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Synthesis of Micron-size Functional Polystyrene Fluorescent Microspheres and their Adsorbability to Human Serum Albumin

Di Qiang WANG, He LI, Jie HU, Xian Feng LIAO, Bai Ling LIU*

Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences, Chengdu 610041

Abstract: Polystyrene microspheres with sulfo- or aldehyde- surface were synthesized through dispersion polymerization. Functional polystyrene fluorescent microspheres were prepared by the way of adding 2, 5-diphenyloxazole (PPO) into the reaction system directly and dying the blank microspheres in the ethanol solution of PPO. The influence of preparing matters on the encapsulating rate of PPO, and the influence of functional groups on the adsorbability to human serum albumin (HSA) were investigated.

Keywords: Polystyrene, fluorescent microspheres, HSA, HTS, functional monomer.

As a kind of special functional microspheres, fluorescent polymer microspheres have important applications in many fields especially in biomedicine. For example, cell can be labeled and separated by specific binding with fluorescent microspheres[1]; region blood flow can be assayed by injecting fluorescent microspheres into vein[2]; flow cytometer can use fluorescent microspheres as marker and standard[3]; chemical reaction can be assayed by using fluorescent microspheres[4]; even the transform and diffusion of particles in soil can be analyzed by fluorescent microspheres[5]. However, one of the most important applications of fluorescent microspheres is in the high-throughput screening of drugs (HTS). Through affinity binding, radioactive ligands (latent drugs) are bound with fluorescent microspheres covered by receptor, and luminescence is caused by radioactivity, so ligands can be assayed and screened[6].

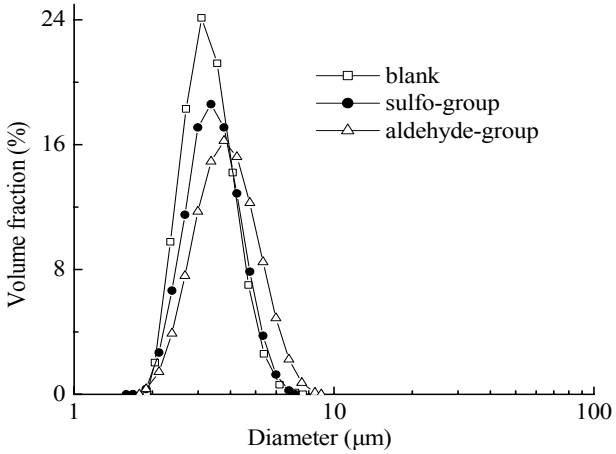
Dispersion polymerization is a very important and available method for preparing micron-sized monodispersion microspheres[7]. Styrene washed by 5% NaOH solution was mixed with stabilizer polyvinyl pyrrolidone, initiator azodiisobutyronitrile and ethanol, and polymerized under nitrogen at 70℃ for 24 hours for preparing the blank polystyrene microsphere. Microspheres with sulfo- or aldehyde- surface were prepared by adding functional monomers 2-acrylamido-2-methylpropane sulfoacid (AMPS) or acrylic aldehyde (AL) in the reaction system after 8 hours.

Figure 1 was the diameter distribution graph of PSt microspheres prepared through dispersion polymerization, it could be seen that the range of the diameter distribution of blank microsphere, the sulfo-group microsphere and the aldehyde-group microsphere were narrow, and accorded with normal distribution. This result was a good foundation

* E-mail: diqiangwang@163.com

of the microspheres' applications.

Figure 1 Diameter distribution of PSt microspheres



There are four methods for preparing fluorescent microspheres[8]. If the structure and properties of the fluorescent materials were very stable, such as PPO, the adsorbing method could be used for preparing polystyrene fluorescent microspheres. In this research paper, two ways were taken, one was direct adding PPO into the dispersion polymerization system (procedure 1), and the other was dying the blank microspheres in the ethanol solution of PPO (procedure 2).

Figure 2 was the ultraviolet absorption spectrograph of PPO in ethanol and in toluene. Despite the obvious absorbance of toluene and (poly)styrene in ultraviolet region, ultraviolet spectrophotometric method could be used for detecting the encapsulating rate of fluorescent materials in polystyrene microspheres, because the absorption wavelength of toluene and (poly)styrene is under 250 nm, and one maximam absorbance of PPO in ethanol and toluene was near 300 nm.

According to the standard curve of the ultraviolet absorbance of PPO in toluene at 306.5 nm, and according to the absorbance of the toluene solution of fluorescent microspheres at 306.5 nm, it could be calculated that the encapsulating rate of PPO were 48% in procedure 1 and 42% in procedure 2, respectively.

