Factors affecting preparation and evaluation of Kitorolac tromethamine microsponges for ocular use.
Wid F. Neamah*, Nidhal K. Maraie**

Ministry of health, Al-Karkh Health Department, Central Child Hospital*
Department of Pharmaceutics, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq**.

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Abstract:

Ketorolac Tromethamine (KT) is prepared for the first time by double emulsion procedure. The current research involves preparation and evaluation of microsponges for ocular applications. This work included preparation of sixteen formulas of KT-microsponges by double emulsion (w/o/w) method using poly lactic-co-glycolic acid (PLGA) as a polymer and poly vinyl alcohol (PVA) as a stabilizer, with different mixer types for different time and power. The prepared microsponges were characterized by Scanning Electron Microscopy (SEM) to investigate the morphology and particle size, the entrapment efficacy and percentage yield were calculated as well as in vitro drug release. Best formula (F14) of KT-microsponges had EE (74%), % yield (83%) with initial drug release (approximately 21% within the first fifteen days) which continued to reach (approximately 86% within 90 days) by using 30% of PLGA concentration with 0.05% of PVA and 200 ml of the external aqueous phase using a probe sonicator for 4 minutes at 200 Watt power. This formulation technique will be the interest of pharmaceutical company.

Key words: Microsponges, Ketorolac Tromethamine, poly lactic-co-glycolic acid (PLGA), poly vinyl alcohol (PVA).

الخلاصة:

يتضمن البحث الحالي إعداد وتقييم إسفنجيات مصغرة للعين من الكيتورولاك تروميثامين. وقد قام هذا العمل بتحضير فترة تركيبة من الكيتورولاك تروميثامين بواسطة طريقة المزدوج المشابه (الماء/الدمي/المائي) باستخدام حامض بولي لاكتيك-جليكوليك كيديكولك وبولي نيل الكيرول كرطب مع أنواع خلطة مختلفة وأختلاف الوقت والقوة. ميزت الاستنتاجات المصنعة المحضة بواسطة المسح المجهرى الإلكترونى الحديث لتحديد الشكل وحجم الجسيمات، تم حساب فعالية التحميل ونسبة الناتج وكذلك في تحيز الدواء مختبرياً. أفضل صيغة (F14) من الاستنتاجات المصنعة للكيتورولاك تروميثامين كانت كفاءة التغليف (74%)، % الناجج (83%) مع تحيز مبدئي للدواء (حوالي 21% خلال الخمسة عشر يومًا الأولي) واستمر التحفيز في الوصول (حوالي 86% خلال 90 يومًا) باستخدام 30% من تركيز البوليمر مع 0.05% من تركيز الميثيل والخماسي المائي المائي الخارجي الذي اختل العشوائي السوسيكين لمدة 4 دقائق في 200 واط بالطاقة. النتيجة لذلك، نقترح الصيغة المئلى لاستخدامها في إعطاء لها العين.

الكلمات المفتاحية: إسفنجيات مصغرة، كيتورولاك تروميثامين، بولي لاكتيك-جليكوليك، بولي نيل الكيرول

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Introduction:
Microsponges have a large impact on the healthcare as they provide sustained release and/or targeted release to specific site in the body. Dosage forms containing microsponge are applied in many pharmaceutical product (prescribed or over the counter) as well as to the cosmetic materials [1]. It is a modern development in microparticulate(s) drug delivery system. Microsponge is a polymeric delivery system consisting of spongy microspheres characterized as a tiny, inert non-collapsible structure with particle size range from 5-300 μm but the nanosponge is usually bellow 1 μm. This type of delivery system has a large impact on the healthcare as it provides sustained release and/or targeted release to specific site in the body [2].

Microsponges have many advantages as they are stable over wide range pH 1-11 and temperature up to 130°C. In addition, it is self-sterilizing since its average pore size is 0.25 μm in which the bacteria cannot penetrate. Furthermore, it is non-irritant, non-toxic and non-allergic as it delivers the drug in programmable manner without reduce its efficacy [3]. They have high loading capacity, free flowing and cost effectiveness [4]. There are many applications for microsponge including: topical application such as beta-methasone [5], oral application for example melatonin to control sleeping problems [6] and ophthalmic application such as acetazolamide microsphere in situ gel formulation [7].

