International Journal of Drug Delivery 3 (2011) 324-334
http://www.arjournals.org/index.php/ijdd/index

Original Research Article

ISSN: 0975-0215

Factorial effect of process parameters on pharmaceutical characteristics of biodegradable PLGA microparticles

K. Derakhshandeh[1,2*],M. Nikmohammadi[1], A. Hosseinalizadeh[1]

***Corresponding author:**

K. Derakhshandeh
1 Department of Pharmaceutics, Faculty of Pharmacy, Kermanshah University of medical science, 67145-1673, Iran
Email: kderakhshandeh@kums.ac.ir
2 Pharmaceutical Science Research Network,Tehran Iran

Abstract

Among the drug delivery strategies intended to increase the bioavailability of drugs, the use of polymeric biodegradable microcarriers has shown a significant degree of success.

The purpose of this study was developing a polymeric drug delivery system for a model drug: furosemide, which belongs to class IV of BCS (low solubility and low permeability), intended to oral administration and improving the stability and intestinal absorption of the drug.

To achieve this goal, furosemide loaded poly (lactic-*co*-glycolic acid) (PLGA) microparticles were prepared by solvent evaporation method and characterized. To obtain an appropriate mathematical model with minimum experiments for optimization of formulation, a 2^4 full factorial design based on four independent variables (amount of polymer, emulsifier, volume of internal and external phases) was used to plan the experiments. The effects of these parameters on the drug loading efficiency were investigated. The release profiles of furosemide from microparticles were examined in simulated gastric fluid (SGF pH 1.2), simulated intestinal fluid (SIF pH 7.4) and phosphate buffer (pH: 7.4).

The results of optimized formulation showed a narrow size distribution with an average diameter of 60 ± 5 µm and a drug loading of more than 60%. In simulated gastric fluid (SGF), less than 8% of furosemide was released from microparticles in 24 h and about 60% and 50% furosemide was released in 24 h in simulated intestinal fluid (SIF) and phosphate buffer, respectively.

Results from this preliminary work showed that furosemide loaded PLGA microparticles can be successfully obtained through solvent-evaporation technique, with good morphological characteristics, high encapsulation efficiency and controlled drug release profile suitable for per oral administration.

Keywords: Furosemide; PLGA microparticles; Full factorial design.

Introduction

Furosemide, 5-(aminosulfonyl)-4-chloro-2-[(furanylmethyl) amino] benzoic acid is a potent and short acting diuretic that widely used in the treatment of edematous states associated with cardiac chronic renal failure and hypertension [1,2].

Although the therapeutic effect is fast and intense after oral administration of furosemide but it was previously reported its oral bioavailability was poor and shows erratic absorption and high intersubject variation in pharmacokinetic parameters [3]; however the causes of these problems remains unclear. Low solubility and

permeability, intestinal secretion by efflux pump; gastric elimination by acid hydrolysis may be causes of this problem [2-5].

Among the recently drug delivery strategies intended to overcome this obstacle and increase the bioavailability of drugs, especially polymeric microparticles has shown a significant degree of success.

Microparticles are one of the multiparticulate delivery systems and are prepared to obtain prolonged or controlled drug delivery, to improve bioavailability or stability and to target drug to specific sites. Microparticles can also offer advantages like limiting fluctuation within therapeutic range, reducing side effects, decreasing dosing frequency and improving patient compliance [6].

The biodegradability or biocompatibility is an essential property for the polymer used for pharmaceutical applications. Among biodegradable polymers, poly (lactic-*co*-glycolic acid) (PLGA) has been studied for many years as a suitable drug delivery material; It can ultimately degrade by hydrolysis of their constituents, which are usual metabolic and non-toxic degradation products [7,8].

Several methods were proposed for the preparation of PLGA microparticles, such as extrusion [9], spray drying [10] and supercritical fluid extraction [11]. The technique mostly used, however, is the emulsification solvent evaporation method. It involves the solution of the PLGA polymer in an organic solvent, emulsifying the PLGA solution in a non solvent (mostly water) and precipitating the PLGA polymer as particles by evaporating the organic solvent (Fig. 1) [12,13].

Several important factors contribute to the effectiveness of solvent evaporation method in preparing particles with acceptable size range, shape and the percentage of the drug load, namely the amount of polymer, percentage of surfactant and volume of organic and aqueous phases. It is difficult to assess the effect of the variables individually or in combination, therefore deriving a mathematical model suitable for establishing a quantitative relationship between the formulation variables is necessary [14]. Factorial design is a very efficient tool to obtain an appropriate mathematical model with minimum experiments for optimization of formulation design. Studies based on factorial designs allow all the factors to be varied simultaneously, thus enabling evaluation of the effects of each variable at each level and showing inter relationship among them [15-22].

