Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 54 (1): 59–64 (2017)

ISSN: 2518-4261 (print), ISSN 2518-427X (online)

Pakistan Academy of Sciences

Research Article

Different Oxidation Treatments on Polystyrene (PS) Microspheres by using an Ultraviolet/Ozone (UVO_3) System

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Abstract: Oxidation is a common physical treatment used to render the surface of polystyrene suitable for cell adhesion and spreading. In this work, the effects of oxidation of polystyrene (PS) microspheres by three different treatments; oxidation by ozone aeration only, UV irradiation only or by the combination of both (UVO_3), by using a self-fabricated UVO_3 system were investigated. Results expressed in carboxylic acid (COOH) concentrations on the surface of microspheres revealed that, the treatment by UVO_3 yielded the highest concentration. Presence of atomic oxygen species in UVO_3 treatment has contributed to higher COOH concentration.

Keywords: Microspheres; oxidation; polystyrene; ultraviolet/ozone

1. INTRODUCTION

Polystyrene (PS) is an inexpensive synthetic polymer made from the monomer styrene, a liquid hydrocarbon derived from petroleum. PS is used in an extremely wide range of applications as it has many interesting properties such as low specific weight (~1.04 g/cm^3), good optical properties, high chemical resistance, mechanical flexibility and biocompatibility [1]. In cell culture, PS has been used as early as in the 1960s for producing tissue culture flasks, roller bottles, vacuum canisters and culture medium filters. However, unmodified PS is unsuitable for cell attachment due to its hydrophobic surface chemistry. PS must undergo surface treatment to render it suitable for cell attachment [2].

Ultraviolet/ozone (UVO_3) treatment has been reported as able to improve the hydrophilicity of PS by introducing polar oxygen functional groups such as hydroxyl, carbonyl and carboxyl on its surface [3]. Hydroxyl and carboxyl functional groups are known to have positive influences on cell attachment on polymer surfaces. Numerous studies have demonstrated that, carboxylic acid enriched PS surfaces allow good adhesion and growth of multiple cell lines [4-6]. The surface of PS may also be derivatized by the incorporation of extracellular matrix and recombinant proteins to facilitate cell attachment. These proteins can be conjugated on PS surfaces by using covalent bonding. This method generally requires the surface of PS to be activated prior to conjugation. One possible way to accomplish that requirement is by introducing carboxyl functional groups on its surface.

In this work, we have fabricated a UVO_3 treatment system that allows oxidation to be done by three different modes; oxidation by ozone aeration only, UV irradiation only or by the combination of both, UVO_3. PS microspheres

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Received, January 2017; Accepted, March 2017
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were treated with all three modes, and their effects on carboxyl functional group deposition on their surfaces were investigated.

2. MATERIALS AND METHODS

2.1 Production of PS Microspheres

PS microspheres were prepared by an oil-in-water (O/W) emulsion solvent-evaporation method. Four grams of PS powder (250 µm, homopolymer) were dissolved in 20 mL chloroform by magnetic stirring to form the oil phase. The organic solution was then emulsified in 100 mL of 0.25 % PVA (partially hydrolyzed, MW = 30 000) solution (water phase). Resulting o/w emulsion was later stirred at 300 rpm (1 rpm = 1/60 Hz) and 80 °C for 6 h to extract chloroform. Next, microspheres were retrieved by vacuum filtration, washed vigorously with distilled water and dried at 50 °C overnight. Finally, dried PS microspheres were stored in vacuum desiccator at room temperature until further use.

2.2 Treatment on PS Microspheres

Surface modification of PS microspheres was carried out in the self-fabricated UVO_3 system. A 250 mL conical flask for containing PS microspheres was fixed on a shaker and shaken at

