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INVESTIGATION OF EFFECTIVE PARAMETERS IN INTELLIGENCE DRUG DELIVERY SYSTEMS PROCESS WITH MICRO ENCAPSULATION METHOD

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ABSTRACT

In this paper, lysozyme as the model peptide was incorporated into poly (d,l-lactictide-coglycolide) or dl-plg microspheres using double emulsion solvent evaporation method in bench scale, has been investigated. The inner aqueous phase volume used for dissolving the model peptide, lysozyme, was 100 μL and the total volume of the double emulsion was about 2 ml. The concentration of poly (d,l-lactictide-co-glycolide) or dl-plg in the middle phase of the water-in-oil-in-water or (w/o/w) emulsion was increased from 4.5% of (wate-in- water) to 36% of (water-in-water). Different compositions of the organic phase were employed. The concentration of lysozyme within the inner water phase was increased in four steps from 1.25% (water-in-water) to 32% (water-in-water). In this research we attempted to evaluate the effect of changing the process parameters in order to achieve the maximum degree of retained biological activity (RBA), a high degree of entrapment efficiency (EE) during the preparation process and then compared with previous similar experimental works, in which a good agreement result is achieved.

Key words: Effective parameters, Intelligence drug delivery, Micro encapsulation method

INTRODUCTION

Interest in the advantages of using controlled drug delivery systems is increasing in modern biomedical and chemical engineering development. The advantages of these systems include-

(i) Location of the drug at the site of action, e.g. in antitumour therapy (Bastian et al., 1998).

(ii) Prolongation of drug release, e.g. in hormone therapy (Zolatex et al., 1998). Incorporation of sensitive drugs such as peptides, a protein, into polymeric microparticles to protect from chemical or enzymatic degradation (Vanberver et al.,

1999 and Winters et al., 1996).

(iii) Increasing the immunogenic response of antigens entrapped in oral microsphere formulations; the particulate nature of these formulations can markedly increase the immunogenic response compared with that to solutions of free antigens (Morris et al., 1994).

In the development of drug delivery systems, biodegradable polymer microspheres are frequently considered as drug carriers for future medical products. There are two main reasons for this, firstly the material is biocompatible and degrades by forming the nontoxic monomers, lactic acid and glycolic acid and secondly, the release rate of the entrapped drugs can be controlled by varying the molecular weight and the copolymer ratio. Furthermore, administration of the drug by injection is possible, if it is dispersed into microsphere. Biodegradable microparticles can be prepared by various methods but perhaps the most thoroughly investigated methods are the single and double emulsion evaporation methods. Drug-loaded microparticles have recently been produced using spray drying technology. This method offers the advantage of being a one-step process, and appears to be superior to other conventional incorporation methods such as emulsion evaporation methods.

Methods of micro encapsulation

A wide range of microencapsulation techniques have been developed. The selection of a particular technique depends on the nature of the polymer and the drug to be incorporated.

Single emulsion-solvent evaporation method

Microencapsulation of the polymer can be prepared using either oil-in-water (o/w) or oil-in-oil (o/o) emulsion systems. In the o/w emulsion technique, the polymer is dissolved in an organic solvent (the oil phase) such as methylene chloride or chloroform. The drug is either dissolved or suspended in this solution, which is then emulsified by adding a larger volume of water containing a suitable emulsifier. The organic solvent is then removed by evaporation or extraction, resulting in phase separation of the polymer and the drug to produce solid microparticles suspended in an aqueous phase. A flow diagram for this technique is shown in Fig. 1. This technique has been used for the incorporation of various steroids by many research groups (Eavalier et al., 1986). However the entrapment efficiency (EE) of hydrophilic drugs is poor using this technique, since they partition out from the organic phase into the aqueous continuous phase.