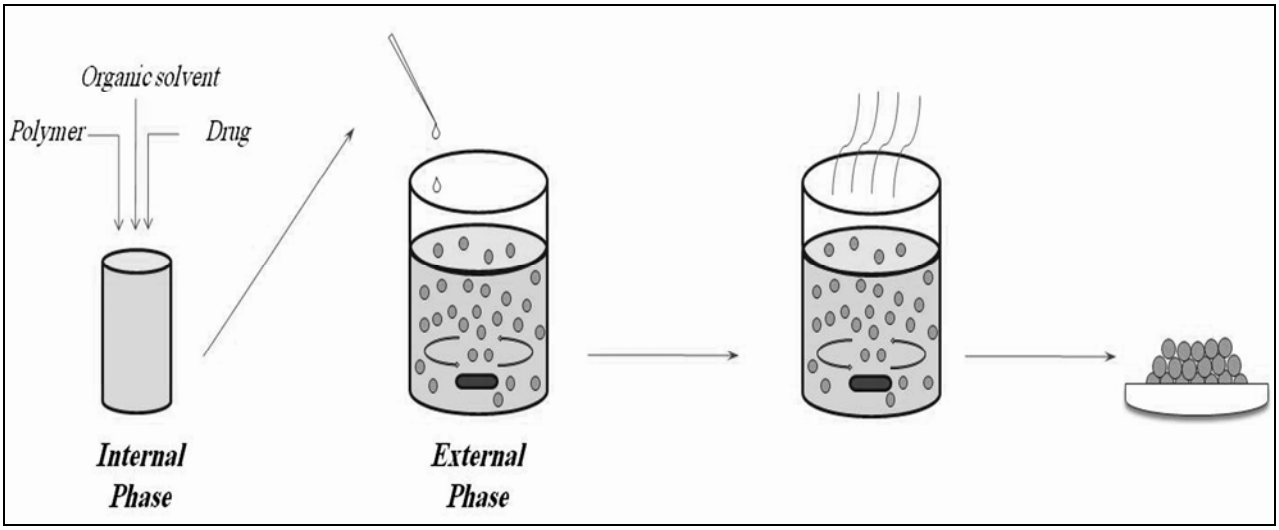


Figure 1.

In this project a two-level factorial design experiment was used for obtaining a mathematical model and prediction of optimized formulations. The resulted furosemide microparticles were characterized with regard to morphology, size, drug loading, in vitro drug release and property in microspheres.

Materials and methods

Materials

Furosemide was purchased from Auro Laboratories (India). poly (lactic-*co*-glycolic acid) (PLGA, 50:50 MW 12,000, inherent viscosity of 0.16–0.24 dl/g) was obtained from Boehringer Ingelheim Co. (Ingelheim, Germany) in the form of Resomer® 502H. Polyvinyl alcohol (PVA, MW 130000 Da, 87% hydrolyzed) was purchased from Mowiol (Germany). Dichloromethane and dimethylformamide were purchased from Merck (Darmstadt, Germany). All other chemical reagents used were of pharmaceutical grade. All aqueous solutions were prepared exclusively in distilled water.

Preparation of furosemide PLGA microparticles

Furosemide loaded PLGA microparticles were prepared by o/w emulsion/solvent evaporation method. Procedure was as follows: exact quantity of PLGA polymer and furosemide (10 mg) was accurately weighted and dissolved in dichloromethane. The organic phase was added drop-wise (0.5 ml/min) into PVA aqueous solution and stirred magnetically at room temperature until complete evaporation of the organic solvent. Subsequently, microparticles were separated by centrifugation at 10000 rpm for 15 min. The separated microparticles were redispersed and centrifuged two times in phosphate buffer (PH: 7.4) and distilled water to completely remove free drug and excess surfactants. Microparticles were then collected and dried under room temperature.

Experimental design

A factorial design is frequently employed for the planning of a research because it provides the maximum of information, requiring the least experiments. A specific purpose of the experiments and a quantitative target function of the system are required to be accurately defined before experiments are designed. Most important variables which affect the system function are selected and systemic experiments are then performed to the specified factorial design. The number of independent variables selected decides the number of experiments that are to be performed.

The response (Y) is measured for each experiment and then either a simple linear, interactive, or quadratic model is generated by carrying out multiple regression analysis and F-Statistics to identify statistically significant terms. Responses Obtained from the reduced equation, an equation containing only statistically significant terms, are used for drawing response surface plots to visualize the impact of changing variables. The optimum point may be identified from the plot and replicate trials may be run to verify the prediction of optimum response [15-22].

Based on preliminary study of the effect of parameters on the drug loading of microsphere by solvent evaporation method, four different factors at two levels and their influence on the microparticles properties were evaluated using a 2^4 Full factorial design. The four factors investigated were the amount of polymer, emulsifier percent and volume of internal and external phases. The different preparations were made in triplicate in order to estimate the experimental error. The design required a total of 2^4 experiments with one replica each or mean of triplicate. For each factor, the lower and higher value of the lower and upper level can be represented by 1 or -1 sign. Values of these selected variables are shown in Table 1.

