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Engineering of HBsAg Antigen Containing Poly (ε- Caprolactone) Microspheres to Tailor Particle Size

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ABSTRACT

The aim of this work was to optimize particle size of Hepatitis B Surface (HBsAg) antigen containing poly(ε-caprolactone) (PCL) microspheres using the water-in-oil-in water solvent evaporation technique. Effects of manufacturing and formulation variables on particle size were investigated. It was observed that microsphere size could be controlled by modification of PCL concentration in the organic phase, polyvinyl alcohol (PVA) concentration in the outer aqueous phase, HBsAg concentration in inner aqueous phase, organic phase volume, and sonication time for primary w/o emulsion preparation. The optimized conditions yielded microspheres with an average particle size of 7.34 µm. Such biodegradable PCL microspheres appear suitable for the use as vaccine adjuvant.

Keywords: Microspheres; HBsAg; Poly(ε-caprolactone); Polyvinyl alcohol

1. INTRODUCTION

Hepatitis B caused by hepatitis B virus (HBV), is one of the most widespread infectious disease and a major public health concern in the world. Since 1991, World Health Organization has recommended that a hepatitis B vaccine be included in routine immunization schedule. The biodegradable and biocompatible polymers are the primary candidates for the development of microparticles as vaccines adjuvant.[1, 2] The particle size is an important factor affecting uptake of microsphere by antigen presenting cells (APC) and their immunogenicity. Microspheres of particle size below 10 µm were found more immunogenic than larger microspheres since these were up taken by APC.[2, 3] Thus the control of particle size is important. Each method of microsphere preparation involves a number of process variables that can be modulated to achieve objective of particle size (less than 10 µm). In the present investigation, HBsAg containing PCL microspheres were prepared by a W/O/W double emulsion and effect of various process and formulation variables on particle size were investigated [4]. The size optimized method was used to prepare final PCL microsphere entrapping HBsAg antigen.

Materials

Poly (ε-caprolactone) (MW 65,000 and 10,000) samples were procured from Sigma Aldrich USA. HBsAg (2.24mg/ml) was generous gift sample from Dr. K.S. Jaganathan, Shantha Biotech Ltd, Hyderabad India. The BCA kit (KT-31) was purchased from Genei (Bangalore, India). All other chemicals and reagents employed were of analytical grade.

Preparation of PCL microspheres

Several batches of HBsAg containing PCL microspheres were prepared by double emulsification solvent evaporation technique.[7] and process parameters were varied. Firstly the oil phase was prepared by dissolving PCL dissolved in 10 ml of methylene chloride. 1 ml of aqueous phase comprising of HBsAg antigen in phosphate buffer saline (pH 7.4) was added to 10 ml of methylene chloride solution and the mixture was sonicated for 10sec using a 250-W probe-type sonicator (Soniweild India) to prepare a primary (w/o) emulsion. The resulting w/o emulsion was then again emulsified with 50 ml of PVA solution in PBS by sonication for 10 sec. The resulting w/o/w emulsion was subjected to mechanical stirring overnight (Remi motors) at room temperature. The solidified microspheres were harvested by centrifugation at 10000 rpm for 10 min. Finally, the microspheres produced were collected by centrifugation, washed with distilled water and freeze-dried to obtain free flowing powder-like PCL microspheres.

Study Design for Optimization of Process Parameters

The processing factors such as PCL concentration in the organic phase, polyvinyl alcohol (PVA) concentration in the outer aqueous phase, HBsAg concentration in inner aqueous phase. organic phase volume, sonication time for primary w/o emulsion preparation were varied and all trials were run in triplicate.

Batches were prepared to optimize process parameters for preparation of PCL microspheres, according to a 3^1 factorial design i.e. 1 independent variable (volume of internal phase of primary emulsion/ or volume of external phase of secondary emulsion or PLGA concentration /or PVA concentration) and 3 levels of study [4, 5& 6].

