# Single carboxyl porphyrin-functionalized chitosan can take shape into polymer molecule microsphere

Chenghong Huang[1,2],Qinlin Yang[2],Chennan Chen[2],BiRong Han[2],YunFei Li[2],Fei Xiong[2]

1,Chongqing Key Laboratory of Micro/Nano Materials Engineering and Technology of Chongqing, Chongqing University of Arts and Sciences,402160, China

2, Chongqing Key Laboratory of Industrial Fermentative Microbes , Chongqing university of Science and Technology, 401331,China

[1]Corresponding email: CHhuang2007@sinano.ac.cn

**Key words:** Porphyrin, Chitosan, Molecule microsphere

**Abstract.** In this study, we utilize EDC/NHS as catalyst to graft Single carboxyl porphyrin molecules onto acidized chitosan for synthesis of porphyrin-functionalized chitosan composite(PFCC) and testified its structure by FT/IR. In liquid phase, we identified the formation mechanism of molecule microsphere with tetrahydrofuran(THF)/$H_2O$ solution that chitosan molecules are on the outside and porphyrin molecules are in the side by laser particle size distribution(LPSD) determination. Scanning electron microscope(SEM) results further confirmed that Single carboxyl porphyrin-functionalized chitosan in liquid phase can form polymer molecule microspheres.

## Introduction

Due to its suitable dimension, the use of micro/nanostructure materials for biomedical applications including drug transportation has substantially increased over the past years[1]. One of the main advantages of these nanoparticles is their properties of controllable size and flexible shape after chemical modification with specific materials or special processing[2]. Porphyrin, a family of macrocyclic organic molecules and a good electronic donor, has been extensively investigated because they possess outstanding ability to convert light energy into electron motion[3]. Metalloporphyrins have also received much attentions as their excellent physical/chemical characteristics[4, 5]. Recently, chitosan, because of its active group carrying the ability of linking with other targets, received more and more attentions. It can be decorated by porphyrin[6] to obtain significant nanomaterials. For example, Shibano reported that chitosan reacted with phenylisothiocyanate to give porphyrin-appended chitosan derivatives[7]. Furthermore, metal tetra(4-carboxyphenyl)porphyrin can also be grafted onto chitosan to form a practical model carrying the structure and function of hydrophobic pocket cavity for cytochrome P-450 enzyme activity[8]. This study, we synthesize single carboxyl porphyrin-functionalized chitosan composite and prepared porphyrin–functionalized chitosan nanoparticle for drug transportation.

## Materials and method

1-ethyl-3-(3-dimetylaminopropyl) carbodiimide hydrochloride(EDC, Lot#BCBF916V) was purchased from Aldrich; N-hydroxysulfosuccinimide sodium salt (NHS,Lot#F20110210) and Chitosan (MW~2×104; 85-90% deacetylation, Lot#F20110519) were both purchased from Sinopharm chemical reagent Co.Ltd.(China); $H_2$CPTPP was self-made by our Lab; All of the other

 

chemicals were analytical grade. Scanning electron microscope (SEM) was completed in Suzhou Institute of Nano-Tech and Nano-Bionics(CAS).

The unsymmetrically phenyl substituted porphyrin $H_2$CPTPP was synthesized according to literature and single carboxyl porphyrin-functionalized chitosan reaction was performed by method[9]. Briefly, total 4g chitosan was dissolved in 10ml deionized water overnight. The solution was adjusted to pH5.0 by 2M HCl. Meanwhile, 2mg $H_2$CPTPP was dissolved in tetrahydrofuran (THF) completely and its carboxyl groups were activated by EDC/NHS mixture (mole ratio=3:1) for 15min. The activated $H_2$CPTPP was then added gradually into the chitosan solution. The reaction solution was stirred for 1h at room temperature and then transferred to a dialysis bag (cut-off MW, 12000) for dialysis against deionized water to remove unreacted molecules or some small molecules. The dialyzed solution was named porphyrin-functionalized chitosan solution. The obtained solution was basified by NaOH to pH=10 and then filtered by nylon membrane overnight. The dried chitosan/$H_2$CPTPP residue was named porphyrin-functionalized chitosan composite. Different ratio THF/$H_2O$ solution was used to explore the liquid shape of porphyrin-functionalized chitosan.

## Results and discussions

In order to confirm whether carboxylic groups in $H_2$CPTPP had been introduced to the chitosan, FT-IR spectroscopy was performed to chitosan/$H_2$CPTPP composite. Fig.1 shows typical FT-IR spectrum. It can be seen that the FPCC have a typical characteristics peaks of 1158cm$^{-1}$, meanwhile, the peak of 1320cm$^{-1}$ and 967cm$^{-1}$ in single carboxyl porphyin molecule are both disappeared. Furthermore, there have additional 932cm$^{-1}$ characteristic peaks carried by FPCC composite, which demonstrated $H_2$CPTPP molecules containing phenyl ring structure were successfully introduced to the chitosan.

