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Preparation of Sustained Release Phenobarbitone Microspheres Using Natural and Synthetic Polymers

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Abstract. Rounded and uniform shape phenobarbitone microspheres were successfully prepared using Eudragit RL. The yield percent, the actual drug content and the incorporation efficiencies of the prepared microspheres increased with increasing the drug: polymer ratio. The particle size analysis data showed an increase in the mean microspheres diameter from 235.54 to 292.58 μm with increasing the amount of polymer from 1:1 to 1:4 ratios. The dissolution studies revealed that the phenobarbitone release was greatly extended and delayed as the drug polymer ratio was decreased due to the increase in the diffusion pathway through the pores of the polymer. Sustained release casein-chitosan microspheres containing phenobarbitone were also prepared using the coacervation technique in completely aqueous conditions. The entrapment efficiencies (83.20-90.60%), particle size (620.5 - 675 μm), flow properties; compressibility and *in vitro* dissolution of the microspheres were variable depending on the preparation conditions. Casein and chitosan concentrations were found to be the main parameters that affect the properties and the performance of the prepared microspheres. Eudragit RL microspheres showed more prolonged and slower drug release than casein-chitosan microspheres, however, the release rates were prolonged from both types of microspheres.

Keywords: Phenobarbitone, Eudragit RL, Casein-chitosan microspheres, Sustained release.

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Introduction

The sustained release of drugs is still one of the main objectives of drug delivery systems. The successful formulation to control drug for the required duration of time with optimum release mode depends on various factors, such as the physicochemical properties of the drug, the nature of drug-carrier matrix, the type of the dosage form and the route of administration[1].

Methacrylic copolymers (Eudragit) are offered in a variety of types with different water solubility and permeability properties and have been used for drug release modification in several oral solid dosage forms[2-4]. Eudragit S100 and L100 have a pH-dependent solubility in water, whereas Eudragit RS and RL are insoluble but water permeable. Because Eudragit L100 is soluble at pH > 6 and S100 at pH > 7, both are used as coating materials resistant to the gastric fluid[5]. Eudragit RS is slightly water permeable, whereas RL is freely permeable to water because of higher content of quaternary ammonium groups. Both Eudragit RS and RL are therefore used for the production of sustained-release formulations[6].

Microspheres carrier systems made from the naturally occurring proteins have attracted a considerable attention for several years as a matrix for controlled and sustained release delivery of many drugs[7,8]. Recently, there have been some interests in the milk protein, casein, as a drug carrier mainly for the sustained delivery of cytotoxic drugs[9]. Glutaraldehyde cross-linked casein microspheres were found to be resistant to proteolytic tract for more than 24 h and it is suggested that cross- linked casein could be used as a matrix for the controlled delivery of oral drugs[10].

Chitosan is a hydrolyzed derivative of chitin, a biopolymer widely distributed in nature. Chitosan has attracted attention as a matrix for controlled release systems since it possesses reactive functionalities, and easily degraded into non-toxic products by enzymes[11].

Phenobarbitone, either alone or in a combination with phenytoin, is used in the treatment of Grand Mal epilepsy and whilst the former material has a natural extended activity, it has been found that the reported contraindications occur less frequently when it is given in microencapsulated forms[12]. It was also desired to combine both

phenytoin and phenobarbitone microcapsules with other barbituric acid derivatives.

The aim of this study was to evaluate the potential use of both synthetic polymers *e.g.,* Eudragit RL and natural polymers *e.g.,* casein and chitosan in the preparation of microspheres as a drug delivery system for sustained release of phenobarbitone and to study some parameters affecting the preparation and performance of the microspheres.

Materials and Methods

Materials

Phenobarbitone, Light liquid paraffin, acetone n- hexane, acetic acid, sodium hydroxide and formaldehyde (BDH, Pool, UK), Eudragit RL (Rohm Pharma, Darmstadt, FRG).

Span 20 (Koch-light laboratories Ltd., England), Bovine milk casein and high molecular weight chitosan (Fluka Chemie, Buchs, Switzerland). The used reagents were of analytical grade.

