Electronic Supplementary Material (ESI) for Polymer Chemistry.

Polymerization-induced self-assembly of Large-scale iohexol nanoparticles as contrast agents for X-ray Computed Tomography Imaging

Yuxun Ding,[a] Xinyu Zhang,[a] Yingjin Xu,[b] Tangjian Cheng,[a] Hanlin Ou,[a] Zhanyong Li,[a] Yingli An,[a] Wenzeng Shen,*[b] Yang Liu*[a] and Linqi Shi*[a]

[a]Key Laboratory of Functional Polymer Materials of Ministry of Education, State Key Laboratory of Medicinal Chemical Biology and Institute of Polymer Chemistry, College of Chemistry, Nankai University, Tianjin, 300071, China.

[b]College of Medicine, The Affiliated Hospital, Hebei University, Baoding, 071000, P.R. China.

*Correspondence and request for materials should be addressed to Y.L (yliu@nankai.edu.cn), W.S (shwz7098@163.com) and L.S (shilinqi@nankai.edu.cn)

Characterization

^{1}H NMR spectra were recorded on a Varian UNITY-plus 400 M NMR spectrometer at room temperature with tetramethylsilane (TMS) as an internal standard. Transmission electron microscopy (TEM) measurements were performed using a Philips T20ST electron microscope at an acceleration voltage of 100 kV. To prepare the TEM samples, the sample solution was dropped onto a carbon-coated copper grid and dried slowly at required temperature. The zeta potential values were measured on a Brookheaven ZetaPALS (Brookheaven Instrument, USA), using phosphate buffer (PB) solution (0.01 M) with a pH 7.4 as the background buffer. The CT imaging was performed using CT scanner (Discovery CT750 HD; GE Medical Systems, Milwaukee, WI, USA). The CT scan was initiated by using dynamic 500 row (Volume Helical Shuttle) mode acquisition (120kV; 150mAs; SFOV, Large body; DFOV, 160mm; matrix, 512×512 pixels; detector coverage, 40.0mm; thickness, 5.0mm; pitch, 1.375:1; rotation time, 0.4s; 31 pass; total exposure time, 50.44s).

Operation of CT scanner

At the time of imaging, the biological function experimental system, peristaltic pump and the entire rabbits were lined into a CT scanner. The rabbits were constrained in the supine head-first with an abdominal compression bandage in order to reduce artifacts caused by unnecessary respiratory movement. Tubing for the contrast material, normal saline, and peristaltic pump

were attached to the ear vein catheter. First, a nonenhanced abdominal CT examination was performed to locate the renal tumor for perfusion imaging. Subsequently, the rabbits of A and B group perfusion CT scan were initiated by using dynamic 500 row (Volume Helical Shuttle) mode acquisition (120 kV; 150 mAs; SFOV, Large body; DFOV, 160 mm; matrix, 512×512 pixels; detector coverage, 40.0 mm; thickness, 5.0 mm; pitch, 1.375:1; rotation time, 0.4 s; 31 pass; total exposure time, 50.44 s).

CT imaging *in vitro*

20 mL INPs solution was added into 50mL macrotube. The samples were constrained and detenimed using a CT scanner. As shown in Figure S11, a very bright and uniform signal was observed, demonstrating that the INPs exhibited remarkable CT imaging capacity *in vitro*.

Cell culture

MCF-7 Cells and 3T3 cells were purchased from Nanjing Kaiji Biotech. Ltd. Co. (Nanjing, China). Cells were cultured in RPMI-1640 medium with 10% (v/v) fetal bovine serum (FBS) and 1% penicillin-streptomycin in a humidified atmosphere at 37 °C with 5% CO_2. The cells were subcultured with 0.25% trypsin-EDTA when reaching 80-90% confluence.

