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Proje Başlığı : Eletriptan Hidrobromür içeren polimerik nanopartiküllerin hazırlanması ve yüksek performanslı sıvı kromatografisi ile in vitro değerlendirilmesine yönelik analiz yöntemi geliştirilmesi

Yukarıda bilgileri yazılı olan projemin sonuç raporunun e-kütüphanede yayınlanmasını;

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Prof. Dr. Sibel Ayşıl Özkan

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Ankara Üniversitesi Bilimsel Araştırma Projeleri
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I. Projenin Türkçe ve İngilizce Adı ve Özetleri

Türkçe Adı : Eletriptan Hidrobromür içeren polimerik nanopartiküllerin hazırlanması ve yüksek performanslı sıvı kromatografisi ile in vitro değerlendirilmesine yönelik analiz yöntemi geliştirilmesi

İngilizce Adı : In vitro evaluation of eletriptan polymeric nanoparticles via high performance liquid chromatohraphic technique

Özetleri : Eletriptan lipofilik, oral olarak uygulanabilen, hızlı etkili ve sumatriptandan 4-8 kat 5-HT 1B/1D reseptör afinitesine sahip bir etken maddedir. Kranyovasküler seçiciliği sumatriptanın 2-3 katıdır. Bu etkinliğin kardiyovasküler yan etkileri arttırdığına dair bir kanıt yoktur. Ancak P-glikoprotein (P-gp) substratı olması ve santral sinir sisteminden dışarı atılması nedeniyle eletriptanın kan beyin bariyerini geçişi diğer maddelerden düşüktür. Bu proje ile migren ataklarında kullanılan eletriptanın etkisini daha uzun süre sürdürebilmek ve daha düşük doz uygulanarak yan etkilerinin azaltılabilmesi amacıyla PLGA polimeri kullanılarak polimerik nanopartiküllerinin hazırlanması ve karakterizasyonu ile daha hassas ve etkin bir ölçüm amacıyla YPSK kullanılarak yeni bir miktar tayini yöntemi geliştirilmesi amaçlanmıştır. PLGA biyolojik uyumlu olan ve kullanımı uygun olan polimerlerden biridir. Biyolojik olarak uyumlu olan bu polimer ile hazırlanan bu polimerlerin ilaç yükleme ve salım özelliklerinin uygun olması nedeniyle birçok farklı uygulama yolu ile eletriptan uygulanması sağlanabilecek ve daha düşük dozla, daha uzun süre etki gösterilerek migren tedavisinde etkisi arttırılabilecektir. Ayrıca sağlanması planlanan daha düşük dozla tedavinin ölçülebilmesi, yine hazırlanacak nanopartiküllerin yükleme etkinliklerinin hassasiyeti ile in vitro salımlarındaki uzun sürecek düşük konsantrasyondaki eletriptan salımının ölçümündeki hassasiyetin arttırılması amacıyla YPSK kullanılarak yeni bir yöntem geliştirilmiştir.

Eletriptan is a lipophilic, orally administered, fast acting and sumatriptan 4-8-fold 5-HT 1B / 1D receptor affinity agent. Krankovascular selectivity is 2-3 times that of sumatriptan. There is no evidence that this effect increases cardiovascular side effects. However, due to the fact that P-glycoprotein (P-gp) is a substratum and is ejected from the central nervous system, eletriptan's blood-brain barrier penetration is lower than other substances. With this project, it is aimed to develop a new quantitative method using HPLC for the preparation and characterization of polymeric nanoparticles using PLGA polymer and for the more precise and effective measurement with the aim of reducing the side effects by applying the Eletriptan effect for migraine attacks for a longer period of time. PLGA is one of biocompatible and suitable polymers. these polymers to be prepared with this biocompatible polymer are suitable for drug loading and release properties, it will be possible to apply Eetriptan through many different applications and to increase the effect in treatment of migraine with lower dose and longer duration. Moreover, a new method using HPLC in order to be able to measure the lower dose treatment is provided, the sensitivities of the loading activities of the nanoparticles to be prepared and the sensitivity in the measurement of Eletriptan release in long-term low concentrations in vitro releases, is developed.

II. Amaç ve Kapsam

Eletriptan lipofilik, oral olarak uygulanabilen, hızlı etkili ve sumatriptandan 4-8 kat 5-HT 1B/1D reseptör afinitesine sahip bir etken maddedir. Kranyovasküler seçiciliği sumatriptanın 2-3 katıdır. Bu etkinliğin kardiyovasküler yan etkileri arttırdığına dair bir kanıt yoktur. Ancak P-glikoprotein (P-gp) substratı olması ve santral sinir sisteminden dışarı atılması nedeniyle eletriptanın kan beyin bariyerini geçişi diğer maddelerden düşüktür. Bu proje ile migren ataklarında kullanılan eletriptanın etkisini daha uzun süre sürdürebilmek ve daha düşük doz uygulanarak yan etkilerinin azaltılabilmesi amacıyla PLGA polimeri kullanılarak polimerik nanopartiküllerinin hazırlanması ve karakterizasyonu ile daha hassas ve etkin bir ölçüm amacıyla YPSK kullanılarak yeni bir miktar tayini yöntemi geliştirilmesi amaçlanmıştır.

Migren tekrarlanan ve iş gücü kaybına yol açan, 2007 yılı verilerine göre Türkiye'de prevalansı 15-55 yaş grubunda kadınlarda % 21.8'i ve erkelerde %10.9 olan ve patofizyolojisi halen tam olarak aydınlatılamamış bir nörolojik bozukluktur. Beyin sapındaki nörolojik bozukluğun migrene neden olduğu düşünülmektedir. Migren üç mekanizma ile gerçekleşir: Kranyal arteryel vazodilatasyon, ekstraserebral nörojenik inflamasyon ve santral ağrı iletiminin inhibisyonundaki azalma. Santral ve periferik olarak trigeminovasküler sistemin aktivasyonu, kalsitonin geni ile ilişkili peptid (CGRP) ve Nörokinin A gibi vasoaktif nöropeptidlerin salımına ve böylece vazodilatasyona, kan damarlarında steril inflamasyona ve ağrı sinyal iletimine neden olur. Bu proje ile migren ataklarında kullanılan eletriptanın etkisini daha uzun süre sürdürebilmek ve daha düşük doz uygulanarak yan etkilerinin azaltılabilmesi amacıyla PLGA polimeri kullanılarak polimerik nanopartiküllerinin hazırlanması ve karakterizasyonu ile daha hassas ve etkin bir ölçüm amacıyla YPSK kullanılarak yeni bir miktar tayini yöntemi geliştirilmesi amaçlanmıştır. PLGA biyolojik uyumlu olan ve kullanımı uygun olan polimerlerden biridir. Literatür incelemesi sonucu eletriptanın PLGA polimeri kullanılarak hazırlanmış bir polimerik nanopartikül formülasyonu ile karşılaşılmamıştır. Biyolojik olarak uyumlu olan bu polimer ile hazırlanacak bu polimerlerin ilaç yükleme ve salım özelliklerinin uygun olması durumunda birçok farklı uygulama yolu ile eletriptan uygulanması sağlanabilecek ve daha düşük dozla, daha uzun süre etki gösterilerek migren tedavisinde etkisi arttırılabilecektir. Ayrıca sağlanması planlanan daha düşük dozla tedavinin ölçülebilmesi, yine hazırlanacak nanopartiküllerin yükleme etkinliklerinin hassasiyeti ile in vitro salımlarındaki uzun sürecek düşük konsantrasyondaki eletriptan salımının

III. Materyal ve Yöntem

Proje sonuçlarını içeren, ISI' nın SCI kapsamında hakemli dergilerde yayınlanmış makalesi, III. Materyal ve Yöntem ve IV. Analiz ve Bulgular bölümleri yerine kabul edilmesinden ötürü yayın ek olarak verilmiştir.

