The Influence of Formulation and Process Parameters on the Morphology, Size and Release Profile of L-Dopa-Loaded Poly Lactic-Co-Glycolic Acid (PLGA) Microspheres

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Abstract: Many existing pharmaceuticals are rendered ineffective in the treatment of central nervous system (CNS) diseases due to the highly challenging aspects of drug delivery to the most delicate organ namely brain. In order to overcome the problems of delivering neuroprotective agents to the CNS, numerous strategies have been proposed. Among the developed drug carrier to the CNS, poly lactic-co-glycolic acid (PLGA) microspheres have shown desirable outcomes because of their biocompatibility, biodegradability, convenient processability and resorbability through natural pathways. Meanwhile, a comprehensive understanding of the factors affecting drug release mechanisms from microspheres is critical to the design of optimal drug-loaded microparticles. In the present study, we investigated the physicochemical and emulsifying properties of synthesized L-dopa-loaded microsphere. A series of microspheres of different compositions were prepared by varying the salt (NaCl) content, stabilizer content and homogenizer speed. The prepared microspheres were loaded with L-dopa and characterized by SEM techniques to gain insights into the structural and morphological features. The microspheres size was also determined to elucidate the influence of varying formulation and dynamic properties on the drug release pattern. After evaluating morphology and size of the microspheres, the optimum formulation and process parameters including speed of stirring applied for emulsification, drug concentration, amounts of surfactant and NaCl content in the solvent, were revealed using taguchi software according to the prolonged drug release pattern of microspheres.

Keywords: Poly(lactic-co-glycolic) acid, L-dopa, Microsphere, NaCl, Homogenizer.

1. INTRODUCTION

Target delivery of drug molecules to central nervous system (CNS) remains one of the most challenging research areas for both neuro- and pharmaceutical scientists. For this purpose, a wide range of strategies have been developed including osmotic disruption of the blood brain barrier [1], infusion pumps delivering drugs into the cerebrospinal fluid [2], intravenous injection of surfactant-coated nanoparticles [3] coupling of drugs to a carrier undergoing receptor-mediated transcytosis through the blood–brain barrier [4], implantation of tissue or cells [5] and gene therapy [6, 7]. Biomaterial-based delivery systems represent an alternative to more traditional approaches, with the possibility of increased efficacy. Among the various strategies have been proposed to improve the delivery of different drugs to CNS, locally controlled drug release by the way of drug-releasing biomaterials in the injectable microspheres form has attracted great attention as promising carriers the release of both small and large molecules. Microspheres made of polylacticco-glycolic acid (PLGA) heteropolymers are very attractive colloidal drug carriers because of their biocompatibility, biodegradability, convenient processability and resorbability through natural pathways. The size of microspheres, the molecular weight, and the composition of PLGA copolymer have been shown to alter the release kinetics of various encapsulated therapeutic agents, suggesting that PLGA is a versatile material for drug delivery applications. The rate of degradation of these nanoparticles depends on four basic parameters: (i) the hydrolysis rate which is affected by the molecular weight, the lactic/glycolic ratio and the morphology, (ii) the amount of absorbed water, (iii) the diffusion coefficient of the polymer fragments through the polymer matrix, and (iv) the solubility of the degradation products in the surrounding aqueous medium [8]. All of these parameters are influenced by temperature, additives (including drug molecules), pH, ionic strength, buffering capacity, size and processing history, steric hindrance etc. Therefore, controlling the formulation and process parameters is of critical importance to obtain desirable drug carriers for the development of future treatments. Further, many neurological disorders such as parkinson's disease (PD) may require a long-term delivery system to target the potential neuroprotective agents. Currently available pharmacological therapies are unable to arrest the progression of this relentlessly progressive and severely devastating disease and L-dopa still remains the backbone of effective drug therapy of PD. The current study investigates the dynamic effects of emulsification process on the morphology, size and drug release pattern of L-dopa-loaded PLGA microspheres to attain more reliable and proper formulation parameters in the preparation of PLGA

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microspheres. Various samples were prepared based on varying physicochemical properties in the synthesis process.