Methods

Preparation of Phenobarbitone Microcapsules using a Synthetic Polymer

Phenobarbitone microcapsules were prepared by adopting an emulsion-solvent evaporation technique[13]. Phenobarbitone and Eudragit RL were used in ratios of 1:1, 1:2, and 1:4 to obtain significant different characteristics. Three grams of the polymer were dissolved in 100 mL of acetone using a magnetic stirrer. The calculated amounts of phenobarbitone powder were dissolved in the polymeric solutions during stirring. Each of these dispersions was slowly poured into 100 mL of light liquid paraffin containing 1% (w/v) span 20 and was emulsified by vigorous stirring at 700 rpm using a three-blade mechanical stirrer. Stirring was continued until all solvent had evaporated; the formed microcapsules were allowed to settle, washed with n-hexane to remove any oil residue. This emulsification was carried out in a water-jacketed beaker at a temperature of 30°C to allow the gradual evaporation of acetone.

Preparation of Phenobarbitone Microspheres using Natural Polymers

Coacervation phase separation technique was used in the preparation of phenobarbitone microspheres[14]. A total of 25 mL of 20% w/v casein solution in 0.5 M NaOH was freshly prepared. The calculated amount of phenobarbitone compared to the different chitosan weights tried in the study (50% w/w) was dissolved in 25-mL chitosan solution in 5% v/v acetic acid. Chitosan solution containing the drug was transferred into 150 mL beaker and stirred at 500 rpm for 5 min. Casein solution was slowly poured into the beaker containing the chitosan-drug solution with stirring for 15 min to form a precipitate at pH 4.9. Formaldehyde (3 mL) was then added and the stirring was allowed to continue for 60 min to increase the rigidity of the formed microspheres. The formed microspheres were then filtered using a Buchner funnel and air dried at ambient temperature for 24 h.

Different concentrations of casein (10, 20, & 30% w/v) were used for the preparation of the microspheres. The effect of chitosan concentration (0.5, 1.0 & 1.5 w/v) on microspheres formation and characteristics were also investigated.

Particle Size Analysis

Eudragit RL or casein-chitosan microspheres containing phenobarbitone were placed separately on a set of standard sieves (British Standard) of size range 63-1250 Um and shaken for 15 min. The resulting fractions remaining on the sieves were weighed to determine the particle size distribution. The mean microspheres diameters were calculated after sieving[15].

Flow Properties

Flow properties of the microspheres were evaluated by determining the angle of repose and compressibility index. Static angle of repose was measured according to the fixed funnel and free standing cone method[16]. A funnel with the end of the stem cut perpendicular to the axis of symmetry is secured with its tip 2 cm height, H, above a paper placed on a flat horizontal surface. The microspheres were carefully poured through the funnel until the apex of the conical pile so formed just reaches the tip of the funnel. The mean diameter, 2R, of H, base of the powder cone,

was determined and the tangent of the angle of repose was given by: tan α = H / R where α is the repose angle.

Compressibility index (I) values of the microspheres were determined by measuring the initial volume (Vo) and final volume (V) after subjecting to 100 tapping in a graduated measuring cylinder using the equation:

$$I = \{1- (V/Vo)\} \times 100$$

Determination of the Total Drug Content

A total of 100 mg of the microspheres were triturated in a dry mortar and triplicate samples of 20 mg of the triturate were vortexed with 1 mL of dimethyl formamide for 40 min. An aliquot of 100 mL of ethyl alcohol was then added to the solution and shaken for another 40 min. The solution was completed with phosphate buffer of pH 7.4 to 250 mL. A total of 10 mL aliquot was filtered through membrane filter (0.22- μm) (Millipore, Bedford, MA USA) and assayed spectrophotometrically for its content of phenobarbitone at 255 nm[12].