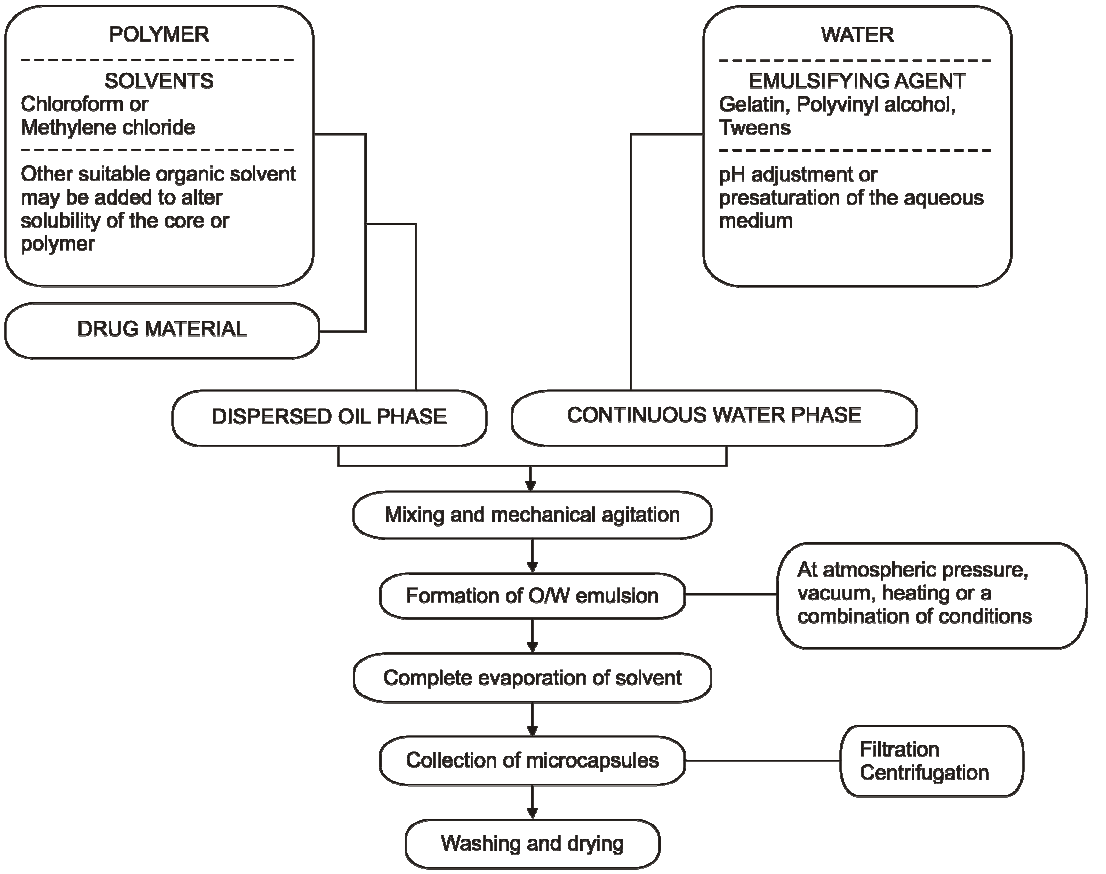

Fig. 1: Schematic diagram of the preparation of drug loaded microparticles by the o/w single emulsion-solvent evaporation method

Oil-in-oil emulsion systems can be used to improve the loading of water-soluble drugs into polymers. In these systems, the polymer is dissolved in acetonitrile (the dispersed phase), which is subsequently emulsified with liquid paraffin (continuous phase) containing an oil soluble emulsifier such as a sorbitan ester. Removal of the volatile solvent (acetonitrile) by heat result in co-precipitation of the polymer and drug to form drug-loaded microparticles. A schematic diagram of this method is shown in fig. 2 The properties of microsphere can be affected by changing various processing parameters, such as emulsifier concentration, emulsifier type, polymer concentration, viscosity, stirring rate and solvent evaporation rate. If polymers of high molecular weights are used, precipitation will be rapid and the resultant microsphere will be porous. The more hydrophilic polymers result is smoother and less porous microspheres (Nixon et al., 1990).