2. MATERIALS AND METHODS

2.1. Material

The poly-D, L-lactide-co-glycolide polymer, Resomer ®RG 506 PLGA with a 50:50 copolymer ratio (MV 13 kDa) and 0.45-0.6 dl/g intrinsic viscosity (in a 0.1 % chloroform solution at 25 °C) was supplied by Boehringer Ingelheim K.G. (Ingelheim, Germany). Dichloromethane (DCM) (MV 84.93 g/mol, solubility in water: 13 g/L at 20 °C), Polyvinylpyrrolidone (PVP) (density: 1.2 g/cm³, molar mass: 2.500 - 2.5000.000 g·mol−1) and Sodium chloride (NaCl) (molar mass: 58.44 g mol−1, density: 2.165 g cm−3) were purchased from Merck. L-DOPA (L-3, 4-dihydroxyphenyl-alanine, molar mass: 197.19 g/mol) was a gift from Alborz Darou pharmaceutical company, Iran.

2.2. Preparation of L-DOPA -Loaded Nanoparticles

In order to investigate the effect of formulation and process parameters including drug concentration in the first aqueous phase, homogenizer speed, concentration of surface active agent and salt concentration in the second aqueous phase, nine samples (L1 to L9) were made by applying the Taguchi method which their properties are presented in Table **1**. Fig. (**1**) shows the Taguchi graphs.

PLGA microspheres containing L-DOPA were prepared under aseptic conditions from PLGA 50:50 using a water-in-oil-in-water (W/O/W) double-emulsion-solvent evaporation method with PVP as a stabilizer. The first emulsion was carried out as previously reported with modifications. Briefly, 1mL of L-DOPA aqueous solution was emulsified with 5 mL PLGA solution (6% w/v) in dichloromethane (oil phase). The obtained particle solution was then centrifuged at 10000 rpm for 2 min. The resulting first emulsion (W1/O) was poured into 30 ml of second aqueous solution containing with varying concentrations of PVA and NaCl in each sample and emulsified for one min at varying homogenizer speeds for each sample using a SilentCrusher M (P/N 596-08010-00) in order to perform the double emulsion (w/o/w). The microspheres were centrifuged at 7500 rpm and resuspended in 30 mL of distilled water three times. The microspheres were then collected by centrifugation at 10,000 rpm for 5 min, washed three times with distilled water to remove residual PVA, resuspended in double-distilled water. The obtained microparticles were placed in a freezer at -20 °C and then were lyophilized by freeze-dryer (at -50 °C and 5×10-3 mbar for 15h). Finally, the microspheres were precisely collected and kept in sterile Falcon tubes at -20 °C.

2.3. Microsphere Morphology

Scanning electron microscopy (SEM, JEOL 6300F FEG HRSEM, USA; 5 kV) was used to determine the shape and size of the nanoparticles. Some of the microspheres were dried under vacuum, fixed with a chemical fixative and coated with gold in coater (Cressington 108 Sputter Coater) and examined by SEM. Image Pro Plus software was used to analyze the SEM photographs. Size of microspheres was calculated by averaging of total size of 50 examined microsphere.

2.4. Preparation of Phosphate Buffered Saline (PBS)

0.6 g of KH2PO4, 6.4 g of Na2HPO4, 5.85 g of NaCl and 1 L of double-distilled water were transferred to the Erhlenmyer flask, rapidly mixed by a magnetic stirrer. The Speed of the magnetic stir bar was adjusted and we ensured that there are no remaining particles of undissolved salts in the solution before adjusting the pH. After proper calibration of pH meter, the pH of the solution was measured and by slowly adding the 0.1 N solutionof HCl and NaOH, we adjusted the pH to 7.4. To prevent microbial contamination, sodium azide was added to the buffer solution at concentration of 0.01 % (w/v).