Ketorolac is a non-steroidal anti-inflammatory drug belonging to nonselective COX inhibitor group. Ketorolac act as NSAID by inhibition of cyclooxygenase enzyme. It is used to control pain in cancer patients. Furthermore, intravitreal ketorolac tromethamine injection (Toradole® 30mg/ml) as anti-inflammatory agent is used to treat chronic cystoid macular edema after complicated cataract surgery. Acular® is a topical dosage form of ketorolac used for ocular condition as an anti-inflammatory and analgesic agent [8]. The aim of this work is the preparation of sustained release microsponges and studying the experimental conditions and factors to optimize the prepared microsponges for potential ocular administration.

Materials and methods:

Materials:
Ketorolac tromethamine (KT) was purchased from Provizer – India. Dichloromethane (DCM) was purchased from Fluka-Germany. Poly vinyl alcohol (PVA) was purchased from GCC, U.K. Poly lactic-co-glycolic acid (PLGA) was purchased from Shanghai, China.

Methods:
Melting point determination:
The melting point of ketorolac tromethamine (KT) was measured using capillary tube method. The capillary tube (sealed from one side) was dipped in the powder of drug then put it inside the apparatus (electrical melting point apparatus) and watching the drug powder through small window till reach the temperature that melted the drug gradually and converted from solid state to liquid one [9].

Determination of UV spectrum for Ketorolac tromethamine:
To prepare stock solution of KT (100 μg/ml), (10 mg) of KT was dissolved in phosphate buffer pH 7.4 (100 ml) then a dilute solution (10 μg/ml) from this stock solution was prepared and scanned against phosphate buffer (as a blank) over range of wavelength from 200-400 nm using UV visible spectrophotometer [10].

Calibration curve of Ketorolac tromethamine:
Calibration curve of KT was obtained in phosphate buffer pH 7.4 by preparing serial dilutions with concentrations (1, 2, 3, 4, 6, 8, 10, 12, 14, 16 and 18 μg/ml) from KT
stock solution. The absorbance was measured at the recommended $\lambda_{\text{max}}$ then plotted against concentration to obtain calibration curve by which $R^2$ value and calibration curve equation was obtained \[11\].

Preparation of microsponge containing Ketorolac tromethamine

Microsponges were prepared by double emulsion (w/o/w) method \[12\]. At first, the following three solutions were prepared individually: internal aqueous phase (IAP) which is composed of 25 mg of KT that dissolved in 1 ml deionized water (DIW), organic phase (OP) which is composed of 500 mg of poly lactic-co-glycolic acid (PLGA) that dissolved in 2.5 ml dichloromethane (DCM) and external aqueous phase (EAP) which is composed of 200 mg of poly vinyl alcohol (PVA) that dissolved in 200 ml deionized water (DIW).

When the solutions prepared each one separately, 250 µl of IAP solution (containing 6.25 mg KT) was added to the OP solution then homogenized with probe sonicator for 4 minutes with 40% power (200 Watt) at 30°C, then the mixture was added drop by drop to 200 ml of the EAP solution under stirring by magnetic stirrer on 800 rpm and 37°C for 12 hours. The microsponge particles were separated from the solution by filtration and washed three times with deionized water and left to dry for 24 hours under atmospheric conditions.

Sixteen formulas (F1-F16) of KT microsponges were prepared (Table 1). In order to choose the best formula, the effect of different variables (included in the preparation method) on the percent (%) yield and encapsulation efficiency of the prepared KT microsponges were studied.

