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Preparation of hemoglobin immobilized self-assembling nanoporous filter material and application for selective reduction of harmful components in cigarette smoke

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Outline

- Introduction
- Hypothesis
- Results and Discussion
- Conclusion

Introduction

- With signature of FCTC, development of safe cigarettes with high quality has already become international direction.
- Hemoglobin (Hb) become the emphasis because its characteristics of strong combination and hard separation with CO,etc.
- In the way as former reported, the activity of Hb and its reduction function could not be convinced.
- Self-assembling nanoparticle has broad application in drug controlled release for its perfect physical, chemical characters and biocompatibility.

Hypothesis

To obtain activity of Hb, 3 kinds of methoxy poly(ethylene glycol)-block-poly (D, L-lactic acid) (PEDLLA) nanoporous particles (PNP) were designed, synthesized and characterized by ^{1}hydrogen-nuclear magnetic resonance spectrometer (^{1}H-NMR), infrared spectra (IR), transmission electron microscopy (TEM) and laser particle size analyzer. After that, Hb was stabilized into nano hole and produced combined filters after granulated.

Then, the corresponding physical characters, selective deduction characters and smoking quality of hemoglobin immobilized nanoporous particles (HINP) combined filters were detected.

Results and Discussion

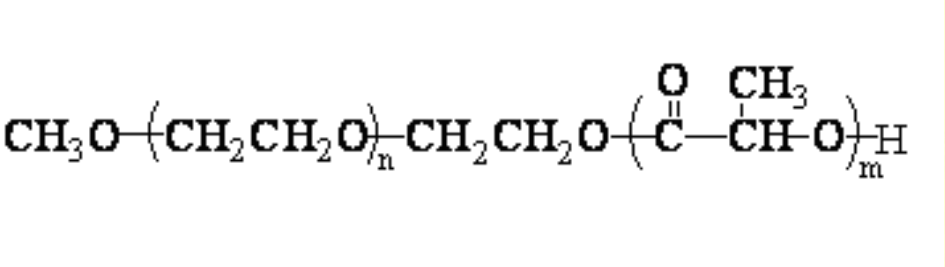
- Synthesis and characterization of methoxy poly(ethylene glycol)-block-poly (D, L-lactic acid) (PEDLLA) nanoporous particles (PNP) .
- Stabilization of Hb
- Characterization of hemoglobin immobilized nanoporous particle (HINP) combined filter

1.Synthesis and characterization of PNP

- Synthesis of PEDLLA
- FTIR characterization of PEDLLA
- ^{1}H-NMR characterization of PEDLLA
- Morphology of PNPs

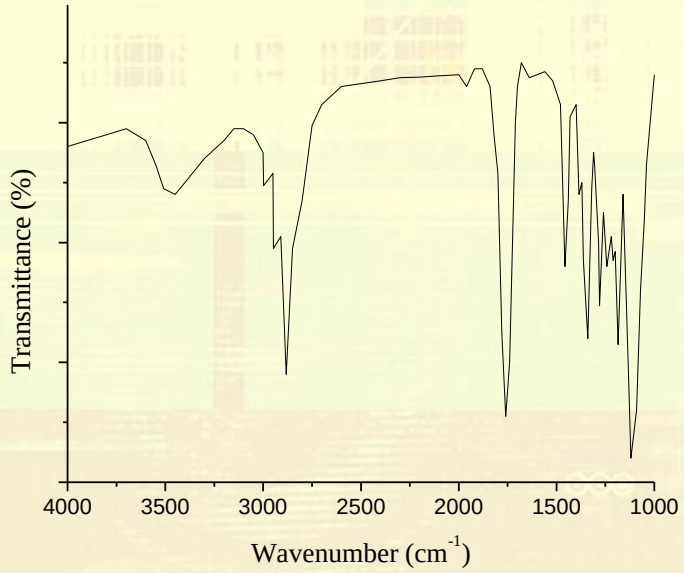
(1). Synthesis of PEDLLA

Monomethoxy Poly(ethylene glycol) (mPEG), and D,L-lactic acid, were polymerized to synthesis PEDLLA. The designed structure was as below:

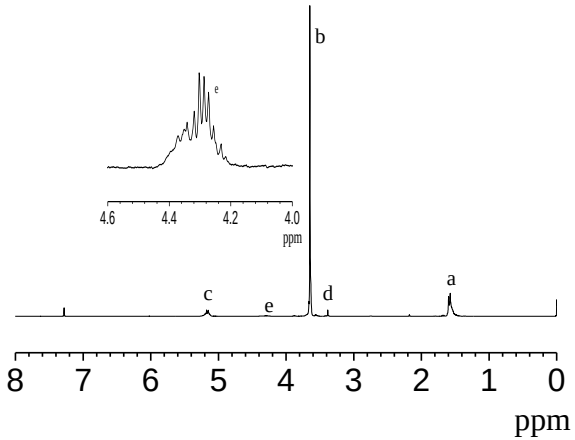


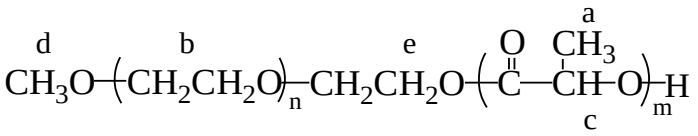
(2). FTIR characterization of PEDLLA

It is indicated that －COO－ ester carbonyl group peak at 1758 without C=O carboxyl group peak at 1725; C－O ester group at 1188, while character peak of hydrogen bond at 2500-3000 disappeared which means polymerization happened and obtain PEDLLA with designed structure.