2.5. *In Vitro* Release Studies

In order to carry out studies on peptide release from microspheres, 30 mg of microspheres were placed in test tubes containing 1 ml of phosphate buffered saline pH 7.4 and incubated at 37 °C under continuous orbital rotation. At certain time intervals, up to 30 days, the samples were centrifuged at 7500 for 10 min. The drug release study was continued after replacement with the same volume of fresh buffer. The collected buffer was transferred to the quartz cuvette (1 ml) and assayed for drug quantification using the Pharmacia Biotech Ultra Spec 2000 UV spectrophotometer

Table 1. Formulation and Process Parameters for Prepared Microsphere Sample

Samples	Homogenizer Speed (rpm)	Drug concentration (mg/ml)	PVP concentration (w/v %)	NaCl concentration (w/v %)
L1	9500	1.5	2.5	2.5
L2	9500	3	5	5
L3	9500	4.5	10	10
L4	13500	1.5	5	10
L5	13500	3	10	2.5
L6	13500	4.5	2.5	5
L7	20000	1.5	10	5
L8	20000	3	2.5	10
L9	20000	4.5	5	2.5

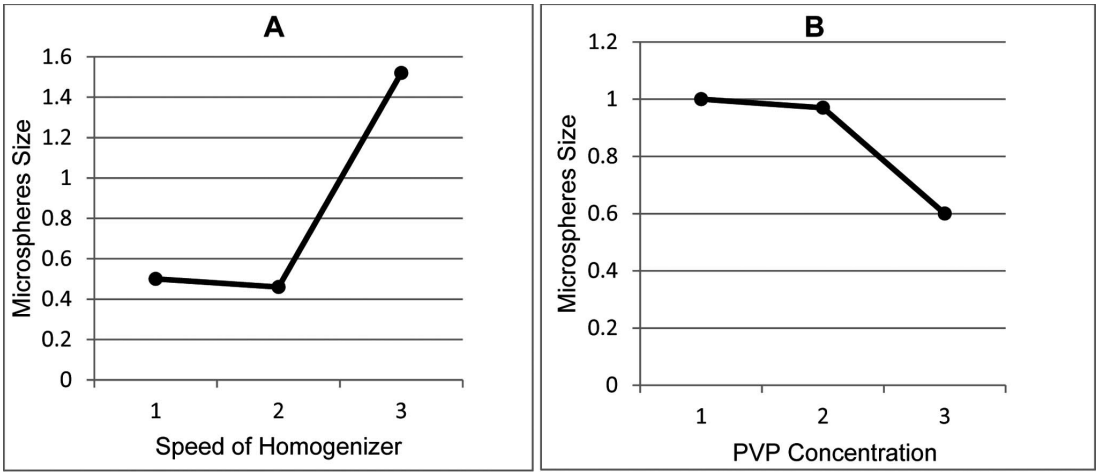


Fig. (1). nine samples (L1 to L9) were made by applying the Taguchi method. Microspheres size are in micrometer and speeds of homogenizer in graph A are in (Krpm).

at 280 nm. Drug release profiles were generated for each microsphere formulation in terms of cumulative peptide release versus time.

3. RESULT AND DISCUSSION

3.1. Microsphere Morphology

In an attempt to optimize the formulation and process parameters like homogenizing speed and concentration of stabilizer, different experimental conditions for the preparation of these particles were evaluated. Scanning electron microscopy showed that freshly prepared PLGA microspheres are fairly spherical (Fig. **2**). As can be seen in Fig. (**2**), L-dopa-loaded PLGA microspheres, depending on the preparation of samples (L1 to L9) exhibited smooth or microporous outer surface. Different parameters have affected this broad range of surface morphology. As shown in Fig. (**2**), there exists no aggregation and attachment of microspheres to each other in samples L2, L4 and L5 because of proper PVA content in relation to the homogenizer speed, therefore the microspheres do not tend to aggregate and prepared in a separate spherical particles. However, it can be clearly seen that samples L1, L6 and L7 show an aggregation which was likely due to the low concentration of PVA in the preparation of solution (2.5 % w/v). Finally, very high PVA content in relation to homogenizer speed (9500 rpm) in the preparation of sample L3 caused that PVA as a stabilizer acted inversely and was led to a strong tendency of microspheres to form clusters (aggregation).

Moreover, a tremendous change in the morphology of the sample L9 (Fig. **2**) was indicated in the SEM micrographs. A discernible surface porosity was observed in the sample L9. There are likely two reasons why such a porous surface morphology was formed: 1) High-speed homogenizer applied an intensive shear force which resulted in a porous surface. Moreover, the more homogenizer speed the more temperature would be generated in the solution so that evaporation speed increased which resulted in a porous surface morphology 2) concentration of drug is 180 times higher than the salt which cause an increase in the osmotic pressure gradient from the oil phase to the inner layer (W1), so that the water diffuse across the oil phase (Semi permeable liquid membrane) to the inner layer and resulted in an inflammation and porosity of inner particles. Meanwhile, the increase of water increases the drying time and cause further porosity.