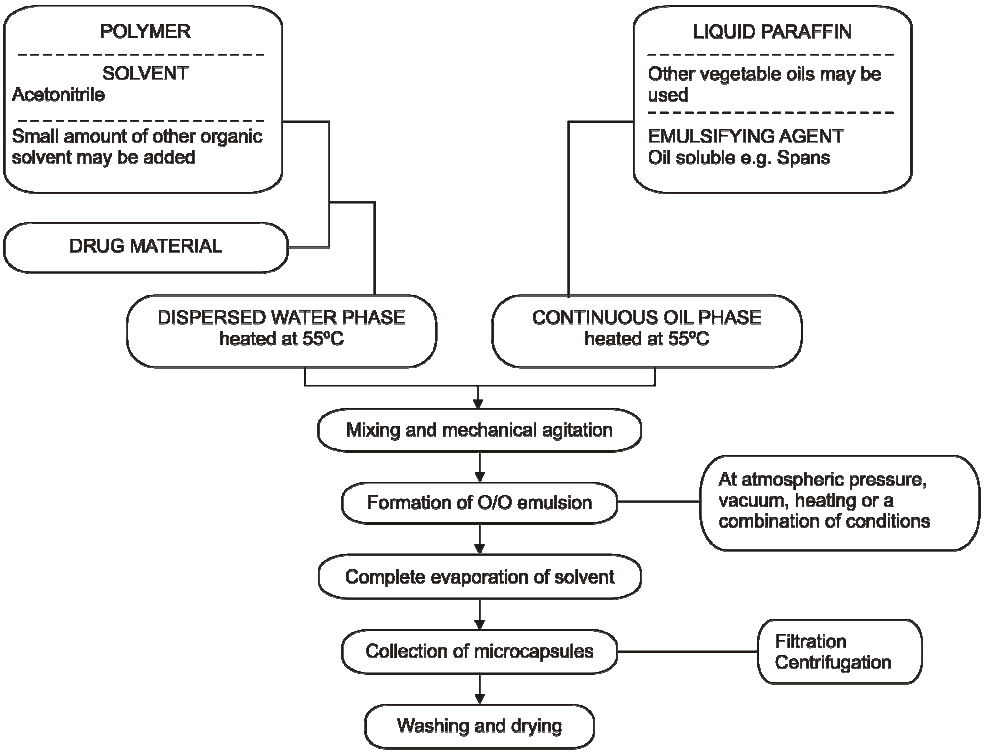

Fig. 2: Schematic diagram of the preparation of drug loaded microparticles by the o/o single emulsion-solvent evaporation method

Double emulsion - solvent evaporation method

Sensitive water-soluble drugs such as proteins can be incorporated into microspheres using an alternative method i.e. double emulsion method. This method decreases contact between the organic phase and the active substance and improves the entrapment efficiency (EE) of water soluble drugs. This preparation method could be also used for incorporation of exclusive substances since it may be performed in a small scale (Sturesson, et al., 1999). Briefly, the water soluble drug is initially dissolved in a small aqueous aliquot, which is subsequently emulsified with the organic polymer solution to form a water-in-oil emulsion. In a second emulsification, this emulsion is added to an aqueous solution containing an emulsifier, and a water-in-oil-in-water (w/o/w) double emulsion is the result. The organic solvent is then removed to leave an aqueous suspension of microspheres containing the drug. The microspheres are isolated by centrifugation, washed in water three to five times depending on the size of the batch, and freeze-dried as

shown in Fig. 3.

