Chapter 4

PREPARATION OF THE FORMULATIONS

4.1 Introduction

A wide range of micro-encapsulation techniques have been developed to date. The selection of the microencapsulation technique depends on the nature of the polymer, the drug and the intended use. When preparing controlled release formulations, the choice of optimal method is most important for the efficient entrapment of the active substance.

The microspheres prepared for the present investigation are intended to be used for intra-articular injection. The desirable properties of the microspheres are the high drug entrapment efficiency, high drug loading and smaller particle size. So the method employed should be able to produce the microspheres having all these properties. In the present investigation, microspheres of three different hydrophilic polymers gelatin, chitosan and bovine serum albumin were prepared using three different anti-arthritic drugs celecoxib, rofecoxib and valdecoxib. Solid lipid nanoparticles of celecoxib were prepared by melt homogenization technique.

4.2 Experimental

4.2.1 Materials

Gelatin USP was a gift sample from Sterling gelatin limited, Vadodara. Chitosan (MW 6.524×10^5, degree of deacetylation 81.5%) was a gift sample from Central fisheries Technology limited, Cochin. Bovine serum albumin was purchased from Loba chemie limited. Formaldehyde was purchased from s.d.fine chem. limited, Boisar, glutaraldehyde was purchased from E.Merck India limited. Tween-80 was purchased from S.D.fine chem. Ltd, Mumbai. Celecoxib was gifted by Sun pharmaceutical Advanced Research centre, Vadodara. Rofecoxib was gifted by Torrent Pharmaceuticals Limited, Ahmedabad, Valdecoxib was gifted by Lyka laboratories limited, Ankleshwar. Compritol 888 ATO(Glycerol Behenate) was gifted by Gattefosse (St Priest, Cedex, France); this material has a melt point of 72°C and is a mixture of 12% to

18% mono-, 52% to 54% di-, and 28% to 32% triglycerides of behenic acid (more than 87% of the fatty acid fraction) Poloxamer 407 was obtained from BASF AG (Ludwig-shafen, Germany)

4.2.2 Equipments

Remi twin blade stirrer, magnetic stirrer with heater, Ultrasonicator, High pressure homogenizer (Avestin Canada), Vacuum filtration assembly, membrane filters, Ultracentrifuge.

4.2.3 Preparation of gelatin microspheres by emulsification solvent extraction method

The microspheres were prepared by slight modification of the method reported by previous workers (Raymond et al, 1990) Weighed amount of gelatin was dissolved in distilled water at 55°C. Tween-80 was added and mixed properly by stirring on a magnetic stirrer. The drug was finely triturated and passed through the sieve no 400. Weighed amount of the drug was then added to the gelatin solution and sonicated to obtain a uniform dispersion. One ml of this dispersion was injected to a mixture of liquid paraffin, span-85 and aluminium tristearate, while stirring at a definite stirring speed Stirring was continued for a definite interval of time and then Isopropyl alcohol was added while stirring to extract water from the internal phase of the emulsion. The formed microspheres were then separated by membrane filtration and washed with petroleum ether to remove the residual liquid paraffin. Each batch was prepared in triplicate.

4.2.4 Preparation of gelatin microspheres by emulsification chemical cross-linking method

In this method, weighed amount of gelatin was dissolved in distilled water at 55°C. Tween-80 and poly-ethylene glycol-400 were added and stirred properly to get a uniform mixture. The drug was finely triturated in a mortar and passed through sieve no 400. Weighed amount of the drug was then added to the above solution and sonicated to obtain a uniform dispersion. One ml of this dispersion was injected to a mixture of 20 ml liquid paraffin and span-85 maintained at 55°C while stirring at 2500 rpm. Cross-linking of the polymer present in the internal phase of the emulsion was done by adding either glutaraldehyde or formaldehyde. Stirring was continued for a definite interval of time and then the formed microspheres were separated by centrifugation and washed with petroleum ether to remove the residual liquid paraffin. 10 ml of 5% sodium bisulphite was then added and stirred on the magnetic stirrer for 10 minutes to remove the residual crosslinking agent. Finally the microspheres were washed with water and dried at room temperature The free drug was removed from the formulation by centrifugation at 5000 rpm at which the microspheres sediments leaving the free drug in the supernatant. Each batch was prepared in triplicate.