Preparation of VX-2 tumor bearing rabbit models

VX-2 tumor cells were recovered at 37 ^{o}C in 2 min and centrifuged for 3 min (1200 r/min). The supernatant was removed and replaced with PBS buffer (2 mL). One New Zealand white rabbit was inoculated VX-2 tumor cells on anterolateral thigh (ALT) using solution of VX-2 tumor cells (1 mL) prepared in advance. The rabbit was cultured until a obvious tumor (2-4 cm) was observed. By this time, the tumor-bearing rabbit was prepared successfully. Two weeks later, the tumor-bearing rabbit was narcotized by 25% ethyl carbamate (EC, 4 mL/kg) injected from ear-vein. The tumor on the thigh was taken down and divode into small block (1 mm). These tissues were saoked in balance salt saline for further application. Subsequently, one rabbit was constrained on operation table and narcotized using 25% ethyl carbamate (EC, 4 mL/kg). A hole in the twelfth left costal margin of rabbit was cut. Whereafter, the tumor tissues were seeded in the left kidney from the incision. Finally, the incision was sutured and disinfected using iodophor. With the same method, other rabbits were dealt in sequence. Within three days, all rabbits were injected penicillin (400 thousand U) respectively to avoid infection. In the end, the growth of tumor was observed using ultrasonic apparatus (GE Voluson E8, USA).

