RESEARCH ARTICLE

Fabrication and Optimization of Prolonged-release Microcapsules Containing Protein Drug (Erythropioten)

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ABSTRACT

Objectives: This study includes preparation, evaluation and *in-vitro* release profiles of erythropioten (EPO) microcapsules using poly lactic-co-glycolic acid (PLGA) polymer as coating material. Sustained release dosage form was grown up especially in the treatments of chronic disease to improve the drug efficacy, reduce side effects and improve patient compliance. EPO is a protein produced by the kidney to respond to a different type of anemia.

Methods: Microcapsules of EPO with prolonged-release profile were prepared by double emulsion method (w\o\w) using probe sonicator to get a homogenous double emulsion. PLGA was used as wall material.

Results: Eleven formulas of EPO-PLGA microcapsules were prepared to study the effect of different variables on the entrapment efficiency (EE) and %yield including the addition of plasticizer, NaCl, PLGA concentration and mixing type's effect. The selected formula containing 95000 IU of EPO gave (89%) EE and (85%) %yield with particle size 293.5 nm, zeta potential -41.33 with prolonged-release profile (99.7% within 6 months). Such formula is suggested to be incorporated in a suitable implantable dosage form to treat anemia associated with renal disorder.

Conclusion: EPO was successfully microcapsulated using PLGA polymer as a coat material by double emulsion method with good EE, %yield, particle size, and prolonged release profile continued up to 6 months.

Keywords: Erythropoietin, Microencapsulation, PLGA, PVA, Release.

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INTRODUCTION

Creating smart drug delivery systems is one of the most important aspects that can be used well and successfully to improve the availability of drugs and reduce the side effects.[1] As these systems guarantee a specific mechanism and control drug release by ensuring that the drugs stay for a longer period of time with reasonable concentration limits inside the body, to get the best results with the least side effects.[2]

Microencapsulation is one of the drug transport systems that help control drug releases and increase the stability of the product, especially if the substance is affected by changing the temperature or pH, such as drugs of a protein in nature or peptides.[3]

Erythropioten (EPO) is a protein made of 165 amino acids with a molecular weight of 40.3 kDa. EPO is produced mainly through the kidneys in the human body, and the secretion of the hormone increases in various type of anemia.[4] The amount of EPO decreases in cases of chronic kidney failure. Therefore, recombinant epotein is used to treat patients with chronic renal disease. The half life of EPO is approximately 4 hours after receiving an intravenous injection and patients require treatment from day-to-day subcutaneous injection or once weekly basis according to the dose approved by doctors.[5] EPO was usually given 2000 to 4000 IU subcutaneously every other.

One of the methods to sustain release of the drug for a long time of treatment is microencapsulation. Microcpsaulation of these types of drugs can give two advantages by improving drug stability and controlling the release of the medication for a long time.[6,7]

This work aims to prepare and evaluate microcapsule containing EPO to control the release for a long period of time to improve patient compliance and minimize the dose, decrease the side effect, and improve the stability of EPO.[8] Such microcapsules can be adapted to prepare a dosage form that may be alternative to the repeated injectable regimen for EPO.

METHODS AND MATERIALS

Erythroepotein (EPO) was purchased from Provizer, India. Cetyl trimethyl ammonium bromide (CTAB) was purchased from Shanghai, China. Dichloromethane (DCM) from Fluka,

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Germany. Glycerol and Poly vinyl alcohol (PVA) from GCC, U.K. NaCl from Alpha Chemical, India. PLGA from Shanghai ruizheng, India. Sucrose from Thomas baker, India. Probe sonicator, model (UCD-150), ultra sound power 150 W.