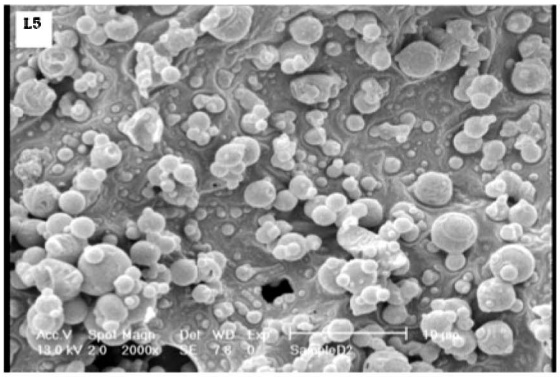
3.2. Microspheres Size

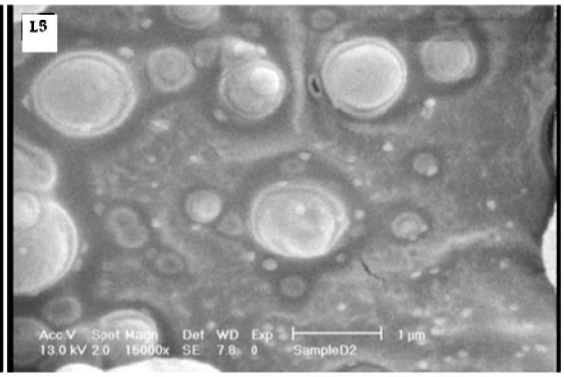
We used the Taguchi software to predict optimum formulation variables to minimize the undesirable conditions. Microspheres size is presented in Table **2**. It is clearly observed in Fig. (**2**) that the optimum homogenizer speed is 13500 because microspheres exhibited the smallest size and lowest porosity. There are several parameters which affect the size of microspheres including, homogenizer speed, solution temperature, concentration of surface active agent and concentration of salt.

By increasing the homogenizer speed, solution temperature was increased and speeds up the evaporation which resulted in an increase of microspheres size. It is worth mentioning that at high temperature, formation of microspheres would be started in lower solution viscosities, in our experiments, however, microspheres were quickly consolidated because of increased temperature and led to increased size of microspheres. This may be attributed to the fact that shear force of the homogenizer do not seriously affect the microspheres which formed in the early steps. Concentration of surface active agent also greatly affected the size of microsphere. As can be seen in Fig. (**3**), size of microspheres decreased with increasing the amount of surface active agent in the second aqueous phase. More importantly, PVA content should be optimum in relation to the homogenizer speed. In the very high concentration of stabilizer and low speed of homogenizing, the homogenizer was not intensely stirred the solution and microsphere aggregated. Moreover, there is an aggregation of microspheres in the very low concentration of PVP and high speed of homogenizer which is occurred in the samples L1, L6, L8. The ratio of drug to salt is also crucial in the size of microspheres. For instance, in sample L9, because of very high ratio of drug to salt (180), larger and porous microspheres were created.

3.3. *In Vitro* Release Studies

Fig. (**3**) illustrates the release profile of L-dopa from microspheres in PBS 7.4 and the quantitative release data are


