Preparation and Evaluation of Microencapsulated Dexamethasone Sodium Phosphate Using Double Emulsion Method

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Abstract:

The need for producing sustained release dosage form was increased (especially for chronic diseases) to decrease side effect, increase efficacy of treatment, so it is now possible to administer the drugs once a week to once a year.

Dexamethasone sodium Phosphate (DSP) is a potent corticosteroid used for the treatment of many conditions like osteoarthritis (OA) and post joint replacement surgery. Microencapsulated dexamethasone sodium phosphate (DSP) having continuous prolonged release was prepared. To achieve that, DSP microcapsules were prepared using w/o/w double emulsion method where PLGA was used as a polymer, PVA and glycerol as plasticizer and Cetyl trimethyl ammonium bromide (CTAB) as emulsifying agent. Nine formulas of (PLGA coated (DSP) (microcapsules) were prepared to study the effect of different variables on the % yield and entrapment efficiency (EE) including the effect of internal aqueous phase volume, presence of NaCl (as osmotic pressure enhancer), PLGA concentration, plasticizer concentration and sonication time. The best formula was found to be formula (F1) containing 16 mg DSP coated with PLGA and found to give 85% EE and 94.19% yield.

Key words: Dexamethasone sodium phosphate (DSP), PLGA, GMO, entrapment efficiency, % yield.

تحضير وتقيم كبسولات مصغره للديكساميثازون فوسفات الصوديوم باستخدام طريقة مستحلب مزدوج

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****فرع الصيدلانيات/كليه الصيدله/ الجامعه المستنصريه***

الخلاصة:

مع زيادة الحاجة إلى إنتاج شكل صيدلاني مديده التحرر (خاصة للأمراض المزمنة) لتقليل الآثار الجانبية وزيادة فعالية العلاج ، كما فأنه من الممكن الآن استخدام العقاقير مرة واحدة في الأسبوع الى مرة واحدة في السنة. ديكساميثازون فوسفات الصوديوم هو كورتيكوستيرويد قوي يستخدم لعلاج العديد من الحالات مثل هشاشة العظام وبعد جراحة استبدال المفاصل. تم تحضير ديكساميثازون فوسفات الصوديوم على شكل كبسولات مصغره له القابليه على التحرر والاستدامه في العمل لمده طويله. ولتحقيق ذلك ، تم تحضير ميكروكابسولات باستخدام مستحلب مزدوج مائي/زيتي/مائي حيث تم استخدام حمض بولي اللاكتيك-غليكوليك كبوليمر ، بولي فينيل الكحول و كليسيرول كملدنات وسيتل بروميد الأمونيوم ثلاثي مثيل كعامل استحلاب. تم إعداد تسع صيغ من كبسولات دقيقة تحوي ديكساميثازون فوسفات الصوديوم المغلفة بماده حمض بولي اللاكتيك-غليكوليك لدراسة تأثير المتغيرات المختلفه على نسبة الإنتاجية والكفاءة بما في ذلك تأثير حجم الطور المائي الداخلي ، وجود كلوريد الصوديوم كمعزز الضغط التناضحي، تركيز حمض بولي اللاكتيك-غليكوليك ، تركيز الملدنات

ومده استخدام جهاز السونيكيشن. تم العثور على أفضل صيغة لتكون صيغة (ف1) والتي تحوي على16 ملغم محاطه بماده حمض بولي اللاكتيك-غليكوليك.وجد ان لهذه التركيبه 85٪ قابليه الحمل و 94.19٪ من نسبه الانتاج.

الكلمات المفتاحية: ديكساميثازون فوسفات الصوديوم ، مستحلب ، قابليه الحمل ، نسبه الانتاج ، كبسولات دقيقه.

Introduction:

Sustained release, sustained action, prolonged action, controlled release, extended release, depot release these are the various terms used to identify drug delivery systems that are designed to achieve a prolonged therapeutic effect by continuously releasing medication over a long period of time after administration of a single dose of drug [1]. The goal in designing sustained release delivery systems is to reduce frequency of dosing or to increase effectiveness of the drug by localization at the site of the action, reducing dose required or providing uniform drug delivery [2].

Sustained release dosage form could be found in many forms like tablets, capsules, granules and even microencapsulated particles.

Microencapsulation is defined as the application of a thin polymeric coating to individual core materials (tiny particles or droplets of liquids and dispersions) that have an arbitrary particle size range from 5-5000 µm to give small capsules with many useful properties [3].