Preparation of Epotein Microcapsules

Three solutions were prepared, and the first solution was internal aqueous phase (IAP) containing 6.25 mg of PVA, 12.5 mg of glycerol, 100 mg of sucrose and 95,000 IU of EPO.[9]

The second solution was the oily phase containing 200 mg of PLGA dissolved in 2 mL of DCM. As for the third solution, the external aqueous solution (EAP), containing 80 mL of distilled water, 80 mg of CTAB and 2.336 g of NaCl.[10]

After preparing each solution separately, 2 mL of the internal aqueous solution that containing 95,000 international units of EPO mixed with 4 ml of the organic solution gradually and the resulting solution was homogenized using a probe sonicator for 4 minutes at a strength of 30% and at a temperature not exceeding 10ºC, using an ice bath during the sonication.[11]

The homogeneous mixture was gradually added to the external aqueous solution using the magnetic stirrer, at a speed of 1250 rpm at room temperature. Stirring continues for two hours to obtain W\O\W emulsion, and then the stirring continues for 12 hours to evaporate the DCM, and the microcapsules containing EPO coated with PLGA were separated and dried.[12] Eleven formulas (F1-F11) were prepared for optimization as shown in Table 1.

Calculation of Entrapment Efficiency and %Yield

The EE test is used to know the ability of the PLGA polymer to encapsulate the EPO to form the microcapsules. The EE can be calculated for all the prepared formulas by resuspending the microcapsules in distilled water and exposing them to the centrifuge at a speed of 6000 rpm for 15 minutes, then the encapsulation efficiency was calculated using the below equation.[13]

$$\%EE = \frac{Actual\ amount\ of\ drug\ in\ microcapsules}{Total\ amount\ of\ drug\ used} \times 100\%$$

Each formula was prepared in three patches and the average of EE and % yield was calculated for F1-F11formulas.

Calculation of %yield for each formula (F1-F11) was done by weighing the final dried microcapsules powder and comparing it with the total weight of the initial drug and polymer. It was calculated using the equation below[14]

$$\%\ yield = \frac{total\ weight\ of\ microcapsules}{total\ weight\ of\ drug\ and\ polymer} \times 100\%$$

The average of three patches of each formula prepared was taken and the % yield was calculated.

Variable Affecting the Entrapment Efficiency and %Yield of the Prepared Erythoepotein Microcapsules

Effect of Internal Aqueous Phase (IAP)

Three different volumes of IAP were used to prepare three formulas (F1-F3) to find out the extent of effect of IAP on the EE and % yield.[15]

Effect of NaCl in External Aqueous Phase (EAP)

Formula F4 was prepared without using NaCl in EAP, while formula F1 was prepared using an osmotic agent NaCl in EAP to observe the effect of the osmotic agent on EE and % yield.[16]

Table 1: Composition of prepared EPO microcapsules, EE and % yield of microcapsules, zeta potential and particle size.

No. of formula	*Volume of IAP(ml)*	*Volume of OP(ml)*	*Volume of EAP (ml)*	*NaCl gm*	*Conc. Of PLGA mg*	*Conc. Of PVA mg*	*Glycerol conc. gm*	*Sugar (mg)*	*Con. Of BSA (%)*	*Sonication time (min.)*	*Sonication power (150 W) (%)*	*EE%*	*% yield*	*Zeta potential*	*PDI*	*P. Size*
F1	1	10	80	2.336	100	6.25	12.5	100	1	4	30	89	85	-41.33	0.005	293.5
F2	2	10	80	2.336	100	6.25	12.5	100	1	4	30	87	79	-6.75	0.226	435.2
F3	3	10	80	2.336	100	6.25	12.5	100	1	4	30	65	74	-6.25	0.005	640.8
F4	1	10	80	–	100	6.25	12.5	100	1	4	30	68.5	76	-29.19	0.241	434.8
F5	1	10	80	2.336	75	6.25	12.5	100	1	4	30	85.2	80.5	12.25	0.493	846.9
F6	1	10	80	2.336	50	6.25	12.5	100	1	4	30	71.5	84.4	-6.5	0.005	514.2
F7	1	10	80	2.336	100	–	–	100	1	4	30	57.6	81.1%	-8.04	0.344	1004.9
F8	1	10	80	2.336	100	6.25	12.5	100	1	4	50	–	–	–	–	–
F9	1	10	80	2,336	100	6.25	12.5	–	1	4	30	39.2	55.9	-41.02	0.005	260.1
F10	1	10	80	2.336	100	6.25	12.5	100	1	6	30	36	62	3.01	0.465	961.3
F11	1	10	80	2.336	100	6.25	12.5	100	1	–	–	40.9	88.6	-6.72	0.005	599