Characterization of furosemide microparticles

Size measurement

The particle size and size distribution of optimum PLGA microparticles were measured by the method of laser light diffraction using a particle size analyzer (Malvern, UK). The particle size was expressed as volume mean diameter in micrometers.

Particle morphology

The morphology of microparticles was determined using scanning electron microscopy (SEM) (Phillips, the Netherlands), the sample were prepared on aluminum stabs and coated with gold prior to examination by SEM.

Differential scanning calorimetry (DSC)

To verify the drug loaded property, the DSC of drug, PLGA polymer, physical mixture of drug with polymer and prepared microspheres. Five to ten milligrams of each sample was put in aluminum pans and hermetically sealed. The heating rate was 10˚C/min, nitrogen served as purge gas and the system were cooled down by liquid nitrogen. The DSC (DSC-60, Shimadzu Co. Kyoto, Japan) instrument was calibrated for temperature using octadecane and indium.

Determination of drug loading and microparticle recovery

Microparticles dried at room temperature were then weighed and the yield of microsphere preparation was calculated using the folowing equation 1:

Equation 1: ***Microparticle recovery:***

Amount of microparticles obtained / amount of polymeric material and drug ×100

The drug content in PLGA and the drug encapsulation efficiency (EE) were measured after extraction from the microparticles. 100 mg of drug-loaded microparticles was dissolved in 1 mL of di-methyl formamide. The mixture was vortexed at 2500 rpm for 1 min and the solution was analyzed by UV/Vis spectrophotometer at a wavelength of 274 nm. Drug content was determined by comparing with a calibration curve of furosemide which had been prepared from DMF solutions with concentrations between 0.5-20 μg/mL.

The drug loading efficiency (DLE) is expressed as follows equation 2:

Equation 2: ***DLE:***

Amount of furosemide in microparticles / amount of furosemide in formulation ×100

In vitro drug release

In vitro release studies were performed by dialysis method using Franz diffusion cell. The release media were simulated gastric fluid: SGF (pH 2.1), simulated intestinal fluid: SIF (pH 6.8) and phosphate buffer (pH 7.4). As regards to sink condition, 1ml of microparticle suspension in each of mediums was placed in donor site and 50 ml desired medium in receptor chamber incubated at 37 ºC under magnetic stirring. At specific time intervals, 500μl of medium was taken and replaced with the same volume of fresh medium. The concentration of released furosemide was determined by UV analysis (λ: 274 nm). The results were expressed as a percentage of the drug released as shown below equation 3:

Equation 3: ***Drug released (%)***

Amount of furosemide released / amount of entrapment furosemide × 100

Different mathematical models may be applied for describing the kinetics of the drug release process from microparticles, the most suited being the one which best fits the experimental results.

The kinetics of furosemide release from PLGA microparticles were determined by finding the best fit of the release data to Higuchi and Korsmeyer-Peppas plots (23- 25).

Results and Discussion

Factorial design

The aim of the present investigation was to formulate and optimize process parameters for encapsulation of furosemide in PLGA microparticles based on a 2^4 statistical factorial design experiments

A technique of two-level factorial design offers the possibility of investigating four independent variables at two levels after performing only sixteen experiments. The selection of factors and levels in the design, which most affects drug loading, would be based on the results of preliminary investigations. Primary independent factors that might affect drug loading in solvent-evaporation method are: amount of polymer (A), percent of surfactant (B), volume of organic phase (C) and volume of aqueous phase (D). We evaluated the influence of these parameters on drug loading by a full factorial experimental design. Sixteen batches of different combinations were prepared by taking values of selected variables: A, B, C and D at two levels as shown in Table 1.

Table 1:- Values and coded units of 2^4 factorial design for preparation of furosemide microparticles by solvent-evaporation method.

Variables	Coded units	Levels	
		1	2
PLGA	A	50	200
PVA (%)	B	0.1	0.2
Organic phase (ml)	C	5	10
Aqueous phase (ml)	D	25	30

To obtain a clearer view, the preparations of the factorial design can also be represented on cubes (Fig. 2), where the different axes represent the different variables. Because there are more factors than axes on a 3D-graph, the different samples can be represented on the corners of two cubes.

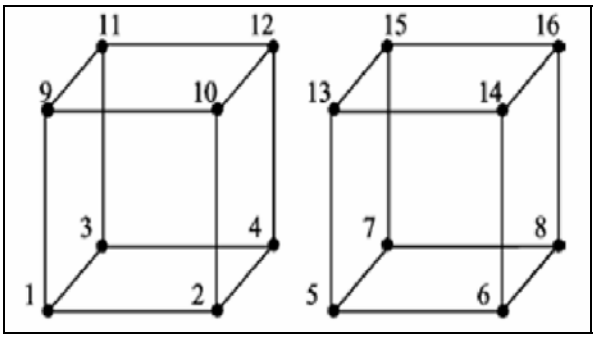


Figure 2.

