Preparation, evaluation and dissolution behaviors of water soluble drug loaded PLGA microspheres.

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الخلاصة:

من اهم انظمة نقل الدواء للمواد الفعالة باستخدام البولمرات سريعة التحلل هي صيغة الكبسولات المصغرة وبالرغم من وجود عدد من الدراسات استخدم فيها البولمر PLGA ولكن مازالت المعلومات الاساسية لتحرر الدواء غير كافية . تم في هذا البحث تحضير مادة اندوميثاسين صوديوم على شكل كبسولات مصغرة باستخدام نسب مختلفة من بولمر PLGA وبطريقتين مختلفتين هما طريقة التغليف وطريقة المستحلب وقد اجريت مقارنة في الشكل , الحجم , الذوبان والتحرر لهذه الكبسولات المصغرة المختلفة وقد لوحظ ان تحرر الدواء يقل كلما زادت كمية البولمر وان الكبسولات المصغرة المحضرة باستخدام نسبة لاتزيد عن 20 % من البولمر اظهرت تدفق سريع اولي عند بداية التحرر وهذا مما يقترح ان ميكانيكية التحرر هي على الاغلب بواسطة التاكل بينما الكبسولات المصغرة المحضرة باستخدام نسب عالية من البولمر تكون ميكانيكية التحرر على الاغلب بواسطة النفوذية. واظهرت النتائج ايضا ان طرق التحضير لها تاثير على شكل وحجم الكبسولات المصغرة حيث اثبتت الدراسة ان الكبسولات المحضرة بطريقة التغليف باستخدام نسبة من البولمر اكبر من 30% يكون شكلها ليس دائريا بشكل كامل وحجمها كبيربالمقارنة مع تلك المحضرة بطريقة المستحلب حيث يمكن استخدام الطريقة الثانية لتحضير كبسولات مصغرة ذات تحرر بطيء وباستخدام نسب اعلى من البولمر ويكون حجمها وشكلها مناسب لتحضير صيغة دوائية بطيئة التحرر تعطى على شكل محاليل زرق.

Abstract

One of important drug delivery system is microencapsulation of bioactive agents using biodegradable polymers. Although a large number of studies, which utilizes PLGA have been performed, however, basic data and information upon drug release are in sufficient. Formulation of indomethacin sodium as microspheres using different percentages of PLGA by applying coacervation / phase separation and emulsification / solvent evaporation methods, were performed. The morphology, particle size and dissolution – release profile of the prepared microspheres have been studied. From both methods, it was noticed that the release of the drug is decreased as the amount of PLGA increased since it affects core: coat ratio. The microspheres prepared with low percentages of PLGA (not more than 20%) showed initial burst effect, which may suggest that erosion, is the major dissolution mechanism while diffusion might be the main mechanism for microspheres prepared with high percentages of PLGA. The shape of microspheres with > 30% PLGA (by coacervation method) has limited sphericity and larger particles size in comparison to that prepared with emulsification / solvent evaporation methods. Therefore, indomethacin – loaded PLGA microspheres (using 50% polymer) prepared by the second method can be used in the formulation of prolonged duration injectable dosage form.

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Introduction

In recent years, much attention has been paid to the controlled release of bioactive agents from microcapsules and microspheres made of biodegradable polymers such as PLGA as one of important drug delivery systems (1, 2, 3). PLGA is one of best known biodegradable polymers and histocompatible aliphatic polyesters which hydrolyzes without enzymes and then matabolised by the body (4,5). It is contributed to reconstruction of deficient or injured organs and to improve galenic formulations. It had been proven non – toxic (6). PLGA is used as release controlling material (7,8). The drug can be released by diffusion through water-filled pores or through the polymer and after hydrolytic degradation / erosion of polymer (9). Microspheres had been prepared by various techniques, this paper focuses on coacervation / phases separation and emulsification /solvent evaporation methods. The first method relies upon a decrease in the solubility of the coating polymer by addition of a third compound to the polymer solution in an origanic solvent (10). The second method consists of four major steps; dissolution or dispersion, emulsification, extraction of the solvent and finally harvesting and drying of the microspheres (11). The model drug used in this study is indomethacin sodium which is acidic non - steroidal anti inflammatory drug that diffuses rapidly across the epithelium and therefore, quickly and completely absorbed from small intestine after oral administration and it is sufficiently soluble; chemically and metabolically stable (12).