<table>
<thead>
<tr>
<th>Formula number</th>
<th>Volume of IAP (µl)</th>
<th>Volume of OP (ml)</th>
<th>Volume of EAP (ml)</th>
<th>% of PLGA in OP</th>
<th>% PVA</th>
<th>Sonicati on type</th>
<th>Sonicati on power (% Watt)</th>
<th>Sonicati on time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>250</td>
<td>2.5</td>
<td>200</td>
<td>20%</td>
<td>0.1%</td>
<td>Probe sonicator</td>
<td>20 (100 W)</td>
<td>4</td>
</tr>
<tr>
<td>F2</td>
<td>250</td>
<td>2.5</td>
<td>200</td>
<td>20%</td>
<td>0.1%</td>
<td>Probe sonicator</td>
<td>30 (150 W)</td>
<td>4</td>
</tr>
<tr>
<td>F3</td>
<td>250</td>
<td>2.5</td>
<td>200</td>
<td>20%</td>
<td>0.1%</td>
<td>Probe sonicator</td>
<td>40 (200 W)</td>
<td>4</td>
</tr>
<tr>
<td>F4</td>
<td>250</td>
<td>2.5</td>
<td>200</td>
<td>20%</td>
<td>0.1%</td>
<td>Probe sonicator</td>
<td>50 (250 W)</td>
<td>4</td>
</tr>
<tr>
<td>F5</td>
<td>250</td>
<td>2.5</td>
<td>200</td>
<td>20%</td>
<td>0.1%</td>
<td>Probe sonicator</td>
<td>60 (300 W)</td>
<td>4</td>
</tr>
<tr>
<td>F6</td>
<td>250</td>
<td>2.5</td>
<td>200</td>
<td>20%</td>
<td>0.1%</td>
<td>Probe sonicator</td>
<td>80 (400 W)</td>
<td>4</td>
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<td>F7</td>
<td>250</td>
<td>2.5</td>
<td>200</td>
<td>20%</td>
<td>0.1%</td>
<td>Probe sonicator</td>
<td>40 (200 W)</td>
<td>6</td>
</tr>
<tr>
<td>F8</td>
<td>250</td>
<td>2.5</td>
<td>200</td>
<td>20%</td>
<td>0.1%</td>
<td>Probe sonicator</td>
<td>40 (200 W)</td>
<td>2</td>
</tr>
<tr>
<td>F9</td>
<td>250</td>
<td>2.5</td>
<td>200</td>
<td>10%</td>
<td>0.1%</td>
<td>Probe sonicator</td>
<td>40 (200 W)</td>
<td>4</td>
</tr>
<tr>
<td>F10</td>
<td>250</td>
<td>2.5</td>
<td>200</td>
<td>30%</td>
<td>0.1%</td>
<td>Probe sonicator</td>
<td>40 (200 W)</td>
<td>4</td>
</tr>
<tr>
<td>F11</td>
<td>250</td>
<td>2.5</td>
<td>200</td>
<td>40%</td>
<td>0.1%</td>
<td>Probe sonicator</td>
<td>40 (200 W)</td>
<td>4</td>
</tr>
<tr>
<td>F12</td>
<td>250</td>
<td>2.5</td>
<td>100</td>
<td>30%</td>
<td>0.1%</td>
<td>Probe sonicator</td>
<td>40 (200 W)</td>
<td>4</td>
</tr>
<tr>
<td>F13</td>
<td>250</td>
<td>2.5</td>
<td>300</td>
<td>30%</td>
<td>0.1%</td>
<td>Probe sonicator</td>
<td>40 (200 W)</td>
<td>4</td>
</tr>
<tr>
<td>F14</td>
<td>250</td>
<td>2.5</td>
<td>200</td>
<td>30%</td>
<td>0.05%</td>
<td>Probe sonicator</td>
<td>40 (200 W)</td>
<td>4</td>
</tr>
<tr>
<td>F15</td>
<td>250</td>
<td>2.5</td>
<td>200</td>
<td>30%</td>
<td>0.5%</td>
<td>Probe sonicator</td>
<td>40 (200 W)</td>
<td>4</td>
</tr>
<tr>
<td>F16</td>
<td>250</td>
<td>2.5</td>
<td>200</td>
<td>20%</td>
<td>0.1%</td>
<td>Vortex</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IAP = Internal Aqueous Phase, OP = Organic Phase, EAP = External Aqueous Phase
Factors affecting the entrapment efficacy (EE) and percentage (%) yield of the prepared KT microsponges:

10 mg of microsponges containing ketorolac tromethamine was suspended in 1 ml dichloromethane then 10 ml of deionized water was added (to extract ketorolac tromethamine). The final solution was mixed by vortex, set aside for few minutes then the upper layer (containing KT) was analyzed spectrophotometrically at 322 nm \[^{[13]}\]. This procedure was triplicated for each formula and the average was calculated by using Equation 1.

\[
\% \text{EE} = \frac{\text{Actual drug entrapped}}{\text{Total amount of drug used}} \times 100\% \quad \ldots \ldots \ldots \ldots 1
\]

In order to determine the % yield of the prepared KT microsponges (F1-F16), the weight of the dry microspnges from each formula was compared with the weight of drug and polymer that was used to prepare microsponges \[^{[14]}\]. This procedure was triplicated for each formula and the average was calculated by using equation 2.

\[
\% \text{yield} = \frac{\text{Practical weight of microsponges}}{\text{Theoretical weight of drug and polymer}} \times 100\% \quad \ldots \ldots \ldots \ldots 2
\]

Effect of sonication power on entrapment efficiency and % yield

Formulas (from F1 to F6) were prepared with different percentages of sonication power to study the effect of sonicator power on entrapment efficacy and % yield.

Effect of sonication time on entrapment efficiency and % yield

Formulas (F7 and F8) were prepared with different sonication time in comparison with (F3) to study the effect of using different sonication time on the entrapment efficacy and % yield.

Effect of mixing type on entrapment efficacy and % yield

Two different types of mixing methods were used to prepare formulas (F3 and F16) to study the effect of mixing types on entrapment efficacy and percentage yield.

Effect of PLGA concentration on entrapment efficacy (EE) and % yield.

Different amounts of polymer (PLGA) were used in the organic phase to prepare formulas (F3, F9, F10 and F11) in order to study its effects on the entrapment efficacy and % yield.

Effect of external aqueous phase (EAP) volume:

Formulas (F12 and F13) in comparison with F10 were prepared with different volumes of (EAP) to study its effect on entrapment efficacy (EE) and % yield.

Effect of stabilizer percentage on entrapment efficacy (EE) and % yield

Formulas (F10, F14 and F15) were prepared with different percentages of poly vinyl alcohol (PVA) in the external aqueous phase (EAP) to study its effect on entrapment efficacy (EE) and % yield.

Selection the optimum formula of KT microsponge

Formulas with highest entrapment efficacy and % yield was chosen as the best formula for further study.

In vitro release

Modified Franz diffusion cell was used to study the in vitro release of KT microsponges \[^{[15]}\]. The cell is composed of donor and acceptor chamber separated from each other by dialysis membrane (molecular weight cut-off (MWCT) 3500). Each chamber had a capacity of 4 ml (equivalent to vitreous chamber volume)\[^{[16]}\]. Pre-determined weight of each of the selected KT microsponge formulas (F10, F13 and F14) was placed in the donor chamber then (4 ml) phosphate buffer pH 7.4 was added in donor and acceptor chamber. The diffusion cell was placed in the shaker water bath at 37 ºC and 25 rpm. At pre-specified time intervals (4 ml) was withdrawn (which is analyzed at λ max 322 nm using UV-visible spectrophotometry) from the acceptor chamber and replaced with fresh buffer solution \[^{[17]}\].

Statistical analysis

Independent sample t-test and one-way analysis of variance (ANOVA) were used
for statistical analysis. Statistically significant of the differences were considered when \((P<0.05)\). SPSS 16 software was used for all data analysis.

**Results and discussion:**

**Melting point determination**
The melting point of ketorolac tromethamine was 160 °C which is within the reference range (160-161 °C) which is identical to the results obtained from evaluation and optimization of fast disintegrating tablets of ketorolac tromethamine\(^{18}\) that indicated the purity of KT powder.

