Materials Science and Engineering C 71 (2017) 268–278

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Extraction and encapsulation of prodigiosin in chitosan microspheres for targeted drug delivery

S.O. Dozie-Nwachukwu [a,b], Y. Danyuo [a,c], J.D. Obayemi [a,d], O.S. Odusanya [a,b], K. Malatesta [d], W.O. Soboyejo [a,d,e,*]

[a] Department of Materials Science and Engineering, African University of Science and Technology (AUST) Abuja, Federal Capital Territory, Nigeria
[b] Biotechnology and Genetic Engineering Advanced Laboratory, Sheda Science and Technology Complex (SHESTCO), P.M.B 186, Garki, Abuja, Federal Capital Territory, Nigeria
[c] Department of Materials Science and Engineering, Kwara State University, Malete, Nigeria
[d] Department of Mechanical and Aerospace Engineering, Princeton University, Princeton, NJ 08544, USA
[e] Princeton Institute of Science and Technology of Materials (PRISM), Bowen Hall, 70 Prospect Street, Princeton, NJ 08544, USA

ARTICLE INFO

Article history:
Received 12 May 2016
Received in revised form 6 September 2016
Accepted 29 September 2016
Available online 30 September 2016

Keywords:
Encapsulation
Prodigiosin
Microspheres
Drug release
Chitosan
Serratia marcescens

ABSTRACT

The encapsulation of drugs in polymeric materials has brought opportunities to the targeted delivery of chemotherapeutic agents. These polymeric delivery systems are capable of maximizing the therapeutic activity, as well as reducing the side effects of anti-cancer agents. Prodigiosin, a secondary metabolite extracted from the bacteria, *Serratia marcescens*, exhibits anti-cancer properties. Prodigiosin-loaded chitosan microspheres were prepared via water-in-oil (w/o) emulsion technique, using glutaraldehyde as a cross-linker. The morphologies of the microspheres were studied using scanning electron microscopy. The average sizes of the microspheres were between 40 μm and 60 μm, while the percentage yields ranged from 42 ± 2% to 55.5 ± 3%. The resulting encapsulation efficiencies were between 66.7 ± 3% and 90 ± 4%. The *in-vitro* drug release from the microspheres was characterized by zeroth order, first order and Higuchi and Korsmeyer-Peppas models.

1. Introduction

One of the biggest challenges of cancer therapy is the issue of non-specific anti-cancer drug delivery. It leads to the destruction of both cancerous cells and healthy cells [1]. Micro-encapsulation is a process by which small particles of solids, liquids or gases are enclosed within the thin walls of microscopic polymeric materials. Micro-encapsulation also enables the controlled release of drugs, with reduced side effects, reduced toxicity of drugs and also prevention of vaporization of many volatile drugs e.g. methyl salicylate and peppermint oil [2]. Micro-encapsulation is also used to mask the organoleptic properties of drugs, such as taste, odor or color of the substance and sometimes, to ensure safe handling of toxic materials [1–6]. Furthermore, the type of coating material used affects the properties of the microspheres. The polymer of choice should exhibit characteristics such as stability, reduced volatility, inertness with the active ingredients, and controlled release under specific conditions [7,8].

To design a drug delivery system, there is need to consider the normal physiological scavenging processes that remove small foreign objects from the blood [9]. Hence, the need for micro-encapsulation. Renal clearance can also be avoided when the nanoparticulates are larger than the glomerular pore sizes [9–10]. Thus, the circulating half-lives of nanoparticles and their associated drugs can be prolonged by controlling their sizes. Controlled release systems maintain the concentration of drug in the blood, or in target tissues over a given period of time [11,12]. This is often achieved by controlling the drug release kinetics [13]. Generally, the initial drug release rate is rapid, in order to achieve the effective therapeutic concentration of the drug. Then, the drug release kinetics follows a well-defined behavior, in order to supply the maintenance dose, thus enabling the attainment of the desired drug concentration [14].

Chitosan is a linear polysaccharide that consists of β-(1–4)-linked D-glucosamine and *N*-acetyl-D-glucosamine. It is produced by alkaline deacetylation of chitin, which is the main component of the exoskeleton of crustaceans, such as shrimps, crabs, and crawfish [15]. Chitin is the second most abundant natural polymer, after cellulose [16,17]. The properties of chitosan depend on the degree of de-acetylation [18]. The degree of de-acetylation (DDA) of chitosan influences its physicochemical characteristics [18], and its biodegradability [19] and immunological activity [20]. Furthermore, chitosan is useful in medicine due to its biocompatibility [21], biodegradability [19,22] and low toxicity [23]. It enhances wound healing and exhibits other biological activities such as anti-microbial properties and the reduction of cholesterol level and antimicrobial properties [24,25]. The positive charge on chitosan, generated under physiological conditions, has been found to be responsible for

* Corresponding author.
E-mail address: soboyejo@princeton.edu (W.O. Soboyejo).

http://dx.doi.org/10.1016/j.msec.2016.09.078
0928-4931/

its enhanced bioadhesion to negatively charged cell membranes. This enables the site-specific applications in controlled delivery systems [26–28].

In forming the microspheres, chitosan is often used, along with glutaraldehyde, as a cross linker. The reaction of chitosan with aldehyde groups often forms covalent imine bonds with the amino groups of chitosan. This is due to the resonance established with adjacent double ethylenic bonds [29] via a Schiff reaction. Covalent crosslinking leads to the formation of a permanent network, allowing for the free diffusion of water and enhancing the mechanical properties of the microspheres. Hence, the structure and mechanical properties of chitosan are in a range that is suitable for the controlled release of chemotherapeutic agents [30] within the therapeutic window.