Although a large number of studies which utilizes PLGA have been performed in the drug delivery system field in the recent years, however, basic data and information upon drug release are in sufficient. In this study low molecular weight PLGA was used to prepare water soluble drug loaded microspheres using coacervation / phase separation and emulsification / solvent evaporation methods. The efficiency of microencapsulation, morphology and the release kinetic of the drug from the microspheres prepared by both methods were investigated. This may contribute to provide more informations about the mechanism of drug release and may lead to the preparation of injectable microcapsules that is able to provide pre- programmed duration of action offering several advantages over the conventional forms of dosage.

Material and Methods

Materials:

The following chemicals were used as received: poly (D, L- lactide – co – glycolide) PLGA (50:50, RG504, M.wt.56,500) (Boehringer, Ingelheim, Germany), silicon oil (1000 cSt) (Dow corning, Seneffe, Belgium), dichloromethan (p.a. 84,93) (Merck, Dramstad, Germany) indomathacin sodium trihydrate ((Merck, Dramstad, Germany), polyvinyl alcohol, n – hexane and methanol (Fisher Scientific UK Limited , U.K.)

Methods:

- **Preparation of microcapsules by coacervation /phase separation technique:**

Indomethacin sodium – loaded PLGA microspheres were prepared by dissolving 10%, 25%, 30% and 50% w/w PLGA in 10 ml dichlormethane, then 50 mg indomethacin sodium dissolved in 0.2 ml water was added with stirring magnetically. Silicon oil was

added dropwise with continuous stirring at 200 r.p.m. until microspheres were obtained. The microspheres then dispersed in n – hexane, stirred, filtered and dried by lyophilzation (10 ,13).

- **Preparation of microcapsules by emulsification / solvent evaporation method:**

Indomethacin sodium – loaded PLGA microspheres were prepared by dissolving 20%, 50%, 60% and 80% w/w PLGA in 20 ml dichloromethane, then 50 mg of indomethacin sodium was added with shaking. The aqueous phase containing 200 ml water, 1.5 ml polyvinyl alcohol and 10 ml methanol was prepared separately and shaked with the organic phase. The organic phase then evaporated by rotary evaporator under reduced pressure. The resulted microspheres dispersed in n – hexane, stirred, filtered and lyophilized (11).

- **Morphology:**

The morphology of the prepared microcapsules was examined by optical microscope (Labophoto – 2, Nikon microscope, EN 29001, ISO 9001 BS 5750, approved by BVQILTD., Nikon corporation Instruments, Division, Yokohama Plant).

- **Measurement of particle size and size distribution:**

Particle size and size distribution of drug – loaded microspheres were detrmined by particle size analyzer (Mazter – sizer X, Malvern Instrument, USA). The uniformity of particle size distribution defined as follows (14):

$$uniformity = \frac{\sum X_i |D - d|}{D \sum X_i}$$

Where: D = median diameter of the distributution.
d = mean diameter of particles.
Xi = the size class in the range.

The particle size distribution presented as the relative frequency of the diameter based on the volume distribution.

- **In vitro release of indomethacin**:

The release of indomethanacin from the prepared microspheres was determined in saline (phosphate buffer) solution at 37^0C and 50 r.p.m. (10) using prolabo dissolution tester with UV/VIS spectrophotometer lambda 40. (Perkin – Elmer spectrophotometer). The release was followed in the salin buffer pH 7.4 for two hours at 320 nm.

Result and discussion

The process of coacervation / phase separation proceeds a long three steps; phase separation of the coating polymer, adsorption of the coacervat on the dispersed phase and hardening of the coating. These steps occur so quickly that they are far from equilibrium and thus sensitive to kinetic influences and system composition (10). The release profiles (fig 1) of microspheres prepared by coacervation technique showed that those prepared using 10% PLGA were characterized by initial burst effect of the drug followed by steady release time this could be attributed to the release of drug from the surface of microcapsules and agreed with the results obtained from diclofenace sodium – loaded PLGA microcapsules (17). It was noticed in this study that large part of the dispersed phase has failed to encapsulate the dispersed aqueous phase for microspheres with 10 % PLGA and this could be due to a mismatching of the water – coacervate and water –

supernatant interfacial tension (10,15). While microspheres prepared with 25 % and 30% PLGA showed no initial burst effect and mor retarded release. The same release profiles was shown for microspheres prepared by emulsification / solvent evaporation method (fig 2) were drug – loaded microspheres prepared with 20 % PLGA showed initial burst effect while those with 50, 60 and 80 % PLGA showed retarded release with no initial burst effect.