L5
Acc.V Spot Magn Det WD Exp
13.0 kV 2.0 2000x SE 7.8 0 SampleD2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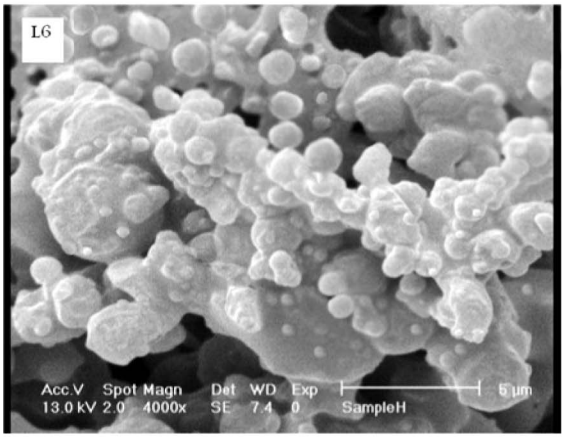
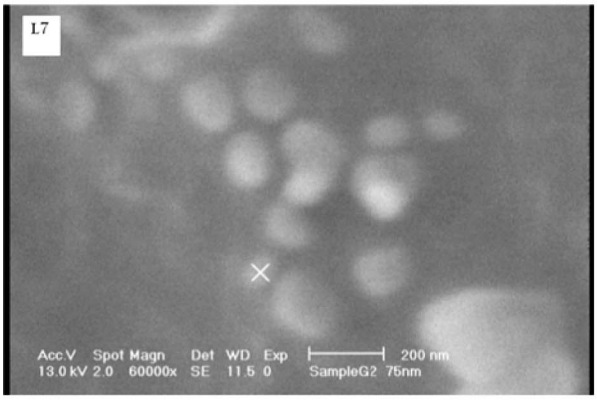