Sixteen batches of microparticles were prepared according to experimental design shown in Table 2 (four variables and one response of drug loading). Each batch was prepared three times and mean drug loading values were determined (Table 2).

Table 2:- Experimental design and percentage of drug loading.

Run	PLGA mg	PVA %	Int. Phase ml	Ext. Phase ml	Loading %
1	50	0.1	10	30	**23±3.3**
2	50	0.2	10	25	**28.6±5**
3	200	0.1	10	25	**41.4±3**
4	200	0.2	5	30	**37.7±4.3**
5	200	0.1	10	30	**47±5**
6	200	0.1	5	30	**60±5**
7	50	0.1	5	30	**47.7±3.54**
8	200	0.2	10	25	**46.7±5**
9	200	0.1	5	25	**48.3±4.23**
10	50	0.2	5	25	**41±3**
11	50	0.2	5	30	**30±2**
12	200	0.2	5	25	**59.3±5.5**
13	50	0.1	10	25	**29.1±3.43**
14	50	0.2	10	30	**27±5**
15	50	0.1	5	25	**37±2.5**
16	200	0.2	10	30	**36**±4

The mathematical modelling of preparation of furosemide microcarriers was carried by the equation 4 in terms of coded factors:
Equation 4:

$$Loading = +39.99 + 7.06\times A - 1.70\times B - 5.14\times C + 1.43\times BC - 4.17\times BD - 2.02\times ABD + 2.70\times BCD$$

Where Y is dependent variable of drug loading. The result of analysis of variance (ANOVA) of the factorial design is presented in Table 3.
Based on the results of ANOVA the Model F-value of 27.06 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, C, BD, ABD, BCD are significant model terms.

The "Pred R-Squared" of 0.8379 is in reasonable agreement with the "Adj R-Squared" of 0.9240. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Our ratio of 17.280 indicates an adequate signal. This model can be used to navigate the design space.

Polymer amount exhibited positive effect on the drug loading of microparticles. A high drug loading obtained with high polymer amount shows that as the polymer concentration is increased in fixed presence of another three factors it favours the formation of high drug loading particles.

The percentage of PVA as internal phase, however, had a negative effect on the drug loading of microparticles.

3D plot generated using Equation 4 is presented in Fig. 3. The 3D plot indicated the percent of surfactant and aqueous phase volume interaction on the drug entrapment efficiency.

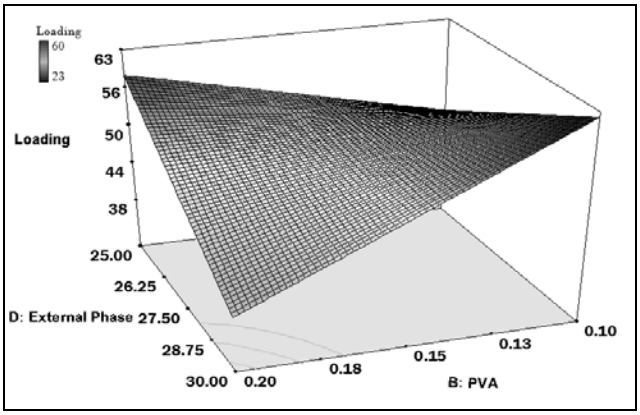


Figure 3.

Table 3:- Analysis of variance for a 2^4 Factorial design experiment for evaluation of some variables effect on the furosemide loaing in PLGA microparticles.

Variables	sum of squares	df	F value	Prob>F
A	797.92	1	85.88	0.0001
B	*46.34*	1	4.99	0.0560
C	422.2	1	45.44	0.0001
BC	32.58	1	3.51	0.0980
BD	278.64	1	29.99	0.0006
ABD	65.49	1	7.05	0.0290
BCD	116.48	1	12.54	0.0076
Std. Dev.	3.05	R-Squared 0.9595		
Mean	39.99	Adj R-Squared 0.9240		
C.V.	7.62	Pred R-Squared 0.8379		
PRESS	297.31	Adeq Precision 17.280		

Normal plot of residuals clearly showed that the variables influencing entrapment efficiency lie far away from the center (Fig. 4).

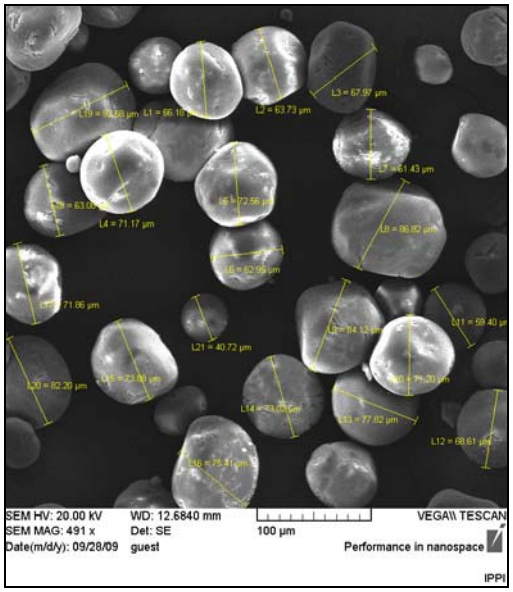


Figure 4.