From both methods, it was noticed that the release of the drug is decreased, as the amount of PLGA is increased since it might affect core: coat ratio (16). Also the microsheres prepared with low percentages of PLGA (not more than 20%) showed initial burst effect while no such effect was observed for higher percentages PLGA microspheres, and this could be attributed to the mechanism of drug release. In general, diffusion and erosion are supposed to be the major mechanisms for drug to release from biodegradable microspheres (18). In this study the observed fast initial burst of the drug may suggest that erosion is the major dissolution mechanism and this agreed with reported data for fast degradation of PLGA (16,17) while diffusion might be the main dissolution mechanism for microspheres prepared with high % of PLGA (> 20%) using both methods, and such mechanism had been reported for the slow release of drug - loaded poly (δ – valerolactone) (PV) microspheres in comparison to PLGA microspheres of the same drug (17).

The amount of PLGA had significant effect on the morphology of microcapsules. The shape of microcapsules prepared with more than 30 % PLGA by coacervation technique has limited sphericity and therefore, they may not be used for parental injection since the sphericity of microcapsules is important from formulation point of view (19). The best regular spherical shape microspheres were obtained with 30 % PLGA with average diameter of 71µm. (fig.3). From these observations, it appears that coacervation method is frequently impaired by residual solvents and coacervating agent found in the microspheres and it depends on the interplay of several kinetic parameters and this agreed with prvious reported data (10, 11). Therefore, the shape and the outer surface is not easily to be controlled, not reproducible and it is not well suited for producing microspheres in the low size range. While drug – loaded PLGA microspheres prepared by emulsification / solvent evaporqation method with 50 % PLGA showed better spherical shape with average diameter of 57µm (fig 4). In the emulsification / solvent evaporation method there is no need for elevated temperature, no phase separation inducing agent, therefore, can induce smaller controlled particle size and provide high encapsulation efficiency as agreed with other reported data (11).

As a conclusion, increasing % of PLGA to prepare drug – loaded PLGA – microspheres using coacervation or emulsification / solvent evaporation technique , will decrease the release of the drug through affecting the mechanism of drug release in addition to its effect on core: coat ration. Besides that, increasing the amount of PLGA had great effect on the morphology and sphericity of microspheres, which may contribute to the preparation of pre – programmed duration injectable dosage form.