L6
Acc.V Spot Magn Det WD Exp 5 µm
13.0 kV 2.0 4000x SE 7.4 0 SampleH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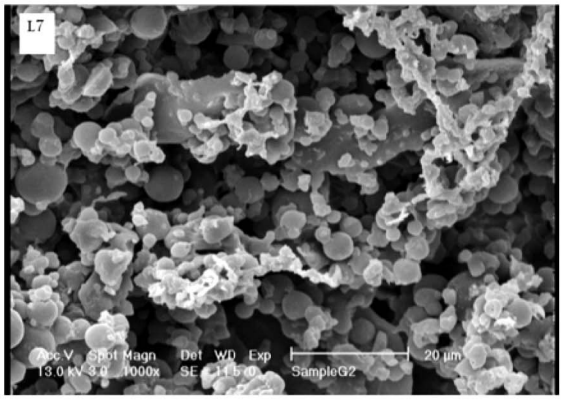
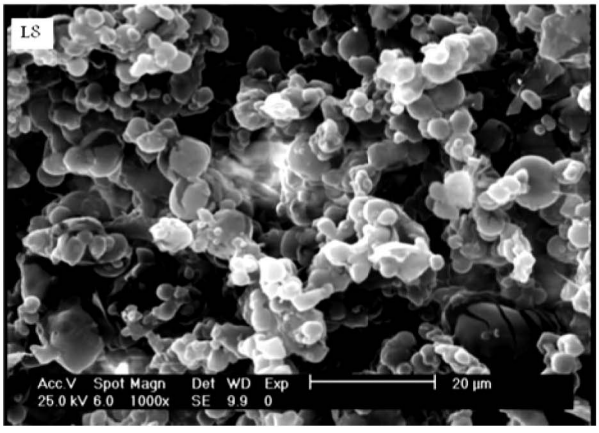
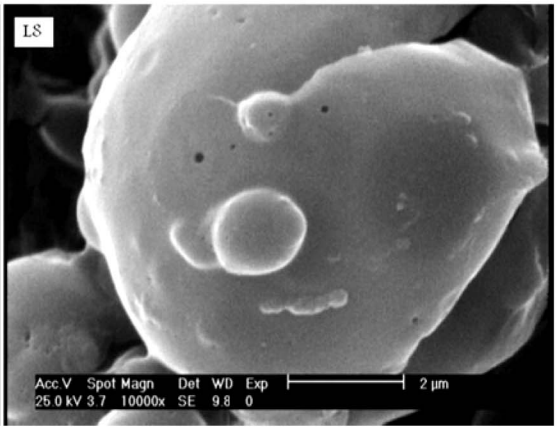
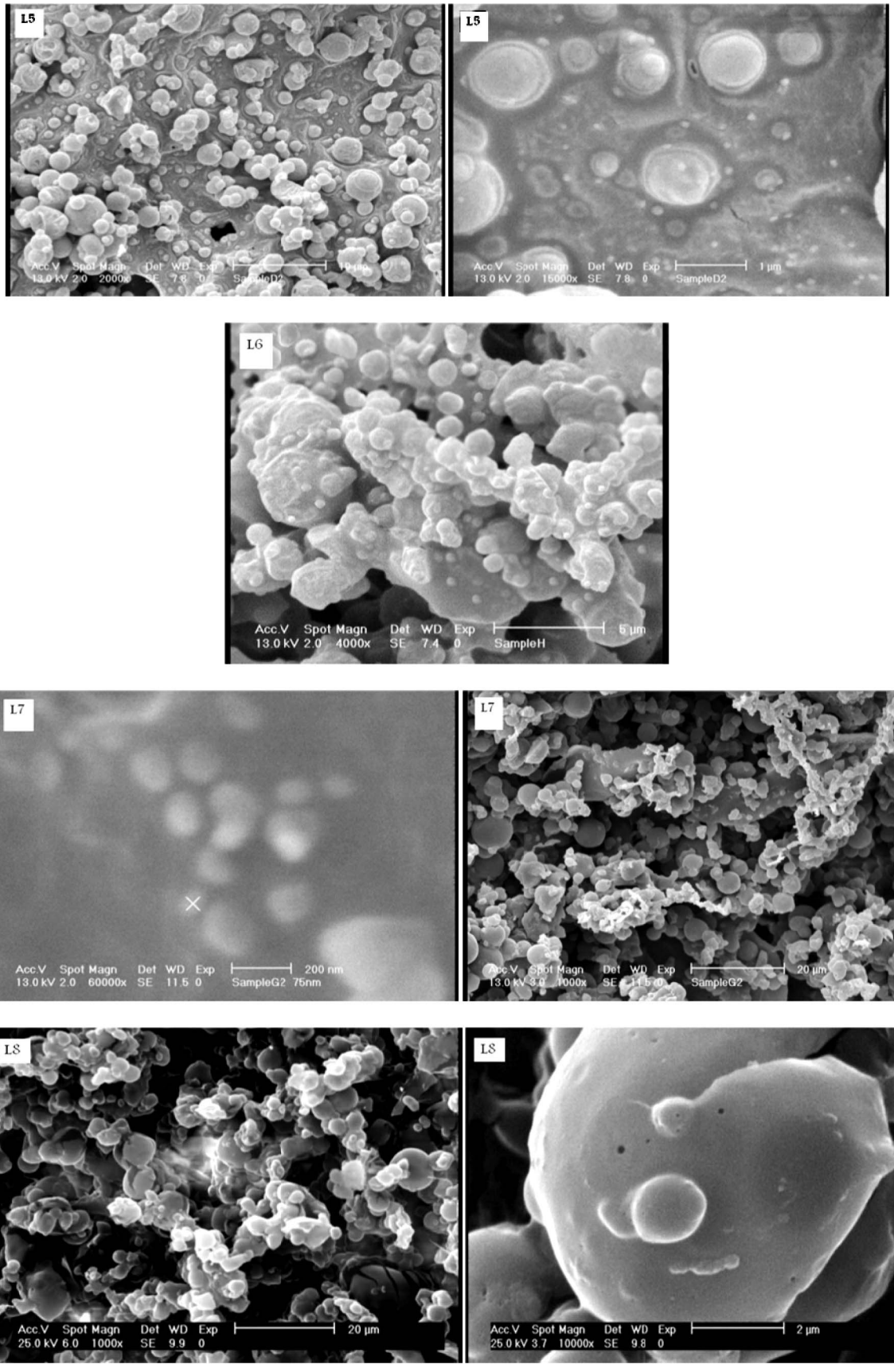

L7
Acc.V Spot Magn Det WD Exp 20 µm
13.0 kV 3.0 1000x SE 11.5 0 SampleG2


L8
Acc.V Spot Magn Det WD Exp 20 µm
25.0 kV 6.0 1000x SE 9.9 0


L8
Acc.V Spot Magn Det WD Exp 2 µm
25.0 kV 3.7 10000x SE 9.8 0

(Fig. 2). Contd…..