Perturbation plot is shown in Fig. 5. This plot compares the effect of all the factors at a particular point in the design space.

Based on the results and mathematical models the software offered optimum setting of formulation as shown in Table 4.

Table 4:- Factorial design optimized formulation.

NO	A	B	C	D	DESIRABILITY	DRUG LOADING (THEORITICAL)	DRUG LOADING (EXPERIMENTAL)
1	185.25	0.1	5.28	29.73	1	60	45±3.54
2	189.91	0.1	5.77	29.97	1	60	52±4.5
3	186.72	0.11	5.02	29.86	1	60	47±5
4	179	0.1	5.01	29.51	1	60	40±4
5	199.38	0.1	5.13	29.19	1	60	60±5
6	187.16	0.1	5.45	29.71	1	60	50±3.23
7	178.44	0.1	5.19	29.84	1	60	57±5
8	200	0.14	5	27.13	0.797	52.5	45±3
9	200	0.14	5	27.07	0.794	52.4	45±5
10	200	0.15	5	26.87	0.79	52.2	43±5

Each formulation was performed three times and means drug loading values were recorded.

The calculated desirability factor for offered formulations was 1.00 indicating suitability of the designed factorial model. As shown in Table 4 the optimum formulation with highest drug loading was achieved by 199.38 mg of polymer, 0.1% of emulsifier, 29.19 ml external phase and 5.13 ml internal phase volumes.

Physicochemical characterization of prepared microparticles

Particle morphology

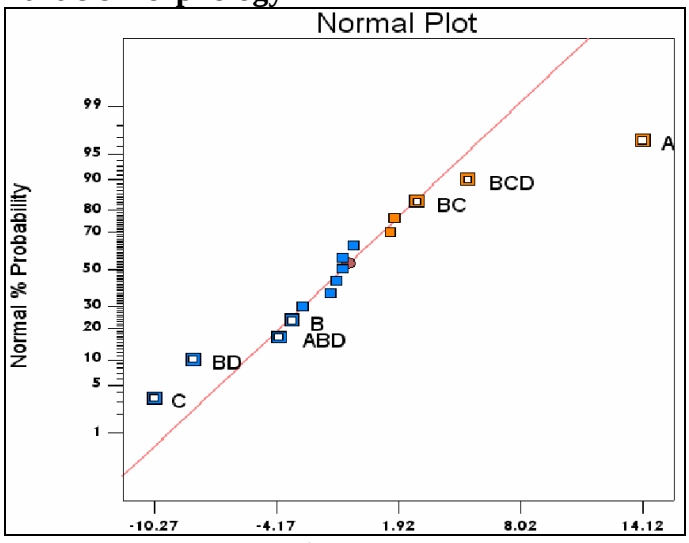
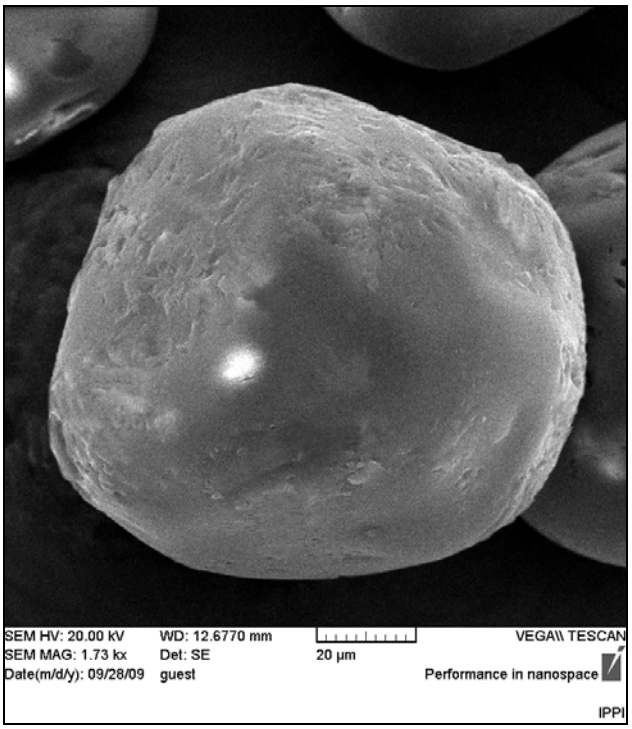


Fig. 5.