(3). ^{1}H-NMR characterization of PEDLLA



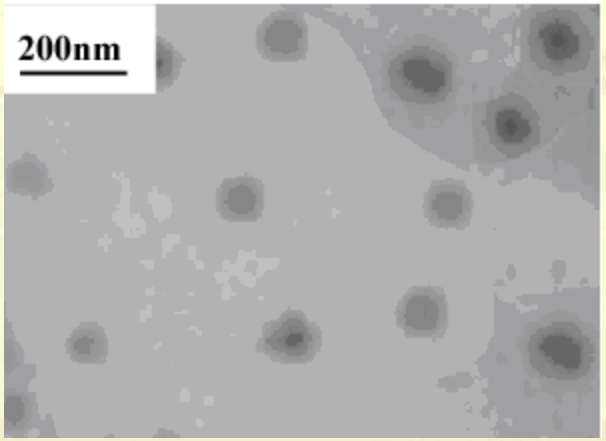


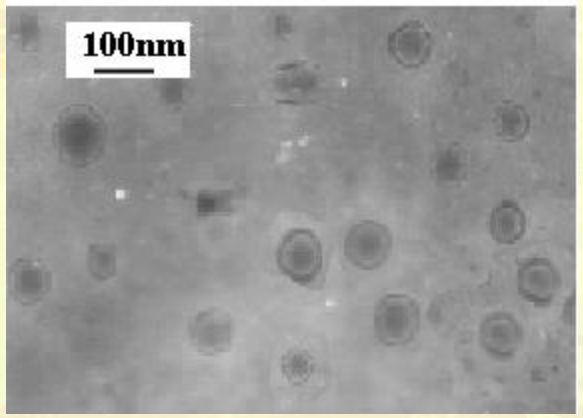
The hydrogen was signed at the figure and structure correspondingly. From the peak area, the m and n can be calculated. So, the average molecular weight was shown as below.

sample	$\overline{Mn}$ (PEDLLA)	$\overline{Mn}$ (mPEG)	$\overline{Mn}$ (PDLLA)
PEDLLA1	3767	2000	1767
PEDLLA2	5820	2000	3820
PEDLLA3	6778	2000	4778

(4). Morphology of PNPs

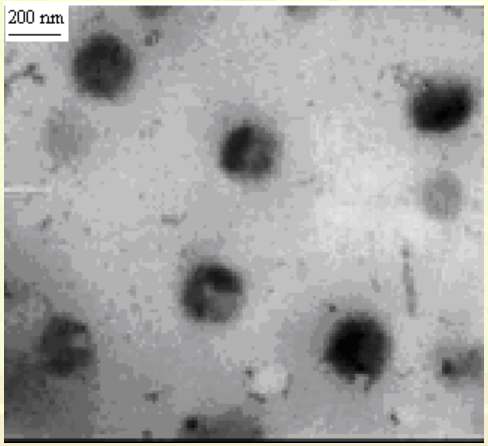
PEDLLA will form nanoporous particless (PNP) self-assembly. Through TEM, it's clear that PNP particles were sphere with inter hallow hole.