Fluorescent microspheres were a key material in HTS, their functional group and binding with receptor proteins were very important. The binding of fluorescent function microspheres with receptor could be simulated by the binding of fluorescent function microspheres with HSA.

Figure 3 was the ultraviolet absorbtion spectrograph of HSA. It showed that there was one maximal absorbance at 278 nm. So the adsorbability of microspheres could be detected indirectly through investigating the ultraviolet absorbance of HSA's aqueous moiety after binding with microspheres.

Figure 2 Ultraviolet absorption of PPO

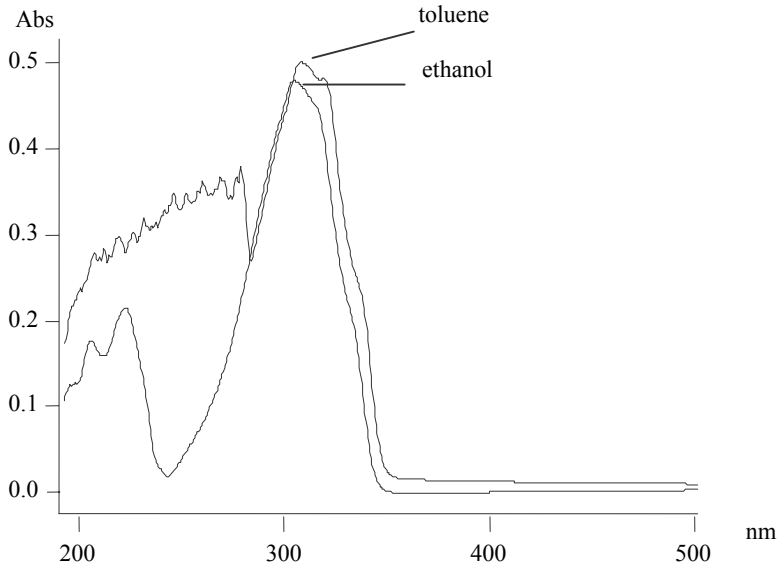


Figure 3 Ultraviolet absorption of HSA

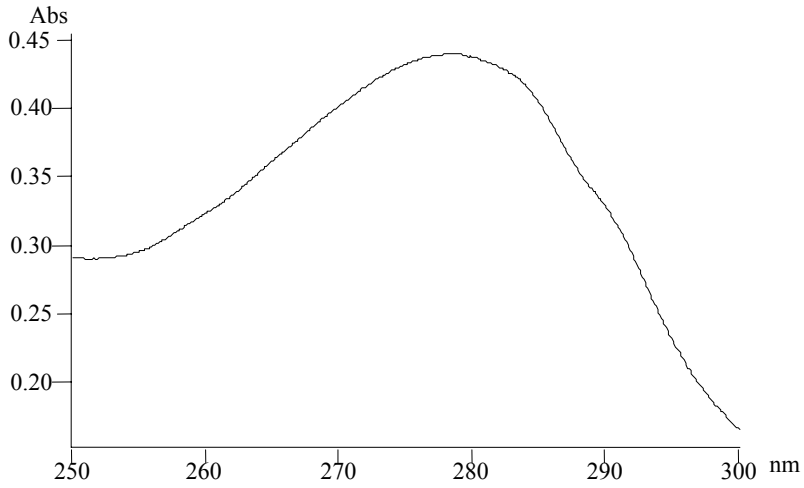
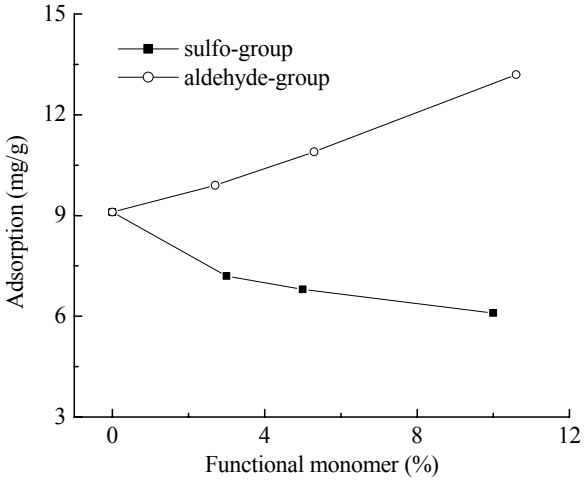


Figure 4 was the relationship of the protein adsorption and functional monomers at pH 7. It showed that the protein adsorption increased with increasing AL resulted from the reaction of aldehyde-group with protein's amino-group, while the protein adsorption decreased with increasing AMPS, because the equivalence potential point of HSA is 4.6, so the adsorption of protein would decrease by the increasing static repulsion between protein and sulfo-group.

Figure 4 Relationship of the protein adsorption and functional monomers



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