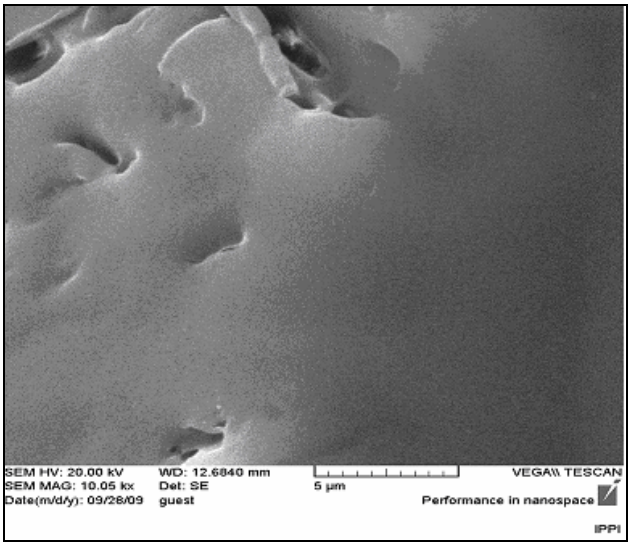


Fig. 6 shows the morphological characteristics of PLGA microparticles. The SEM photomicrographs of the microparticles reveal that they are spherical, nonporous, and uniform with a smooth surface.

Size distribution

The prepared microparticles revealed a unimodal size distribution. A classical size distribution curve of PLGA microparticles is presented in Fig. 7.

Differential scanning calorimetery (DSC)

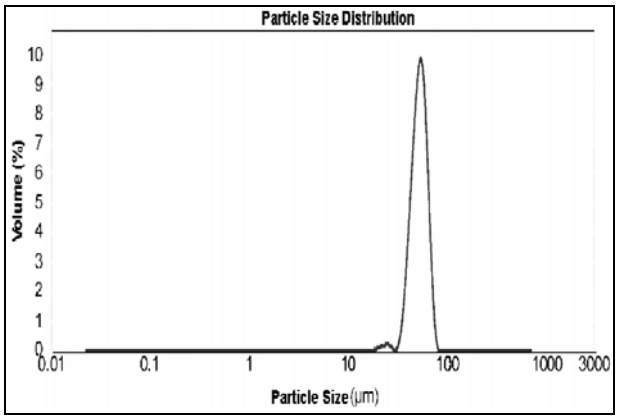


Figure 7.

The graphs of DSC were shown in Fig. 8. As demonstrated in the graphs (a) and (b), furosemide exhibited a characteristic, sharp exothermic peak at 220 ºC which is associated with the melting point of the drug and indicates the crystalline nature of the drug and the PLGA polymer had a peak at 45 ºC. From the graph (c) we can find easily that the physical mixture of drug with carrier material had two peaks at 220 ºC and 50 ºC respectively. These results indicated that their properties were kept in physical mixture. According to the graph (d), the peak which represents the properties of drug disappeared. The reason should be explained that the microparticles were formed and the drug crystal structure changed to amorphous state.

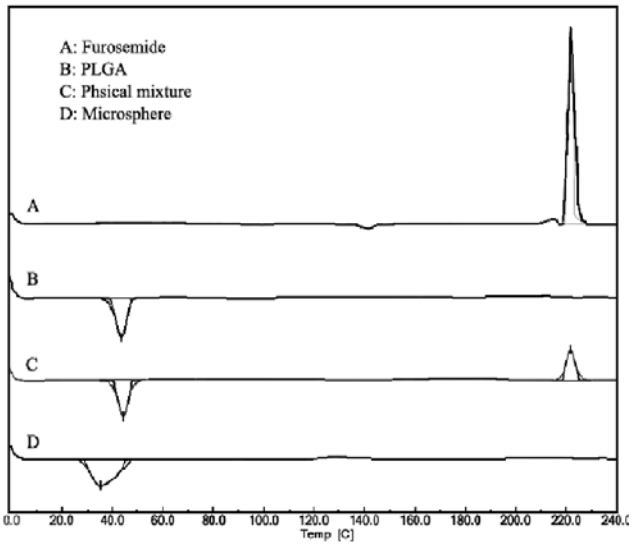


Figure-8

In vitro drug release

The drug release is another important goal for microparticle formulation. The amount of furosemide released from microparticles during 24 hr was evaluated using a dialysis technique. Dialysis membrane with a molecular weight cut-off of 12,000 Da (Sigma) was fixed on Franz diffusion cell and donor and acceptor medium were PBS (pH 7.4), SIF (pH 7.4) and SGF (pH 1.2).

Fig. 9 showed the release behavior of furosemide from PLGA microparticles in various medium. Approximately, 8% of the drug was released in the SGF (pH 1.2) over a period of 24 hr and 60% in SIF (pH 7.4) and 50% in phosphate buffer (pH 7.4).

It was observed that in each three medium the release consisted of an initial rapid release phase, followed by a sustained release period for 24 hours. It was expected because the adsorbed furosemide bound weakly to the surface making a platform value known as a burst effect (initial rapid release). Remained furosemide which was captured in the structure was released in a controlled fashion for 24hr.