**\(\lambda_{\text{max}}\) of KT determination**
The diluted solution of KT with a concentration 10 µg/ml in phosphate buffer pH 7.4 was scanned using UV spectrophotometer. The spectrum showed \(\lambda_{\text{max}}\) of KT at 322 nm that agreed with the reported data \(^{19}\), as shown in Figure 1. Which indicated the purity of our product?

![UV spectrum of ketorolac tromethamine in phosphate buffer pH 7.4.](image_url)

**Calibration curve of KT in phosphate buffer pH 7.4**
The calibration curve of KT in phosphate buffer pH 7.4 obtained by plotting absorbance versus concentration, and the concentrations range used obeys Beer's law, as shown in Figure 2. This results is similar to HPTLC determination of ketorolac tromethamine \(^{20}\).
Factors affecting the entrapment efficacy (EE) and percentage (%) yield of the prepared KT microsponges

Table 2 shows the entrapment efficacy and percentage yield of each resultant formula, where formulas (F10, F13 and F14) achieved the highest entrapment efficacy and percent yield in comparison with other formulas, while formula (F9) had the lowest entrapment efficacy and percent yield.

Sixteen formulas of KT microsponges were prepared to study the effect of different factors on entrapment efficacy (EE) and percentage yield (%). Entrapment efficiency is the percentage of the drug encapsulated (entrapped) in the microsponges [21]. This result is similar to ketorolac tromethamine-loaded albumin microspheres for potential intramuscular administration. The percentage of yield related to the recovery of the product, the largest percentage yield indicates the minimum drug loss during the preparation of microsponges [22].

Effect of sonication power on entrapment efficiency and % yield

Six sonication power (100, 150, 200, 250, 300 and 400 Watt) were used to prepare the primary emulsion of the six formulas (F1-F6) respectively. It was found that as the sonication power increases for (F1-F3) the entrapment efficacy increases (P<0.05) as shown in Table 3. Increasing sonication power leads to finer droplet size of the primary emulsion leading to increasing the entrapment of the drug within the emulsion droplets but further increase in sonication power may start decreasing the entrapment efficacy (F4-F6) since the temperature increased upon using high sonication power that may destroy the obtained primary emulsion [23] as seen from previous study of biodegradable poly(lactic acid) and poly(lactide-co-glycolide) microcapsules. The results showed no significant difference in the percentage yield in all formulas (F1-F6). Therefore, the optimum sonication power 200 Watt was used during further investigation in this study.

Effect of sonication time on entrapment efficacy and % yield

The results showed that increasing the sonication time from 2 minutes for (F8) to 4 minutes for (F3) there was significant increase (P<0.05) in the entrapment efficiency since there was good homogenization of the primary emulsion that might reduce particle size and improved drug encapsulation [24]. Further increase in sonication time up to 6 minutes for (F7) caused decrease in entrapment.
efficiency since excessive sonication might cause further reduction in particle size leading to aggregation and forming larger one and might cause the drug to leach out from the microsponges. Same results observed with liposomes encapsulating cyclodextrin containing paclitaxel \[25\]. Accordingly, 4 minutes was selected as the optimum stirring time and adapted for further work in this study. The results showed that there was no significant difference in the % yield upon increasing stirring time.

**Effect of mixing type on entrapment efficacy and % yield**

Two formulas (F16 and F3) with different mixer type (Vortex and Probe sonicator) respectively were used to study their effects on the primary emulsion. There was significant increase in entrapment efficacy (\(P<0.05\)) when probe sonicator used to prepare the primary emulsion of formula (F3) in comparison with formula (F16) in which vortex was used for preparing the same step due to the lack of energy (using vortex mixer) required to get good emulsification that might lead to polymer aggregation \[26,27\] which was seen from results of sonication parameters for the preparation of biodegradable nanocapsules of controlled size by the double emulsion method. Microsponges from both F3 and F16 were examined using SEM which revealed that F16 microsponges were larger in size and not uniform in shape.

Table (2): The entrapment efficiency and percentage yield of all KT microsponges formula.