Particle Size Analysis

Each batch was evaluated for microsphere size. The microspheres were sized by laser diffraction using a Particle Size Analyzer CILAS 1064 (CILAS Instruments France).[8] The average particle size and standard deviation was expressed as the volume mean diameterat 90% from 3 different microsphere batches.

3. RESULTS AND DISCUSSION

Physical characteristics of microspheres such as morphology and size distribution, influence antigen release kinetics, microsphere injectibility and interaction with antigen presenting cells affect vaccine formulation. It has been shown that smaller particles (typically below 10 µm) are more readily taken up by phagocytic antigen presenting cells than larger particles (typically above 10 µm).[9] Therefore the aim was to optimize method for preparation of microspheres sized below 10 µm. Effect of manufacturing and formulation variables on the particle size were investigated The influence of these parameters on the microsphere size is here as under.

a. Influence of PCL concentration in the organic phase on microsphere size

It was observed that the size of microspheres enlarged from 8.54 µm to 13.71 µm, with an increase in PCL concentration from 100 mg to 500 mg in the organic phase (Figure 1). These results are in accordance with earlier findings using a similar method. This was anticipated also because higher the polymer concentration in the organic phase in primary emulsion, higher will be the viscosity of the organic phase, thus the force that need to be overcome will also be higher.

b. Influence of PVA concentration on microsphere size

When the concentration was varied from 1% w/v to 8% w/v, the microsphere size was reduced from 12.54 to 6.21 µm (Figure 2). Since with 5% w/v PVA in the outer aqueous phase, the desired size of microspheres (size 9.93) was found, 5% w/v PVA kept constant for further optimization studies.

c. Influence of HBsAg concentration in aqueous phase of primary emulsion on Average Diameter

As the HBsAg concentration was increased from 0.5% w/v to 5% w/v, the average diameter of the microspheres increased from 7.54 µm to 38.46 µm. Further HBsAg concentration increase showed no significant effects (Figure 3).

d. Influence of organic phase volume on Average Diameter

The effect of organic phase volume was studied by keeping the amount of PCL concentration constant, HBsAg concentration and other parameters constant. The size of microspheres increased from 8.33 µm to 14.37 µm as the organic phase volume decreased from 3 ml to 0.5 ml (Figure 4). This may be due to decrease in viscosity of organic phase due to increase in organic phase volume.

e. Influence of inner aqueous phase volume on Average Diameter

As the inner water phase volume was increased from 0.5 ml to 3 ml, microsphere size increased from 8.13 µm to 13.93 µm.

However, further increasing the inner water phase volume showed only negligible size changes (Figure 5).

f. Effect of sonication time in preparation of primary w/o emulsion

The desired size was obtained when sonication time was 10sec. The final optimized method of fabricating microspheres is shown in Table 1. Microspheres prepared with final optimized parameters were of 7.34 µm size.

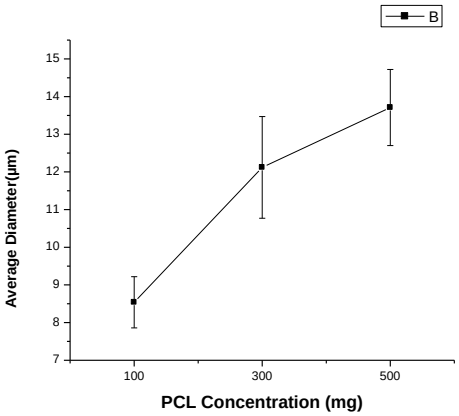

Fig 1: Influence of PCL concentration in the organic phase on microsphere size

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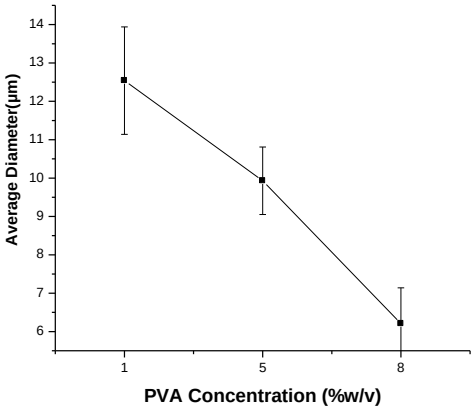

