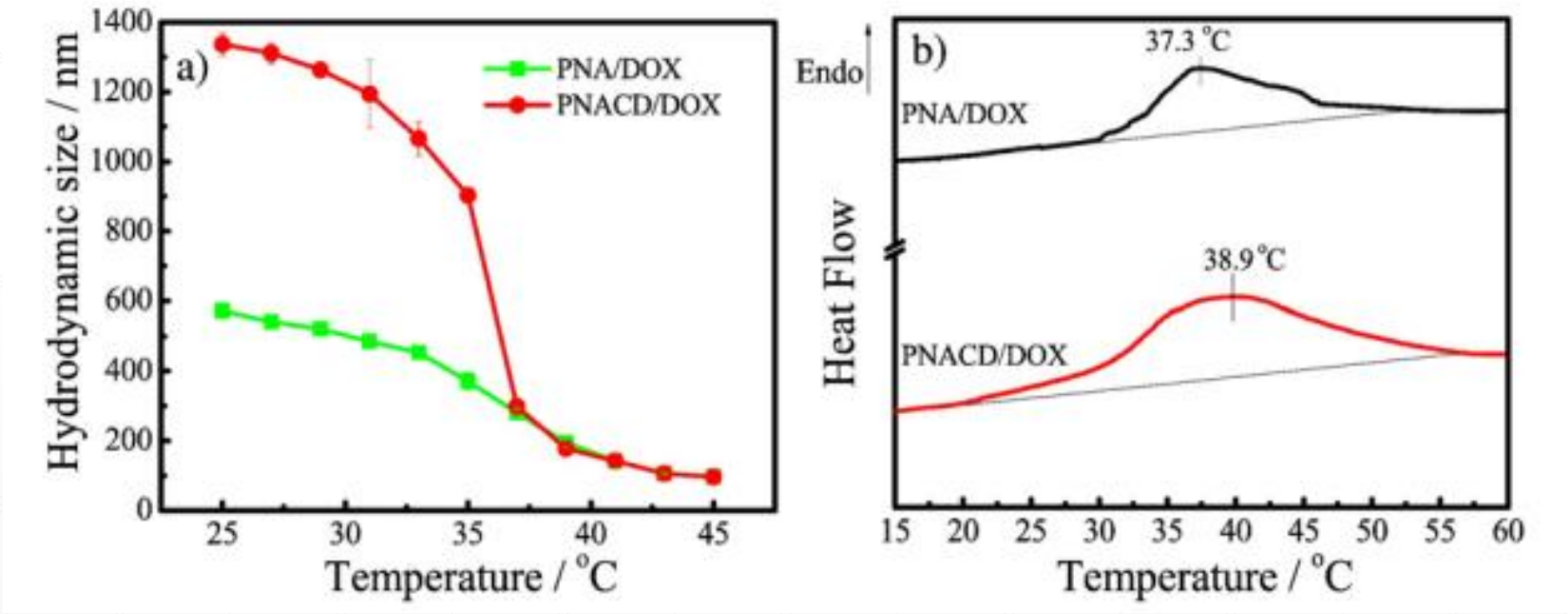


Fig. 3. TEM images of (a) PNA and (b) PNACD nanogels.