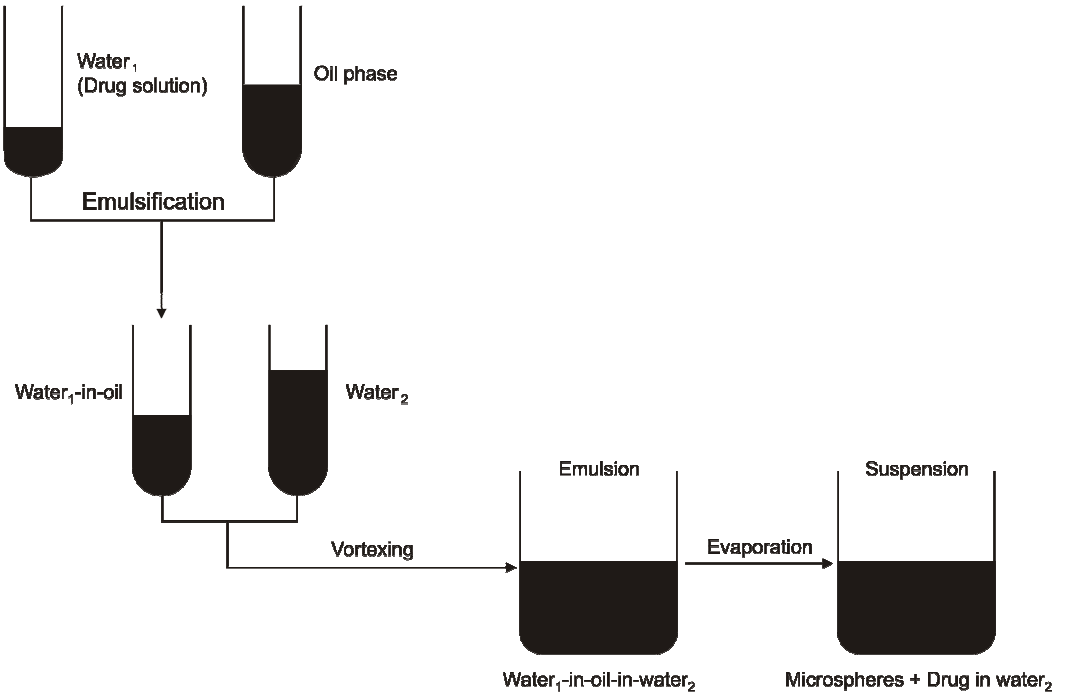

Fig. 3: Schematic diagram of the preparation of drug loaded microparticles by the w/o/w double emulsion-solvent evaporation method

Table 1: Entrapment efficient of manitol^{14}C in microsphere prepared with different DL-PLG concentration in the oil phase using different internal aqueous volumes, $V_{i(aq)}$

$V_{i(aq)}$(μl)	Average entrapment efficiency (%)		
	4.3% PLG	27% PLG	43% PLG
50	1.25 (0.0)	10 (1.8)	18 (4.0)
100	1.50 (0.5)	11 (0.5)	25 (4.3)
200	0.70 (0.0)	9 (0.08)	23 (0.6)
400	0.50 (0.0)	14 (0.7)	27 (8)

Standard deviations are given in brackets

Table 2. The entrapment efficiency (E.E.) and retained biological activity (RBA) of lysozyme entrapped in PLG microsphere

Concentration					
PLG %	**LYSOZ %**	**RBA[d] %**	**E. E. %**	**E. E. x RBA %**	**M. F.[e] x RBA %**
4.5[a]	1.25	54 (0.8)	4.6 (0.9)	2.5 (0.58)	0.051 (0.018)
4.5[b]	1.25	75 (11)	16 (2.2)	13 (2.7)	0.3 (0.06)
4.5[c]	1.25	31 (9.3)	5 (0.15)	1.1 (0.36)	0.02 (0.008)
4.5[a]	32	53 (5.6)	0.4 (0.18)	0.30 (0.089)	0.15 (0.061)
36[a]	1.25	60 (4.8)	56 (7.6)	32 (2.1)	0.07 (0.006)
36[b]	1.25	58 (0.7)	25 (4.0)	14 (2.1)	0.04 (0.01)
36[c]	1.25	33 (1.5)	4.4 (0.55)	1.9 (0.9)	0.0085 (0.00)
36[a]	1.25	79 (10.2)	10 (5.1)	8.2 (6)	0.60 (0.35)

[a]0 : 1 = Acetone: CH_2Cl_2
[b]1 : 1 = Acetone: CH_2Cl_2
[c]2 : 1 = Acetone: CH_2Cl_2
[d]Standard deviations are given in brackets
[e]Mass fraction (lysozym/ lysozym + PLG)

Spray drying method

In this technique, the drug is dissolved or suspended in the organic polymer solution and the resultant mixture is spray dried to form microsphere. The advantage of this technique is that water soluble and insoluble compounds can be incorporated into the spheres, in contrast to the single oil-in-water emulsion system, which is unsuitable for water soluble compounds. Progesterone and theophylline have been incorporated into polylactide microparticles using this method. This system is, however, associated with some drawbacks. For example, needle shaped crystals were formed, when caffeine was incorporated using this method into a polylactide polymer, possibly the result of incompatibility between the drug and the polymer. The choice of organic solvent is also important; the polymer should be dissolved in solvent such as methylene chloride and ethyl acetate solvent, because these solvents evaporate quickly in the heated air in the drying phase and because the polymers used are often insoluble in common organic solvents. Particles of 5 μm to 125 μm in diameter are produced using this method.