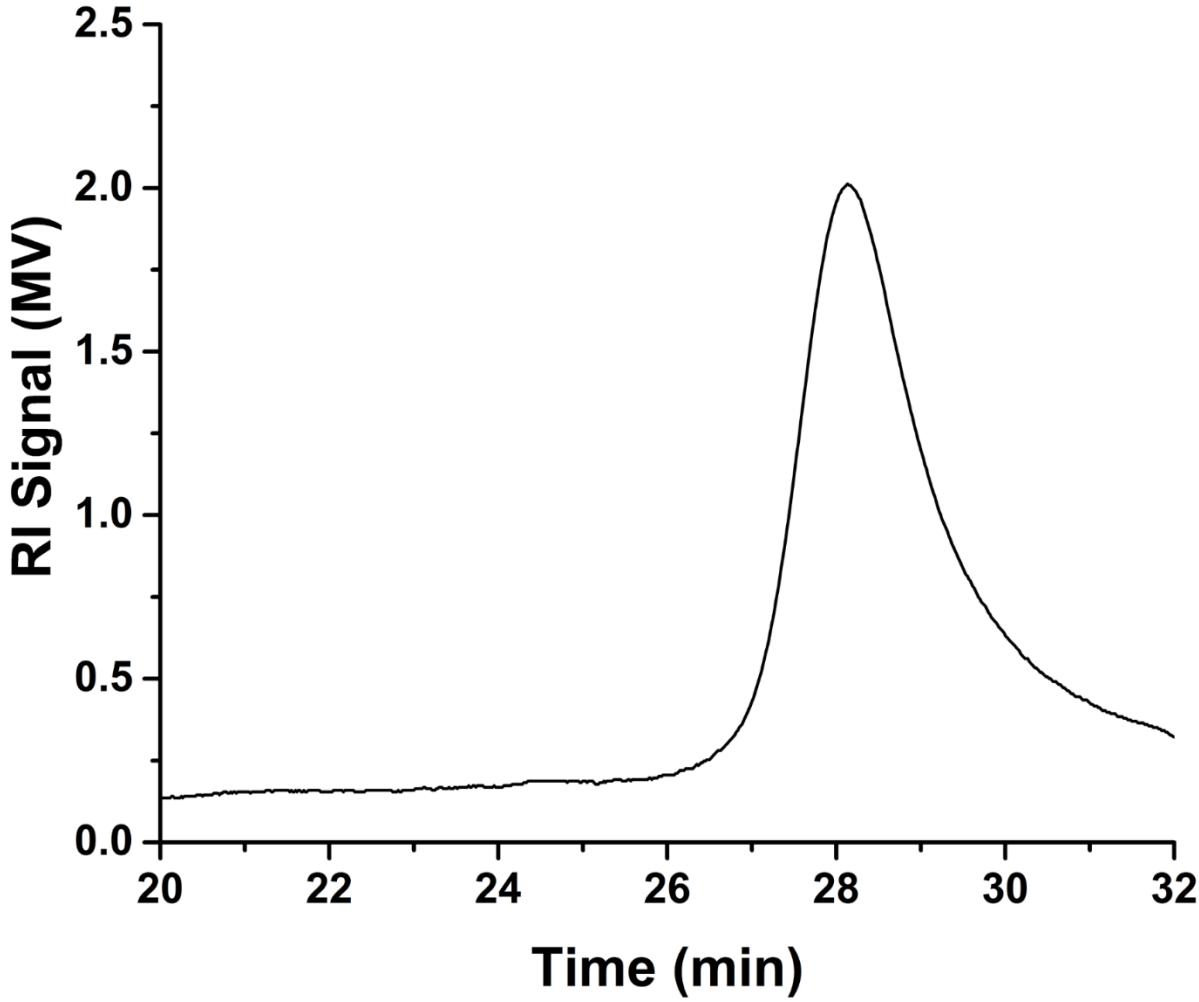

Fig. S1. The GPC traces of the PEG_{113}-TTC.

