

Preparation and characterization of the anti-virus and anti-bacteria composite air filter materials

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The anti-virus and anti-bacteria active components were extracted from some Chinese medicine, such as the honeysuckle, forsythia and the licorice. Using a w/o/w emulsion method, the active components were fabricated to uniform particulate microcapsule with sustained-release properties. The polypropylene punched felt was finished with the finishing agent of microcapsule, nano ZnO and TiO₂ and polymer adhesive, and the composite air filter with anti-virus and anti-bacteria properties were formed, staphylococcus aureus, colibacillus and candida albicans were applied to antibacterial experiments. The results indicate that the anti-bacteria rate are all 100%, and the virus inactivation rate also reaches 100% to pandemic influenza A virus.

microcapsule, nano, Chinese medicine, anti-virus, anti-bacteria

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1 Introduction

With the development of the international economy, the overall industrial pollution has become serious day by day, the indoor air quality deteriorates resulting in a series of Building Related Illness (BRI) and Sick Building Syndrome (SBS) [1]. The main factors influencing indoor air quality is the respirable particulates (such as dust, smoke) and Living Creature Gas Gum (for example, germ, virus, mold, original living creature). The particle diameter less than 10 μm in the respirable particulates will result in respiratory disease, skin infectious disease. An enormous allergy source is composed of dust, germ, hair and scurf, wall louse, smoke and the harmful material in the air. According to statistics, there might be more than 350 kinds of volatility organic compounds (VOC) in the office, some VOCs are thought to be carcinogens or the trigger factor of asthma [2, 3]. For a conservative estimate, the premature death people are up to more than 110000 due to the indoor pollution annually [4].

Since “9-11” terrorist attacks in 2001, the biochemical weapon attack from terrorists has continuously happened. With opening the letter of anthrax spores sent by terrorists, anthrax spores spread to other rooms of building through the return air of the air conditioning system, which caused the whole staff to face huge danger [5]. One of the main dissemination paths of the atypical pneumonia SARS coronavirus in 2003, which infected many people and even caused death is air. An air condition system becomes the main channel of the SARS virus and bacteria. Therefore, how to control the virus and bacteria in the indoor environment, and how to prevent the sudden attack of the bacteria (or virus), have become the important issues in protecting people's life and health [6].

In the paper, the microcapsules composed of active ingredients extracted from natural medicine plant material were fabricated by a w/o/w emulsion method. The finishing agents were obtained by mixing the medicine microcapsules and nano ZnO/TiO₂ with non-toxic, efficient and safe properties, and were employed to finish air filter, and the corresponding anti-virus and anti-bacteria properties were

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systematically analyzed.

2 Experiment

2.1 Materials

The honeysuckle, forsythia and the licorice were purchased from a Chinese medicinal shop, and dried to a constant weight at 40°C in the oven, then smashed and sieved through 40 mesh.

Testing instruments: The DHP-9052 electro-heating standing-temperature cultivator; the KQ-100E ultraviolet ray disinfecting device; the CA-920-3 perpendicular layer flowing cleaning work set, the 5417R centrifugal machine; the HB-202 thermostat water bath cauldron; the 7500 Real-time Sequence Detection System.

2.2 Extracting technology of compound Chinese medicine and the preparation of the medicine microcapsules

2.2.1 Extracting technology of compound Chinese medicine

The extracting technology of compound Chinese medicine was based on the ultrasonic backflow method. The honeysuckle, which is known as the antibiotic of the Chinese medicine, has good function of cleaning hut and anti-virus, chlorogenic acid is the chief ingredient among the honeysuckle. Forsythia [7, 8], as the daily medicine, has the unique effect of anti-virus and anti-bacteria, Forsythin is the main component [9]. The licorice with the main component of glycyrrhizic acid can effectively adjust some Chinese medicine efficacy and reduce the effect of medicine poison [10]. According to the previous reports [11, 12], the compound extracting technology was investigated based on the proportion of honeysuckle, forsythia and licorice being 2:2:1.

2.2.2 Preparation of the medicine microcapsules

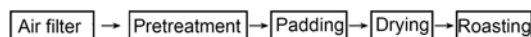
Firstly, ethyl cellulose was dissolved in methylene chloride to form a definitely viscosity macromolecular solution. The W/O pre-emulsion was prepared by adding definitely emulsifiers, the compound extracted solution to the ethyl cellulose-methylene chloride solution. Secondly, the W/O pre-emulsion was dispersed to protective colloidal solution to form a relatively steady emulsion. Finally, the medicine microcapsules was prepared with continuously vaporizing methylene chloride, the ethyl cellulose was deposited and solidified in the internal water, with a high speed stirring, then rinsed with distilled water, and separated by using a centrifugal machine [13].