IV. Analiz ve Bulgular

Proje sonuçlarını içeren, ISI' nın SCI kapsamında hakemli dergilerde yayınlanmış makalesi, III. Materyal ve Yöntem ve IV. Analiz ve Bulgular bölümleri yerine kabul edilmesinden ötürü yayın ek olarak verilmiştir.

V. Sonuç ve Öneriler

Bu proje ile daha önce hazırlandığı tespit edilemeyen eletriptan PLGA nanopartikülleri emülsifikasyon çözücü uçurma (s/y ve s/y/s) yöntemiyle hazırlanmış ve salım özellikleri ile yükleme etkinlikleri gibi özellikleri incelenmiştir. PLGA biyolojik olarak uyumlu olmasının yanında polimerik nanopartikül hazırlanmasında en yaygın kullanılan polimerlerden biridir. Bu özellikleri ile ilaç sanayisinde kullanıma uygundur. Uygun nanopartiküllerin üretimi ve istenilen özelliklerin sağlanması ile eletriptan etkinliği daha da artmış olması nedeniyle daha sonra yapılacak uygun tasarımlar ve in vivo deneyler için büyük bir ilerleme sağlamıştır. Ayrıca geliştirilen yeni YPSK yöntemi ile daha hassas ve hızlı eletriptan miktar tayini yapılabilmiştir. Çeşitli hareketli faz kompozisyonları denenerek maddelerin ayırımları için en iyi koşullar belirlenmiş ve farklı tip tampon çözeltileri, değişik pH değerleri, sıcaklık, modifiye edici ajanlar, akış hızı etkileri gibi deney şartları ile yöntem optimize edilmiştir. Sonuçlarımız SCI de taranan Journal of Pharmaceutical and Biomedical Analysis dergisinde yayınlanmıştır.

VI. Geleceğe İlişkin Öngörülen Katkılar

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VII. Sağlanan Altyapı Olanakları ile Varsa Gerçekleştirilen Projeler

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VIII. Sağlanan Altyapı Olanaklarının Varsa Bilim/Hizmet ve Eğitim Alanlarındaki Katkıları

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IX. Kaynaklar

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X. Ekler

a) Mali Bilanço ve Açıklamaları:

Bütçe Kodu	Açıklama	Önceki Yıldan Devir	Başlangıç Ödeneği	Eklenen Aktarma	Düşülen Aktarma	Eklenen Ödenek	Düşülen Ödenek	Net Ödenek	Harcanan	Bloke Edilen (Avans)	Bloke Edilen (Diğer)	Kalan
03.2	TÜKETİME YÖNELİK MAL VE MALZEME ALIMLARI	0,00	19.999,00	0,00	0,00	0,00	0,00	19.999,00	18.880,00	0,00	0,00	1.119,00
	Toplam	**0,00**	**19.999,00**	**0,00**	**0,00**	**0,00**	**0,00**	**19.999,00**	**18.880,00**	**0,00**	**0,00**	**1.119,00**

Tüm malzemeler alınmıştır.

b) Makine ve Teçhizatın Konumu ve İlerideki Kullanımına Dair Açıklamalar: -
c) Teknik ve Bilimsel Ayrıntılar (varsa Kesim III'de yer almayan analiz ayrıntıları): -
d) Sunumlar (bildiriler ve teknik raporlar) **(Altyapı Projeler için uygulanmaz):** Sevinc Kurbanoglu, Ozgur Esim, Cansel Kose Ozkan, Ayhan Savaser, Sibel A. Ozkan ,Yalcin Ozkan, Determination of anti-migraine drug from loaded polymeric nanoparticles via high performance liquid chromatography, Euroanalysis 2017, 26 Ağustos-1 Eylül 2017, İsveç
e) Yayınlar (hakemli bilimsel dergiler) ve tezler **(Altyapı Projeler için uygulanmaz):** Ozgur Esim, Ayhan Savaser, Sevinc Kurbanoglu, Cansel Kose Ozkan, Sibel A. Ozkan, Yalcin Ozkan Development of assay for determination of eletriptan hydrobromide in loaded PLGA nanoparticles Journal of Pharmaceutical and Biomedical Analysis 142 (2017) 74–83

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Development of assay for determination of eletriptan hydrobromide in loaded PLGA nanoparticles

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ABSTRACT

Eletriptan Hydrobromide is a serotonin 5-HT_1 receptor agonist and it used for the treatment of migraine headaches with or without aura. Even if the drug is well absorbed after oral administration, it has some drawbacks like first pass metabolism and decrease in bioavailability after migraine attacks. Encapsulation of drug into polymeric nanoparticles is one of the methods for protecting the drug against degradation. The present work described a preparation of Eletriptan Hydrobromide loaded poly (D,L-lactide-co-glycolide) nanoparticles prepared using o/w single emulsion solvent evaporation method. In order to determine the factors affecting the physicochemical properties of the nanoparticles on the particle size of poly (D,L-lactide-co-glycolide) nanoparticles, D-Optimal design is used. Moreover, novel, simple, sensitive, selective, and fully validated chromatographic technique for the quantification of Eletriptan Hydrobromide from Eletriptan Hydrobromide loaded poly(D,L-lactide-co-glycolide) nanoparticles was developed. Poly(D,L-lactide-co-glycolide) concentration, sonication time and sonication energy were found as significant factors ($p < 0.05$) on particle size of nanoparticles. Limit of detection and limit of quantification values were calculated as 0.28 $\mu g\,mL^{-1}$ and 0.86 $\mu g\,mL^{-1}$, respectively.

1. Introduction

Eletriptan Hydrobromide is a second generation triptan class drug designated as R-3-[(1-methyl-2-pyrrolidinyl) methyl]-5-[2-(phenyl sulfonyl) ethyl]-1hindole mono hydrobromide with a molecular weight of 463.40 g/mol. It is used to treat migraine but not prevention. Eletriptan Hydrobromide is readily soluble in water and methanol and well absorbed after oral administration [1]. Following oral administration plasma peak levels occurs after 1.5 h and mean absolute bioavailability is approximately 50% in healthy subjects. Even though the drug is well absorbed after oral administration, it undergoes first pass metabolism leading to reduce the oral bioavailability of approximately 50%. After migraine attack, mean bioavailability also falls approximately 30% and T_{max} increases to 2.8 h. It has a half-life of 4 h which is short for migraine with a characteristic recurrent headaches last 4–72 h [2].