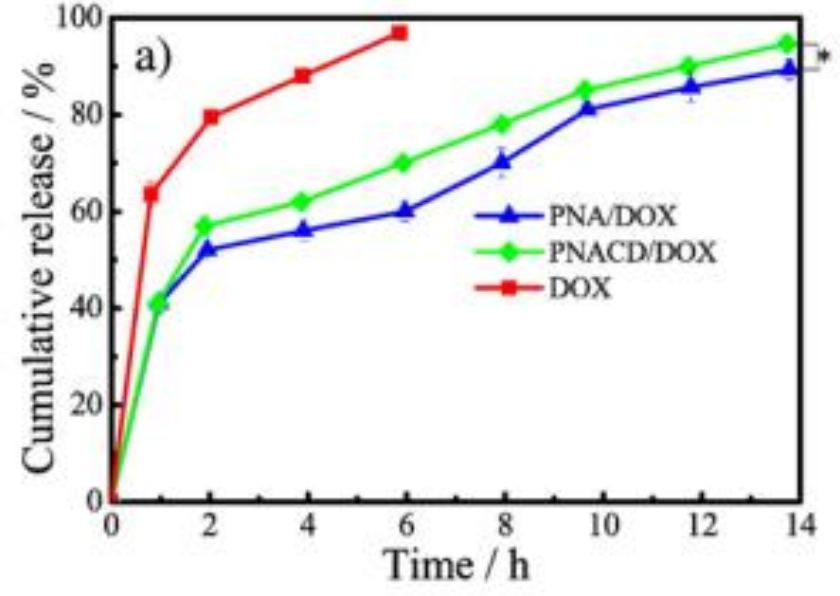
Results and discussion

- Interestingly, after drug loading, both PNA/DOX and PNACD/DOX presented LCST of 37.3 and 38.9 °C, which are slightly higher than biological temperature.



Results and discussion

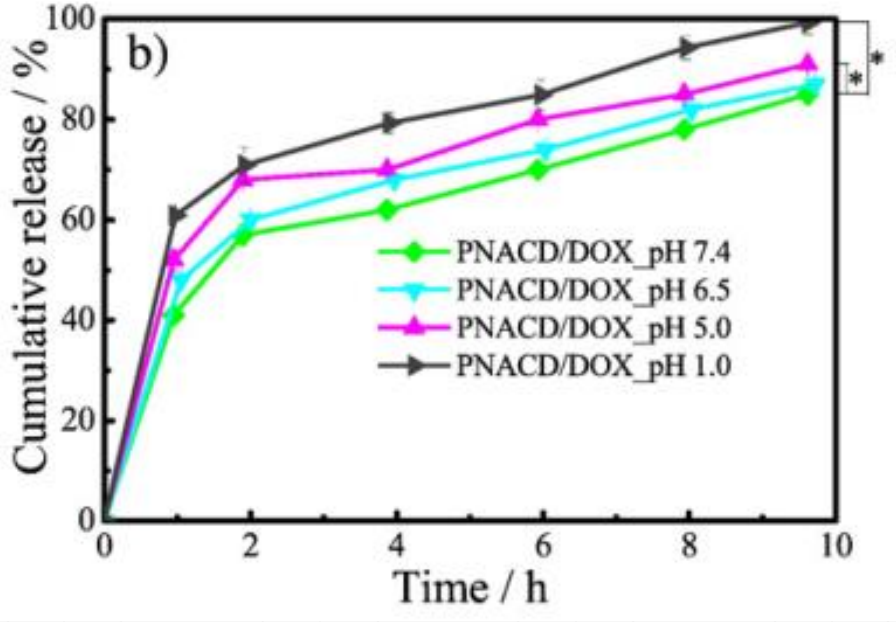
- In vitro drug release of DOX-loaded nanogels
- the nanogels can maintain a long-term release till 14 h, while almost all drug completely released in the case of free DOX sample. Compared to PNA/DOX nanogels, the PNACD/DOX nanogels produced higher drug release efficiency. For example, the drug cumulative release of PNACD/DOX was 96 ± 2% (14 h), higher than that of PNA/DOX nanogels (89 ± 2%).



