OPTIMIZATION OF ULTRAVIOLET/OZONE (UVO_3) PROCESS CONDITIONS FOR THE PREPARATION OF GELATIN COATED POLYSTYRENE (PS) MICROCARRIERS

Mohd Azmir Arifin[1*], Maizirwan Mel[2], Nurhusna Samsudin[3], Yumi Zuhanis Has-Yun Hashim[3], Hamzah Mohd. Salleh[3]

[1]*Faculty of Chemical and Process Engineering Technology, Universiti Malaysia Pahang, 26300 Gambang, Pahang, Malaysia.*
[2]*Department of Biotechnology Engineering, Faculty of Engineering,* [3]*International Institute for Halal Research and Training, International Islamic University Malaysia, Jalan Gombak, 53100, Selangor, Malaysia.*
*Corresponding author: mazmir@ump.edu.my

Extended Abstract

Abstract
The aim of this study was to develop gelatin coated polystyrene microcarriers with good cell adhesion and proliferation properties. Polystyrene (PS) microspheres, prepared using oil-in water (o/w) solvent evaporation method, were loaded with oxygen containing functional groups using an ultraviolet/ozone (UVO_3) system. Using water soluble carbodiimide chemistry, gelatin was subsequently immobilized on UVO_3 treated PS microspheres. The amount of immobilized gelatin was found to be directly proportional to the surface carboxyl (COOH) concentration on PS microspheres. Face Centered Central Composite Design (FCCD) under the response surface methodology (RSM) was employed to optimize the process conditions of UVO_3 treatment in order to maximize the surface COOH concentration on PS microspheres for allowing higher gelatin immobilization. Statistical analysis of results revealed that, the optimized process conditions were ozone flow rate of ~64,603 ppm, exposure time of ~60 minutes and sample amount of 5.05 g. Under these conditions, the surface COOH concentration on PS microspheres was ~1,505 nmol/g with the corresponding amount of immobilized gelatin was ~2,725 µg/g. Characterization analyses strongly suggest that the optimized UVO_3 treatment and successive gelatin immobilization have successfully improved surface wettability and dispersion stability of PS microspheres. Moreover, spinner vessel experiments demonstrated that gelatin coated PS microcarriers were able to support the growth of CHO-K1 cells to high cell density with results that were highly comparable to commercial microcarriers.

Keywords: Microcarrier, Polystyrene, Ultraviolet/ozone, Gelatin immobilization, Optimization, Cell culture

1. Introduction

Animal cell culture has emerged as one of the most important tools used in life sciences today. Techniques of cell culture are essential for studying biochemical and physiological processes, and large scale cultures of animal cells have become the preferred system for commercial production of many biological products such as recombinant proteins, monoclonal antibodies, viral vaccines and gene therapy vectors.[1-3] While some cell types such as lymphocytes can grow in suspension, there are significant number of cell lines with industrial potentials that require attachment to solid substratum for their survival and replication.[4-5] For mass production of biologics using these 'anchorage dependent' cells, an economical and efficient cultivation system with extensive surface area must be established. Several systems that were examined to fulfill such requirements include spiral films, multiple plates, hollow fiber beds, and small beads.[5] Among these, microcarrier suspension culture that was first conceived by Van Wezel in 1967, appeared to be the most successful approach.[6] Microcarriers offer extremely high surface area to volume ratio that enables anchorage dependent cells to grow to high density in suspension cultures while maintaining their normal adherent mode.[7]

The main aim of this study was to produce microcarriers with good cell adhesion and proliferation properties using polystyrene and bovine gelatin as the main materials. Polystyrene microspheres with the size of 150-200 µm were self-prepared using the convenient oil-in water (o/w) solvent evaporation method. The surface of untreated PS microspheres was functionalized by incorporating oxygen functional groups via UVO_3 treatments. The treatment was selected because it can be carried out at atmospheric pressure, does not require wet chemicals, involves simple procedures and inexpensive equipment, and produces non-polluting reaction by-products.[8,9] Subsequently, gelatin was immobilized on the surface of PS microspheres by chemical coupling to the grafted COOH groups using water soluble carbodiimide chemistry method. Surface COOH concentration on PS

microspheres was systematically optimized using response surface methodology in order to achieve high gelatin immobilization. Surface chemistry and elemental composition of PS microspheres pre- and post- UVO_3 treatment and after gelatin immobilization were evaluated using Fourier transform infrared spectrum (FTIR) analysis, scanning electron microscopy (SEM), energy dispersive x-ray spectroscopy (EDX), contact angle and zeta potential analysis. In vitro assessment of gelatin coated PS microspheres was also performed by growing CHO-K1 cells in spinner vessel experiment to determine its cytocompatibility.

2. Materials and Methods

Polystyrene (PS) microspheres were produced by oil-in-water (o/w) solvent evaporation method adapted from Zhu et al. [10]. Firstly, the oil phase was prepared by dissolving 60 g of PS in 300 mL of chloroform at room temperature. Next, the resulting clear solution was added drop-wise into 2 liters of 1% poly(vinyl alcohol) (PVA) solution (aqueous phase) at 80˚C and agitated at 330 rpm. Agitation was continued for 6 hours to completely evaporate the chloroform. Microspheres were collected by vacuum filtration, washed several times with distilled water and dried at 50°C overnight. Dried microspheres were sieved to collect between 150 and 200 µm microspheres and were later stored in a cool dry place until further use.

3. Conclusions

In this study, microcarriers with good cell adhesion and proliferation properties were successfully prepared by using optimized UVO_3 process conditions and successive gelatin immobilization. By using the second order polynomial model developed from statistical methods, maximum surface COOH concentration of ~1,505 nmol/g was achieved when ozone flow rate of ~64,603 ppm, exposure time of ~60 minutes and 5.05 g of samples were applied. As a result, high amount of gelatin were able to be immobilized on the surface of PS microspheres through cross linker reagents, EDAC/NHS. Contact angle and zeta potential measurements revealed that the surface modified PS microspheres have improved surface wettability and dispersion stability. Moreover, its cytocompatibility was clearly demonstrated by the culture of CHO-K1 cells in spinner vessel experiments with results that were highly comparable to that of the existing commercial microcarriers.

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