Prodigiosin, a secondary metabolite, can be produced by the bacteria, *Serratia marcescens* (SM) and other bacteria such as *Zooshikella rubidus*, *Vibrio* sp., *Streptomyces griseoviridis*, and *Hahella chejuensis* [31–38]. It is a natural compound with a broad range of cytotoxic, anti-fungal, anti-bacterial, algicidal, anti-protozoal, anti-malarial, immunosuppressive, anti-cancer and anti-proliferative activities [39–41]. Prodigiosin has been reported to effectively induce apoptosis in hematopoietic cancer cells, as well as cells derived from other human cancers (e.g. gastric and colon cancers), with no marked toxicity in nonmalignant cell lines [42–44]. Furthermore, Francisco and co-workers [45] have reported the effects of PG on different human neuroblastoma cell lines (i.e. SH-SY5Y, LAN-1, IMR-32 (N-type) and SK-N-AS (S-type). Our interest is in its anti-cancer property.

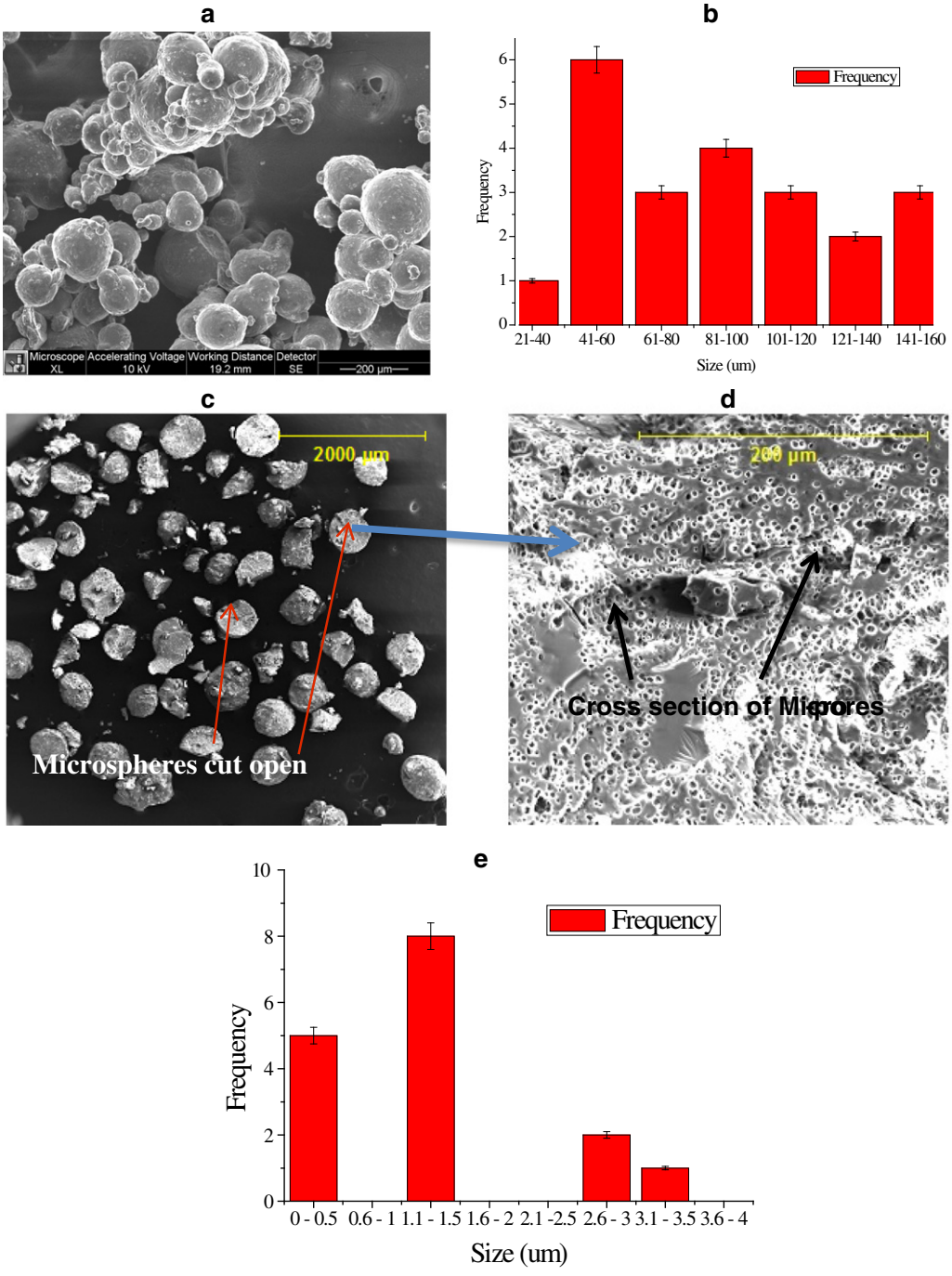

Fig. 1. SEM images of (a) spherical shaped chitosan microspheres, (b) size distribution of chitosan microspheres, (c) microspheres cut open, (d) enlarged micropores within the microspheres, and (e) histogram of size distribution for the micro-pores.