Microencapsulation is applied to protect core material from the environmental factors like light, moisture, temperature or oxygen [4], to stabilize the core material in order to increase shelf-life of the products [5], and to control release of the core material.

Dexamethasone Sodium Phosphate (DSP) is a sodium phosphate salt form of dexamethasone, a synthetic adrenal corticosteroid with potent anti-inflammatory properties. DSP has oral bioavailability of 61%. It is removed rapidly from the blood stream and distributed to muscle, liver, skin, intestines, and kidneys [7]. It has many uses, one of them is to reduce swelling and pain and also reduce opioid consumption after joint replacement surgery by inhibiting prostaglandins E2 and F2 which is produced by fibroblasts and also reduce the pro-inflammatory enzyme, which present in high levels in the traumatic tissue [7]. It is usually used in high doses to become a very strong anti-inflammatory agent – even more than NSAID. It also reduces scar formation in order to keep the joint mobility and flexibility in movement.

The aim of this work is to prepare and optimize microcapsules containing DSP that can be continuously release the drug for a long time in order to reduce the side effect of this drug and to minimize the repeated dose to improve patient compliance.

Materials and Methods:

Materials: Dexamethasone sodium phosphate (DSP) was purchased from Provizer , India. Cetyl trimethyl ammonium bromide (CTAB) was purchased from Shanghai, China. Dichloromethane (DCM) from Fluka – Germany. Glycerol and Poly vinyl alcohol (PVA) from GCC, U.K. NaCl from Alpha chemical – India. Poly lactic-co-glycolic acid (PLGA) from Shanghai ruizheng, India. Sucrose from Thomas baker, India.

Construction of calibration curve:

Calibration curve of DSP in phosphate buffer pH 7.4 was obtained by preparing serial dilutions of the drug from concentration (5µg/ml) to (40 µg/ml). The absorbance of these diluted solutions was determined spectrophotometrically at 242 λ_{max} and plotted against concentration to get a calibration curve. The R^2 value and calibration curve equation were obtained [8].

Preparation of DSP Microcapsules Coated with PLGA:

The following three solutions were prepared:

1) Internal aqueous phase (IAP): which contain 6.25 mg Poly vinyl alcohol (PVA), 12.5 mg glycerol, 100 mg sucrose and 176 mg DSP dissolved in 1 mL distilled water.
2) Organic phase (OP): in which 200 mg Poly lactic-co-glycolic acid (PLGA) was dissolved in 2 mL dichloromethane (DCM).
3) External aqueous phase (EAP): which contain 80 mg Cetyl trimethyl ammonium bromide (CTAB) and 2.336 g of NaCl were dissolved in 80 mL distilled water.

After preparing each solution alone, (100µL) of IAP solution (containing 17.6 mg DSP which is equivalent to 16 mg dexamethasone [9]) was added to (2 mL) of the organic phase (OP) and homogenized using probe sonicator for 4 minutes under 60% powers and 30°C, then this mixture was added drop by drop to 80 mL of the external aqueous phase (EAP) solution with stirring using magnetic stirrer on 1500 rpm and 40°C , then the stirring continued for 12 hrs under the same condition [10] , where W/O/W dispersion containing microcapsules of DSP coated with PLGA was obtained which was subjected to centrifugation in a fixed-angle rotor for 15 min at 6000 rpm and the microcapsules were separated and washed with 15 mL distilled water and re-centrifuged again [11]. The obtained particles were dried. Nine formulas (F1-F9) of DSP microcapsules coated with PLGA (DSP microcapsules) were prepared as shown in table (1) to study the effect of different variables included in the preparation method on the % yield and entrapment efficiency (EE) of the prepared DSP microcapsules in order to optimize the method and select the best formulation for further study.

Variables Affecting the Entra-Pment Efficacy and % Yield of the Prepared DSP Microcapsules:

Entrapment efficacy (EE) was used to describe the efficiency of PLGA in the applied method to encapsulate DSP microcapsules. This test was performed by suspending the prepared microcapsules from each formulation F1-F9 in 15 mL distilled water, the dispersion was centrifuged under 6000 rpm for 15 min, and then takes 5 mL from the supernatant which contain the free drug. The amount of free DSP in the supernatant solution was analyzed by UV-spectrophotometry and subtracted from the total amount of drug used to get the actual amount of drug encapsulated then the following equation was applied [12].

$$\%EE=\frac{Actual\ amount\ of\ drug}{Total\ amount\ of\ drug\ used}\ X\ 100\%$$

Three patches from each formulation were prepared and the average entrapment efficacy for each formula was calculated.

