Marine Pollution Bulletin 131 (2018) 515–524

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Comparison of six digestion methods on fluorescent intensity and morphology of the fluorescent polystyrene beads

Fengmin Li[a,b], Fuyun Li[a,b], Xiaodong Hou[c], Xianxiang Luo[a,b], Haifeng Tu[a,b], Yadan Zou[a,b], Cuizhu Sun[a,b], Mei Shi[a,b], Hao Zheng[a,b,*]

[a] Institute of Coastal Environmental Pollution Control, and Ministry of Education Key Laboratory of Marine Environment and Ecology, Ocean University of China, Qingdao 266100, China
[b] College of Environmental Science and Engineering, Ocean University of China, Qingdao 266100, China
[c] Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550000, China

ARTICLE INFO

Keywords:
Fluorescent PS beads
Digestion
Fluorescence intensity
Morphology
Extraction efficiency

ABSTRACT

Effect of digestion methods on fluorescence intensity of fluorescent polystyrene (PS) beads was poorly understood, which may affect the accuracy of toxicity test of the fluorescent PS beads exposed to marine organisms. Therefore, six digestion approaches were compared on fluorescence intensities and properties of three commercial fluorescent PS beads. Among all the protocols, the digestion using KOH (10% w/v, 60 °C) (KOH-digestion) had no effect on the fluorescence intensity, morphology and composition of the three fluorescent PS beads. Moreover, the extraction efficiency ≥ 95.3 ± 0.2% of fluorescent PS beads in *Daphnia magna* and zebrafish, confirming its feasibility in fluorescent PS beads quantitative analysis. However, the fluorescence intensities of fluorescent PS beads digested by other five protocols were significantly decreased, as well as the change of morphology and composition on fluorescent PS beads. Overall, the KOH-digestion is an optimal protocol for extracting fluorescent PS beads in biological samples.

1. Introduction

Plastics are widely used around the world due to its light weight, long durability, strong plasticity, and low production cost (Ivar and Costa, 2014). Global virgin plastic production reached 8300 million metric tons as of 2017, and approximately 60% of all the plastics ever produced were discarded and are accumulated in landfills or in the natural environment (Geyer et al., 2017). Approximately, 4.8–12.7 million metric tons of plastic waste generated on land of 192 coastal countries in 2010 due to mismanagement entered into oceans (Jambeck et al., 2015). Plastics debris eventually fragment into powdery or microsized fragments in the beach and seawater environment, due to ultraviolet radiation and waves, typically not visible to the naked eye, are called microplastics (MPs), of which the size is < 5 mm (Barnes et al., 2009; Andrady, 2011). Occurrence of MPs in oceans has been reported throughout the world. For example, the mean abundance of MPs was 2080 ± 2190 items m^{-3} in the Pacific Ocean (Desforges et al., 2014). And the MPs (38–234 items m^{-3}) even appeared in the ice cores collected from remote locations in the Arctic Ocean (Obbard et al., 2014). The maximum concentrations of MPs have reached 100,000 items m^{-3} in Swedish harbour area adjacent to a polyethylene production plant (Noren and Naustvoll, 2010). Therefore, the fate and ecotoxicity effects of MPs in marine environment had become one of the hottest environmental issues (Kanhai et al., 2017).

Because of the small particle size and high content MPs are easily ingested by aquatic organisms such as copepods (Cole et al., 2013) and fish (Luís et al., 2015). Ingestion and accumulation of MPs can cause adverse effects on organisms, such as blocking alimentary canal (Wright et al., 2013), restricting food intake (Cole et al., 2013), causing neurotoxicity (Barboza et al., 2018), thus inhibiting body growth and development (Sussarellu et al., 2016). Furthermore, MPs can act as vectors for hydrophobic organic contaminants in aquatic environment because of their large surface area to volume ratio and hydrophobic properties (Ogata et al., 2009). As a result, it is important to investigate the ingestion of MPs by marine organisms and examine their impact on the organisms (Rist et al., 2017).