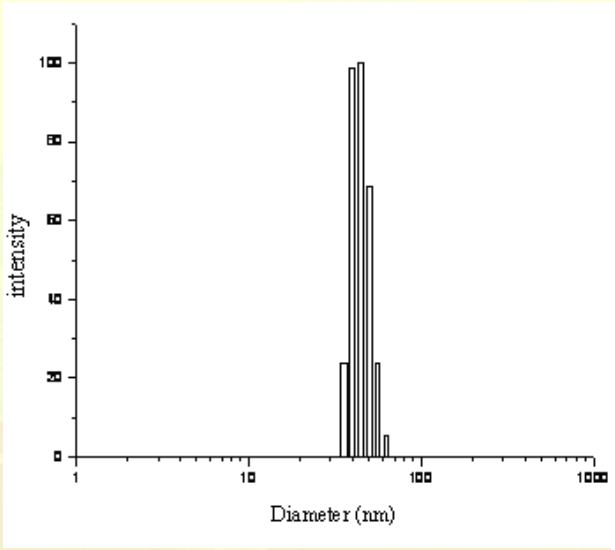


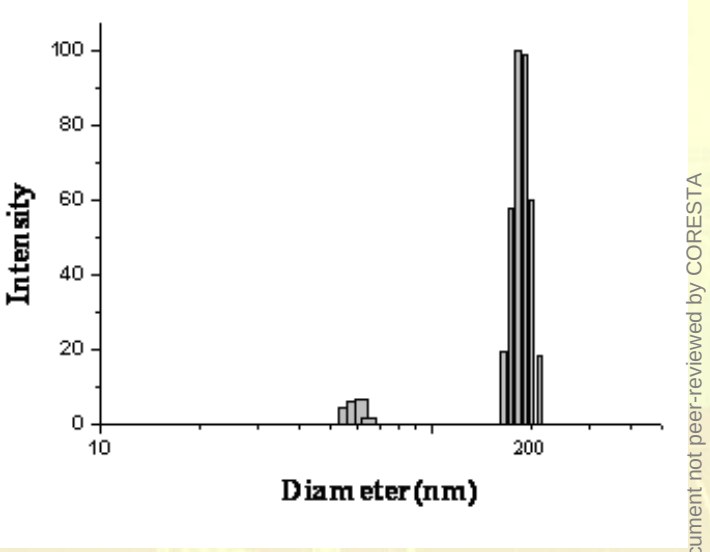


2. Stabilization of Hb

Hb was stabilized with PNP, and formed stabilized HINP. By laser particle size analyzer, the particle size was increase from 100 nm of PNP to 200 nm of HINP, which indicate that PNP loaded Hb successfully. Through TEM, we can see the HINP were sphere without hollow hole.







Hb loading content (LC%) and entrapment efficiency (EE%) were calculated by following formula:

$$\text{load content (LC\% W/W)} = \frac{\text{quality of Hb in NINP}}{\text{quality of NINP}} \times 100\%$$

$$\text{entrapment efficiency (EE\%)} = \frac{\text{original quality of Hb} - \text{quality of Hb in precipitation}}{\text{original quality of Hb}} \times 100\%$$

It is clearly that with increase of LC, the size of HINP and EE were increased. For PEDLLA, the longer molecular chain, the bigger EE.

Samples	Polymers	Theory loading content	Actual loading content （LC %）	Entrapment efficiency （EE %）	Particle size (nm)
HINP-1	PEDLLA1	2%	1.70%	85.00%	225
HINP-2	PEDLLA2	2%	1.75%	87.50%	224
HINP-3	PEDLLA3	2%	1.77%	88.50%	227
HINP-4	PEDLLA1	5%	2.97%	59.40%	269
HINP-5	PEDLLA2	5%	3.28%	65.60%	279
HINP-6	PEDLLA3	5%	3.58%	71.60%	283

3. Characterization of HINP combined filter

- Physical characters of HINP combined filter
- Selective deduction characters of HINP combined filters
- Smoking quality evaluation of HINP combined filters

(1). Physical characters of HINP combined filter

After treatment of HINP with different loading content, combined filters were produced. According to different LC, combined filters made from HINP-3 and HINP-6 were named as HINP combined filter-1 and 2.

The results show that physical characters of HINP combined filters were close to blanks which obey enterprise standards.

Filter	Length (mm)	Adding amount (mg/mm)	Draw resistance (mmH_2O)	Circumference (mm)	Hardness (%)
25mm blank	100	/	302	23.84	90.0
HINP combined filter-1	100	2± 0.2	318	23.95	90.1
HINP combined filter-2	100	2± 0.2	339	24.18	90.3

(2). Selective deduction characters of HINP combined filters

The results show that HINP can reduce tar, nicotine, CO and HCN contents. With the increase of Hb loading content, the reduction of these harmful contents was increased. The reduction of CO and HCN was much greater than tar and nicotine which indicted HINP combined filters had selective deduction characters.

No. of Cigarette	1	2	3
Filter	Blank acetate fiber	HINP combined filter-1	HINP combined filter -2
Weight（g）	0.92±0.02	0.93±0.02	0.93±0.02
Draw resistance （mmH_2O）	121±5	121±5	121±5
Puff number（puffs）	7.9	7.8	8
TPM（mg）	18.8	18.08	17.15
Reduction of TPM（%）	/	3.83	8.78
Tar（mg）	15.2	14.6	14.1
Reduction of tar（%）	/	3.95	7.24
Nicotine（mg）	1.2	1.17	1.12
Reduction of nicotine（%）	/	2.5	6.67
water（mg）	2.4	2.4	2.5
CO content（mg）	16.4	14.2	11.8
Reduction of CO（%）	/	13.41	28.05
HCN content（μg）	113	98	89
Reduction of HCN（%）	/	13.27	21.24

(3). Smoking quality evaluation of HINP combined filters

It is clear that the evaluation of HINP combined filter was close to blank which indicated there was no obvious difference in total quality and cigarette style. In detail, cigarettes with HINP filters was lighter.

Cigarette	Filter acetate fiber	Lust	Aroma	Balance	Off-taste	Irritation	After-taste	Total
Blank	25mm blank	5.0	29.9	5.5	10.5	17.4	23.0	91.3
1	HINP combined filter -1	5.0	29.8	5.5	10.5	17.3	22.8	90.9
2	HINP combined filter -2	5.0	29.6	5.5	10.4	17.4	22.7	90.6

Conclusion

The HINP was firstly introduced into harm deduction in cigarette research and developed new combined filters. This kind of filters can reduce harmful components of cigarettes, especially selectively to reduce CO and HCN greatly with few influence of smoking quality which indicated that it had broad application.

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Thank you!