2.3 Finishing process of air filter material

The finishing agents of anti-virus and anti-bacteria fabric

were prepared with the microcapsules solution, nano ZnO and nano TiO₂ compound gum (it was made with sol-gum, its particle diameter was 46.35 nm), and water polyurethane with high-speed stirring.

The finishing process is as follows:



2.4 Anti- bacteria testing

2.4.1 Preparation of anti-bacteria filter sample

Each filter sample with an area of 2 cm×2 cm and loaded separately was sterilized with high pressure vapor at 121°C for 30 min, then dried at 60°C, respectively. The No.3 sample was put in a living creature Safety Storage Cabinet, with an opening ultraviolet lamp (the power 20 W, the wavelength 253 nm), to activate a photosensitive substance at 35 cm distance for 20 min.

2.4.2 Anti- bacteria experimental

1) Preparing of bacteria solution

The experiment bacteria were recovered and cultivated for passage (transfer of culture). The bacteria were inoculated into the LB liquid culture medium (it was made with tryptone of 10 g L⁻¹, yeast extract of 5 g L⁻¹ and NaCl 5 g L⁻¹), at 37°C for 20 h. The amount of bacteria was tested with the standard barium sulfate turbidity tube turbidimetry method, and diluted into 1.5×10⁶ CFU mL⁻¹ with deionized water. The prepared solution must be used within 2 h.

2) Bacteria solution and sample treated

A piece of filter sample was put into a 100 mL sterile conical flask, then marked it. After adding 200 μL with 1.5×10⁶ CFU mL⁻¹ bacteria solution to the filter sample by a small pipette, the conical flask was sealed and placed at room temperature for 1 h.

3) The dilution of bacteria solution

Filter sample and the bacteria solution were respectively treated in a sterile conical flask for 1 h, 20 mL sterile deionized water was put into the sample, the conical flask was placed in a constant temperature shaker, oscillated and rinsed for 10 min at 20°C and 250 times min⁻¹. Then, the rinsing solution was taken to another sterile conical flask and diluted with sterile deionized water for 10 times.

4) Culture of bacteria

1.0 mL of the diluted rinsing solution and 20 mL of culture solution heated to melt and cooled to 45°C were mixed in a culture dish, and then congealed at room temperature and cultured in a incubator at 37°C for 48 h. Each dilution gradient solution was repeated for three times.

MacConkey Agar was used in culture solution of *Escherichia coli*, high salt nutrient agar was used for *Staphylococcus aureus*, and the Sabouraud's agar culture solution was used for *Candida albicans*.

5) Amount of bacteria tested

To count the amount of bacteria, the culture dish was put into a bacterium calculating instrument. When the number of bacteria was greater than 300 per dish, the count was in non. The number of bacteria ranged from 5 to 200 per dish. The average amount of bacteria could be calculated for each sample (CFU mL⁻¹).

6) Calculation of the ratio of anti-bacteria

The ratio of anti-bacteria = (the amount of control sample-the amount of anti-bacteria filter sample)/the amount of control sample × 100%

2.5 Anti- virus testing

2.5.1 The virus specimen

Influenza A virus strain PR8 (A/PR8/34/N1H1) was purchased from the Peking Virus Institute of the Chinese Academy of Preventive Medicine.

2.5.2 Preparation of anti- virus filter sample

The preparation method of anti-virus filter sample is the same as Section 2.4.1.

2.5.3 Anti-virus experiment

1) The virus recovers and passages

Influenza A virus strain PR8 was taken out from refrigerator at -80°C and diluted with Hank's solution with a proportion of 1:100. The corresponding diluted solution with 1 mL was inoculated into the allantoic cavity of a 10 day's chick embryo, and the virus solution of the allantoic cavity was collected after hatching at 36°C for 72 h. The virus titer (HC50 CFU mL⁻¹) was measured with Coombs experiment. The virus solution was subpackaged and kept at -80°C. Before the experiment, the virus solution was removed out, and diluted to 1.0 × 10³ HC50 CFU mL⁻¹ with Hank's solution.

2) Culture and passage of MDCK cell

The MDCK cell was put into a T25 cell culture flask, to which added 10 mL RPMI1640 cell culture solution (new-born bovine serum of 10%, glutamine of 0.6 mg mL⁻¹, penicillin of 100 uL, streptomycin of 100 ug L⁻¹, 0.02 mol L⁻¹ HEPES).