Currently, Eletriptan Hydrobromide is available in oral formulations. Yet, due to problems like twice in a day administration, side effects at higher doses and gastrointestinal effects like first pass metabolism, tablet dosage form may not be the ideal route of administration. In order to enhance the bioavailability and reduce the side effects, it is beneficial to use prolonged release formulations or other routes [3].

Over the past few years there has been a great interest to patient-friendly and compliant dosage forms. Nano and micro sized particles are examples of these dosage forms and can be used as sustained release preparations which provide constant and prolonged action with reduced gastrointestinal toxic effects. In addition, these systems allow drug applications other than oral route. Taken into account the possibility to enhance the drug action and reduced side effects, Eletriptan Hydrobromide micro and nano-sized drugs have been developed as described in the literature aiming to apply the drug to various administration types [3,4]. However no method was found for the Poly (D,L-lactide-co-glycolide) (PLGA) nanoparticles of Eletriptan Hydrobromide.

Poly (D,L-lactide-co-glycolide) is a highly used polymer in medical industry due to its number of advantages over other poly-

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mers, including biodegradability, biocompatibility, and approval for human use granted by the US Food and Drug Administration [5]. It can be used as drug carrier especially in the formation of nanoparticles. The PLGA copolymers degrade in the body to lactic and glycolic acid. These two monomers are easily metabolized and eliminated as carbon dioxide and water [6]. As a result of these properties PLGA is an excellent choice for drug delivery.

Emulsification solvent evaporation is one of the most commonly used methods for preparation of polymeric nanoparticles due to formation of narrow sized small nanoparticles at the end of the process [7]. In this method, the polymer and drug are dissolved in a water-immiscible organic solvent, and this organic solvent is emulsified in an aqueous solution containing stabilizer. High-energy source such as an ultrasonic device, homogenizer, or colloid mill can be used for this process. The result particles varying sizes from a few nanometers to micrometers by controlling the applied energy and conditions can be obtained after the evaporation of organic solvent [8].

Analytical determination is an important topic in dosage form design. A suitable, precise, reproducible and validated method has to be offered for the qualitative and quantitative analysis of drugs and pharmaceutical substances starts from bulk drugs to the dosage forms [3]. Previously, many works related to determination of Eletriptan Hydrobromide in different biological matrix and pharmaceutical formulations using various analysis methods were described [2,9–11]. Among them HPLC is a mostly used method in the quantification of drugs because of their sensitivity, reproducibility and specificity [12]. Choosing the right method and validation for the intended purpose is crucial in drug development. Validation gives information about suitability of the method for intended application. HPLC is an important technique for fast and sensitive determination of drugs [13]. Eletriptan Hydrobromide is water soluble drug resulting limited drug entrapment in polymeric nanoparticles. Consequently development of a simple and reliable method with high sensitivity is a requirement.

The aim of this study is to optimize the formulation of PLGA nanoparticles, containing Eletriptan Hydrobromide, by trying to determine the factors affecting the physicochemical properties of the nanoparticles. For the determination of Eletriptan Hydrobromide, from its loaded form of PLGA nanoparticles, validated a novel simple, sensitive, selective, rapid RP-HPLC analytical technique also proposed. The prepared nanoparticles were evaluated by particle size, polydispersity index (PDI) and encapsulation efficiency. D-optimal response surface methodology was used perform the experiments. This design allows to determine the effect of different factors on the properties of nanoparticles, with minimum experiments [14].

2. Experimental

2.1. Materials and methods

Eletriptan Hydrobromide was purchased from Alfa Chemistry (USA). HPLC grade acetonitrile, methanol, orthophosphoric acid, PLGA (50:50), polyvinylalcohol (PVA) (Mowiol 4–88) and analytical grade potassium hydrogen phosphate obtained from Sigma-Aldrich (Munich, Germany). Dichloromethane was purchased from Merck (Darmstadt, Germany). Phosphoric acid and double distilled water were used for preparing the mobile phase solutions.

2.2. Equipment

The chromatographic system used in the proposed work was Agilent 1100 series LC system (Wilmington, DE, USA), equipped with a G1379A degasser, G1311A quaternary pump, G1313 auto injector and G1315B diode array detector (DAD). Meterlab Benchtop pH meter (PHM240, France) was used for pH measurements. Probe ultrasonicator (Bandelin Sonoplus, Berlin, Germany) and rotary evaporator (Buchi, Rotavapor R-215, Germany) were used for sonication and evaporation processes at nanoparticle preparation. The ultrapure water was obtained from Type I ultrapure water system (ELGA, USA). Centrifuge (IEC Centra MP4R, USA) was used to separate nanoparticles from aqueous phase. Particle size characterization of polymeric nanoparticles was performed with a Dynamic Light Scattering particle size analyzer (PSS, Nicomp nano, Z3000, USA).For scanning electron microscopy (SEM) images; samples were covered with gold solution using AMITECH K 550X instruments and SEM images were obtained using ZEISS EVO 40 (Merlin, Carl Zeiss).

2.3. Chromatographic conditions

An isocratic RP-HPLC method with a mobile phase containing a mixture of acetonitrile: potassium phosphate buffer (500 mmolL^{-1}, pH 3.5): water (30:6:64 v/v/v) was used. Detection wavelength of 225 nm was set with mobile phase using 1 mLmin^{-1} flow rate. Agilent Zorbax SB-C8 150 × 4.6 mm, 3.5 μm column was used as stationary phase. Mobile phase was filtered through 0.2 μm nylon filter (Alltech, USA) and degassed in ultrasonic bath for 5 min. The sample injection volume was kept at 10 μL and column oven temperature maintained to 25 °C.

2.4. System suitability tests and analytical method validation

Method validation is a necessary procedure in developing an analytical method studies to show developed method's reliability, accuracy, precision. In another words, it is important to show that analyses of all processes applied in the separation methods are selectable and feasible at an accurate, reliable and sufficient level. In this research, optimized chromatographic conditions were validated according to the International Conference on Harmonization (ICH) guideline [15,16]. Regarding to the ICH guidelines, evaluated parameters were system suitability tests (such as capacity factor, symmetry at 10% height, USP tailing, theoretical plate number, selectivity, and resolution) specificity, linearity and range, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy.

2.5. Nanoparticle sample preparation

Encapsulation efficiency of Eletriptan Hydrobromide from the optimum formulation which has the lowest PDI value was calculated by determining the free drug amount present in supernatant through RP-HPLC method. The Eletriptan Hydrobromide-PLGA nanoparticle suspension was centrifuged at 10000 rpm at 4 °C for 30 min, washed three times with distilled water and supernatant was collected. The total amount of unencapsulated drug was measured by the developed RP-HPLC method. Moreover, the percentage of drug encapsulation and drug loading were calculated by using the following equations:

$$\text{Encapsulation efficiency (\%)} = (\text{total amount of the drug} - \text{amount of the free drug}) / \text{total drug} \times 100$$

$$\text{Drug Loading(\%)} = (\text{total amount of the drug} - \text{amount of the free drug}) / \text{mass of the nanoparticles recovered} \times 100$$

2.6. Experimental design method: D-Optimal design

In this study, D-Optimal design which is a widely used form of Response Surface Methodology (RSM), was employed for constructing a model for optimization of Eletriptan Hydrobromide-PLGA nanoparticles prepared by o/w emulsification solvent evaporation technique. D-Optimal design was selected for the study as it generates fewer runs with independent variables. In order to evaluate the influence of operating parameters on the particle size of Eletriptan Hydrobromide-PLGA nanoparticles, the concentration of the polymer (X_1), surfactant (X_2) and drug ratio (X_3) and operational factors sonication energy (X_4) and sonication time (X_5) are chosen on the basis of experimental space. A D-optimal design is employed which refers to a design with a total of 33 experiments containing 5 factors and 3 levels for each.