The percentage yield of the DSP microcapsules coated with PLGA prepared from nine formulations (F1-F9) were calculated using the weight of the final dry microcapsules powder compared to the initial total weight of the drug and polymer used for the preparation of the microcapsules and the percentage yield was calculated by using the equation below [13]:

$$\%\ yield=\frac{Total\ Weight\ of\ microcapsules}{Total\ weight\ of\ the\ drug\ and\ polymer}\ X\ 100\%$$

Three patches from each formulation were prepared and the average % yield for each formula was calculated.

Effect of IAP Volume on Entrapment Efficacy (EE) and % Yield:

Formulations (F1 – F3) were prepared with different volume of internal aqueous phase (IAP) to study the effect of increasing internal aqueous phase (IAP) volume on entrapment efficacy (EE) and % yield.

Table 1: - Compositions of Microencapsulated DSP Formulas.

No. of Formula	volume of IAP µL	Volume of OP mL	volume of EAP mL	NaCl g	concentration of PLGA in OP mg / mL	PVA mg / mL	Glycerol mg / mL	Sucrose mg	Sonication time Min.
F1	100	2	80	2.336	100	6.25	12.5	100	4
F2	200	2	80	2.336	100	6.25	12.5	100	4
F3	300	2	80	2.336	100	6.25	12.5	100	4
F4	100	2	80	---	100	6.25	12.5	100	4
F5	100	2	80	2.336	75	6.25	12.5	100	4
F6	100	2	80	2.336	50	6.25	12.5	100	4
F7	100	2	80	2.336	100	---	---	100	4
F8	100	2	80	2.336	100	6.25	12.5	100	---
F9	100	2	80	2.336	100	6.25	12.5	100	10

Effect of Adding NaCl in the EAP on Entrapment Efficacy (EE) and % Yield:
Formula (F1) was prepared containing 2.336 g NaCl as osmotic pressure enhancer in comparison to Formula (F4) which did not contain NaCl.

Effect of Polymer Concentration on Entrapment Efficacy (EE) and % Yield:
Formulas (F1, F5 and F6) were prepared with different amount of polymer (PLGA) in the organic phase to study the effect of polymer concentration on entrapment efficacy (EE) and % yield.

Effect of Plasticizer on Entrapment Efficacy (EE) and % Yield:
Formulation (F7) was prepared without using PVA and glycerol (both as stabilizer) to study their effect on entrapment efficacy (EE) and % yield in comparison to formula (F1) which contain both.

Effect of Sonication Time on Entrapment Efficacy (EE) and % Yield:
Formulations (F1, F8 and F9) were prepared by applying different sonication time to study the effect of sonication time on entrapment efficacy (EE) and % yield.

Selection of the Optimum Formulation for DSP Microcapsules:
The selection of the optimum formulation was based on the formulation that gave highest % entrapment efficacy and highest % yield and its morphology studied by SEM with determination of its particle size.

Statistical Analysis:
Statistical analysis was done by using one-way analysis of variance (ANOVA). The differences were considered statistically significant when ($p < 0.05$). All data analysis was performed using SPSS software.