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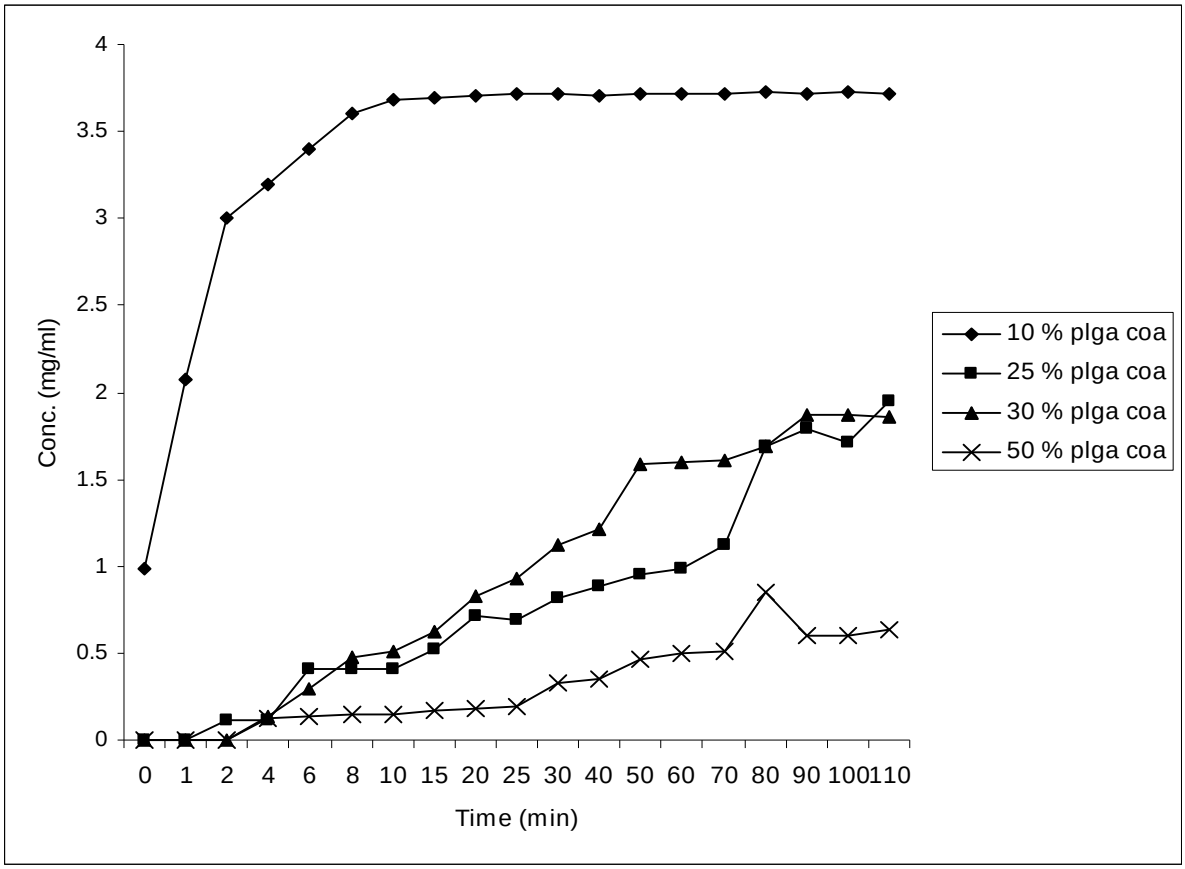

Fig.1 – Release of indomethacin sodium from microspheres prepared by coacervation / phase separation method.