The followed delayed release may be attributed to diffusion of the dissolved drug within the PLGA core of the microparticle into the dissolution medium.

The release data were analyzed on the basis of Korsmeyer- Peppas equation and Higuchi kinetics. The release rates k and n of each model were calculated by linear regression analysis using Microsoft Excel 2007 software. Coefficients of correlation (R^2) were used to evaluate the accuracy of the fit. The R^2, k and n values are given in Table 5.

On calculating and comparing R^2 (0.8763 and 0.9835) values for Higuchi and Peppas kinetic models optimum formulation gave good fit to the Peppas model. Also because the release exponent: n is less than 0.5, the release

mechanism follows Fickian diffusion. Fickian drug release is characterized by a linear dependence of the released drug with the square root of time that is concentration dependent. The fundamental of diffusion is based on Fick's laws, which describe the macroscopic transport of molecules by a concentration gradient.

Table 5:- Values of R^2, k, and n for optimum Formulation.

	Higuchi	Korsmeyer-Peppas
R^2	0.8763	0.9835
Y	0.0762+0.508	0.4218x-1.5293
K or n	K=0.0762	n=0.42

Conclusion

Together with a good toxicity profile, adequate biopharmaceutical /pharmacokinetic characteristics are essential for the clinical success of drug candidates. Currently, a high percentage of drugs with good efficacy/toxicity ratio fail to go through the drug discovery pipeline due to their low intestinal absorption.

The two main causes of low oral bioavailability of drugs are poor solubility and low permeability through biological membranes. The origin of the biopharmaceutic classification system (BCS), which categorises drug molecules on the basis of solubility and permeability, has had a strong impact on drug research, development and regulatory approvals. This new concept has not only generated the need for extensive database regarding solubility and permeability coefficients of pre-existing molecules, but it has also made the determination of these values an imperative step in the basic drug development process. Drug molecules with low solubility and poor permeability through biomembranes (Class IV drugs) are the most difficult to formulate in conventional drug delivery systems [26-29].

furosemide which most used in the treatment of edematous states associated with chronic heart failure[1] is One of these potent drugs that belong to Class IV of BCS and has poor and low oral absorption due to its low solubility and low permeability.

CHF is a major public health problem in most industrialized countries where the elderly population is rapidly increasing and is the leading cause of mortality in most developed countries [30].

It was previously reported that the oral bioavailability of furosemide was poor and shows erratic absorption and high intersubject variation in pharmacokinetic parameters; however the causes of these problems remains unclear. Low solubility and permeability, intestinal secretion by efflux pump; gastric elimination by acid hydrolysis may be causes of this problem [2, 4-5]. Recently increasing attention has focused on formulating such therapeutic agents in biocompatible microparticles to enhance bioavailability, sustain drug release, and improve Stability from enzymatic degradation [6,31]. The aim of this work was to study the factorial effect of some process parameters on the pharmaceautical characteristics of PLGA microparticles containing furosemide to achieve a suitable carrier to improve intestinal absorption and subsequently oral bioavailability of drug.

From the experimental results obtained with respect to particle size, drug loading and prolonged drug release, it may be concluded that the developed PLGA microparticles could be useful for oral administration of furosemide. Pharmacokinetic and pharmacodynamic studies are required to confirm the efficacy of these oral microparticles and its effect on clinical use of this potent drug.

Aknowledgments

This work was supported by a grant from Kermanshah university of Medical sciences, Faculty of Pharmacy, Iran.