The fluorescent polystyrene (PS) beads are a type of artificially manufactured commercial plastic microbeads with stable fluorescence that enable a clear distinction from other environmental plastic particles (Cole et al., 2013; Watts et al., 2016). And the fluorescent PS beads

* Corresponding author at: Institute of Coastal Environmental Pollution Control, and Ministry of Education Key Laboratory of Marine Environment and Ecology, Ocean University of China, Qingdao 266100, China.
E-mail address: zhenghao2013@ouc.edu.cn (H. Zheng).

https://doi.org/10.1016/j.marpolbul.2018.04.056
Received 7 February 2018; Received in revised form 23 April 2018; Accepted 24 April 2018

Table 1
The details of six selected digestion protocols for extracting fluorescent PS beads.

Reagents	Concentration[a]	Volume[b] (mL)	Temperature[c] (°C)	Duration time[d] (h)	References
KOH	10% (w/v)	40.0	60	24	Dehaut et al., 2016
NaOH	10 M	40.0	60	24	Cole et al., 2014
H_2O_2	30% (w/w)	40.0	65	24	Li et al., 2015
HNO_3	69% (w/w)	1.0	70	2	Lu et al., 2016
HNO_3:HCl	1:1 (v/v)	1.0	80	1/2	Desforges et al., 2014
HNO_3:$HClO_4$[e]	4:1 (v/v)	1.0	20/90	12/(1/6)	De Witte et al., 2014

[a] 10% (w/v) indicated the concentration of KOH was 100 g L^{-1}; 30% and 69% (w/w) indicated the mass fraction of H_2O_2 and HNO_3 was 30% and 69%, respectively; 1:1 (v/v) and 4:1 (v/v) indicated the volume ratio of HNO_3:HCl and HNO_3:$HClO_4$ was 1:1 and 4:1, respectively, and the mass fraction of HCl and $HClO_4$ was 37% and 71%.
[b] Volume indicated the amount of the reagent that used in the digestion.
[c] Temperature indicated the constant temperature during digestion.
[d] Duration time indicated the duration time used in the digestion.
[e] The fluorescent PS beads were digested using HNO_3:$HClO_4$ at 20 °C for 12 h, then at 90 °C for 1/6 h.

are often used in laboratory toxicity experiments to investigate their accumulation and transfer in marine organisms (Lu et al., 2016; Rist et al., 2017). Thus the quantification of fluorescent PS beads ingested by organisms is crucial to assess their potential impacts to marine ecosystems. One of the commonly used quantitative methods of fluorescent PS beads ingested by organisms is the observation of the tissue sections using fluorescence microscopy (Batel et al., 2016). However, this method is less applicable for quantifying fluorescent PS beads ingested by organisms, because it is complex, time-consuming, and are likely to cause omission of fluorescent PS beads (Rist et al., 2017). Additionally, quantification using fluorescence microscopy is limited by the particle size of fluorescent PS beads and the resolution of the microscope (Rist et al., 2017). Consequently, a viable quantitative method via measuring the fluorescence intensity of the fluorescent PS beads has been used to determine the content of MPs in biological samples (Rosenkranz et al., 2009; Lu et al., 2016). Nevertheless, the measurement of fluorescence intensities of the fluorescent PS beads was based on thoroughly digestion of the biological samples (Rist et al., 2017). Studies had shown that the digestion reagent such as strong acidic (e.g., HCl, HNO_3, and $HClO_4$) and alkali (e.g., NaOH and KOH) solution, strong oxidizing reagent (e.g., H_2O_2), and Protease K could effectively remove the organic matters from the biological samples (Lusher et al., 2017). Among these methods, the protease K was not recommended because of its complicated procedures and lower extraction efficiency (Dehaut et al., 2016). Moreover, the extraction efficiency of fluorescent PS beads was also related to the digestion conditions and the chemical resistance. However, little was known about the effect of the digestion approaches on the fluorescence intensity and extraction efficiency of fluorescent PS beads in the biological samples.