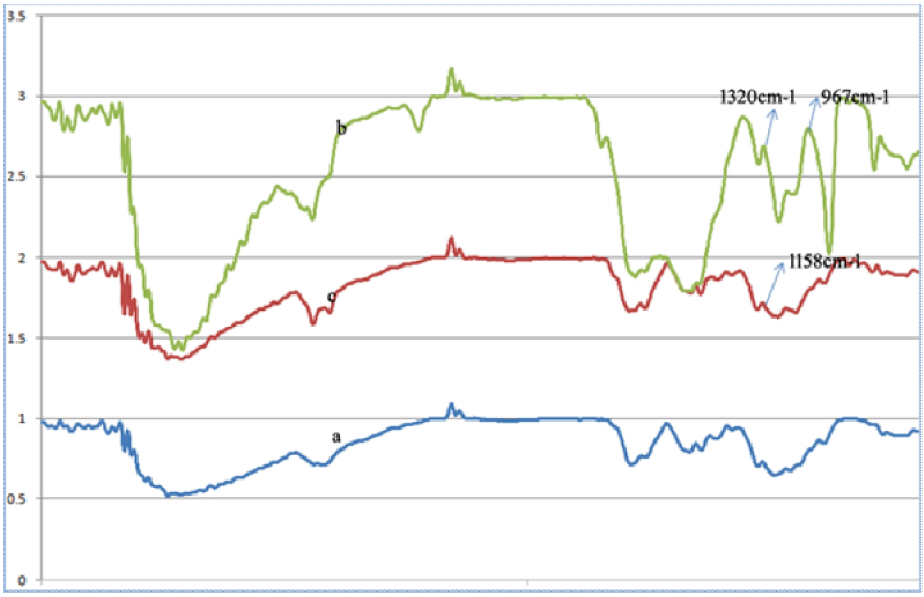


Fig.1 FT/IR spectra of chitosan(a), $H_2$CPTPP(b) and FPCC composite(c).

Chitosan is water insoluble. Acidification chitosan possesses excellent solubility that can be processed easily[10]. Hydrophobic porphyrin can dissolve in organic solvent such as THF solution. It can be speculated that the status of porphyrin-functionalized chitosan may be great disparity in

different solutions. As hydrophilic group(-OH) existence , the FPCC may be represent loosely that these groups scattered randomly on the outside, but, porphyrin molecule will huddle up in the inside. On the other hand, the FPCC may be tightly wrapped into a small mass when added more organic solvent in the mixture. We hence Proportionally prepared mixtures with THF and $H_2O$ by a series of ratio 100% , 80% , 60% , 40% , 20% , 0% and measured its Laser particle size distribution(PSD).

Result is shown in figure 2. There have two size distribution when the FPCC was dissolved in 100% $H_2O$ with scales of about 2.0μm and 200μm (Fig2a). With the increase of THF, size distribution gradually turned narrow (fig2b-e) with a average of near 2-10μm. This basically coincides with reported experimental results that porous and non-porous chitosan-supported Me-TCPPs can take shape into hydrophobic pocket cavity of cytochrome P-450 enzyme for aerobic oxidation of cyclohexane[8].

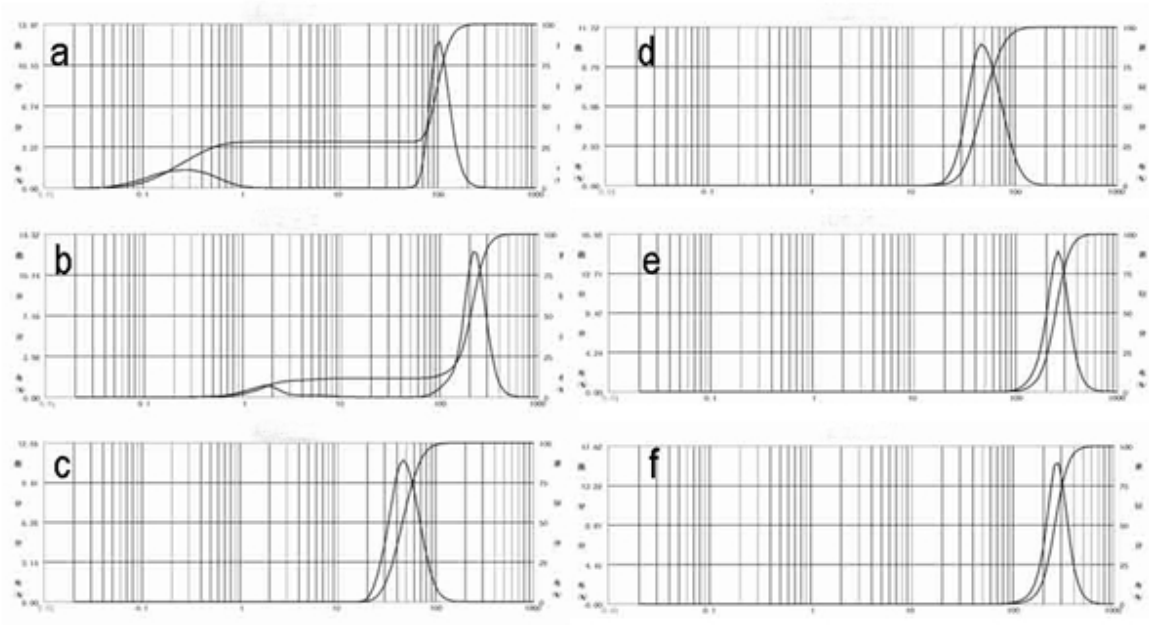


Fig.2 Laser Particle size distribution(LPSD) of porphyrin functionalized-chitosan in THF/$H_2O$ with proportion(a,100%;b,80%;c,60%;d,40%;e,20%;f,0%).

