Materials Science and Engineering C 48 (2015) 391–399

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Influence of excipients on characteristics and release profiles of poly(ε-caprolactone) microspheres containing immunoglobulin G

Özge Erdemli [a,1], Dilek Keskin [a,b], Ayşen Tezcaner [a,b,*]

[a] Department of Engineering Sciences, Middle East Technical University, Ankara, Turkey
[b] Biomaterials and Tissue Engineering Center of Excellence, Middle East Technical University, Ankara, Turkey

ARTICLE INFO

Article history:
Received 21 July 2014
Received in revised form 14 October 2014
Accepted 9 December 2014
Available online 10 December 2014

Keywords:
Protein delivery
Microspheres
Polymeric drug delivery
Excipients
γ-Sterilization
In vitro release

ABSTRACT

Protein instability during microencapsulation has been one of the major drawbacks of protein delivery systems. In this study, the effects of various excipients (poly vinyl alcohol, glucose, starch, heparin) on the stability of encapsulated human immunoglobulin G (IgG) in poly(ε-caprolactone) (PCL) microspheres and on microsphere characteristics were investigated before and after γ-sterilization. Microspheres formulated without any excipients and with glucose had a mean particle size around 3–4 μm whereas the mean particle sizes of other microspheres were around 5–6 μm. Use of PVA significantly increased the IgG-loading and encapsulation efficiency of microspheres. After γ-irradiation, IgG stability was mostly maintained in the microspheres with excipients compared to microspheres without any excipients. According to the μBCA results, microspheres without any excipient showed a high initial burst release as well as a fast release profile among all groups. Presence of PVA decreased the loss in the activity of IgG released before (completely retained after 6 h and 15.69% loss after 7 days) and after γ-irradiation (26.04% loss and 52.39% loss after 6 h and 7 days, respectively). The stabilization effect of PVA on the retention of the activity of released IgG was found more efficient compared to other groups formulated with carbohydrates.

1. Introduction

Monoclonal antibodies (mAbs) comprise an important class of therapeutic proteins used in the treatment of a wide variety of diseases, including leukemia and many other types of cancer, asthma, inflammation, arthritis, psoriasis, transplant rejection, neuroimmunological diseases and cardiovascular diseases [1–5]. All of the approved therapeutic mAbs belong to the γ-immunoglobulin or immunoglobulin G (IgG) family [6] and there are currently more than 25 approved mAbs worldwide [7]. As in many protein based drugs, there are several problems associated with the applications of the therapeutic mAbs; among them their inherent physical and chemical instability and the need of frequent administrations due to their short biological half-lives are considered as the most important ones [8–10]. Besides these, production of protein drugs is very costly and multiple and high-dose injections further increase the cost of treatment. Because of these facts, studies related with the development and optimization of various drug delivery systems are needed to improve the potential for clinical use of mAbs.

Using biodegradable microspheres as carriers for therapeutic protein and peptides is one of the most widely studied approaches in delivering them to the target site in a controlled release fashion [11]. Other advantages of these systems are easy injection and providing high bioavailability [11]. One of the most widely used techniques for formulating protein containing polymeric microspheres is the water/oil/water (w/o/w) double emulsion method because of its relatively simple process and suitability in controlling process parameters [12].

Poly(ε-caprolactone) (PCL) has been a major area of interest to develop controlled delivery systems for peptides and proteins due to its biodegradability and biocompatibility [13–15]. PCL is a highly biocompatible polymer and there exists PCL based drug delivery devices that were approved by the U.S. Food and Drug Administration (FDA) [14,16]. There are many studies related with drug loaded PCL microspheres [13,14,16].