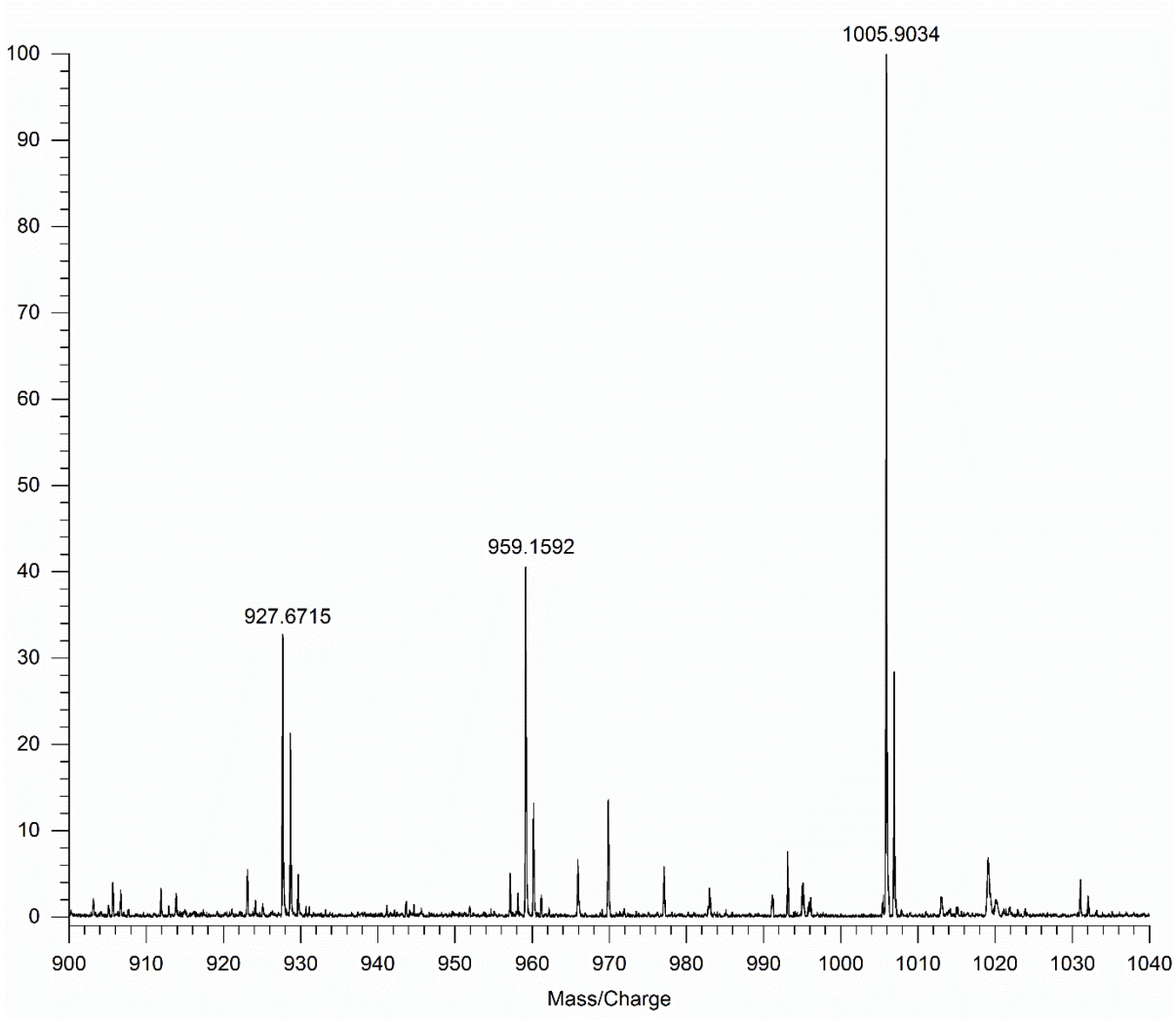

Fig. S2 The MS spectra of iohexol acrylate (the peak of 1005.9034 belong to triacrylate of iohexol).

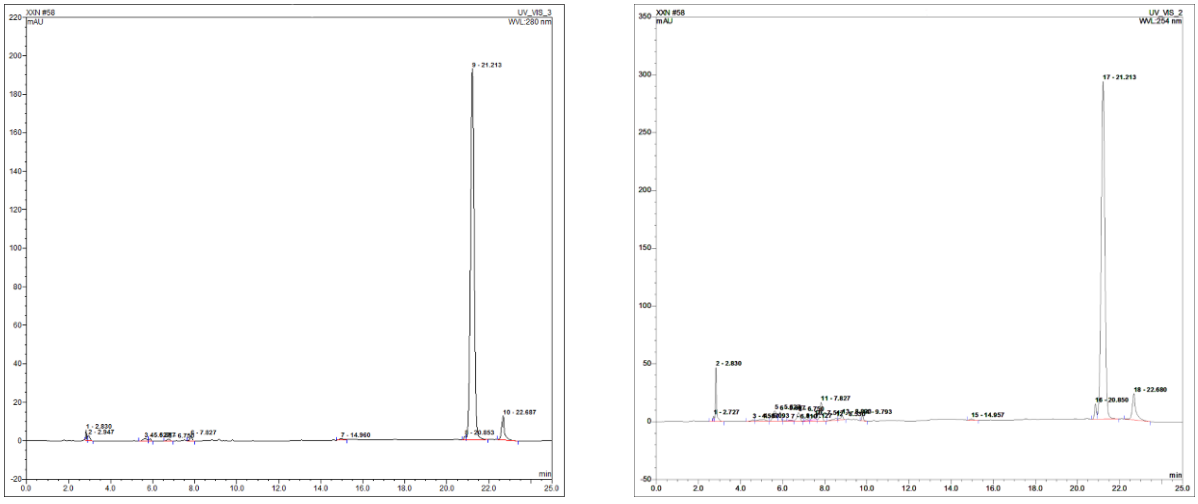

Fig. S3 The HPLC of iohexol acrylate at 280nm (left) and 254nm (right).

Table S1 The HPLC of iohexol acrylate.