References

1. Aceves JM, Cruz R, Hernandez E. Preparation and characterization of Furosemide-Eudragit controlled release systems. Int J Pharm. 2000;195:45-53.
2. Beyers H, Malan S, Van der watt JG, De villiers MM. Structure–solubility relationship and thermal decomposition of furosemide. Drug Dev Ind Pharm. 2000;26(10):1077-1083.
3. Hua A, Jones SA, Villiers MMD, Lvov YM. Nano-encapsulation of furosemide microcrystals for controlled drug release. J Control Rel. 2003;86:59-68.
4. Flanagan ShD, Benet LZ. Net secretion of furosemide is subject to indomethacin inhibition, as observed in caco-2 monolayer and excised rat jejunum. J Pharm Res. 1999;16:221-224.
5. Bundgaard H, Noorgaard T, Nielsen NM. Photodegradation and hydrolysis of furosemide and furosemide esters in aqueous solutions. Int J Pharm. 1988;42:217-224.
6. Haznedar S, Dortunc B. Preparation and in vitro evaluation of Eudragit microparticles containing acetazolamide. Int J Pharm. 2004;269:131-140.
7. Li M, Rouaud O, Poncelet D. Microencapsulation by solvent evaporation: State of the art for process engineering approaches. Int. J. Pharm. 2008;363(1-2):26-39.
8. Dawes GJS, Fratila-Apachitei LE, Mulia K, Apachitei I, Witkamp G, Duszczyk J. Size effect of PLGA spheres on drug loading efficiency and release profiles. J Mater Sci: Mater Med. 2008. DOI: 10.1007/s10856-008-3666-0.
9. Zhang X, Wiss U, Pichora D, Goosen MFA. A mechanistic study of antibiotic release from biodegradable poly (D, L-lactide) cylinders. J. Control. Rel. 1994;31:129-144.
10. O'Hara P, Hickey AJ. Respirable PLGA microparticles containing rifampicin for the treatment of tuberculosis: Manufacture and characterization. J. Pharm. Res. 2000;17(8):955-961.
11. Kompella UB, Koushik K. Preparation of drug delivery systems using supercritical fluid technology. Crit. Rev. Ther Drug Carrier Syst. 2001;18(2):173-199.
12. Vandervoort J, Ludwig A. Biocompatible stabilizers in the preparation of PLGA nanoparticles: a factorial design study. Int J Pharm. 2002;238(1-2):77-92.
13. O'Donnell PB, McGinity JW. Preparation of microspheres by the solvent evaporation technique. Adv Drug Delivery Rev. 1997;28:25-42.
14. Derakhshandeh K, Erfan M, Dadashzadeh S. Encapsulation of 9- nitrocamptothecin, a novel anticancer drug, in biodegradable nanoparticles: Factorial design, characterization and release kinetics. Eur J pharm Biopharm. 2007;66:34-41.
15. Gohel M, Amin A. Formulation optimization of controlled release diclofenac sodium microparticles using factorial design. J Control Rel. 1998;51(2-3):115- 122.
16. Mayank D, Sandip B, Mansoor M. Formulation optimization for the nanoparticles-in-microsphere hybrid oral delivery system using factorial design. J Control Rel. 2006;110:422-430.
17. Mathew ST, Devi SG, KV S. Formulation and Evaluation of Ketorolac Tromethamine-loaded Albumin Microspheres for Potential Intramuscular Administration. AAPS Pharm Sci Tech. 2007;8(1):Article 14.
18. Rawat M, Saraf Sh, Saraf S. Influence of Selected Formulation Variables on the Preparation of Enzyme-entrapped Eudragit S100 Microspheres. AAPS Pharm Sci Tech. 2007;8(4):Article 116.
19. Patel JK, Patel RP, Amin AF, Patel MM. Formulation and Evaluation of Mucoadhesive Glipizide Microspheres. AAPS Pharm Sci Tech. 2005;6(1):49-55.
20. Dillen K, Vandervoort J, Mooter GVD, Verheyden L, Ludwig A. Factorial design, physicochemical characterization and

activity of ciprofloxacin-PLGA nanoparticles. Int J Pharma. 2004;275:171-187.
21. Bozkir A, Saka OM. Formulation and investigation of 5-FU nanoparticles with factorial design-based studies. J Farmaco. 2005;60:840-846.
22. Singh D, Saraf Sh, Dixit VK, Saraf S. Formulation Optimization of Gentamicin Loaded Eudragit RS100 Microparticles Using Factorial Design Study. Biol Pharm Bull. 2008;31(4):662-667.
23. Krznar DB, Filipovic J, Zorc B, Zoyko M. Dissolution of celecoxib from mucoadhesive disks based on polyaspartamide derivatives. Acta. Pharm. 2006;56:463-471.
24. Sancho CM, Herrero-Vanrell R, Negro S. Vitamin A palmitate and acyclovir biodegradable microspheres for intraocular sustained release. Int J Pharma. 2006;326:100-106.
25. Tanwar YS, Naruka PS, Ojha GR. Development and evaluation of floating microspheres of verapamil hydrochloride. J Rev Bras Cienc Farm. 2007;43:529-534.
26. Emami J. In vitro – In vivo Correlation: from theory to applications. J Pharm Pharmaceut Sci. 2006;9:169-189.
27. Yu LX, Amidon GL, Polli JE, Zhao H, Mehta MU, Conner DP, Shah VP, Lesko LG, Chen ML, Lee VHL, Hussain AS. Biopharmaceutics Classification System: The scientific basis for biowaiver extensions. J Pharm Res. 2002;19:921-925.
28. Martinez M, Augsburger L, Johnston T, Jones WW. Applying the Biopharmaceutics Classification System to veterinary pharmaceutical products: Part I: Biopharmaceutics and formulation considerations. J. Advanced Drug Delivery Reviews. 2002;54(6):805-824.
29. Sharma P, Chawla HPS, Panchagnula R. Analytical method for monitoring concentrations of cyclosporin and lovastatin in vitro in an everted rat intestinal sac absorption model. J Chromatography B. 2002;768:349-359.
30. Nessler J, Skrzypek A. Chronic heart failure in the elderly: a current medical problem. Pol. Arch. Med. Wewn. 2008;118(10):572-580.
31. Davis SS. The design and evaluation of controlled release systems for gastrointestinal tract. J Control Rel. 1985;2:27-38.