Proteins are large macromolecules and their activity depends on their secondary, tertiary and even quaternary structures. The protein structure is sensitive to external conditions such as pH, temperature and surface interaction. Therefore, the stability and biological activity of the encapsulated protein should be maintained during the preparation and handling process of protein loaded microspheres. In double-emulsion solvent evaporation method, encapsulated proteins may undergo aggregation and lose their biological activity due to the harsh processing conditions. The presence of water/organic solvent interfaces causes protein denaturation and aggregation. Besides these, proteins

* Corresponding author at: Middle East Technical University, Department of Engineering Sciences, 06800 Ankara, Turkey.
E-mail address: tezcaner@metu.edu.tr (A. Tezcaner).
[1] Present address: Laboratoire de Chimie Physique et Microbiologie pour l'Environnement CNRS UMR7564, France.

http://dx.doi.org/10.1016/j.msec.2014.12.044
0928-4931/

can be adsorbed to the hydrophobic polymer and the shear stress used for the formation of the emulsion may result in proteins unfolding [17]. In addition to the microencapsulation methods, sterilization of the protein loaded microspheres is also critical for preserving the protein structure and bioactivity. Among the different sterilization procedures, γ-irradiation has been widely used for sterilization of the biodegradable microspheres [18–20]. However, γ-irradiation can induce denaturation and degradation that affect the integrity and bioactivity of the proteins [21–23]. Considering all, it is important to preserve protein structure and bioactivity during microencapsulation and γ-sterilization to obtain a functional treatment system.

The use of excipients during microencapsulation is the most common strategy to prevent aggregation and to stabilize the folded protein structure. Many excipients such as carbohydrates in the form of sugars [11,24], starch [25] and heparin [24,26] and water soluble polymers including poloxamers [24] and poly(vinyl alcohol) (PVA) [11,27,28] have been used in the formulations to increase the stability of proteins through various mechanisms. Carbohydrates could efficiently maintain the stability of proteins by forming a hydration layer around the protein which means that more water molecules are found on the surface of the protein than in the bulk [29]. Some other excipients such as PVA could protect protein stability competitive adsorption on the oil–water interface.

Research on development of drug delivery systems has gained increasing attention in recent years. The main concern in this field is the retention of the stability of protein drugs during microencapsulation and release. Excipients play an important role for this end. In this study, effects of various excipients on the stability of IgG encapsulated in PCL microspheres were investigated. In addition, the effect of the excipients on the stability of encapsulated IgG during γ-irradiation and the changes in the release patterns after γ-irradiation were investigated. For this reason, the encapsulation efficiencies and in vitro release profiles of IgG, a model therapeutic mAb, were compared before and after γ-irradiation of microspheres. Protein stability was evaluated by using Fourier transform infrared (FT-IR) spectroscopy for encapsulated IgG and retained activity during release using enzyme-linked immunosorbent assay (ELISA).

2. Materials and methods

2.1. Materials

Poly(ε-caprolactone) (M_w 65,000, Aldrich, Germany), human immunoglobulin G (IgG, Sigma, USA), poly(vinyl alcohol) (PVA, Mowiol® 4-98, Aldrich, Germany), D (+) glucose (Merck KGaA, Germany), starch (Merck KGaA, Germany), heparin sodium (Neuparin, Mustafa Nevzat, Turkey), dichloromethane (DCM, Merck KGaA, Germany), Tween 20 (Sigma, Germany), sodium azide (Sigma, Germany), sodium dodecyl sulfate (SDS, Bio-Rad, USA), and bicinchoninic acid (BCA) reagent (Sigma, Germany) were used as received. Human IgG enzyme-linked immunosorbent assay (ELISA) was purchased from RayBiotech (USA). Hydrophilic polyvinylidene difluoride (PVDF) membrane was obtained from Millipore (Ireland). All other chemicals were of analytical grade and were used as received.