Effect of PLGA Concentration

The PLGA concentration 100 mg/mL was used to prepare formula F1 while formula F5 and F6, containing 75 mg/mL and 50 mg/mL, respectively. These formulas (F1, F5 and F6) were used to study the effect of PLGA concentration on EE and % yield.[17]

Effect of Plasticizers

PVA and glycerol were used in preparation of microparticles as plasticizer. Formula F1 was prepared using 6.25 mg PVA and 12.5 mg glycerol, in other hand Formula F7 was prepared without plasticizers. These formulas (F1and F7) were used to find out the effect of PVA and glycerol on the EE and %yield.[18]

Effect of Sucrose Addition.

Formula F9 was prepared using no sugar to study the effect of adding sugar (sucrose) on the EE and %yield compared to formula F1 containing sucrose in its preparation.[19]

Effect of Mixing Type

Formula F1 was prepared using probe sonicator for 4 minutes of sonication, while Formula F11 was prepared using magnetic stirrer to study the effect of mixing on EE and %yield.[20]

Effect of Sonication Power

Formula F8 was prepared using 50% (75 W) of probe sonicator power in comparison with formula F1 prepared with 30% (50 W) of probe sonicator power.[21]

Effect of Sonication Time

Formula F10 was prepared using sonication for 6 minutes, to study the effect of sonication time on the EE and yield in comparison with formula F1 prepared with 4 minutes sonication time.[22]

Particle Size and Zeta Potential Analysis

Laser diffraction technique is usually used for determining the particle size in the pharmaceutical industry, and it can be used for drug production, development and quality assurance. The 100 mg of microcapsules of each formulas (F1–F11) were separately examined by a laser diffraction particle size analyzer. The particles were suspended in distilled water and characterized through the number and volume distribution using laser diffraction and polarization intensity differential scattering.[23]

***In-vitro* Release Study**

The in vitro drug release study was performed in a modified Franz diffusion cell for the prepared microcapsules (F1–F11) except formula F8.[22,23] This cell has donor and acceptor site separated by dialysis member (MWCO 3500). Each site had a capacity of 10 mL.[24] Each of the prepared microcapsules formulas (F1–F11) (equivalent to 95000 IU) was placed into the donor site to which 10 mL of phosphate buffer (pH 7.4) was added in both sides.

The diffusion cell was covered with an opaque cover to prevent the light since EPO is sensitive to light and placed in a water bath (37ºC) and stirred at 50 rpm using magnetic stirrer. The 10 mL were withdrawn from the acceptor site at defined time points and replaced with fresh buffer solution.[25] The samples were analyzed by UV-visible spectrophotometer at λ_{max} 280 nm. The release was followed for 6 months.

Selection of the Optimum Formula for EPO Microcapsules

The process of selecting the best formulas for microcapsules through this study relied mainly on the EE, %yield, the release profile of the formulas, and the particle size of the resulting microcapsule.

Morphological Characterization

The morphological characterization of the selected microcapsules Formula (F1) was determined by scanning electron microscopy (SEM).[22]

Statistical Analysis

The statistical analysis was performed using a single-variable analysis method (ANOVA). The statistical differences were taken when ($p < 0.05$). Data analysis was done by using the SPSS program.

RESULTS AND DISCUSSIONS

Variables affecting the EE and % yield for the prepared EPO microcapsules were studied using eleven formulas to determine the effect of different factors. The EE represents the amount of drug entrapped within the carrier covered by PLGA polymer. Different ways were used to indicate the EE, either directly or indirectly. An indirect method was applied in this study to calculate the EE, by measuring the free drug, which was not entrapped, and subtracting the results from the total amount of the drug used to calculate the capsulated drug.[26]

The %yield was calculated to indicate the least wastage of encapsulated drug prepared of microcapsules.[27] Table 1 shows EE and %yield results where the optimum EE and %yield appeared with formula F1 and the minimum EE showed by formula F10 while F9 showed minimum %yield.