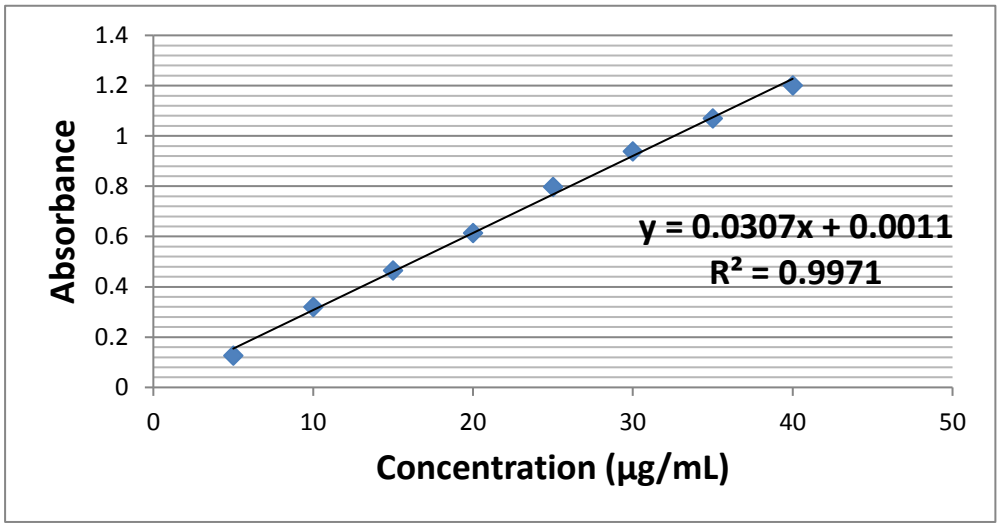


Figure 1: - Calibration curve of DSP in Phosphate Buffer pH 7.4

Results and Discussions:

Calibration curve of DSP in phosphate buffer pH 7.4:

The Calibration curve of DSP in phosphate buffer pH 7.4 obtained from plotting absorbance versus concentration with high regression coefficient (R2) proves that it follows (Beer's law) within the concentrations range used as shown in Figure (1) [14].

Variables Affecting Entrapment Efficacy and % Yield of the Prepared DSP Microcapsules:

Nine formulations for DSP micro-encapsulated particles were prepared in order to investigate the effect of different variables on entrapment efficacy and % yield. Entrapment efficiency used for the description of drug loading which is the percentage of drug that associated with the carrier which encapsulate or cover it. Many methods used to measure EE may be direct or indirect method, however the indirect method is much easier, in which the free dug (i.e. the one which was not encapsulated) were measured and subtracted from the total amount of drug used to get the encapsulated drug [15]. Percentage yield related to the product recovery and was mainly determined from the weight of the collected microencapsulated powder by finding the ratio of the weight of the resulting powder to the amount of the total weight and polymer used in the preparation of this microencapsulated particles. So, the highest % yield indicates the minimum loss of the encapsulated particles during the preparation [16].

The result of measuring encapsulation efficiency and % yield is shown in table (2) in which the highest entrapment efficacy and % yield was obtained from formula (F1) while the lowest Entrapment efficacy and % yield was obtained from formula (F8). 3.2.1 Effect of changing internal aqueous phase (IAP) volume on entrapment efficacy and % yield:

Concerning the effect of IAP volume on entrapment efficacy and % yield,

formulations (F1-F3) were prepared in which IAP volume was 100 µL for formula (F1), 200 µL for formula (F2) and 300 µL for formulation (F3). The results showed that the increase in IAP caused a decrease in the entrapment efficacy and % yield while there is no significant difference in % yield as shown in Figure (2).This can be explained on the basis that the drug was dissolved in the (IAP) so the increase in IAP volume caused a decrease in the concentration of the drug [17], where the drug concentration for formulations (F1, F2 and F3) were equal to 176, 88 and 58.6 mg/mL respectively. The decrease in the

concentration of the drug and polymer in the mixture was the main cause for the increase in the amount of free drug in it and cause the decrease in the entrapment efficacy [18].

Table 2: - The Entrapment Efficiency and %Yield for Each Formula

Formula number	Entrapment efficacy	% yield
F1	85%	94.19%
F2	78%	93.62%
F3	75%	91.89%
F4	70%	89.18%
F5	78%	92.19%
F6	69%	94.20%
F7	45%	89.95%
F8	33%	87.23%
F9	38%	%89.35

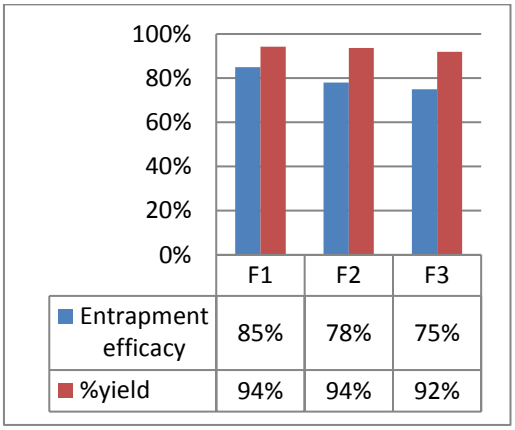


Figure 2: - The Effect of Increasing IAP on the Entrapment Efficacy and % Yield of Formulas F1, F2 & F3

Effect of Adding NaCl in the External Aqueous Phase (EAP) on the Entrapment Efficacy and % Yield:

The effect of adding NaCl to EAP was studied by comparing the result between formulation (F1) with formulation (F4) which was prepared without adding NaCl in the EAP, the results showed that the addition of NaCl to the EAP had significant increase ($P < 0.05$) in the entrapment efficacy and % yield as shown in Figure (3).