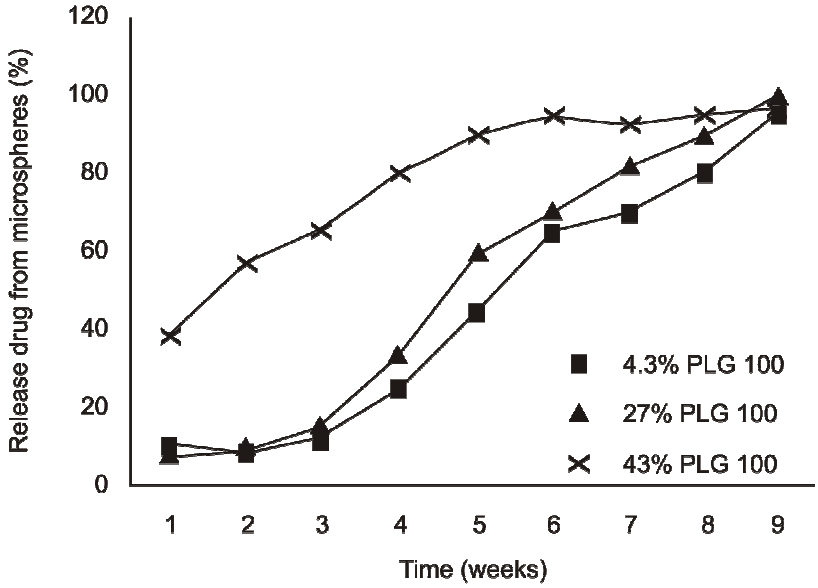

Fig. 4: Cumulative release of drug from microsphere with prepared different concentration PLG in the oil phase

RESULTS AND DISCUSSION

The particle size increased with increasing polymer concentration. The results were in an agreement with earlier findings using a similar method of preparation (Yan et al., 1994). The RBA and E.E of lysozyme were improved, when the PLG concentration in the organic phase of the emulsion was increased. As the concentration of polymer in the organic phase was increased, the E.E increased more than tenfold. A high lysozyme concentration in the inner water phase of the emulsion resulted in decreased EE and an increase in the proportion of fragmented particles. Furthermore, the external porosity of the microspheres increased with increasing concentrations of lysozyme. The RBA of lysozyme in the microsphere varied between 32 and 80% with change to the process. When the DL - PLG concentration was increased from 4.5 to 36%, the RBA of the entrapped lysozyme was increased from 54 to 79%. Fig. 4 and Table 1 show that the higher is the internal volume of microparticles produced by the second encapsulation method, the faster is the enclosed drug released. It is also shown that increasing the proportion of polymer to drug improves the entrapment efficiency and decreases the release rate for this method. It is important to optimize the process of manufacturing microspheres in order to give the entrapped peptide maximum protection against chemical degradation and denaturalization

during the preparation process.