Results and discussion

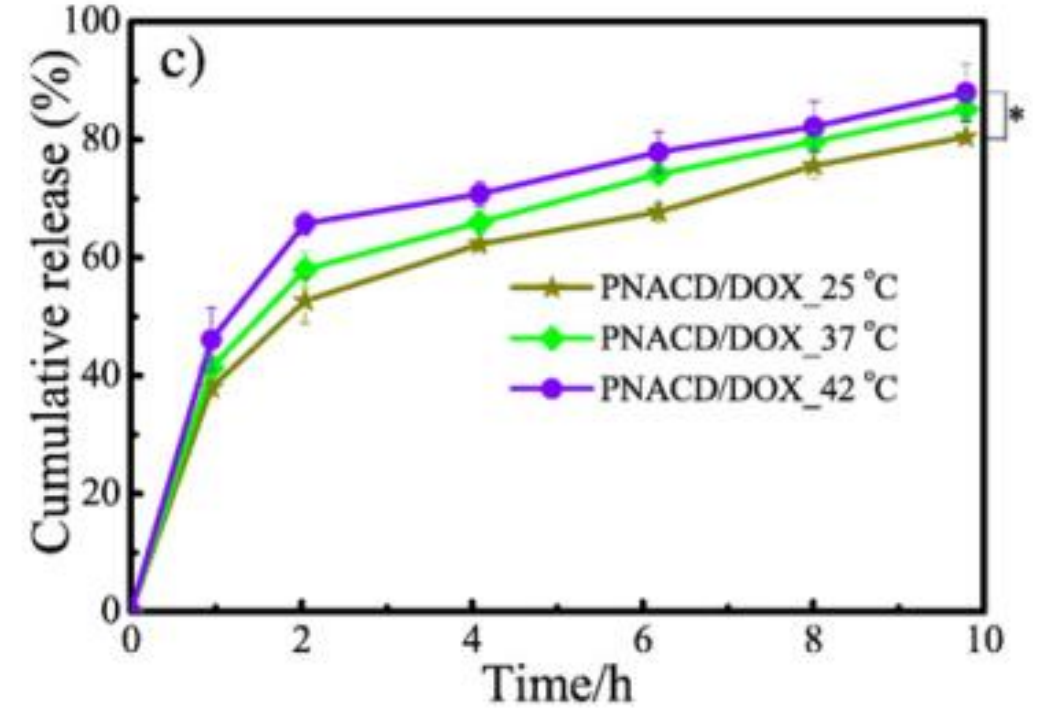
- tumor tissues (pH 6.5) similar to that of colon juice // compared to neutral one (pH 7.4) in normal tissues and intestinal juice // Cells (about 5.0) in their endo-lysosomal compartments // simulated stomach (pH value of 1.2).

- the cumulative release values of the nanogels were 85 ± 2% (pH 7.4), 87 ± 1% (pH 6.5), 91 ± 1% (pH 5.0) and 99 ± 2% (pH 1.2), respectively, during the release period of 10 h.



Results and discussion

- the increase of temperature promoted the DOX release rate from the PNACD/DOX nanogels. At 10 h, their accumulative drug release values at 25, 37 and 42 °C were 80 ± 0%, 85 ± 2%, 88 ± 5%, respectively.