Effect of IAP Volume on Entrapment Efficacy and %Yield

F1-F3 formulas were prepared in which IAP volume was 1-ml for formula (F1), 2 ml for formula (F2) and 3 ml for formula (F3). Table 1 shows that increasing IAP volume caused a decrease in the EE, this can be explained because the drug was dissolved in (IAP), so upon increasing IAP volume, the drug concentration decreased.[26,27] The drug concentration for formula (F1, F2 and F3) equal to 125, 90 and 62.5 µg/mL, respectively. A similar result was observed with the microencapsulation of insulin.[28]

Effect of Adding NaCl in External Aqueous Phase (EAP)

F4 was prepared without adding NaCl in EAP, in comparison to F1, which contains NaCl in EAP. The results show that the addition of NaCl to the external phase led to an increase in the EE with no significant difference in %yield since the addition of salt to the external phase led to an increase osmotic pressure in the polymer phase that prevents the escape of drug from IAP to EAP.[28,29] The result agreed

with that reported for dexamethasone sodium phosphate microencapsulation.[30]

Effect of PLGA concentration.

Formulas F6 (50 mg/mL), F5 (75 mg/mL) and F1 (100 mg/mL) containing different concentrations of PLGA were prepared to study the effect of PLGA concentration on EE and %yield. The result in Table 1 shows that as the polymer concentration increased, leading to a significant ($p < 0.05$) increase in EE and no significant difference in %yield. This can be explained because when the PLGA concentration increased, the precipitation of polymer became faster on the surface of the dispersion phase preventing the drug from escaping. Also, the viscosity was increased upon increasing the concentration of the polymer leading to a delay in the drug diffusion through the polymer, which produces no significant effect on %yield. Same results were observed with encapsulation of celocoxib.[31]

Effect of Stabilizer on Entrapment Efficacy and %Yield

The presence of stabilizers (PVA and Glycerol) showed a significant effect ($p < 0.05$) on the EE of the prepared formula but no significant change in the % yield where F1 (containing plasticizers) showed significantly $P < 0.05$ higher EE than F7. The presence of a stabilizer increases the viscosity of IAP, leading to a decrease in the drug leakage from microcapsules.[32] PVA also plays an important role in the formation and stabilization of the microencapsulated particles as it works as a surfactant and causes reduction in the surface tension at the interface between oil phase and water phase and prevents droplet coalescence in the oil medium. This stabilized the emulsion and facilitated the coating of the particles.[33] The same result was observed with the addition of a stabilizer in the internal water phase in the encapsulation of dexamethasone phosphate.[30]

Effect of Sugar (Sucrose)

Formulas F1 and F9 were prepared to study the effect of sucrose. Where formula F1 containing sucrose (100mg) while formula F9 has no sucrose. The EE of formula F1 (89%) and %yield (85%) were significantly ($p < 0.05$) higher than formula F9, since sucrose may prevent coalescence in the emulsion system by the formation of a protective film around the droplets, which provided less surface area for drug escape to the external processing medium.[32] The same results were observed with orange oil microencapsulation.[33]

Effect of Mixing

The results in Table 1 show that the encapsulation efficacy increased after 4 min sonication using probe sonicator for formula F1 (EE 89%) compared to formula F11 (EE 40.9%), which was prepared without sonication, while there was no significant effect on %yield. Sonication led to the production of homogenous dispersion and a decrease in particle size with increased surface area, which made the coating process much easier and gave high entrapment efficacy.[34,35] Same results were obtained with microencapsulation of Jussara pulp and cisplatin.[36,37]

Sonication Power Effect

Formula F8 was prepared using 50% (75W) of probe sonicator power compared to formula F1 prepared with 30% (50W) of probe sonicator power. The results show that increasing sonication power to 50% (75W) may lead to complete degradation of PLGA and converting the product to black color; therefore, no EE and %yield was calculated; similar results were observed in the preparation of PLGA nanoparticles.[38]

Sonication Time Effect

Increasing the sonication time up to 6 minutes as in formula (F10) caused a dramatic decrease in the EE compared to formula F1-indicating that excessive sonication might cause degradation of the polymer PLGA leading to a decrease in its capability for complete coating of the drug particles and leaching the entrapped drug.[39] Similar result was observed through varying the sonication time during the preparation of vicrestine and verapamil HCl nanoparticles.[40]