A cell monolayer of MDCK was formed on the cell culture flask wall at 37°C with 5% CO₂ for 6 days. The monolayer cells were digested by TPCK (tosyl-phenylalanine chloromethyl-ketone) pancreatin and 25 mL cell culture solution was added, thus the monolayer cell suspension was formed. The cell suspension was put to a 12-hole tissue culture plate with 1.0 mL/hole, and cultured at 37°C with 5% CO₂ for 4 days. When the hole bottom of the culture plate was overgrown, the monolayer cells were transferred to a hatchery at 35°C.

3) Sample treating

A piece of filter sample was put into a 100 mL sterile conical flask. 200 μL 1.0 × 10³ HC50 CFU mL⁻¹ of virus so-

lution was added to filter sample by a little pipette, then sealed and placed in a refrigerator at 4°C for 1 h

4) Collection and dilution of the virus solution

Filter sample and virus solution had been treated each other for 1 h. After the treating described above, 20 mL sterile Hank's solution was added to the sample, then the sample was placed in a constant temperature shaker, oscillated and rinsed for 10 min at 20°C. The rinsing solution was taken out from a sterile conical flask, diluted with sterile Hank's solution to continuous gradient 10 times.

5) Measurement of the virus lacuna

The solution of MDCK tissue culture plate with the monolayer cells was drained. The diluted rinsing solution with 200 μL was added respectively into 3 holes of MDCK tissue culture plate and placed at 37°C for 1 h. The un-adsorbed virus solution was drained, and rinsed with sterile Hank's solution for 3 times. Agarose and deionized water were mixed with mass ratio 1:4 and heated to dissolve. The corresponding solution was diluted continuously to 0.8% of the solution, then blended with the lacuna culture solution (RPMI1640 cell culture solution consisting of 10 ug mL⁻¹ DEAE, 1.25 ug mL⁻¹ TPCK pancreatin, 2% of new-born bovine serum). The 1 mL blended solution was added quickly to the hole of tissue culture plate absorbing virus. After the condensation of the culture solution, the tissue culture plate was transferred to a CO₂ incubator at 34°C with 5%CO₂ for 72 h.

The tissue culture plate was taken out and dyed with 0.2% of the neutral red formalin solution. The number of virus lacuna was counted by a microscope, and the virus titer (HC50 CFU mL⁻¹) was obtained.

6) Calculation of virus inactivation rate

Virus inactivation rate = (The virus titer of control sample-the virus titer of anti-virus filter sample)/the virus titer of control sample × 100%.

3 Results and discussion

3.1 Analysis of the extracting process of compound Chinese medicine

After a series of experiments, the optimum extracting process of compound Chinese medicine is described below. The extracting temperature is 60°C, ethanol 60% (v/v) and extracting times 3 h. 10.00 g of dried medicine material was weighted with the proportion of the honeysuckle: forsythia: the licorice as 2:2:1, and was extracted by the ultrasonic backflow method for 3 times. The results of the extracted compound Chinese medicine are shown as Table 1.

As shown in Table 1, the ultrasonic backflow method has some advantages, such as high extracting ratio and stability. The extracting ratios of Forsythin and Chlorogenic acid are 85.7% and 61.6%, respectively. The result is higher than that of the general water extracting process [14–16].

Table 1 Results of the extracted compound Chinese medicine

Samples	Extracted medicine (%)			Average (%)	Ratio of extraction (%)
Chlorogenic acid	3.17	3.19	3.21	3.19	61.6
Forsythin	0.52	0.54	0.55	0.54	85.7
Glycyrrhizic acid	1.95	1.97	1.93	1.95	48.0

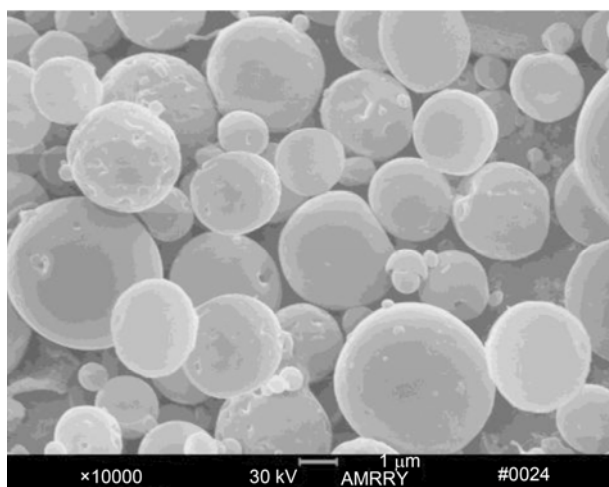
3.2 Preparation of medicine microcapsules and finishing agents