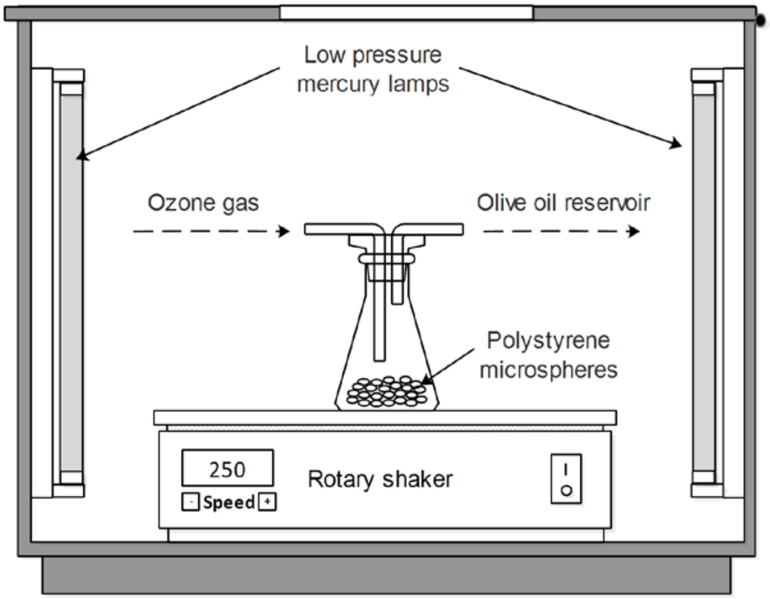

Fig. 1. Treatment of PS microspheres in UVO_3 system.

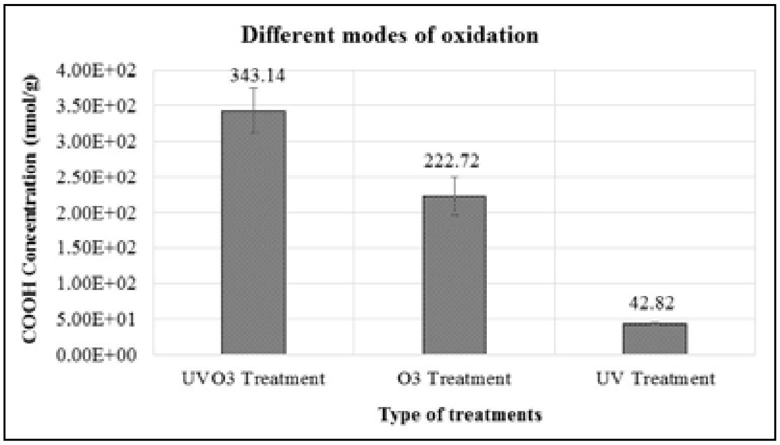

Fig. 2. Effect of different treatments on COOH deposition on PS microspheres.

250 rpm for homogenization during treatment. For treatment using both UV and ozone, 1 g of PS microspheres were aerated with ozone concentration of 42 494.57 mg kg^{-1} (in O_2 flow rate of 2 rpm) and irradiated with UV lights from two oppositely located low pressure mercury lamps with wavelengths of 254 nm. Ozone was produced by silent discharge of oxygen (99.9 %) while irradiation from both UV lamps to the sample was measured to be at 0.343 mW cm^{-2}. Samples were treated by the UVO_3 system for 10 min. For treatment by using ozone only, all parameters were set identical as the previous UVO_3 treatment but the UV lamps were not turned on and for treatment by using UV only, the ozone was not supplied but UV lamps were turned on. Setup of the treatment is illustrated in Fig. 1.

2.3 Measurement of Carboxylic (COOH) Functional Group Concentration

Toluidine blue O (TBO) assay was used to determine the amount of carboxyl (COOH) functional group introduced on the surface of PS microspheres. The assay was adapted from the article published by Rodiger [7] with various modifications. One gram of microspheres was incubated in 10 mL of toluidine blue O (TBO) solution (1 mM NaOH, 0.1 % TBO) for 30 min at 40 °C and was shaken at 400 rpm. After that, the microspheres were washed with 1 mM NaOH solution until the rinsing solution become colourless. Dyed microspheres were later recovered by using vacuum filtration. Next, TBO was desorbed by incubation of microspheres in 10 mL of 20 % sodium dodecyl sulfate (SDS) solution (30 min, 40 °C, shaken at 1 300 rpm). Microspheres were then pelleted by centrifugation. Finally, TBO absorption of SDS supernatants was measured for absorbance at a wavelength of 625 nm using a spectrophotometer. Amount of COOH surface concentration on PS microspheres was determined by comparison to the standard curve.

3. RESULTS AND DISCUSSION

Fig. 2 shows the concentration of carboxyl (COOH) functional group per gram of PS microspheres after different treatments as measured using TBO assay. It can be observed that UVO_3 treatment has introduced the highest concentration of COOH functional group on the surface of PS microspheres with 343.16 nmol g^{-1}. This is followed by the treatment of PS microspheres with ozone only which is 222.72 nmol g^{-1} and the least concentration of COOH was achieved when PS microspheres were treated with UV only with concentration of 42.82 nmol g^{-1}. This result is in accordance with the result reported in reference [8] in which treatment of polystyrene by both UV and ozone have yielded higher COOH concentration on the surface of polystyrene as compared to treatment by using ozone or UV only.