Particle Size and Zeta Potential

From the observation of the results in Table 1 (which shows the particle size, PdI and zeta potential of the prepared microcapsules (F1–F11)), it is found that the greater the volume of the internal aqueous phase in formulas F2 and F3 (2 mL, 3 mL), the larger the size of the microcapsules in comparison with formula F1 (1-mL). It means that a larger volume of internal phase embedded into the PLGA solution matrix could induce a looser cross-linking structure of the drug-loaded PLGA microparticles.[41,42] Same result was shown in microencapsulation of hydrophilic drug.[43]

The results also showed a significant ($p < 0.05$) increase in particle size of microcapsules in F7, which did not contain plasticizer in comparison with F1 (containing plasticizer) since the presence of PVA as a plasticizer which also act as a surface-active agent produced homogenous dispersion leading to decrease in the size of microparticles.[44] The same results were found with metoprolol sustained-release microspheres prepared by solvent evaporation method.[45]

Formula F11 (prepared without sonication) shows larger particle size than formula F1. The ultrasonic process is used as homogenizer to produce small particles in a liquid to improve uniformity and stability. Ultrasonic homogenizer sheds shear stress to break particles into smaller ones in which sonication time play a vital role. It is known that the size of the microcapsules formed is related to the size of the particles in the primary emulsion, so all the parameters such as solvent volume, drug polymer ratio, and solubility of organic solvent in water that affect the droplet size in the primary emulsion affect the particle size in the final emulsion.[46] These data agreed with data obtained from sylamarine microencapsulation.[47]

Zeta potential can be defined as the electrical potential at the slipping plane. This plane is the interface that separates the mobile fluid from fluid that remains attached to the surface. The zeta potential is an important and readily measurable indicator of the stability of colloidal dispersions. The magnitude of the zeta potential indicates the degree of electrostatic repulsion

between adjacent, similarly charged particles in the dispersion. For molecules and particles that are small enough, a high zeta potential will confirm stability, i.e., the solution or dispersion will resist aggregation. When the potential is small, attractive forces may exceed this repulsion, and the dispersion may break and flocculate. So, colloids with high zeta potential (negative or positive) are electrically stabilized, while colloids with low zeta potentials tend to coagulate or flocculate.[48] The zeta potential results in Table 1 revealed that; all prepared formulas showed moderate stability except formula F1 and formula F9 showed good stability that led to more homogenous dispersion and smaller particle size, indicating the efficiency of the applied microencapsulation method. The same results were observed in preparing hyaluronids loaded PLGA nanoparticles.[49]

In-vitro Release Study

The in vitro release was studied by drawing the percentage of the cumulative amount of drug released from EPO microcapsules versus time. Different variables affecting the release profile, including IAP volume, NaCl concentration, PLGA concentration, sonication effect and absence or presence of plasticizer had been investigated as the follows:

Effect of IAP on Release Profile.

The effect of internal aqueous phase volume on the in vitro release of microcapsule is shown in Figure 1. The drug release profile was found to be increased evidently from F3 (3 mL IAP 98.9% within 3 months) than F2 (2 mL IAP 99.6% within 5 months) and to formula F1 (1-mL IAP 99.7% within 6 months). The significantly rising drug release profile ($p < 0.05$) was caused by the more porous surface structure of microspheres prepared with larger internal aqueous phase volume. Moreover, the larger internal phase volume would lead to the thinner polymer layer of microspheres, inducing a faster drug release rate within first 10 days,[50] where F3 gave approximately 50% release, F2 gave approximately 35%, while F1 gave 15%. Same results were observed with Bioactive long-term release from biodegradable microspheres preserves implanted ALG-PLO-ALG microcapsules.[51]

Effect of NaCl Concentration on Release Profile

In general, release occurs in three phases: First, rapid release (initial release) followed by a second phase (lag-phase), characterized by a slow-release rate related to drug diffusion into the medium, and the third phase with a rapid release resulting from polymer erosion. Phase one and in some cases phase two can be influenced by the preparation method, whereas the erosion phase is dominated by the polymer.[52]