The optimum fabrication parameters of the medicine microcapsules are 3% (w/v) of the ethyl cellulose, 4% (v/v) of emulsifiers, 1500 r min⁻¹ of emulsify speed, protection colloid PVA for 1.5% (w/v). The average particle size of the microcapsules is 2.76 μm, and extracting ratio is 75.4%. Figure 1 is the micrographs of the medicine microcapsules with spherical structure, smooth surface. The particle sizes of the medicine microcapsules are distributed from 0.3 to 3.0 μm.

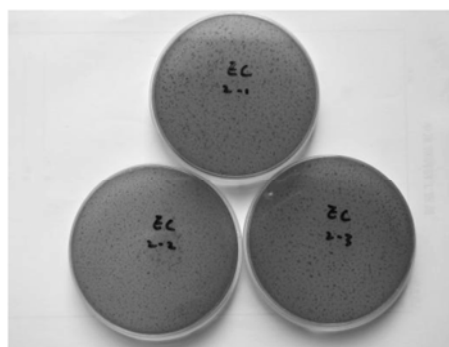
The optimum fabrication parameters of the finishing agent are 30% (w/v) of the medicine microcapsules, 30% of nano ZnO and nano TiO₂ compound gum and 40% of water polyurethane. The polypropylene punched felt was finished with finishing agent, and the original properties, such as appearance, handle and basic physical machine characterization were kept.

3.3 The anti-bacteria rate of finished filters

The anti-bacteria rates of the finished filters are shown as Table 2. Figures 2 and 3 are the images of the finished and control samples treated with the colibacillus, respectively. *Staphylococcus aureus* and *Candida albicans* have the same results. As shown in Figure 2, many living bacteria are growing in the control sample, but no living bacteria are observed in the finished sample. Therefore, the anti-bacteria rate of *Staphylococcus aureus*, *Colibacillus* and *Candida albicans* are all 100%.

**Figure 1** SEM micrographs of the medicine microcapsules.**Table 2** The anti-bacteria rate of finished filters

Bacterial kinds	Amount of bacteria treated for 1h (CFU mL ⁻¹)		Anti-bacteria rate (%)
	Control sample	Finished sample	
<i>Staphylococcus aureus</i> ATCC25922	1.08×10 ⁴	0	100
<i>Colibacillus</i> ATCC25925	1.36×10 ⁴	0	100
<i>Candida albicans</i> CMCC85021	1.03×10 ⁴	0	100

**Figure 2** *Colibacillus* (control sample).**Figure 3** *Colibacillus* (finished sample).

3.4 The anti-virus rate of finished filters

The anti-virus rate of the finished filters are shown as Table 3. The influenza A virus PR8(A/PR8/34/N1H1) inhibition ratios are all 100%.

Table 3 The anti-virus rate of finished filters

Diluted time	Number of virus lacuna (hole)							
	control sample				finished sample			
	1	2	3	average	1	2	3	average
10 ⁻¹	cannot count	cannot count	cannot count	—	0	0	0	0
10 ⁻²	22	19	21	2.067	0	0	0	0
10 ⁻³	2	2	2	2.0	0	0	0	0
PFU mL ⁻¹	2.0335×10 ³				0			
Virus inactivation rate (%)					100			

4 Conclusions

The extracting process of compound Chinese medicine (the honeysuckle, forsythia and licorice) was investigated by the ultrasonic backflow method, and using ethyl cellulose as external water-phase, the extracted oils as internal water-phase, the medicine microcapsules were fabricated by a w/o/w mutually-phase emulsion method. The average particle size of the prepared microcapsules is 2.76 μm .

The anti-virus and anti-bacteria finishing agents were prepared with the Chinese medicine microcapsules, nano ZnO and nano TiO₂ compound gum and water polyurethane. The anti-virus and anti-bacteria air filters were obtained by padding the finishing agents onto polypropylene punched felt. The testing results show that the anti-bacteria rates of *Staphylococcus aureus*, *Colibacillus* and *Candida albicans* are all 100%, and the influenza A virus inactivation rate of air filters is 100%, which are all attributed to cooperative effect of the Chinese medicine and nano material.

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