<table>
<thead>
<tr>
<th>Formula number</th>
<th>Entrapment efficacy % ± SD</th>
<th>Percentage yield ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>52 ± 2.5</td>
<td>75 ± 2.09</td>
</tr>
<tr>
<td>F2</td>
<td>63 ± 1.2</td>
<td>77 ± 1.1</td>
</tr>
<tr>
<td>F3</td>
<td>66 ± 2.6</td>
<td>76 ± 1.05</td>
</tr>
<tr>
<td>F4</td>
<td>60 ± 1.5</td>
<td>76 ± 2.2</td>
</tr>
<tr>
<td>F5</td>
<td>58 ± 0.9</td>
<td>77 ± 1</td>
</tr>
<tr>
<td>F6</td>
<td>55 ± 2.3</td>
<td>75 ± 1.11</td>
</tr>
<tr>
<td>F7</td>
<td>59 ± 1.6</td>
<td>72 ± 1.3</td>
</tr>
<tr>
<td>F8</td>
<td>37 ± 1.7</td>
<td>74 ± 2.6</td>
</tr>
<tr>
<td>F9</td>
<td>16 ± 1.3</td>
<td>61 ± 1.5</td>
</tr>
<tr>
<td>F10</td>
<td>76 ± 2.7</td>
<td>85 ± 2.8</td>
</tr>
<tr>
<td>F11</td>
<td>50 ± 3.1</td>
<td>68 ± 3.2</td>
</tr>
<tr>
<td>F12</td>
<td>60 ± 2.6</td>
<td>87 ± 1.4</td>
</tr>
<tr>
<td>F13</td>
<td>72 ± 1.03</td>
<td>81 ± 2.3</td>
</tr>
<tr>
<td>F14</td>
<td>74 ± 1</td>
<td>83 ± 1.5</td>
</tr>
<tr>
<td>F15</td>
<td>64 ± 1.4</td>
<td>80 ± 1.9</td>
</tr>
<tr>
<td>F16</td>
<td>59 ± 1.8</td>
<td>79 ± 1.3</td>
</tr>
</tbody>
</table>

**Effect of mixing type on entrapment efficacy and % yield**

As seen in Figure 3, two formulas (F16 and F3) with different mixer type (Vortex and Probe sonicator) respectively were used to study their effects on the primary emulsion. There was significant increase in entrapment efficacy (\(P<0.05\)) when probe sonicator used to prepare the primary emulsion of formula (F3) in comparison with formula (F16) in which vortex was used for preparing the same step due to the lack of energy required to get good emulsification that might lead to polymer aggregation \[28\] (using vortex mixer) so the resulting microsponge was larger and not uniform in shape when prepared using vortex than by probe sonication \[29\].
Figure (3): SEM pictures for (A) microsponges of formula (F16) which was prepared by vortex, (B) microsponges of formula (F3) which was prepared by probe sonicator.

Effect of polymer concentration on entrapment efficacy and % yield
Four concentrations of PLGA polymer were used during the preparation of F9 (10%), F3 (20%), F10 (30%) and F11 (40%). Significant increase in entrapment efficacy (EE) and % yield was noticed ($P<0.05$) with increasing polymer concentration in formulas F9, F3 and F10, this could be attributed to the increase in the viscosity of organic phase and availability of polymer for coating the drug $^{[30]}$. Entrapment efficacy and % yield were decreased with further increase in polymer concentration (F11) since too sticky viscous polymer solvent obtained and this may lead to non-uniform dispersion of the drug in the coating solution and aggregation of the particles, same results were obtained with polymeric PLGA nanoparticulate drug and PLGA nanoparticles loaded with Vincristine sulfate $^{[31]}$. 

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Effect of external aqueous phase (EAP) volume on entrapment efficacy and %yield
Formulas (F10, F12 and F13) were prepared with different continuous phase (EAP) volume keeping other variables constant. It was found that with increasing external aqueous phase volume from (F12; 100ml) to (F10; 200ml) significant increase in entrapment efficiency ($P<0.05$) from (60% to 76%) was obtained due to the fact that low continuous phase gave opportunity to the microsponge particles to aggregate and collide then fused to form larger microsponges so decreasing entrapment efficiency, these results matched with the results of microspheres prepared by double emulsion method\[32\].