Fig. 3 shows one of the possible reactions when PS is treated with ozone alone as reported by Kefeli [9]. C-H bonds available on polystyrene polymer chain are accessible to ozone as reactive sites to form peroxides and its derivatives [10]. Kefeli [9] has demonstrated that, the C-H bond that is linked to the tertiary carbon as the most reactive site while ozone interaction with aromatic rings of PS represents no more than 1 % to 2 % in the total interactions. The treatment of polystyrene with ozone give rise to the formation of polymer peroxy radicals (POO•) which occurs in a rapid reaction. In the presence of ambient oxygen molecules, POO• radicals subsequently form carbonyls, carboxylic acids or hydroperoxides in slow reactions which may involve chain-scissions [11].

In the treatment of PS with UV radiation (λ = 254 nm) only, absorption of UV by the aromatic ring provides the energy for the formation of alkyl radicals by the dissociation of aromatic ring from the tertiary carbon or from the scission of the C-C or C-H bonds. These radicals later crosslinked to each other, disproportionate or may form polymer peroxy radicals (POO•) by reacting with ambient oxygen molecules [12]. Similar to the treatment of PS with ozone alone, POO• that resulted from the interaction of PS and UV radiation may also undergoes slow reactions to form carbonyls, carboxylic acids or hydroperoxides, in the presence of oxygen molecules. Apart from this mechanism, there are still many possible reactions that may occur when PS is exposed to UV radiation. In Fig. 4, possible reaction mechanisms that may occur when PS is treated with UV are presented.

PS treated with UVO_3 was modified to a greater extent when compared to the treatment that uses ozone aeration or UV radiation only. Apart from modifications mentioned in the treatment of PS with ozone or UV only, polymer chains of PS

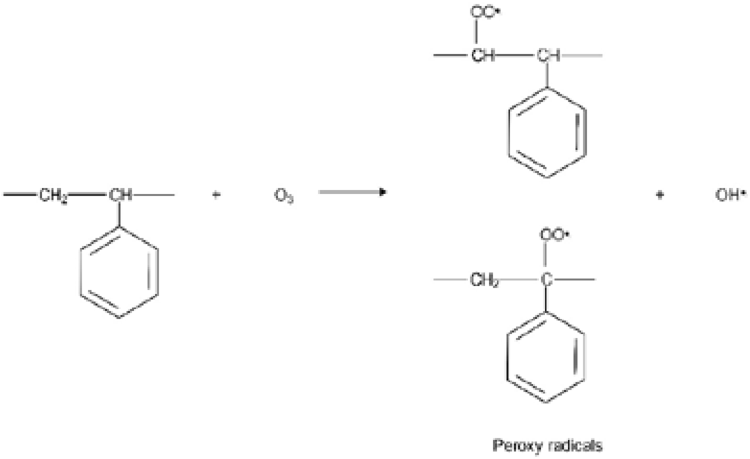

Fig. 3. Formation of POO• from the interaction of polystyrene with ozone.