Therefore, six digestion reagents were selected to study the effect of different digestion approaches on the fluorescence intensity, surface morphology, and extraction efficiency of three common commercial fluorescent PS beads. The specific objectives were to: (1) investigate the effects of the six published digestion methods that commonly used in literatures on the fluorescence intensity and morphology of fluorescent PS beads; (2) explore the effects of the volume of the digestion reagents, digestion temperature and duration time on the fluorescence intensities of fluorescent PS beads; (3) evaluate the extraction efficiency of fluorescent PS beads in the biological samples by the optimal method; and (4) elucidate the underlying mechanisms responsible for the decreased fluorescence intensity of fluorescent PS beads resulted from the digestion protocols. These findings will screen an optimal digestion protocol for quantitative analysis of the fluorescent PS beads in the biological samples using fluorescence spectrophotometer, which is helpful for investigating and predicting the ecotoxicity effects of fluorescent PS beads in marine ecosystems.

2. Materials and methods

2.1. Fluorescent PS beads preparation

Three kinds of fluorescent PS beads were purchased from Thermo Fisher Scientific (MA, United States), Baseline ChromTech Research Centre (Tianjin, China), and Big Goose ChromTech Research Centre (Tianjin, China), respectively. The properties of the pristine fluorescent PS beads were shown in Table S1. All of the stock solutions were dispersed in 2 L Milli-Q water and sonicated for 15 min at 120 W (FB 120, Fisher Scientific, USA) to avoid aggregation. The composition of the pristine fluorescent PS beads without treatment was confirmed by Fourier transform infrared spectroscopy with attenuated total reflectance accessory (ATR-FTIR, L1600401, Spectrum, UK), and there was no significant difference in the composition of the pristine fluorescent PS beads (Fig. S1).

2.2. Digestion and determination of the fluorescent PS beads

Six digestion methods were selected according the published studies to investigate their effects on fluorescence intensity of the fluorescent PS beads, and each group set 3 parallels. Briefly, the fluorescent PS beads (1.0 mg) and the desired volume of digestion solution were added to 10 mL or 100 mL glass bottles with caps to avoid contamination. Then, the samples were digested in a constant temperature water bath. The specific procedures for the six digestion methods were shown in Table 1. Subsequently, the pristine fluorescent PS beads and digested fluorescent PS beads were diluted with Milli-Q water to a final volume of 100 mL and the fluorescence intensity was measured using a fluorescence spectrophotometer (F-4600, Hitachi, Japan).

To investigate the effect of digestion conditions including volume of the digestion reagent, digestion temperature, and duration time on the fluorescence intensities of fluorescent PS beads, the experiments were divided into three groups and each group was set for triplicates. In the first group, the effect of the digestion reagent volume on the fluorescence intensity of fluorescent PS beads was explored. The volumes for these reagents are shown in Table S2, i.e., HNO_3: 0.2–1.5 mL, HNO_3:HCl: 0.2–1.4 mL, HNO_3:$HClO_4$: 0.5–2.0 mL, H_2O_2: 2.0–90 mL, NaOH: 10–60 mL and KOH: 10–60 mL, the digestion temperature was 60 °C and duration time was 24 h. In the second group, the effect of the digestion temperature from 20 °C to 90 °C on the fluorescence intensity of fluorescent PS beads was investigated. The volumes of HNO_3, HNO_3:HCl, HNO_3:$HClO_4$ were 10 mL and the volumes of H_2O_2, NaOH and KOH were 60 mL, the fluorescence intensity of the fluorescent PS beads had reached a steady state in the above volumes, and the duration time was 24 h. In the third group, the effect of the duration time from 10 min to 72 h on the fluorescence intensity of fluorescent PS beads was compared. The digestion reagent volumes were same as the