Figure 2 shows the effect of NaCl on the release profile of EPO microcapsules. The release profile from Formula F1 (containing NaCl) with formula F4 (no NaCl) shows that within the first month, F1 showed higher drug release than F4 because the presence of NaCl leads to osmotic pressure gradients between the two aqueous phases and water flux during microcapsules formation causing to the more porous surface of microcapsules that enhance drug release. Similar results were observed with a model protein where the porosity and the initial burst release were correlated.[53]

Effect of PLGA Concentration on Release Profile

Figure 3 shows the release profile of EPO microcapsules formulas (F1, F5 and F6) in which different concentrations of PLGA were used. Formula F1 (containing 100 mg/mL PLGA) showed slower release than formula F5 and formula F6 containing 75 and 50 mg/mL PLGA, respectively since PLGA (Lactide:Glycolide; 50:50) microcapsules displayed a typical three-phase release and slow degradation rate for its high lactic acid content. Therefore, formula F1 showed a slower release profile rate mainly at the second phase release (drug diffusion) due to thicker PLGA coat resulting from the disposition of a higher amount of polymer used. These results were observed in the preparation of three months injectable microsphere of super agonist leprolin.[54]

Effect of Plasticizer on Release Profile

Figure 4 shows the effect of plasticizer addition on the release profile of formula F1, which contain plasticizers (PVA and

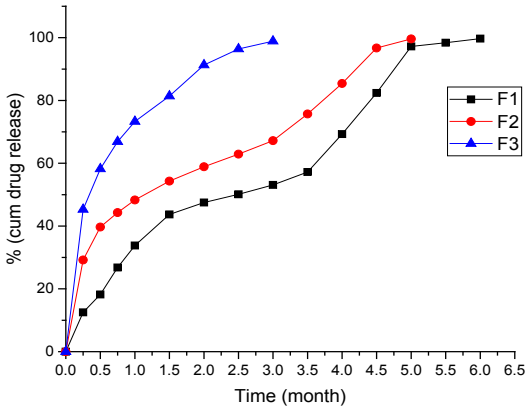


Figure 1: Effect of IAP volume in F1-F3 on release profile in phosphate buffer pH 7.4.

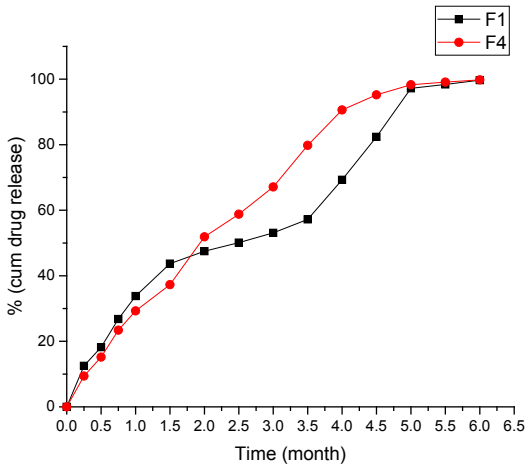


Figure 2: Effect of NaCl on release profile of EPO microcapsules (F1 and F4) in phosphate buffer pH 7.4.

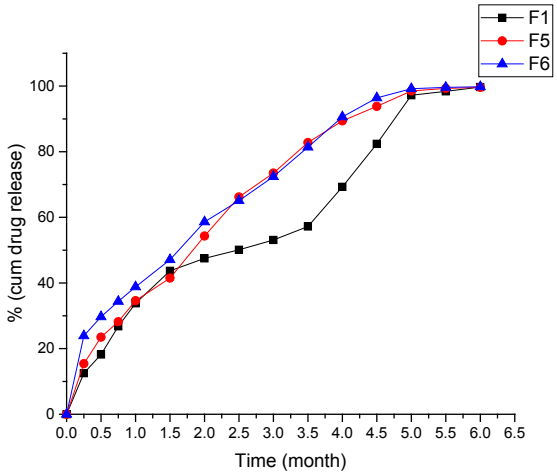


Figure 3: Effect of PLGA concentration on the EPO microcapsules (F1, F5 and F6) release profiles in phosphate buffer pH 7.4.