The fitness of the model among the linear, two-factor interaction model and quadratic model was assessed on the basis of the analysis of variance p value and determination coefficient r^2, to predict the best suitable formulation. The p-value less than 0.05 were considered to be statistically significant and maximal R^2 value was the indicator of good quality. Optimization was executed by using a desirability function to obtain the optimal points concerning the predetermined constraints in which the mean diameter particle size and polydispersity index in minimum levels [17].

2.7. Preparation of nanoparticles

Eletriptan Hydrobromide-PLGA nanoparticles were prepared using o/w single emulsion solvent evaporation method [18]. In the process, the organic phase was prepared by dissolving accurately weighed PLGA and Eletriptan Hydrobromide in dichloromethane (1 mL) as organic solvent. The organic phase was then poured into an aqueous phase containing polyvinyl alcohol dissolved in water (10 mL). This particles of emulsion are broken down by applying external energy using a probe sonicator. The organic solvent was evaporated using rotary evaporator. The colloidal nanosuspension was centrifuged at 10000 rpm for 30 min at 4 °C to get the final nanoparticulate containing pellet as encapsulated Eletriptan Hydrobromide. The pellets were washed with deionized water three times to remove unencapsulated drug from the surface of nanoparticles. Finally, pellets were redispersed in water.

2.8. Characterization of nanoparticles

2.8.1. Measurement of particle size and polydispersity index

Average particle size and polydispersity index of the developed nanoparticles were determined by laser dynamic light scattering. Particle size measurements were performed in triplicate by diluting NPs suspension to in water. The polydispersity index value shows the particle size distribution of nanoparticles in a sample. Higher value of polydispersity index indicates the distribution of nanoparticles with variable size range which results in the formation of aggregates and could result in low stability of particle suspension and low homogeneity [19].

2.8.2. Scanning electron microscopy

The three different nanoparticle formulations which have various particle size and polydispersity index were examined under scanning electron microscope at 30000 magnification. Samples were analyzed after gold sputtered [20].

Table 1
Statistical evaluation of the calibration data by RP-LC.

Compound	Eletriptan Hydrobromide
Retention time(min)	6.25
Linearity range ($\mu g\,mL^{-1}$)	5–1000
Slope (mAU μg^{-1} mL)	3084.8
SE of slope	1.42×10^1
Intercept (mAU)	−3.738
SE of intercept	3.063
Determination coefficient	0.999
LOD ($\mu g\,mL^{-1}$)	0.28
LOQ ($\mu g\,mL^{-1}$)	0.86
Within day Repeatability [a] (RSD%)	0.341
Between day Repeatability [a] (RSD%)	0.564

3. Results

3.1. Analytical method validation

From the RP-HPLC results, it was observed that the Eletriptan Hydrobromide eluted at a retention time of 6.25 min (Fig. 1). Chromatogram did not present any interference effect in the retention time of Eletriptan Hydrobromide when the blank PLGA nanoparticles were injected. The specificity of the method was investigated by observing the absence of interferences of the excipients for nanoparticle preparation and none of the peaks were observed at the same retention time as the Eletriptan Hydrobromide peak. System suitability test were further performed related to USP criteria. Capacity factor (>2), symmetry at 10% height (<2), USP tailing (<2), theoretical plate number (>2000), selectivity >1, and resolution >2 parameters were examined. They were all in acceptable limits with values of capacity factor as 3.44, symmetry at 10% height as 1.036, USP tailing as 1.22, theoretical plate number as 16114, and selectivity as 10.304, and resolution as 16.258.

Linearity of the method was investigated using eight standard solutions of Eletriptan Hydrobromide that were freshly prepared in the concentration range of 5–1000 $\mu g\,mL^{-1}$. The obtained calibration curve was linear in the above mentioned concentration range. The mean values of slope and intercept were 3084.8 and −3.738, respectively. The determination coefficient was 0.999 for the Eletriptan Hydrobromide.

The limit of detection (LOD) and limit of quantification (LOQ) was determined by the analysis of samples with known concentrations of Eletriptan Hydrobromide and by establishing the minimum level at which this analyte can be reliably detected with the equation LOD = 3.3 s/m and LOQ = 10 s/m using the standard deviation of the lowest response (s) and the slope (m) of the calibration curve [15,21].

LOD was found as 0.28 $\mu g\,mL^{-1}$ and LOQ was found as 0.86 $\mu g\,mL^{-1}$. Standard deviation of slope and intercept values were also calculated. The accuracy was demonstrated by the recovery of known amounts of Eletriptan Hydrobromide. Recoveries from 95.0 to 105.0% of the added amounts are recommended in dissolution tests [13] and the results were reliable with the recommend values. The intra-day precision was evaluated at three different concentration levels. The intermediate precision was evaluated in the same solutions at different days. Values presented in Table 1 show the good precision of the method with relative standard deviation values lower than 2%. All the statistical parameters and recovery result were summarized in Tables 1 and 2.

3.2. Drug encapsulation efficiency

The drug encapsulation efficiency of Eletriptan Hydrobromide-PLGA nanoparticles was determined as described in the method section and using the analytical methodology of this study. The