We also investigated the solid shape of porphyrin-functionalized chitosan with scanning electron microscope (SEM) observation. Figure 3 showed the detailed morphologyafter the composite dried into powder. Global or ellipsoidal, as well as irregular bodies (fig3b) are all exhibited. These spheriod can break up when the temperature was up to 100□. A large cavity became visible in figure3d. The spheriod can also be wrapped into a much small ball with different morphology such as prismatic and bar. These cavity structures are beneficial to medicine accommodation for particular organ absorption.

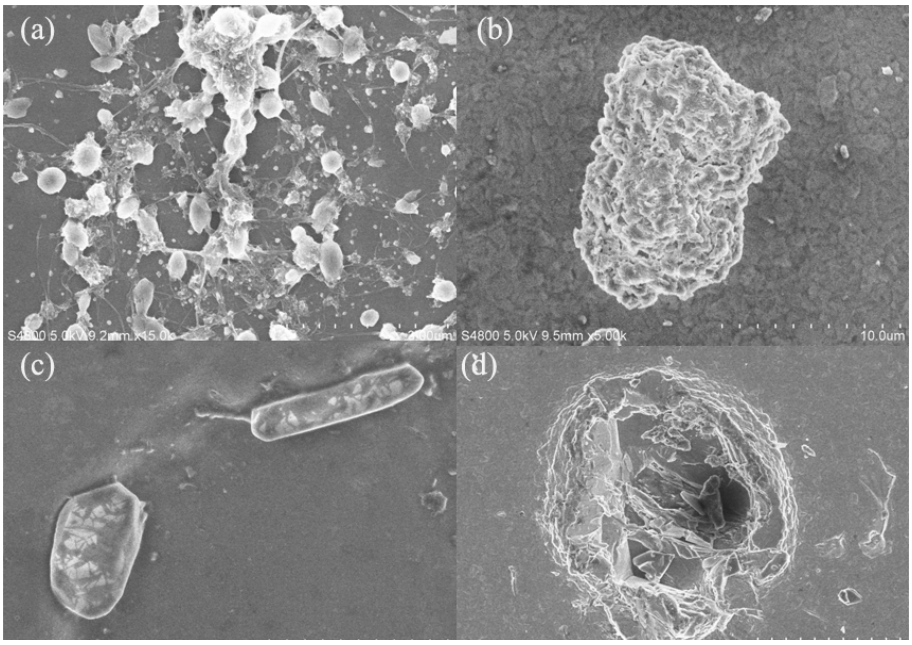


Figure 3 SEM morphology of porphyrin-functionalized chitosan composite with different ratio (a-c,4:1,1:1 and 9:1) of THF/$H_2O$ solution. The microsphere will split by 100□ treatment(d).

## Conclusions

Single carboxyl porphyrin can be grafted to acidized chitosan by EDC/NHS to form porphyrin-functionalized composite FPCC, which was confirmed by FT/IR measurement. This composite represents microsphere structure in THF/$H_2O$ solutions with characteristic of bar, sphere, ellipsoid, or irregular morphology testified by LPSD determination and SEM observation, respectively. The cavity structure has the potential of drug transportation.

## Acknowledgement

This work was financially supported by the open-fund Chongqing Key Laboratory of Micro/Nano Materials Engineering and Technology and Chongqing program of Application Foundation and Advanced Technology (cstc2014jcyjA50032).

## References

[1]. Sandeep M., Neeraj K.,and Chetan N: J Magnetism Mag Mats Vol.404(2016),p. 166-169.
[2]. L. Li.,A. Ruotolo.,C.W. Leung: Microelectronic Eng Vol.144.(2015), p.61-67.
[3]. Synytsya, A: Biomacromol Vol.13(2012),p. 489-498.
[4].Sessler,J.L.,Karnas E., and Sedenberg E: SupraMol Chem Vol.124(2012),p.24-27.
[5]. Bradley L.,Elke F.,Hasan S: Photochem and Photobiol Vol.88(2012),p.527-536.
[6]. Mirela S.,Ioana B.,Simona S: Int J Dermatol Vol.50(2011),p.280-286.
[7]. Masaya S.,Shouko N.,Yasuko S.: Carbohyd Polym Vol.113(2014),p.279-285.
[8]. C.H.Huang.,J.X.Li.,Y.Tang: Materials Sci and Eng C Vol.49(2015),p.844-850.
[9]. C.H.Huang.,C.J.Hou.,J.Li:Advanced Materials Res Vol.535-537(2012),p.1591-1596.
[10]. Y.Z. Chen.,Y.K.Huang.,Y.Chen: Chinese Chem Let Vol.26(2015), p.909-913.