280nm

No.	**Ret.Time**	**Peak Name**	**Height**	**Area**	**Rel.Area**	**Amount**	**Type**
	min		**mAU**	**mAU*min**	**%**		
1	2.83	n.a.	5.400	0.271	0.63	n.a.	BM
2	2.95	n.a.	2.478	0.321	0.74	n.a.	MB
3	5.63	n.a.	1.365	0.302	0.70	n.a.	BM
4	5.88	n.a.	1.241	0.137	0.32	n.a.	MB
5	6.75	n.a.	1.118	0.191	0.44	n.a.	BMB
6	7.83	n.a.	2.400	0.293	0.68	n.a.	BMB
7	14.96	n.a.	0.817	0.146	0.34	n.a.	BMB
8	20.85	n.a.	1.894	0.185	0.43	n.a.	BM
9	21.21	n.a.	192.918	39.260	91.05	n.a.	MB
10	22.69	n.a.	12.657	2.014	4.67	n.a.	BMB
Total:			222.289	43.120	100.00	0.000	

254nm

No.	Ret.Time	Peak Name	Height	Area	Rel.Area	Amount	Type
	min		mAU	mAU*min	%		
1	2.73	n.a.	2.806	0.155	0.20	n.a.	Ru
2	2.83	n.a.	46.934	3.316	4.20	n.a.	BMB
3	4.59	n.a.	1.238	0.189	0.24	n.a.	BM
4	5.09	n.a.	1.578	0.656	0.83	n.a.	M
5	5.63	n.a.	8.819	1.963	2.49	n.a.	M
6	5.88	n.a.	7.963	0.886	1.12	n.a.	MB
7	6.41	n.a.	0.617	0.109	0.14	n.a.	Ru
8	6.75	n.a.	7.351	1.433	1.81	n.a.	BMB
9	7.13	n.a.	1.267	0.166	0.21	n.a.	Ru
10	7.52	n.a.	2.828	0.553	0.70	n.a.	Ru
11	7.83	n.a.	16.234	2.690	3.41	n.a.	BMB
12	8.53	n.a.	1.901	0.438	0.55	n.a.	BM
13	8.82	n.a.	3.949	0.571	0.72	n.a.	MB
14	9.79	n.a.	4.611	0.427	0.54	n.a.	BMB
15	14.96	n.a.	1.038	0.186	0.24	n.a.	BMB
16	20.85	n.a.	13.174	1.349	1.71	n.a.	BM
17	21.21	n.a.	292.047	59.301	75.13	n.a.	MB
18	22.68	n.a.	22.928	4.548	5.76	n.a.	BMB
Total:			437.283	78.936	100.00	0.000	