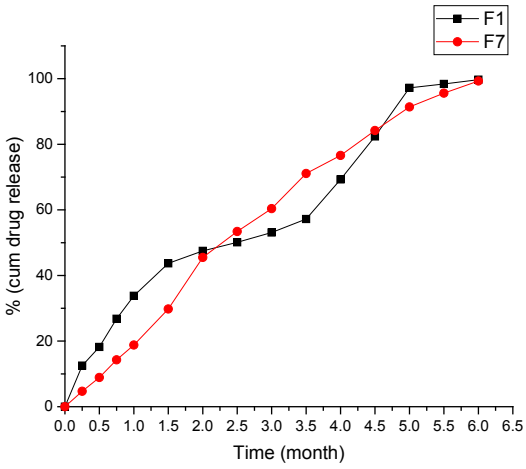
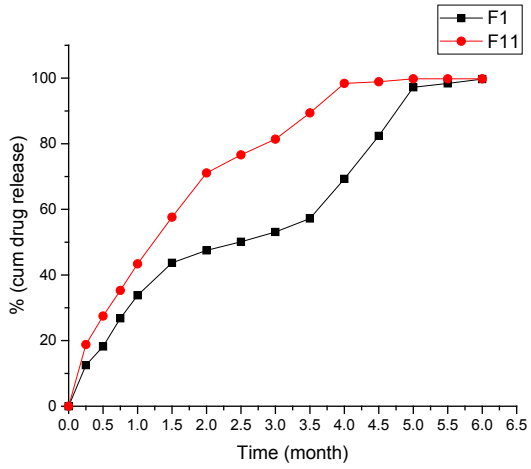


Figure 4: Effect of plasticizer on EPO microcapsule (F1and F7) release profiles in phosphate buffer pH 7.4.



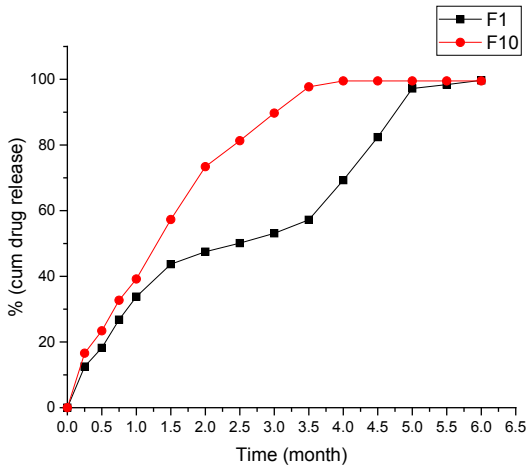


Figure 5: *A:* to the left effect of mixing on EPO microcapsules (F1 and F11) release profiles in phosphate buffer pH7.4, *B:* on to the right: Effect of sonication time on release profile of EPO microcpasules (F1 and F10) in phosphate buffer pH 7.4.

glycerol) in comparison to formula F7, which not contain plasticizer. The results showed that formula F1 gave a significantly ($p < 0.05$) higher release profile in the first two months than formula F7 and within first month (F1 gave 33.8%, F7 gave 18.8%) since the presence of PVA and glycerol led to the formation of pores on the surfaces of microcapsules attributed to leaching of plasticizers during dissolution so enhancing drug release. The presence of plasticizer lead to produce smaller particle size and higher EE of formula F1. Similar results were observed with the production of curcumin-biopolymer microspheres.[55]

Effect of Mixing Method on EPO Microcapsules Release Profiles

Figures 5 (A and B) show the effect of the sonication process on the release profiles of prepared microcapsules. It was reported that microcapsule production using ultrasound waves is efficient in terms of simplicity, reproducibility and process time. Ultrasound exerted a great impact on the properties of the prepared microcapsules. Scale-up procedure and commercialization would be possible if ultrasound technique is employed to produce microcapsule.[56]

Figure 5A shows the effect of using different mixing methods on the release profile of EPO where formula F1 (prepared using probe sonicator for mixing) showed a slower release profile in comparison with formula F11 (prepared using a magnetic stirrer). Using a magnetic stirrer may lead to the incomplete coating of the drug (also verified by low EE) during preparation which lead to faster release profile. Sonication leads to smaller particle size and higher EE and complete homogenous coated microcapsules for formula F1. Similar result was obtained from study of shear force to make a primary w/o emulsion on the morphology and protein release.[57]