Fig 2: Influence of PVA concentration on microsphere size

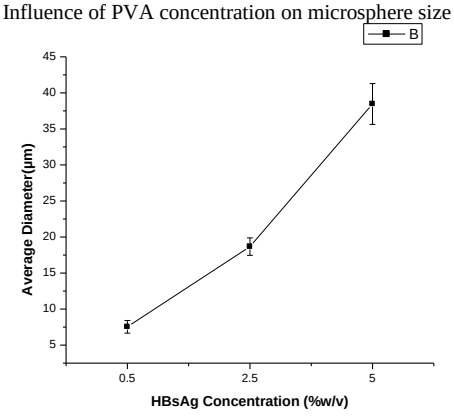

Fig 3: Influence of HBsAg concentration in aqueous phase of primary emulsion on average size

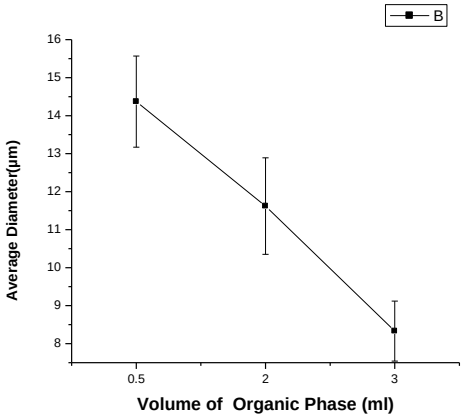

Fig 4: Influence of organic phase volume on Average Diameter

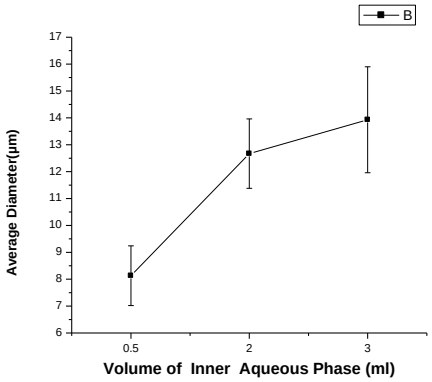

Fig 5: Influence of inner aqueous phase volume on Average Diameter

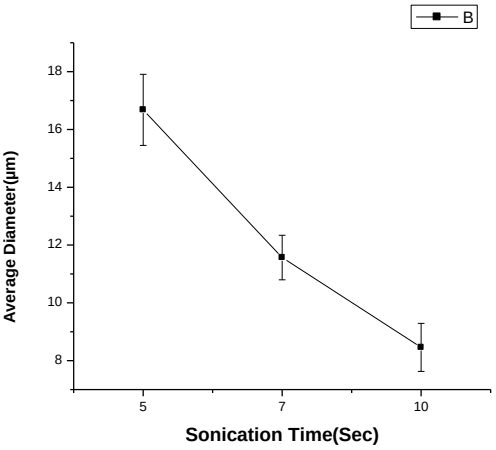

Fig 6: Effect of sonication time in preparation of primary w/o emulsion

Table 1: Final optimized process parameters

S. No.	Parameters	Value
1.	PCL concentration	3.5% w/v
2.	Organic phase volume	3 ml
3.	HBsAg concentration	0.5% w/v
4.	Inner aqueous phase volume	0.5 ml
5.	Sonication time	10 sec
6.	PVA concentration	5% w/v

4. CONCLUSION

This work has dealt with the optimization of the preparation of microparticles based on poly (ε-caprolactone) using a (water-in-oil)-in water emulsion solvent evaporation method. These investigations have provided an understanding of the influence of some process parameters which exhibit a strong influence on particle size.

5. ACKNOWLEDGEMENT

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