L5
Acc.V Spot Magn Det WD Exp
13.0 kV 2.0 2000x SE 7.6 0 SampleD2
1 µm
L5
Acc.V Spot Magn Det WD Exp
13.0 kV 2.0 15000x SE 7.8 0 SampleD2
1 µm
L6
Acc.V Spot Magn Det WD Exp
13.0 kV 2.0 4000x SE 7.4 0 SampleH
5 µm
L7
Acc.V Spot Magn Det WD Exp
13.0 kV 2.0 60000x SE 11.5 0 SampleG2 75nm
200 nm
L7
Acc.V Spot Magn Det WD Exp
13.0 kV 3.0 1000x SE 11.5 0 SampleG2
20 µm
L8
Acc.V Spot Magn Det WD Exp
25.0 kV 6.0 1000x SE 9.9 0
20 µm
L8
Acc.V Spot Magn Det WD Exp
25.0 kV 3.7 10000x SE 9.8 0
2 µm

(Fig. 2). Contd…..

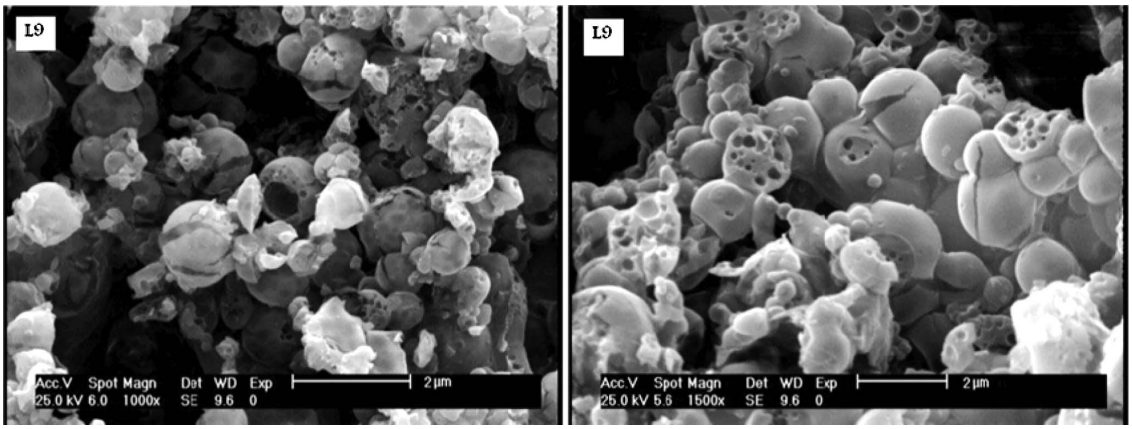


Fig. (2). SEM micrographs of synthesized samples (L1 to L9) indicating the effect of different parameters on the surface morphology of microspheres.

Table 2. Microspheres Size

Samples	L1	L2	L3	L4	L5	L6	L7	L8	L9
Microspheres size(µm)	0.568	0.85	0.172	0.250	0.520	0.690	1.3	1.778	1.8

Table 3. Drug Release from L-Dopa-Loaded Microspheres

Time (day)	Cumulative Release (%)								
	L1	L2	L3	L4	L5	L6	L7	L8	L9
0.04	0.1	2.15	0.07	1.35	2.65	1.15	1.2	2.3	5.5
0.12	0.79	3.366	0.553	2.04	3.866	2.366	1.6	2.7	5.9
0.21	1.816	3.866	1.2712	3.066	4.366	2.866	2.2	3.3	6.5
0.34	1.98	4.56	1.386	3.23	5.06	3.56	12.7	13.8	17
0.5	2.7	5.6	1.89	4.5	6.1	5.89	14.3	19.8	25
1	3.4	7.1	2.38	5.77	7.6	9.12	19.3	23.5	29.2
2	4.3	10.8	3.01	9	12.3	13	21.8	26.3	35.1
4	6	12.4	4.2	10.27	14.4	15	24.9	28	40.9
7	7.2	13.3	5.04	10.69	15.3	15.6	27.9	32	43.7
14	7.5	18.1	5.74	16.39	20.4	19.7	34.9	37.7	49.1
21	9.1	19.6	7.63	17.79	22.8	23.7	38.2	41.6	50
30	11.2	23.2	11	22.43	26.3	28.5	40.1	44.7	50