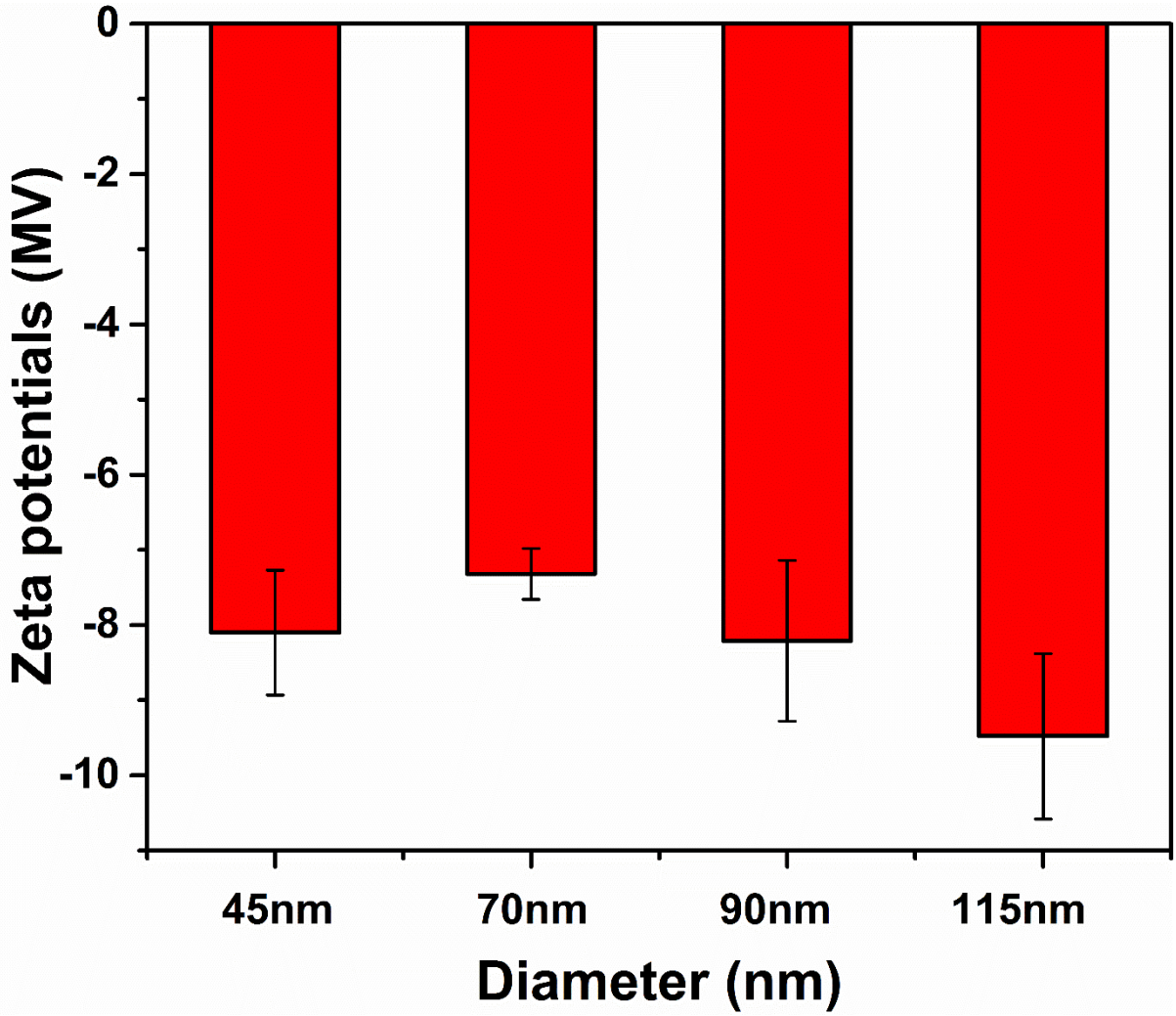

Fig. S4 Zeta potentials of INPs with different size measured at pH 7.4.