2.2. Preparation of immunoglobulin G loaded PCL microspheres

IgG and different excipient loaded PCL microspheres were prepared by the double emulsion-solvent evaporation method used in our previous studies [30,31]. Briefly, 100 μL of the internal aqueous solutions (phosphate buffered saline (PBS), 10 mM sodium phosphate, 145 mM NaCl, pH 7.2) containing 10 mg/mL IgG and different excipients such as PVA (Group P), glucose (Group G), starch (Group S) and heparin (Group H) with known concentrations (Table 1) were added to 2 mL of PCL (5%, w/v) solution in DCM. The mixture was sonicated for 60 s on ice using a sonicator (Sonorex, Bandelin, Germany). The resulting primary water-in-oil (w/o) emulsion was added to an external 40 mL PVA solution (1%, w/v) and stirred with a magnetic stirrer (Schott, Australia) at 1100 rpm for 15 min. The double emulsion (w/o/w) was poured into 180 mL of PVA solution and stirred at 14,000 rpm for 3 min in an ice bath with a homogenizer (Ultraturrax T-25, IKA, Germany). After this homogenization step, emulsion was stirred at 1100 rpm for 3 h to evaporate dichloromethane. Finally, formed microspheres were collected by filtration through a 0.45 μm hydrophilic PVDF membrane, washed with distilled water and stored at 4 °C. As a control group, IgG loaded microspheres formulated without any excipients (Group E) in the internal aqueous phase were also prepared by using the same procedure.

Table 1
Particle sizes at 10%, 50%, and 90% percentiles and SPAN values of IgG loaded PCL microspheres formulated with different excipients.

Formulation code	Internal aqueous phase	d[0.1] (μm)	d[0.5] (μm)	d[0.9] (μm)	SPAN
Non-irradiated					
E	Only PBS	1.34	3.01	5.74	1.46
G	Glucose (2.5% w/v) in PBS	1.07	3.44	6.09	1.46
S	Starch (2.5% w/v) in PBS	1.71	5.29	9.96	1.56
H	Heparin (2.5% w/v) in PBS	1.57	4.83	9.81	1.71
P	Poly(vinyl alcohol) (2.5% w/v) in PBS	2.73	5.36	9.63	1.29
γ-Irradiated					
E	Only PBS	1.46	3.01	5.70	1.41
G	Glucose (2.5% w/v) in PBS	1.13	3.45	6.10	1.44
S	Starch (2.5% w/v) in PBS	1.69	5.20	9.95	1.59
H	Heparin (2.5% w/v) in PBS	2.14	4.70	9.88	1.65
P	Poly(vinyl alcohol) (2.5% w/v) in PBS	2.86	5.06	9.33	1.28

Microspheres were sterilized by exposure to γ-irradiation (25 kGy) from ^{60}Co γ-source (Gamma-cell 220, MDS Nordion, Canada) at ambient temperature and fixed dose rate (1.74 kGy/h) in the Turkish Atomic Energy Authority [18,30,31].

2.3. Surface morphology and particle size

The surface morphology of γ-irradiated and non-irradiated microspheres formulated with different excipients was examined by scanning electron microscopy (SEM, JSM-6400, JEOL, Japan). Prior to the examination, microspheres were mounted onto metal stubs using carbon tape and coated with gold by using sputter coating device (Hummel VII, Anatech Electronics, USA).

The mean particle size and particle size distribution of γ-irradiated and non-irradiated microspheres formulated with different excipients were determined from SEM images by measuring the diameters of 500 microspheres for each group using ImageJ analysis software (NIH, USA). The resulting microsphere size distribution was plotted as a histogram with an equal number of bins between the largest and smallest values and as a total cumulative percent arithmetic curve. Measure of the width of the distribution of particle size (SPAN) values of microspheres was obtained from cumulative (% undersize) microsphere size distribution curves by using Eq. (1):

$$\mathrm{SPAN} = (d[0.9] - d[0.1])/d[0.5] \quad (1)$$

where d[0.9], d[0.5], and d[0.1] are the particle diameters determined at the 90th, 50th, and 10th percentiles of undersized particles, respectively.

2.4. Protein loading and encapsulation efficiency

The encapsulation efficiency of microspheres formulated with different excipients was determined by using hydrolysis of the microspheres with a strong base and the extraction of the protein with SDS as described in detail in our previous study [30]. The amount of IgG