REFERENCES

1. S. Aftabroshad, and E. Doclker, Factors Influencing the Entrapment of a Water Soluble Model Drug into Injectable Microparticle Prepared Using Solvent Evaporation and Phase Separation Techniques. Eur. J. Pharm. Biopharm., **40 (40),** 237-242 (1994).
2. A. -C. Albertsson, J. Carlfors and C. Stturesson, Preparation and Characterisation of Poly (Adipic Anhydride) Microshphres for Ocular Drug Delivery, J. Appl. Polym. Sci., **62**, 695-705 (1996).
3. A. -C. Albertsson, A. Lofgren, C. Sturesson, and M. Sjoling, "Chapter 14: Design of New Building Blocks in Resorbable in Resorbable Polymers - Application in Drug Delivery Microspheres." in Ploymeric Drugs and Drug Delivery Systems, R. Ottenbrite, (Ed.), ACS Symposium series (1994) p. 545.
4. M. J. Alonso, S. Cohen, T. G. Park, R. K. Gupta, G. R. Siber, and R. Langer, Determinants of Release of Tetanus Vaccine from Polyester Microspheres, Pharm. Res., **10**, 945-953 (1993).
5. P. Bastian, R. Bartkowski, H. Kohler and T. Kissel, Chemo-Embolization of Experimental Liver Metastases. Part I: Distribution of Biodegradable Microspheres of Different Sizes in an Animal Model for the Locoregional Therapy, Eur. J. Pharm. and Biopharm., **46(3)**, 243-254 (1998).
6. S. Benita, J. P. Benoit, F. Puisieux and C. Thies, Characterization of Drug Loaded Poly (d,l-lactide) Microspheres, J. Pharm. Sci., **73(12)**, 1721-1724 (1984).
7. J. -P. Benoit, N. Faisanta, M. -C. Venier-Julienne and P. Meneib, Development of Microspheres for Neurological Disorders: from Basics to Clinical Applications, J. Controlled Release, **65(1-2)**, 285-296 (2000).
8. J. Bleich, P. Kleinebodde and B. W. Muller, Influence of Gas Density and Pressure on Microparticles Produced with the ASES Process, Int. J. Pharm., **106**, 77-84 (1994).
9. R. Bodmeier and J. W. McGinity, Solvent Selection in the Preparation of Poly (d,L-lactide) Microspheres Prepared by the Solvent Evaporation Method, Int. J. Pharm., **43**, 179-186 (1988).
10. R. Bodmeier, and J. W. McGinity, Poly (lactic acid) Microspheres Containing Quinidine Base and Quinidine Sulphate Prepared by Solvent Evaporation Technique. I. Methods and Morphology, J. Microencapsulation, **4(4)**, 279-288 (1987 a).

11. R. Bodmeier, and J. W. McGinity, The Preparation and Evaluation of Drug Containing Poly (d,l-lactide) Microspheres Formed by the Solvent Evaporation. Pharm. Res., **4**, 465-471 (1987 b).

12. G. Braun, H. Schmitz., K. Kuhlein, and R. Steiner, German Patent Appl. No. DE 3323940 Al, (1985).

13. L. Fredriksen, K. Anton, P. V. Hoogevest, H. R. Keller and H. Leuenberger, Preparation of Liposomes Encapsulating Water-Soluble Compounds using Supercritical Carbon Dioxide, J. Pharm. Sci., **86(8)**, 921-928 (1997).

14. P. M. Gallagher, V. J. Krukonis and G. Botsaris, Gas Antisolvent Recrystallization: Application to Particle Design, AIChE Annual Meeting in Chicago. IL (1990).

15. P. Gal Lagher-Wetmore, M. P. Coffey and V. Krukonis, Application of Supercritical Fluids in Recrystallization: Nucleation and Gas-Antisolvent (GAS) Techniques. In: Tespiratiory Drug Delivery IV, P. R. Byron, R. N. Dalby, and S. J. (Eds.) Farr, Interpham Press, Inc., Buffalo Grove, IL, (1994) pp. 287-295.

16. D. L. Wise, G. J. McCormick, G. P. Willet, and L. C. Anderson, Sustained Release of an Antimalarial Drug using a Co-polymer of Glycolic/Lactic Acid, Life Sciences, **19**, 876-874 (1976).

17. C. Yan, J. H. Resau, J. Hewetson, M. West, W. L. Rill and M. Kende, Characterization and Morpholofical Analysis of Protein-Locaded Poly (Lactide-co-glycolide) Microparaticles Prepared by Water-in-oil-in Water Emulsion Technique.

18. F. Morris and A. W. Francies, Ternary Systems of Liquid Carbon Dioxid, J. Phys. Chem., **58**, 109 -114 (1994).

19. P. Vanberver and A. Dillow, Bacterial Inactivation by Using Spray Drying Method, J. Controlled Release, **24**, 27-44 (1999).

20. K. Winters and J. W. Fong, Evaluation of Biodegradable Microcapsules Prepared by Solvent Evaporation Process Using Oleate as Emusifier, J. Controlled Release, **24**, 27-44 (1996).

21. L. Zolatex and D. Patterson, The Preparation and Evaluation of Drug Containing Poly (d,l-lactide) Microspheres Formed by the Solvent Evaporation, Pharm. Res., **4**, 465- 467, (1998). Bodmeier

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