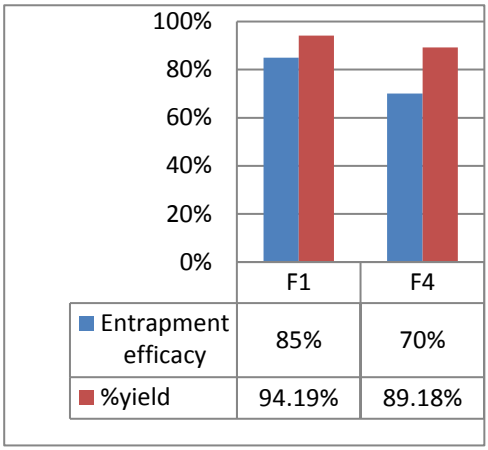
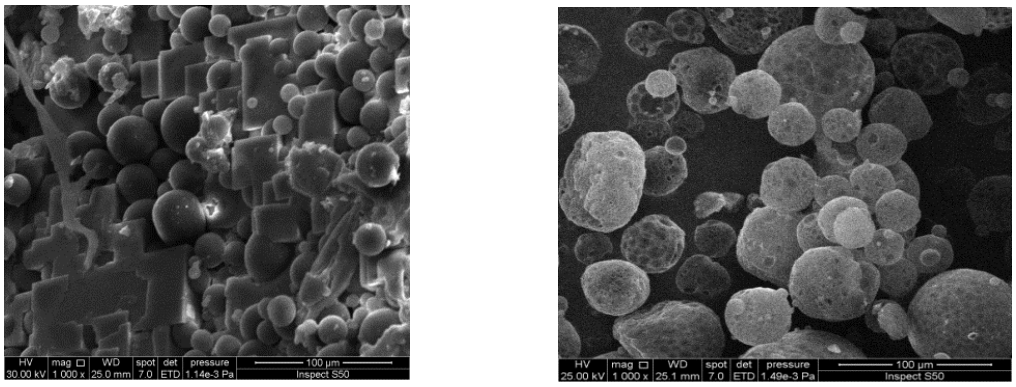


Figure 3: - Effect of Addition NaCl to the EAP on the Entrapment Efficacy and % Yield of Formula F1 and F4



A **B**

Figure 4: - SEM Pictures for (A) Microcapsules of Formula F1 (with NaCl), (B) Microcapsules of Formula F4 (without NaCl).

The addition of salt to the EAP caused an increase in the osmotic pressure in the polymer phase which prevented the escape of the drug from the IAP to the EAP by reducing the formation of water channels across the OP [19]. This difference in the osmotic pressure caused adhesion of the microcapsules. Into larger (accumulation) particles which also prevented the escape of the drug from the IAP into EAP. The resulting microcapsules prepared from formula (F1 and F4) were dried and seen under SEM to see the difference between them as shown in Figure (4).

The Effect of Polymer Concentration on the Entrapment Efficacy and % Yield:

Figure (5) shows the effect of increasing polymer concentration on the entrapment efficacy (EE) and % yield for the prepared formulas (F1, F5 and F6). The results showed that as the polymer concentration was increased, the EE was significantly increased ($P < 0.05$) while no significant changes in the % yield.

These results can be explained based on the increase in polymer concentration which cause faster precipitation of the polymer on the surface of the dispersed phase and prevented the drug diffusion across it [20].

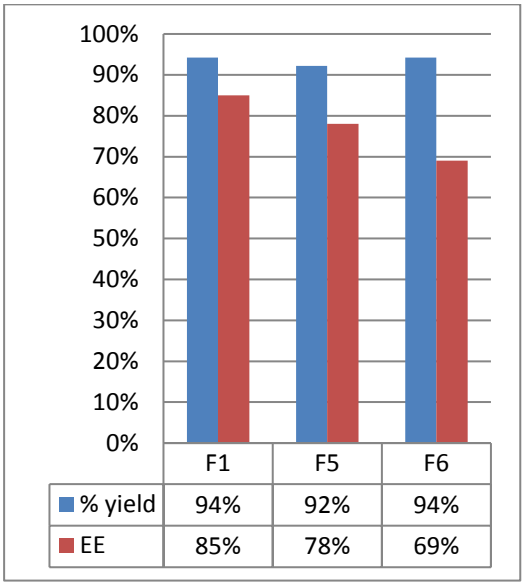


Figure 5: - The Effect of Increasing Polymer Concentration on the Encapsulation Efficiency and % Yield of Formula F1, F5 and F6.