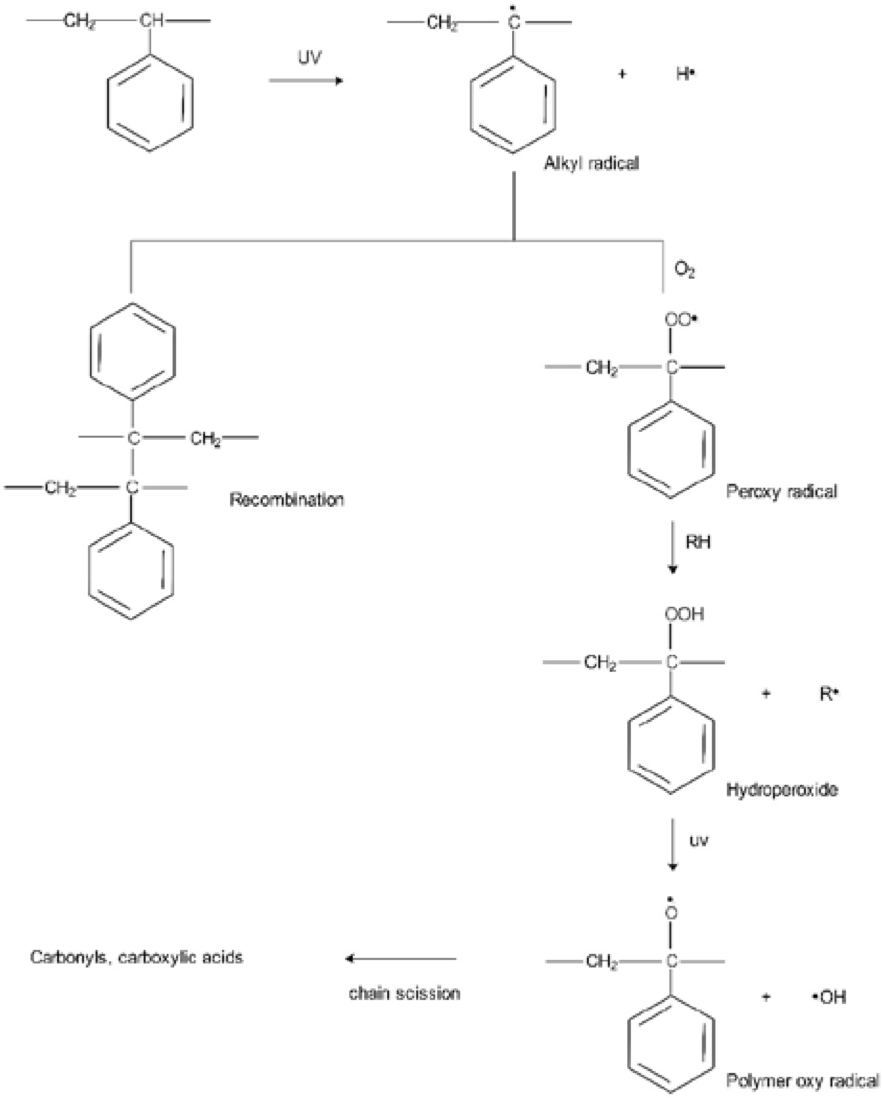

Fig. 4. General interaction mechanism between PS and UV radiation; R represents carbon containing substituents [12].

were also modified by atomic oxygen, O(^{1}D) which arises as one of the co-products alongside molecular oxygen, O_2 ($^1\Delta_g$ or $^1\Sigma_g^+$) from the decomposition of ozone after absorbing UV radiation (λ=254 nm). Walzak et al. [11] has reported that, in the presence of ozone, the interaction between polymer chains with UV radiation reduced significantly, since most of the radiation has been absorbed by ozone. O(^{1}D) is a very reactive oxygen species and may react with the polymer chain, ozone, ambient oxygen and water vapor in many possible reactions resulting in various oxygen functional groups. Interactions of O(^{1}D) with ozone, oxygen and water vapor present during the treatment are presented in the following equations [13]:

$$O(^1D)+O_2 \rightarrow O(^3P)+O_2(^1\Sigma_g^+) \quad (1)$$

$$O(^1D)+O_3 \rightarrow O_2+2O(^3P) \quad (2)$$

$$O(^1D)+H_2O \rightarrow 2\bullet OH \quad (3)$$

The interaction of ground state atomic oxygen atoms, O(^{3}P) with the polymer chain may produce alkyl radicals and subsequently form hydroxyl groups when combined with hydroxyl radicals. While for molecular oxygen, interactions with alkyl radicals may produce polymer peroxy radicals (POO•) which eventually can turn into carbonyls, carboxylic acids or hydroperoxides in slow reactions in the presence of oxygen. The interaction of O(^{1}D) with the polymer chain may also produce hydroxyl groups from mass insertion of the reactive groups into C-H bonds or forms ether when inserted into C-C bonds. Further oxidation of these groups may result in the formation of higher oxidized functional groups, ketones and ester [13].

4. CONCLUSIONS

As the conclusion, among three different oxidation treatments applied on PS microspheres, the combination of both UV and ozone (UVO_3) has yielded the highest COOH concentration. The result obtained by UVO_3 was 54 % higher than that achieved by the treatment by ozone only and 701 % higher when compared to the treatment by UV only. High surface COOH concentrations on UVO_3 treated PS microspheres may render them suitable as cell microcarriers for suspension culture of adherent cells.

5. ACKNOWLEDGEMENTS

This work was funded by the Ministry of Education Malaysia (MOE) under the Prototype Research Grant Scheme (grant no. PRGS 11-001-0001).

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