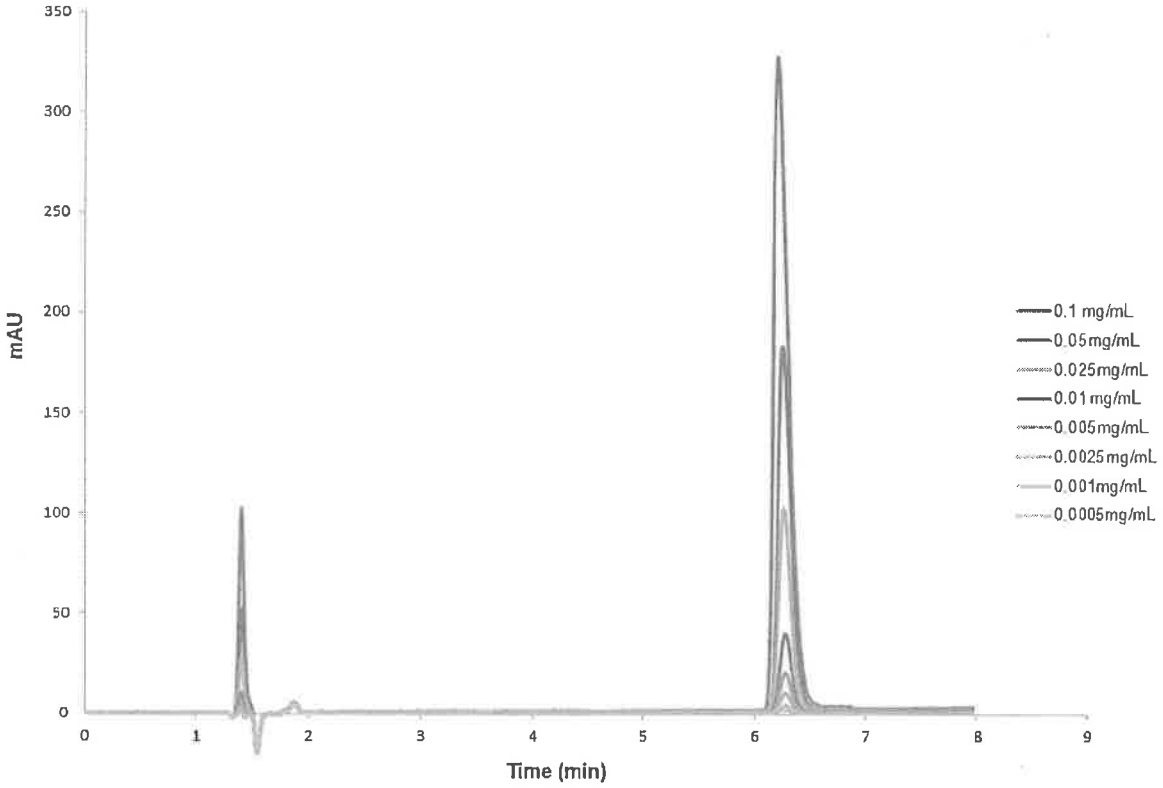

Fig. 1. Chromatograms of increasing concentrations of EletriptanHydrobromide.

Table 2
Recovery studies from Eletriptan Hydrobromide loaded nanoparticles.

Loaded (mg)	0.248
Found (mg)	0.251
Recovery (%)	101.21
RSD (%)	0.484
Bias (%)	0.011

encapsulation efficiency of the optimum formulation which has the lowest PDI value (formulation 8) was found as 6.70 ± 0.84% and the drug loading was found as 0.57 ± 0.05%. The encapsulation of water soluble drugs as well as Eletriptan Hydrobromide generally results low encapsulation by the conventional O/W solvent evaporation method due to rapid partitioning of the drug from the organic phase and into the aqueous phase. In order to overcome this problem innovative modifications to the conventional O/W solvent evaporation method have been reported [22].

3.3. Experimental design

Response Surface Methodology (RSM) is a combination of statistical and mathematical techniques used for designing and building of experimental models involving many interactive parameters. Moreover, RSM has been applied to calculate the relationship between multiple independent variables and optimization for a specific desired response. Classically, the application of RSM techniques in the development of the drug formulation can result in reduced process variability combined with the requirement of less resources (time, reagents and experimental work) by reducing the number of experimental runs that need to be performed [23].

Nanoparticles must be fabricated with an ideal ratio of drug and polymer for the highest encapsulation efficiency to prevent polymer toxicity [24]. Obtaining the effects of formulation variables on nanoparticle properties is important for providing maximum encapsulation efficiency and reduced particle sizes. It has been reported that smaller sized nanoparticles had a higher bioavailability than did larger particles [25].

Application of response surface methodology can be successfully applied for identification of descriptive variables that have significant effect on response properties [17]. D-optimal design is one of the response surface methodologies and can be applied for the optimization.

The application of experimental design methods in optimization of nanotechnology is useful due to high cost of materials and complex experimentation [26]. Experimental design studies can be applied to aim at finding the factors of main effects from larger pool of factors, finding a description of how factors affect the response, formulation optimization, calculation of the testing errors and mathematical modeling of the process. This study aims to enlighten which factors and their levels effecting the nanoparticle preparation. Specifically, D-Optimal Design is adapted to calculate the effects the parameters of o/w emulsification solvent evaporation process on particle size and polydispersity index of Eletriptan Hydrobromide-PLGA nanoparticles [19].

The effect of interaction between the independent variables on the size and encapsulation efficiency of Eletriptan Hydrobromide-PLGA nanoparticles was determined by D-Optimal Design. Based on this method, the interaction effect as indicated in the experimental runs can be explained through the analysis of variance (ANOVA) of the model [23]. Furthermore, it is essential to check the suitability of the model using the diagnostic graphs and to validate the model by checking the optimum experimental conditions [27].

The effects of the independent variables on the size of Eletriptan Hydrobromide-PLGA nanoparticles were investigated using the

Table 3
D-optimal design of nanoparticle preparation with responses.

Run	X1: PLGA (mg)	X2: PVA (%)	X3: EH (mg)	X4: Sonication Energy (W)	X5: Sonication Time (sec)	Y1: Z-Ave (nm)	Y2: PDI
1	75.00	1.00	15.00	30.00	60.00	438.7 ± 270.7	0.381
2	75.00	5.00	5.00	70.00	30.00	411.6 ± 195.9	0.227
3	75.00	5.00	15.00	30.00	30.00	316.8 ± 124.2	0.154
4	75.00	1.00	5.00	70.00	30.00	605.4 ± 372.0	0.377
5	25.00	1.00	5.00	30.00	30.00	265.6 ± 137.1	0.266
6	25.00	5.00	15.00	30.00	60.00	233.1 ± 129.2	0.307
7	25.00	1.00	5.00	30.00	90.00	289.7 ± 141.8	0.241
8	75.00	1.00	10.00	30.00	30.00	418.1 ± 61.0	0.021
9	25.00	1.00	5.00	70.00	30.00	312.1 ± 88.0	0.080
10	75.00	1.00	5.00	50.00	90.00	544.8 ± 384.1	0.497
11	75.00	1.00	15.00	30.00	60.00	324.5 ± 113.6	0.122
12	75.00	5.00	5.00	70.00	90.00	607.8 ± 211.5	0.121
13	25.00	5.00	5.00	30.00	30.00	299.6 ± 142.9	0.228
14	25.00	5.00	5.00	50.00	90.00	390.7 ± 177.0	0.205
15	50.00	3.00	12.50	50.00	60.00	454.0 ± 205.9	0.218
16	25.00	1.00	15.00	70.00	30.00	419.1 ± 281.6	0.452
17	50.00	3.00	12.50	50.00	60.00	426.6 ± 193.7	0.206
18	50.00	5.00	15.00	30.00	90.00	289.8 ± 53.6	0.034
19	75.00	5.00	15.00	70.00	90.00	872.5 ± 411.8	0.223
20	75.00	5.00	10.00	30.00	90.00	412.8 ± 61.1	0.022
21	50.00	5.00	5.00	30.00	90.00	352.9 ± 122.8	0.121
22	50.00	1.00	5.00	70.00	90.00	877.1 ± 761.3	0.753
23	75.00	3.00	15.00	70.00	30.00	384.7 ± 210.4	0.299
24	25.00	1.00	15.00	70.00	90.00	788.5 ± 500.7	0.403
25	75.00	3.00	5.00	30.00	30.00	372.7 ± 146.5	0.154
26	25.00	5.00	15.00	70.00	30.00	309.0 ± 91.5	0.088
27	25.00	1.00	15.00	30.00	30.00	317.1 ± 108.8	0.118
28	25.00	5.00	15.00	70.00	90.00	1029.6 ± 898.8	0.762
29	75.00	5.00	15.00	30.00	30.00	289.0 ± 87.6	0.092
30	50.00	1.00	15.00	30.00	90.00	331.0 ± 170.4	0.265
31	25.00	5.00	5.00	30.00	30.00	284.9 ± 110.2	0.150
32	25.00	5.00	5.00	70.00	60.00	360.8 ± 193.7	0.288
33	25.00	5.00	15.00	50.00	30.00	217.3 ± 106.7	0.241

Table 4
ANOVA results of the model.