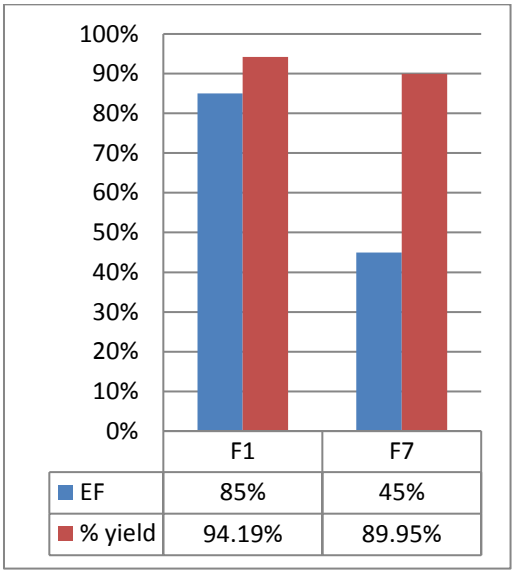


Figure 6: - Effect of Plasticizer on the Encapsulation Efficacy and % Yield of Formula F1 and F7

Effect of Plasticizer on Entrapment Efficacy and % Yield:
The presence of plasticizers (PVA and Glycerol) showed a significant effect on the EE of the prepared formulation while there was no significant change in the % yield Figure (6), where F1 (containing plasticizers) showed significantly higher EE than F7 (without plasticizers).The main effect of the plasticizers was due to their effect on the viscosity of the IAP (increase its viscosity) leading to a decrease in the drug leakage from the microcapsules [21]. PVA also play 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 as well as it prevents droplet coalescence in the oil medium. This stabilized the emulsion and facilitated the coating of the particles [22].

Effect of Sonication Time on Entrapment Efficacy and % Yield: The results in Figure (7) showed that there was a significant increase in the encapsulation efficacy after 4 min sonication for formula F1 (85%) comparing with formula F8 (38%) which was prepared without sonication. Sonication caused decrease in particle size and increased surface area which made the coating process much easier and gave high entrapment efficacy [23]. On the other hand, increasing the sonication time up to 10 min as in formula (F9) caused a dramatic decrease in the EE (33%) indicating that excessive sonication might cause degradation of the polymer PLGA leading to decrease in its capability for complete coating the drug particles [24]. Regarding % yield, there was no significant difference in the % yield of formulation F1 (4 min sonication) and F8 (no sonication) since % yield is a percentage ratio between weight of microcapsules obtained and total weight of materials used, while formula F9 (10 min sonication) showed a significant decrease. This proved that PLGA is degraded partially upon excessive sonication leading to lose some of its molecular weight. Same

results obtained with microencapsulation of Jussara pulp and cisplatin [25,26].

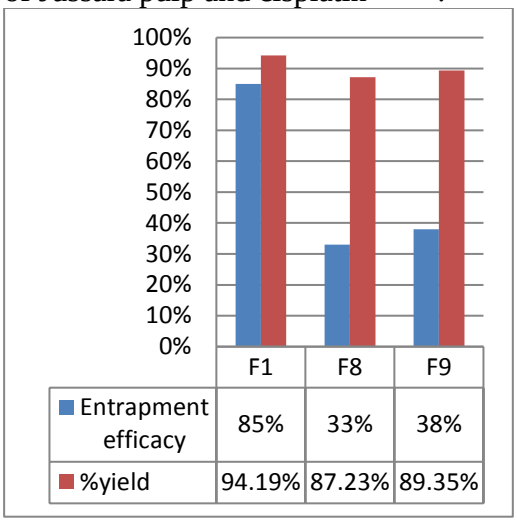


Figure 7: - The Effect of Sonication time on the Encapsulation Efficiency and % Yield for Formula F1, F8 and F9

Conclusion:

Depending on the results obtained in this study; this work succeeded in preparing microencapsulated DSP particles with PLGA. The optimum formulation was ready to be incorporated in any suitable dosage form.

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