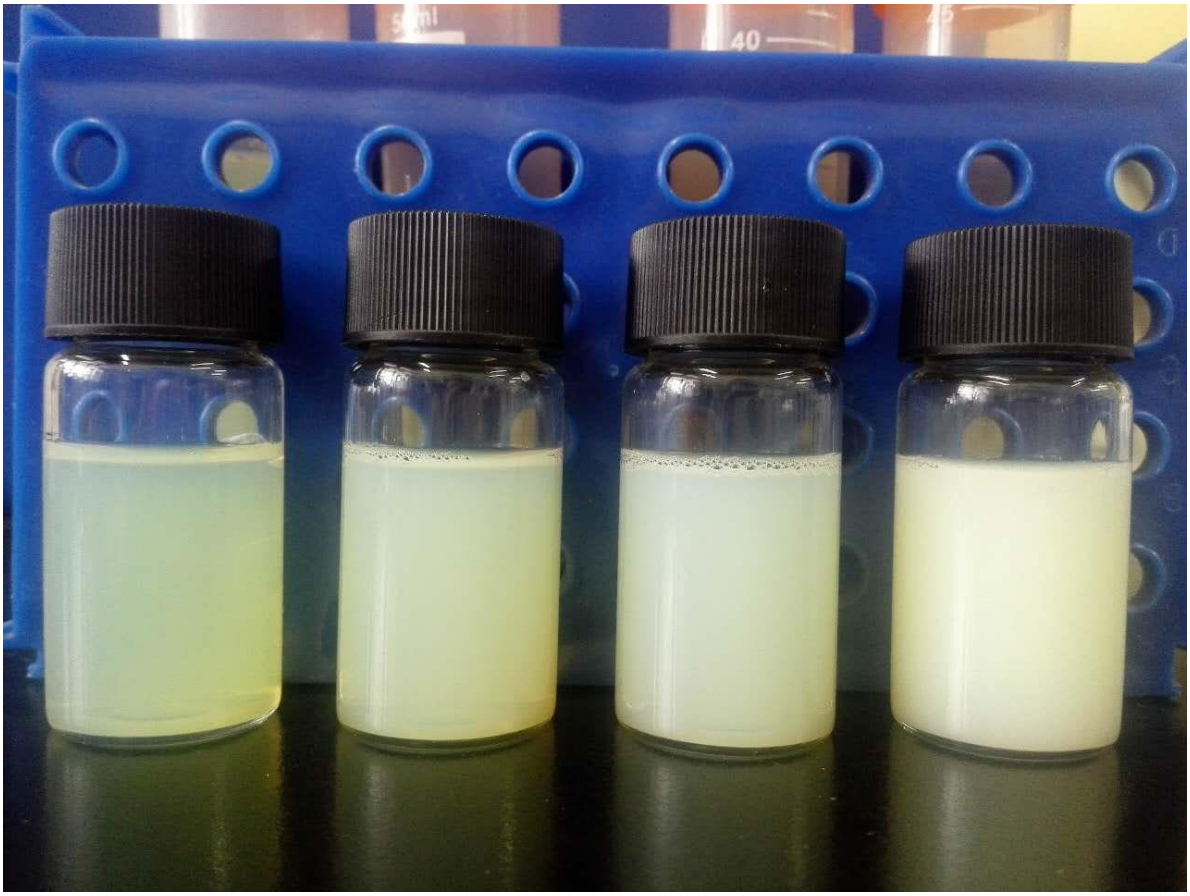

Fig. S5 The picture of INPs solution with different sizes.