	Mean Particle Size (Model: 2FI)					Polydispersity Index (Model: Linear)				
Source	Sum of Squares	df	Mean Square	F Value	p-value	Sum of Squares	df	Mean Square	F Value	p-value
Model	1131361.54	15	75424.10	9.84	<0.0001	0.3800	5	0.0760	3.24	0.0204
X1-PLGA Concentration	47107.51	1	47107.51	6.14	0.0240	0.0250	1	0.0250	1.06	0.3120
X2-PVA Concentration	32755.52	1	32755.52	4.27	0.0543	0.0870	1	0.0870	3.71	0.0647
X3-Drug Concentration	10037.22	1	10037.22	1.31	0.2684	0.0018	1	0.0018	0.078	0.7821
X4-Sonication Energy	398801.18	1	398801.18	52.01	<0.0001	0.1800	1	0.1800	7.85	0.0093
X5-Sonication Time	294744.64	1	294744.64	38.44	<0.0001	0.0740	1	0.0740	3.14	0.0874
X1*X2	4925.89	1	4925.89	0.64	0.4339					
X1*X3	8372.15	1	8372.15	1.09	0.3107					
X1*X4	51.05	1	51.05	0.01	0.9359					
X1*X5	8970.89	1	8970.89	1.17	0.2945					
X2*X3	5379.52	1	5379.52	0.70	0.4139					
X2*X4	7635.41	1	7635.41	1.00	0.3323					
X2*X5	8285.72	1	8285.72	1.08	0.3131					
X3*X4	24577.81	1	24577.81	3.21	0.0912					
X3*X5	27055.71	1	27055.71	3.53	0.0776					
X4*X5	241119.46	1	241119.46	31.44	<0.0001					
Residual	130362.84	17	7668.40			0.6300	27	0.0230		
Lack of Fit	122972.17	13	9459.40	5.12	0.0635	0.5900	23	0.0260	2.62	0.1808
Pure Error	7390.67	4	1847.67			0.0390	4	0.0098		
Cor Total	1261724.38	32				1.0100	32			

quadratic model. Mean diameter particle size and polydispersity index were shown in Table 3 and the analysis of variance based on ANOVA for this regression model is listed in Table 4. Two selected dependent variables particle size and Polydispersity index values were found to be in the range of 233.1–1029.6 nm and 0.021–0.762, respectively. Mathematical relationships were calculated by response surface regression analysis D-optimal design model using Design-Expert® 7.0 software and founded as follow:

$$\begin{aligned}\mathbf{ParticleSize} = {} & +457.41 + 46.60 * X_1 - 35.24 * X_2 \\ & + 20.22 * X_3 + 131.57 * X_4 + 107.12 * X_5 - 14.78 * X_1 * X_2 \\ & - 19.59 * X_1 * X_3 + 1.60 * X_1 * X_4 - 21.23 * X_1 * X_5 + 14.54 * X_2 * X_3 \\ & - 18.39 * X_2 * X_4 + 19.07 * X_2 * X_5 + 32.79 * X_3 * X_4 \\ & + 33.91 * X_3 * X_5 + 104.09 * X_4 * X_5\end{aligned}$$

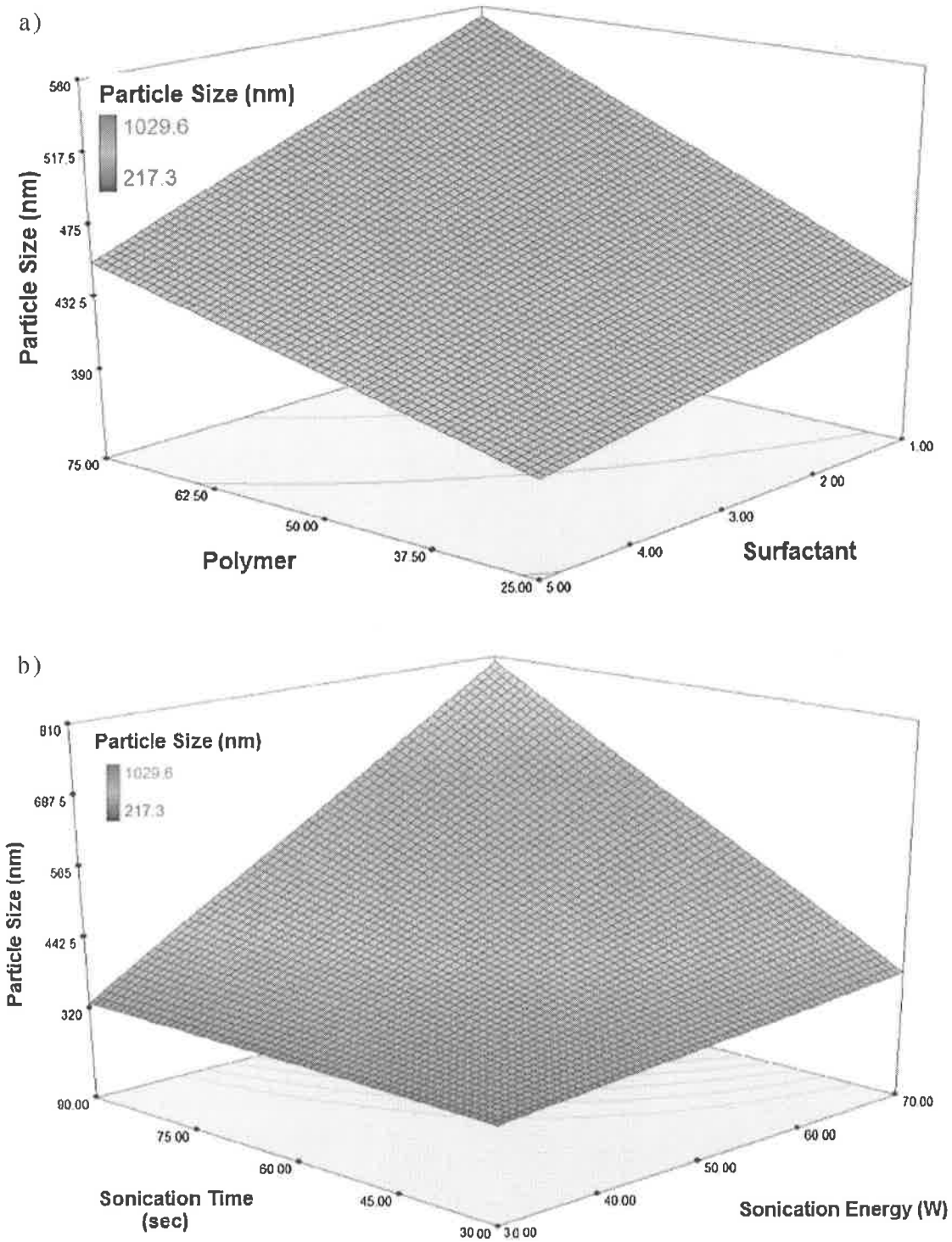

Fig. 2. Effect of polymer and surfactant concentration (a), sonication time and energy (b) on mean particle size.