Figure 5B shows the release profile of formula F10, which was sonicated for 6 minutes, compared to formula F1, which was sonicated for 4 minutes only. Where formula F10 gave 70% release within 2 months while formula F1 gave a significantly lower release profile (48% within 2 months) since excessive

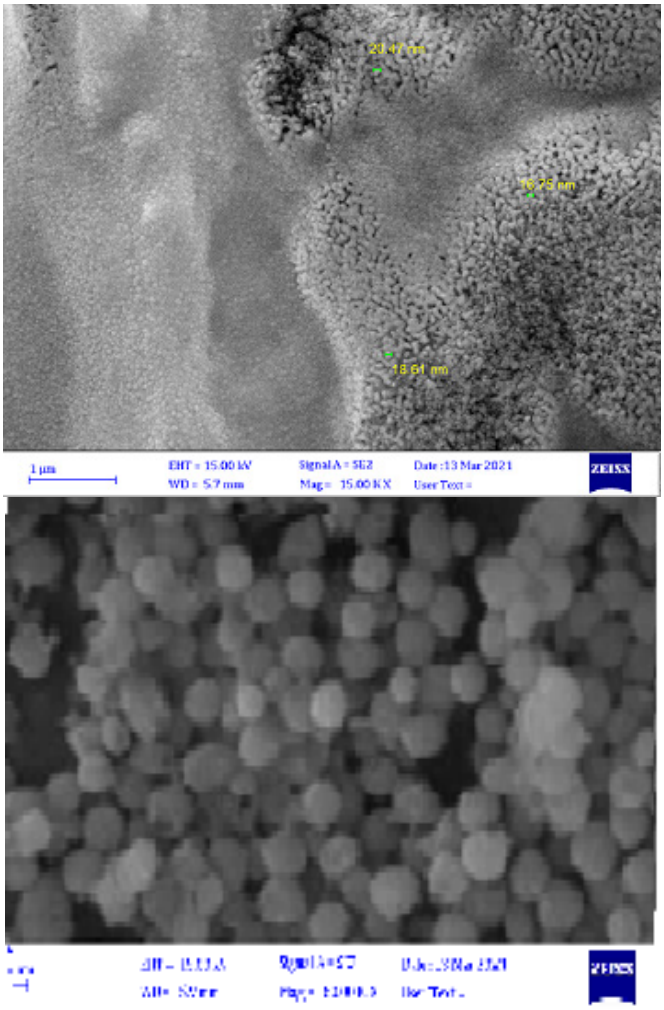


Figure 6: SEM image of formula F1: to the Left in 100 µm scale, and to the Right in 1 µm scale.

sonication time may lead to cracks and gaps in the coat layer, leading to increased release rate. The same result was obtained with mesoporous silica nanoparticles.[58]

Selection of the Best Formula

The selection of the optimum formulation was based on highest% entrapment efficiency and highest %yield, particle size, and in vitro release profile. Formula F1 gave the high EE (89%) and %yield (85%) as well as has a particle size (293 nm). The release profile of formula F1 within 2 months reached 47.5% and continued up to 100% after 6 months. Therefore F1 was selected as the best formula that may accomplish the aim of this work.

Morphological Evaluation for the Selected Formula

The morphology of selected microcapsules formula F1 is shown in Figure 6 using electron scanning microscopy. The EPO microcapsules of formula F1 had smooth surface with spherical shape, indicating efficient deposition of PLGA on the surface of the drug particles with complete coating and efficient conditions used to optimize the method applied. Similar observations were found with microcapsules prepared using the double emulsion method and with encapsulation royal jelly.[59,60]

CONCLUSION

EPO was successfully microencapsulated using PLGA polymer as a coat material by double emulsion method with good EE, % yield, particle size and prolonged-release profile continued up to 6 months. These microcapsules may be adapted to develop a prolonged-release dosage form for EPO that could be an alternative to the repeated EPO injectable regimen used in treating anemia associated with chronic renal disease. Such alternative dosage form may improve patient compliance, reducing the drug dose and its side effects.

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