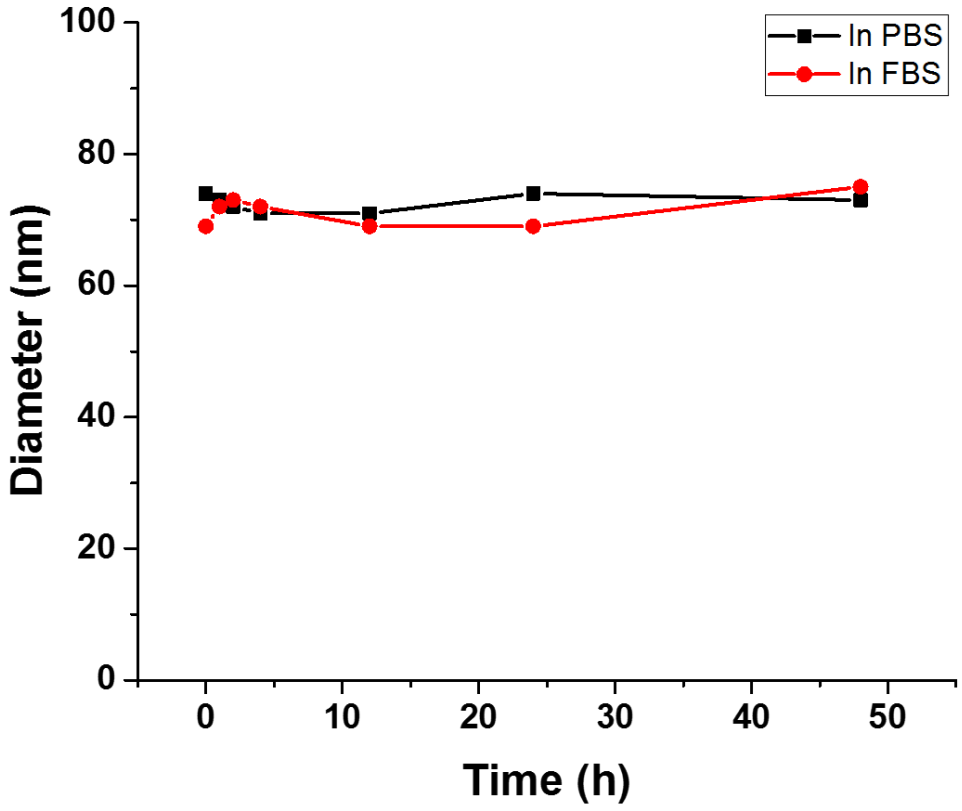

Fig. S6 The stability in PBS and fetal bovine serum (FBS).

Standard curves of CT value and iodine content

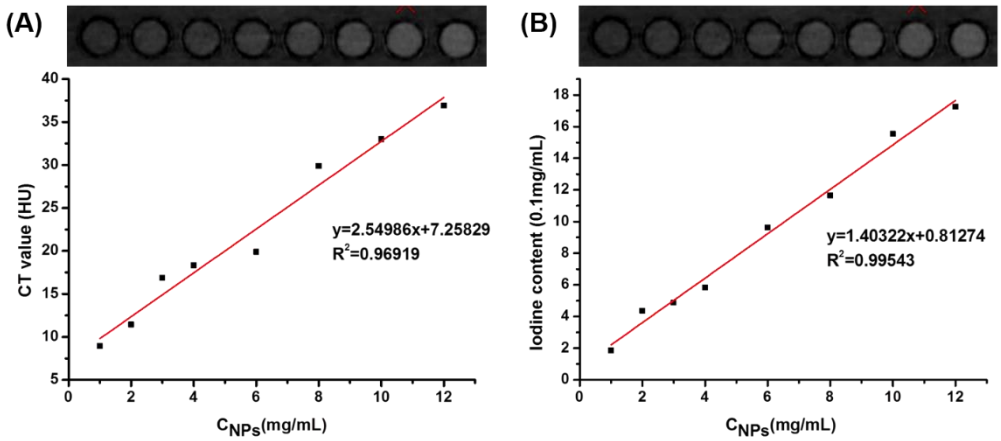

Fig. S7 The statistical characteristic of INPs. (A) CT value standard curve as a function of concentration. (B) Iodine contents standard curve as a function of concentration.

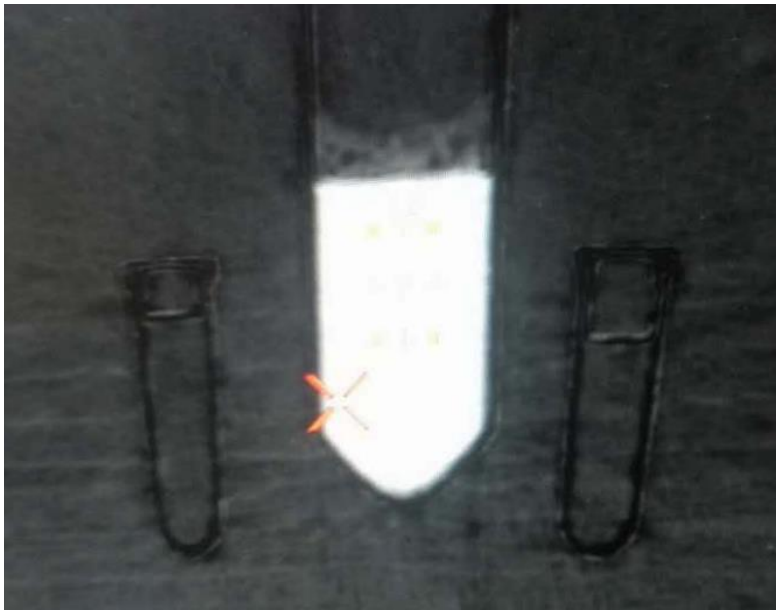

Fig. S8 CT imaging of INPs solution.