$$PDI = +0.27 - 0.031 * X_1 - 0.055 * X_2 + 70.0079 * X_3 + 0.082 * X_4 + 0.053 * X_5$$

The model equation for the Eletriptan Hydrobromide-PLGA nanoparticle preparation was well described within the range of the independent variables. Model is considered to be significant when p value falls below 0.05. P values of models indicate that polydispersity index and particle size models are significant according to the p values of 0.0204 and <0.0001, respectively (Table 3). Moreover, high lack of fit values show insignificant relation to the pure error. In addition, the model's lack of fit with F-value of 5.12 and 2.62 appears to be insignificant relative to the pure error [27].In

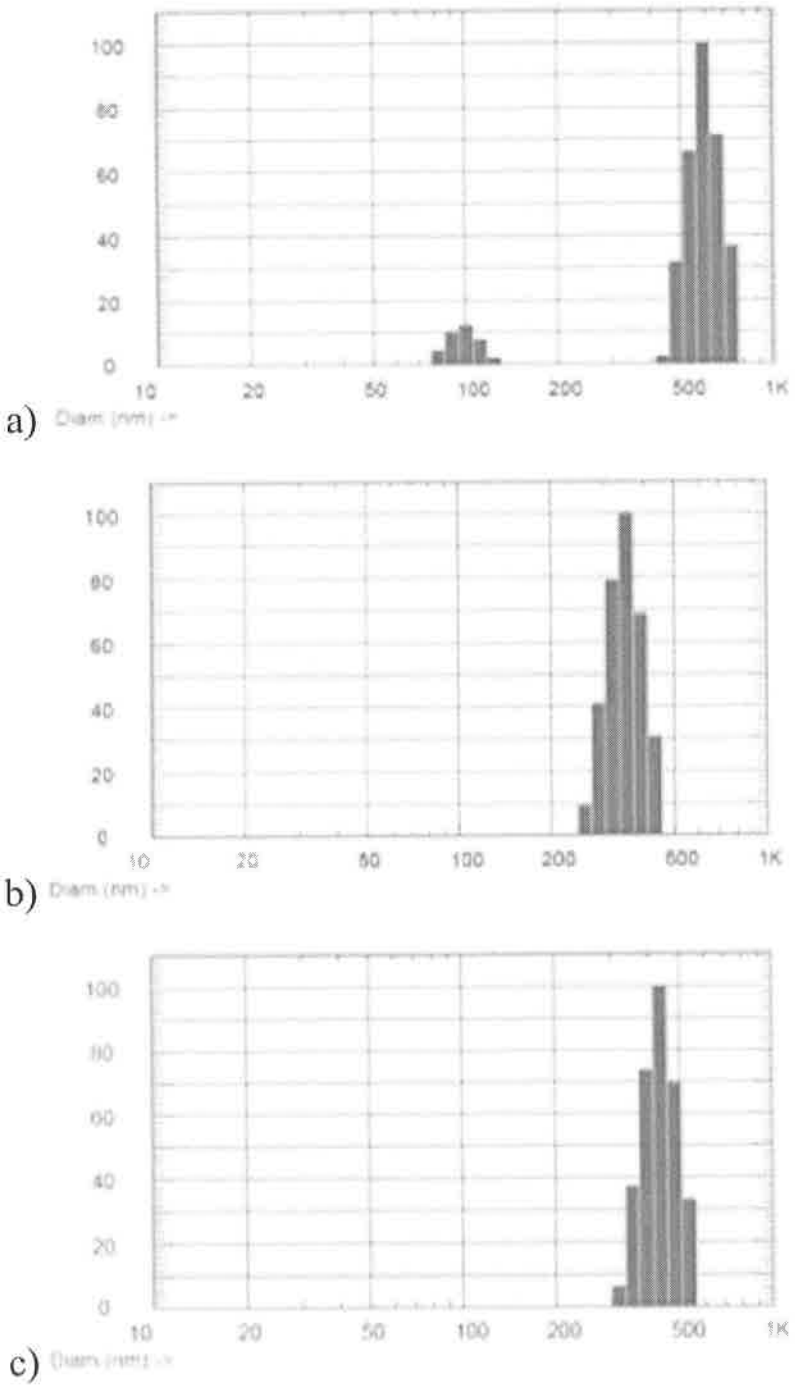

Fig. 3. Particle size distributions of formulations 28 (a), 25 (b) and 8 (c).

both responses p value of drug concentration are higher than 0.05. Due to this high results model is simplified by eliminating drug concentration [28].

The three dimensional response surface plots for significant factor variables on the particle size and polydispersity index are shown in Fig. 2. Fig. 2 showed the effect of drug, PLGA, PVA concentrations and sonication time and energy on the mean particle size of prepared Eletriptan Hydrobromide-PLGA nanoparticles.

As illustrated in Fig. 2a, the mean particle size was increased with the raise of polymer content. Moreover, the particle size was decreased with the higher level of surfactant concentration. Fig. 2b also showed that, higher sonication energy and time, increases the particle size. This result may be due to insufficient formation of particles as shown in polydispersity index results. As seen in Fig. 3, after higher sonication bimodal distribution was observed with the formation of smaller sized nanoparticles. As a result of this formation high polydispersity index results were observed [29].

In Fig. 4, the effect of independent variables on polydispersity index of nanoparticles is demonstrated. Polymer and surfactant content extensively effect the PDI as shown in Fig. 4a. When poly-