Upon further increase in EAP volume (F13; 300ml), there was a decrease in entrapment efficiency as compared to (F10; 200ml) due to increasing the hydrophilic drug partitioning into the continuous phase during hardening phase\[33\]. As noticed from the results, there was no significant decrease in percentage yield ($P<0.05$) with increasing continuous phase volume and this decrease can be attributed to the escape of the drug (highly water soluble) from the inner phase to the large outer one and/or faster polymer precipitation at large external water phase so less porous spheres were created\[34\]. Therefore, (200ml) was selected as the optimum external aqueous volume.

Effect of stabilizer on entrapment efficacy and %yield
Different concentrations of polyvinyl alcohol PVA (as stabilizer) were used with formula (F10, F14 and F15) containing (0.1, 0.05 and 0.5% respectively) to investigate their effects on EE and %yield. The results showed that there was no significant increase in entrapment efficacy upon increasing PVA concentration from (F14; 0.05%) to (F10; 0.1%) although increasing PVA concentration may increase the stability of primary emulsion droplets against coalescence leading to decrease in particle size\[35\]. Meanwhile, upon further increase in PVA concentration (F15; 0.5%), the entrapment efficacy was significantly decreased in comparison to (F10 and F14) which could be attributed to the high solubilizing effect of PVA that may lead to squeezing and partitioning of the drug from microsponges into the external aqueous phase leading to reduction in drug entrapment within the polymer vesicle. Same result was reported with olanzapine PLGA nanoparticles\[36\]. Concerning the percentage yield, there was no significant increase between the three formulas upon changing PVA concentration this may be due to the washing of PVA during microsponge collection where PVA removed during this step leaving few residual amount which has no effect on the net yield \[37\]. Therefore, 0.1% PVA concentration was selected as the optimum concentration in the external aqueous phase.

Selection of the optimum formula of KT microsponge
Upon subsequent evaluation for the variables used in this study; three formulations (F10, F13 and F14) were selected as the optimum formulas depending upon their highest entrapment efficiency and % yield.

In vitro release of KT from the prepared microsponges:
The in vitro KT release from the three microsponges formulas (F10, F13 and F14) was followed for (3 months) as seen in Figure 4. The drug release exhibited biphasic pattern described by initial release then followed by slow continuous release phase, the initial one expected to be due to the release of free drug available on the surface of microsponges\[38\]. Formula (F10) with highest entrapment efficacy (EE = 76%) showed the lowest initial release (15%) within (35 days) in comparison to formulas (F13 and F14) and this initial release followed by slow continuous release reaching 74% within 90
days which may be attributed to the high viscosity due to high PVA concentration in addition to PLGA that creating small internal pores with high tortuous network that slow down the release of the drug from the internal pores of the microsponges\textsuperscript{[39,40]}. The initial effect of formula (F13) was about (17\% within 35 days) followed by continuous release reached (63\% within 90 days). This could be due to the external aqueous volume of (F13; 300ml) which may cause increase in external pores that may lead to drug redistribution during the hardening step and located mainly close to the microsponge surface. The larger EAP volume may lead to less internal porosity in the prepared microsponges due to faster polymer precipitation\textsuperscript{[41]} resulting in lower release in comparison with the two other formulas (F10 and F14). Formula (F14) showed the highest initial release approximately (21\% within 15 days) and the release continued to reach (86\% within 90 days). The reason is low PVA concentration in the external aqueous phase which provided more rapid release since the viscosity increased with higher PVA concentration in the external aqueous phase leading to difficulties for drug aqueous solution to diffuse out while low PVA concentration enhanced drug exchanging with the surrounding. Similar results were reported with FITC-BSA microspheres\textsuperscript{[42]}. All these facts proposed that the microsponge's porosity (external or internal) plays a significant role in restriction of drug release.

**Conclusion:**
This work is suitable for ophthalmic application (implantation) due to the reduction of dose frequency as a result to sustained released up to 3 months so enhance patient compliance.

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