Results and discussion

- In vitro cytotoxicity and cellular internalization of DOX-loaded nanogels

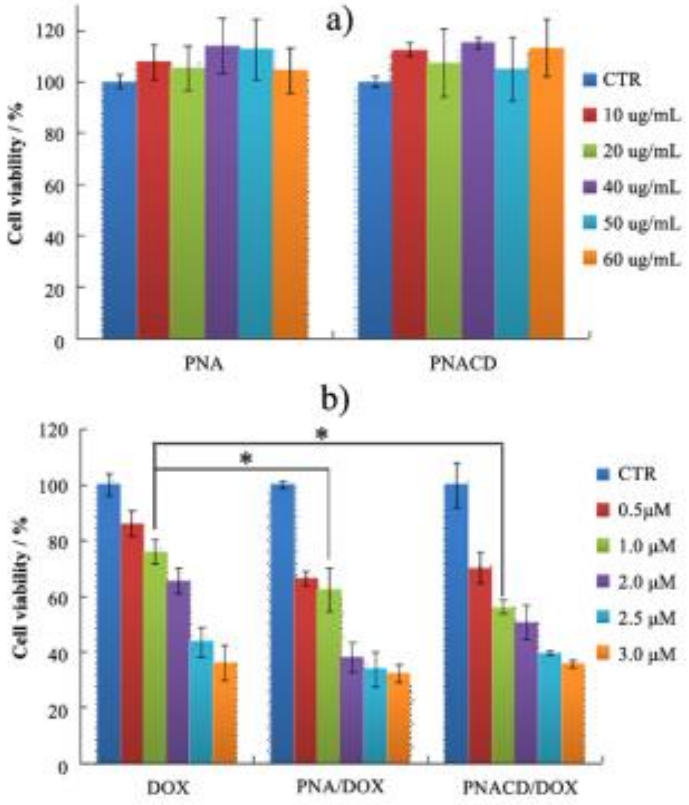
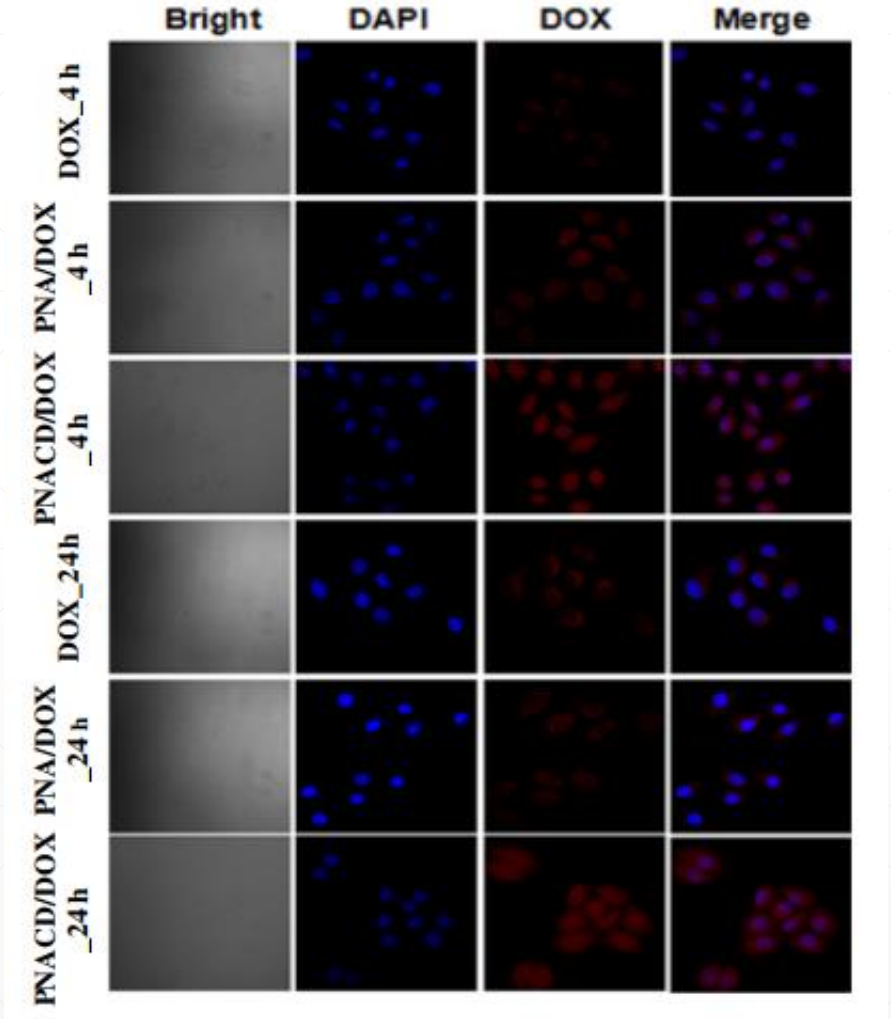


Fig. 6. Cytotoxicity of (a) DOX-free PNA and PNACD nanogels, and (b) free DOX, PNA/DOX and PNACD/DOX nanogels (with equivalent DOX concentration) after 48 h of cell culture using KB cells (± standard deviation, n = 3, *P < 0.05).

Results and discussion



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