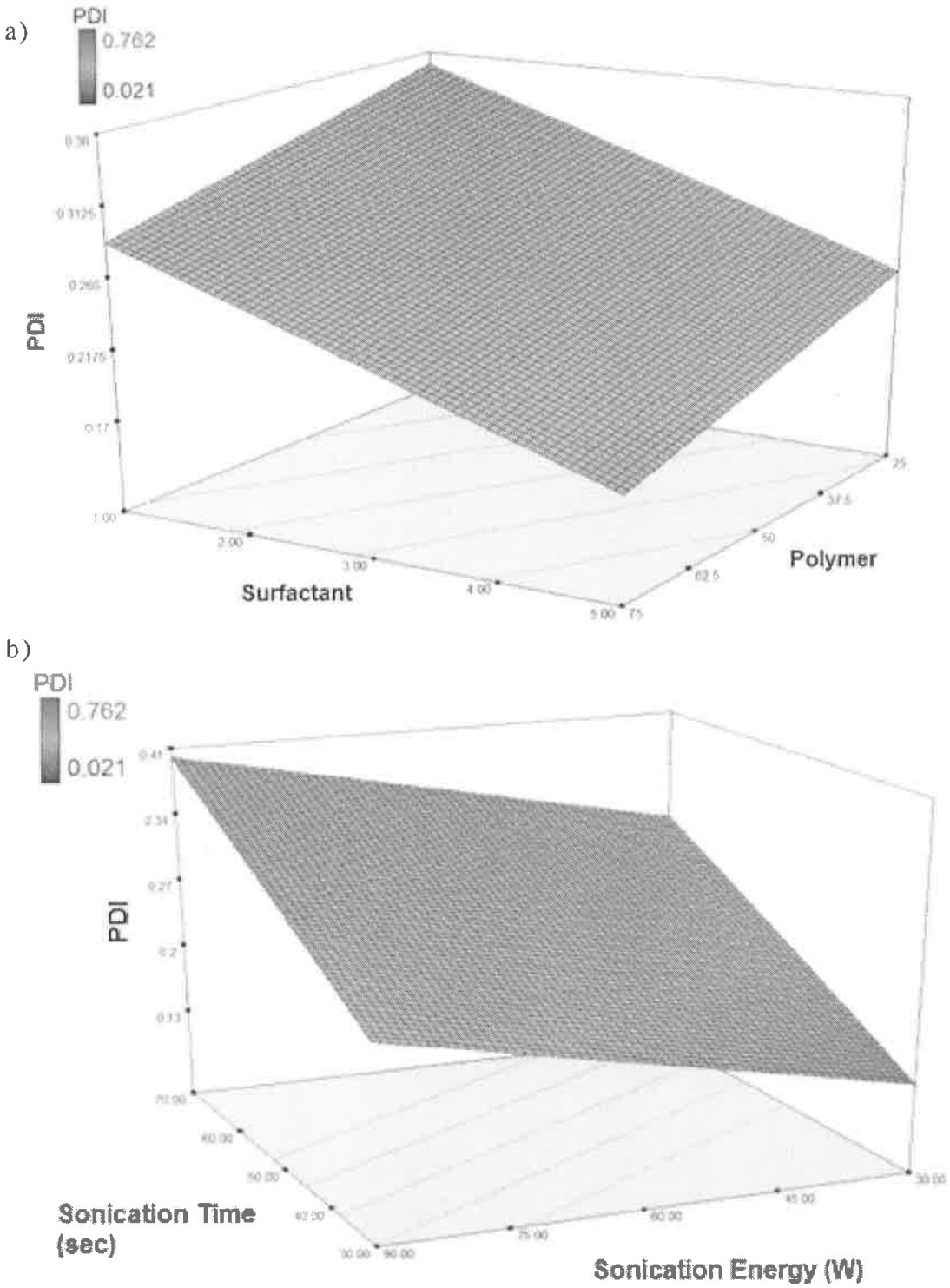

Fig. 4. Effect of polymer and surfactant concentration (a), sonication time and energy (b) on PDI.

mer or surfactant content increases there is an increase in PDI of nanoparticles. All other independent variables also influenced the PDI of nanoparticles. As sonication time and energy increased there was a decrease in polydispersity index of nanoparticles as shown Fig. 4b. After selecting constraints of desirability within the range, the optimum level of polymer content, surfactant concentration, sonication time and energy were found to be 75 mg, 1% (w/v), 30 W and 30 s, respectively. At these levels of independent variables, the predicted value of dependent variables mean particle size and the polydispersity index were generated to be 418.1 nm and 0.021, respectively [17].

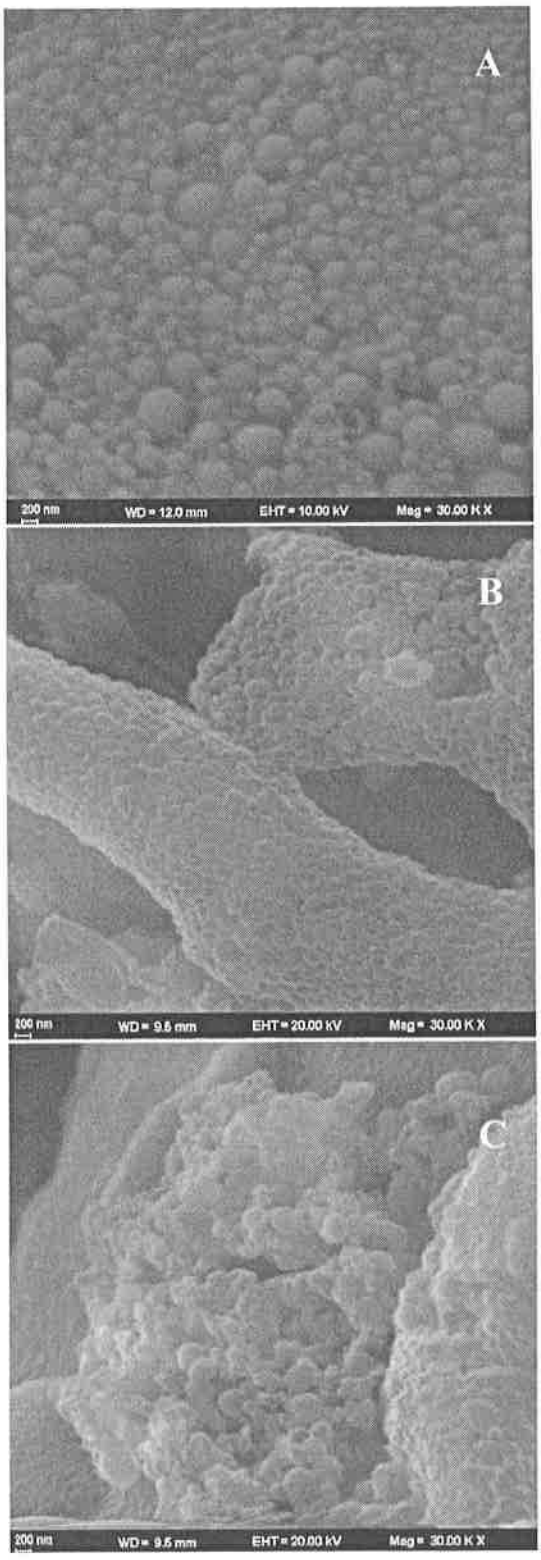

Fig. 5. SEM imagesof formulations 28 (a), 25 (b) and 8 (c).

3.4. Characterization of nanoparticles

Results from particle size analysis and SEM analysis are presented in Fig. 5. The morphological analyses of the nanoparticles showed that spherical shaped nanoparticles were formed (Fig. 5). The closeness of calculated and experimental values indicates the validity of the generated mathematical model for the prediction of particle size of particles [30].

5. Conclusion

It can be concluded from the study that it is possible to prepare Eletriptan Hydrobromide –PLGA nanoparticles using emulsification solvent evaporation method. The experiment was designed using D-Optimal design and the effects of variations in the drug, polymer, surfactant concentrations, sonication time and energy were evaluated through changes in the size of the nanoparticles. Formulation 8 was predicted as the optimal formulation with minimum polydispersity index. Moreover, novel, simple, sensitive, selective, and fully validated RP-HPLC technique for the quantification of Eletriptan Hydrobromide from drug loaded PLGA nanoparticles was developed. However encapsulation efficiency was found% 6.70 ± 0.84 and this result can be enhanced by changing polymers, surfactants or preparation methods. As further studies, this nanoparticles can be used in various root of administration for drug delivery studies.

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