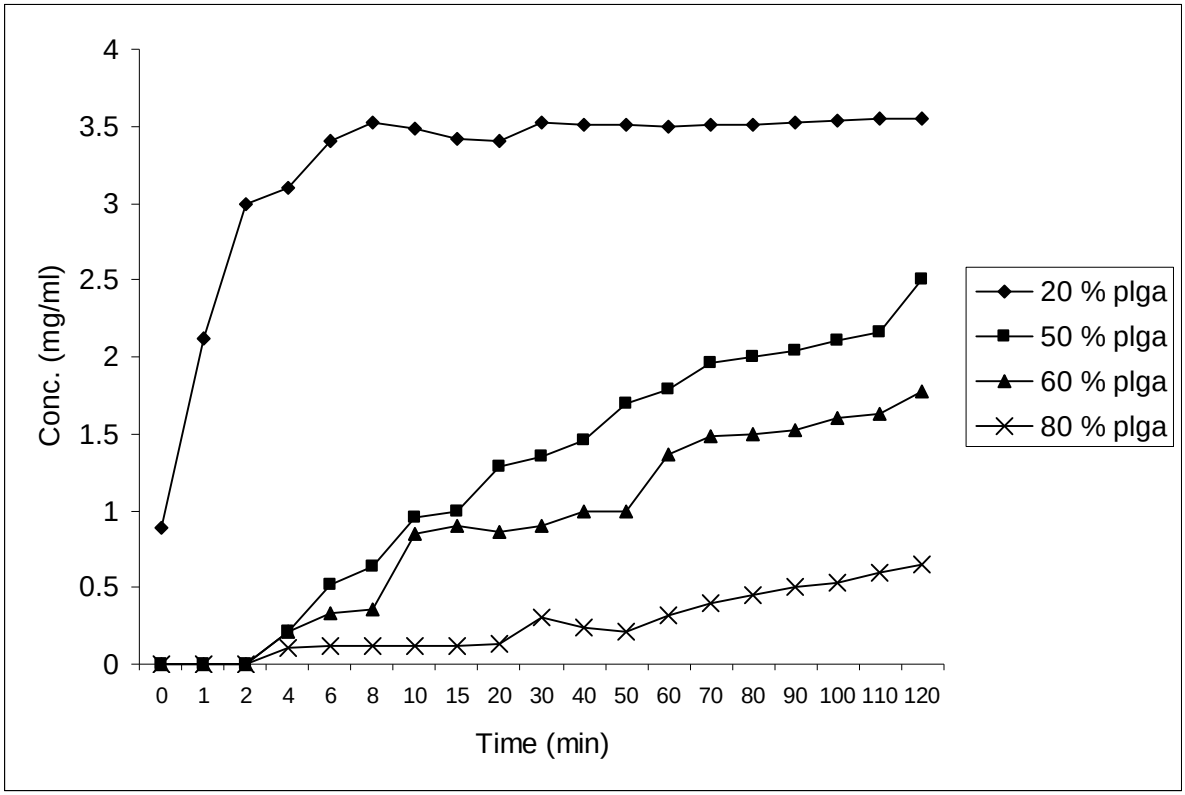

Fig. 2 – Release of indomethacin sodium from microspheres prepared by emulsification / solvent evaporation method.

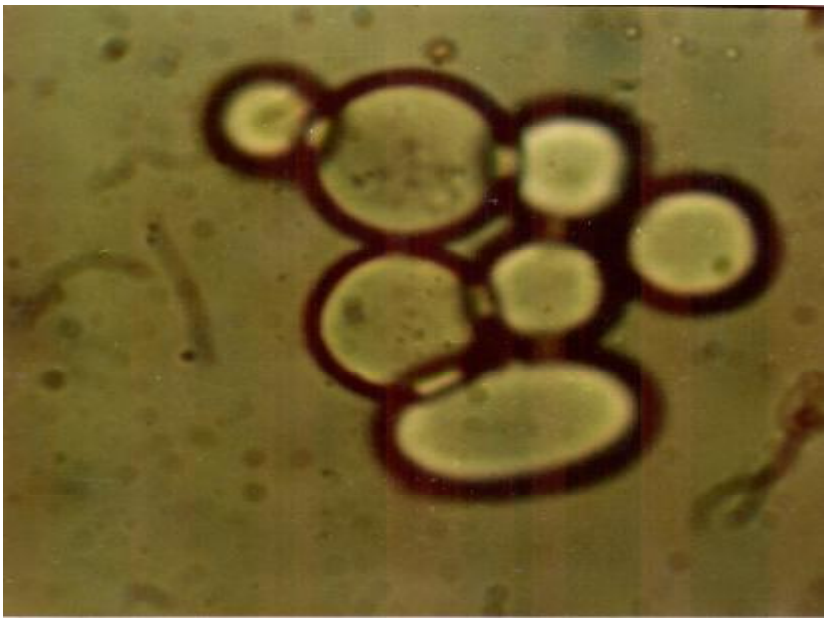

Fig – 3 – Picture of microspheres prepared by coacervation / phase separation method using 30% PLGA

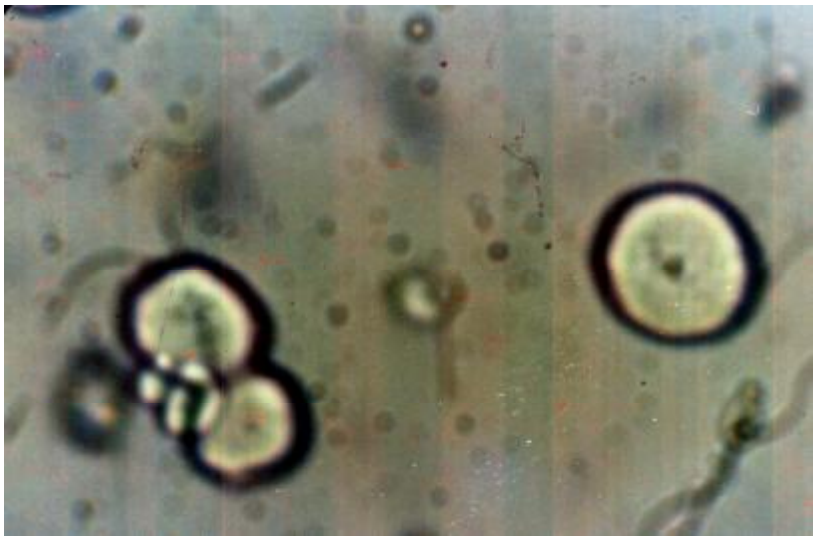

Fig – 4 – Picture of microspheres prepared by emulsification / solvent evaporation method using 50% PLGA