presented in Table **3**. The release rate is strongly influenced bysurface morphology of drug-loaded microspheres. As can be observed, the release behavior of L-dopa from the L7, L8, L9 exhibited a burst pattern characterized by a fast initial release during the first 8 h. This initial burst was more intensive in L9 and 35-40% of the drug was released during this phase. This rapid initial release of L-dopa in L9 was probably due to the drug which was adsorbed or close to the surface of the microspheres and was not remained after washing. It may also be due to the extensive surface porosity which resulted from high homogenizing speed. High ratio of drug to salt and excessive homogenizer speed caused not only highly porous surface but also decreased the performance of sample preparation so as 50% of drug was released from sample L9 and no drug release was observed after 3 weeks. There can be seen a continuous and linear drug release from samples L1, L2, L4, L5 most likely because of smooth surface and delivery mechanism was probably surface erosion of PLGA microspheres so fast release of drug was not seen. Polymer degradation initiated release of acidic group and lowered solution pH and speeded up drug release. L3 showed a slow release pattern which is

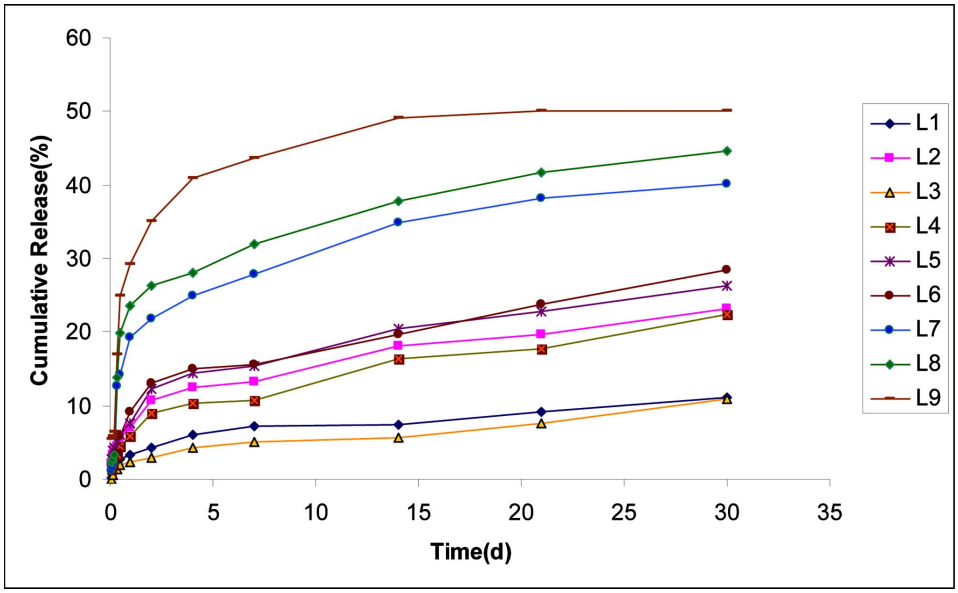


Fig. (3). Cumulative drug release from microspheres.

presumably due to the aggregation of its microspheres. In spite of high drug content in L6, its release pattern similar to L2, L4, L5 which may be attributed to the microsphere aggregation that resulted from low PVA content. Moreover, L2, L5 retained their slow, continuous and linear drug release which can be a promising sample in prolonged delivery systems for long term therapeutics. Finally, drug release stopped in sample L8 because of highly porous surface and low encapsulation efficiency which stemmed from high homogenizer speed and low PVA content. All in all, from SEM investigation, taguchi method and *in vitro* release studies indicated that L2, L4 and L5 are more desirable samples than the other.

CONCLUSION

In the present work the effect of preparation parameters including homogenizer speed, PVP content, drug and salt concentration on the encapsulation efficiency of PLGA microspheres containing L-dopa has been evaluated using taguchi method. The result presented herein showed that the optimum homogenizer speed was 13500 rpm and an increase in the homogenizer speed increases surface porosity and microsphere size. PVA content have also affected the morphological and size characteristics. Meanwhile, it came to light that PVA content depends on the homogenizer speed and should be optimum in relation to stirring intensity. High ratio of drug to salt resulted in a greatly porous surface and larger microspheres. Meanwhile, microspheres with highly porous surface exhibited initial rapid drug release. According to the results, some samples showed a prolonged drug release because of proper formulation parameters and can be a favorable microsphere in long term therapeutic applications.

CONFLICT OF INTERESTS

None declared.

ACKNOWLEDGEMENTS

None declared.

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