4.2.5 Preparation of chitosan microspheres using emulsification cross-linking method

Chitosan microspheres were prepared using emulsion cross linking technique (Wang et al, 1996). Weighed amount of chitosan was dissolved in 2%w/v acetic acid to give a 3%w/w solution. Tween-80 was added at a concentration of 2%w/w. The drug was finely triturated in a mortar and was sieved through sieve no. 400 10 mg of finely powdered drug was added to the above solution and sonicated to obtain a uniform dispersion. One ml of this dispersion was injected to a mixture of 20 ml heavy liquid paraffin and a designated amount of span-85 while stirring at 2500 RPM. Stirring was continued for 10 minutes to obtain a w/o emulsion. The chitosan in the internal phase of

the w/o emulsion was cross-linked using either i) chemical treatment or by ii) Heat treatment as explained below:

1) To produce chemically cross-linked microspheres glutaraldehyde or formaldehyde were used. Cross linking of the chitosan present in the internal phase of the w/o emulsion was done by adding a designated volume(0.5 ml or 1.0 ml) of glutaraldehyde(25%w/w aqueous solution) or formaldehyde(37%w/w aqueous solution). Stirring was continued for a definite interval of time (1 hour or 3 hours) and then the microspheres formed were separated by centrifugation and washed with petroleum ether to remove the liquid paraffin. The microspheres were then suspended in 5%w/v sodium bisulphite solution and stirred on a magnetic stirrer for 10 minutes followed by washing with water to render them free from residual glutaraldehyde. Finally the microspheres were dried at room temperature and stored in a desiccator till further use.

2) To produce heat cross-linked microspheres, the temperature of the emulsion was raised gradually while stirring at 2500 RPM to either 70°C or 90°C. Stirring was continued till the emulsion attained room temperature. Microspheres formed were then separated by centrifugation, washed with petroleum ether to remove the liquid paraffin and air dried. Each batch was prepared in triplicate.

4.2.6 Preparation of albumin microspheres using thermal denaturation method

The microspheres were prepared by a modification of the method reported by previous workers (Filipovic et al, 1993)

In this method, weighed amount of bovine serum albumin was dissolved in distilled water. Tween-80 was added to the solution and stirred properly for uniform mixing. Weighed amount of the drug, finely triturated and passed through sieve no 400 was added to the above solution and sonicated to obtain a uniform dispersion. One ml of this

dispersion was added to a mixture of 20 ml liquid paraffin and span-85 while stirring at 2500 rpm. Stirring was continued for a definite interval of time and then the temperature of the emulsion was raised to 100°C. This temperature was maintained for 10 minutes and then the temperature of the emulsion was brought down to room temperature while stirring. The formed microspheres were separated by membrane filtration and washed with petroleum ether to remove the residual liquid paraffin and air dried. Each batch was prepared in triplicate.

4.2.7 Preparation of albumin microspheres using emulsification chemical cross-linking method

In this method, weighed amount of bovine serum albumin was dissolved in distilled water. Tween-80 was added to the solution and stirred properly for uniform mixing. Weighed amount of the drug, finely triturated and passed through sieve no 400 was added to the above solution and sonicated to obtain a uniform dispersion. One ml of this dispersion was added to a mixture of 20 ml liquid paraffin and span-85 while stirring at 2500 rpm. Stirring was continued for a definite interval of time. Cross-linking of the albumin present in the internal phase of the emulsion was carried out by adding either glutaraldehyde or formaldehyde. Stirring was continued for a definite interval of time and the formed microspheres were separated by centrifugation and washed with petroleum ether to remove the residual liquid paraffin. The residual cross-linking agent was removed by reaction with sodium bisulphite and finally washed with water and dried at room temperature. Each batch was prepared in triplicate.

4.2.8 Preparation of solid lipid nanoparticles of celecoxib

Solid lipid nanoparticles of celecoxib were prepared by hot melt homogenization technique (Utreja and Jain, 2001). The lipid glycerol behenate was heated to 95°C. Weighed amount of celecoxib was dissolved in the molten lipid. This solution was then dispersed in hot aqueous solution containing poloxamer while stirring at 4000rpm. The pre-mix formed is then passed through a thermostated high pressure homogenizer. Then the hot dispersion was cooled to room temperature. The SLN dispersion was lyophilized using sucrose as a cryoprotectant. Each batch was prepared in triplicate.

4.3 References

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