In vitro Dissolution Studies

Dissolution of phenobarbitone from the prepared microspheres of particle size range 250-800 um was carried out using microspheres equivalent to 100 mg of the drug. The dissolution apparatus (USP II) with 50 rpm paddle rotational speed was used in the studies. Distilled water was used as a dissolution medium. The drug concentration and the percentage released were determined with respect to time at 255 nm using Philips PU 8620 spectrophotometer (Cambridge, England). The *in vitro* release studies were performed in triplicate for each of the samples.

Statistical Analysis

Data of phenobarbitone release from the prepared microspheres were compared statistically using analysis of variance (ANOVA) and unpaired "Student's" *t*-test at a significant level $p \leq 0.05$.

Results

The variation in the preparation condition of the microspheres affected the resultant physical and chemical properties of the products to a large extent. Accordingly, the selection of the appropriate polymeric

material has special importance for the preparation of efficient sustained release drug delivery system. The following lines explain the role of each of the ingredients used in the preparation of microspheres and the required precautions to be adopted to obtain satisfactory microspheres.

The emulsification/solvent evaporation technique used in this study for the preparation of phenobarbitone microspheres firstly, depends on the emulsification of Eudragit RL solution containing phenobarbitone into a second immiscible liquid phase (liquid paraffin) containing an emulsifier (1% w/v span) to form a dispersion of drug-polymer solvent droplet. Secondly, the solvent is removed from the dispersed droplets by application of heat. The formed microspheres were separated by filtration.

A small amount of acetone had to be added to the liquid paraffin to avoid the rapid diffusion of the organic solvent into the oil phase and the immediate polymer precipitation before the organic solution could be dispersed as droplets, thus achieving a stable emulsion[17,18].

The addition of span in a concentration of 1% (w/v) to the external phase of the emulsion was helpful for the stability of the formed emulsion. Ruiz *et al.*[19] reported that, the role of the emulsifier was to provide an additional protective sheath around the polymer droplet and to prevent droplets from coalescence during the formation of the microspheres.

The physical characteristics of the prepared Eudragit RL microspheres containing different drug: polymer ratios (1:1, 1:2 and 1:4) were studied. The produced microspheres showed a uniformly rounded appearance (Fig. 1).

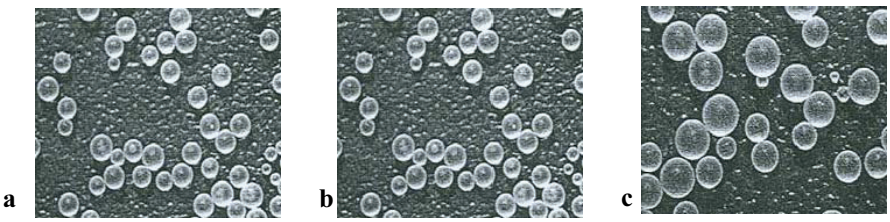

Fig. 1. Microscopic pictures of Eudragit RL microspheres containing phenobarbitone prepared at different drug: polymer ratios (a) 1:1, (b) 1:2, (c) 1:4, (× = 22).

The results in Table 1 show that the yield percent, the actual drug content and the incorporation efficiencies of the prepared microspheres increased with increasing the drug: polymer ratio. This could be attributed to the increase in the amount of solids in the dispersed phase. These results are in agreement with those obtained by Vanichtanukl *et al.*[20] and Horoz *et al.*[21]. The prepared microspheres also show fair flow and compressibility properties.

Table 1. Effect of drug: polymer ratios on the physical characteristics of Eudragit RL microspheres containing phenobarbitone.

Microspheres properties (Mean ±SD)	Drug: Polymer ratios		
	1 :1	1 : 2	1 : 4
Yield %	97.00	93.50	90.46
Actual drug content (%)	48.65	32.45	18.91
Incorporation efficiency (%)	97.30	92.45	91.0
Angle of repose (θ)	36.50 ± 0.90	34.10 ± 20	29.45 ± 48
Compressibility (%)	17.45	9.50	11.00
Mean microspheres Diameter (μm)	235.54 ± 4.25	258.56 ± 3.25	292.58 ± 5.58

The particle size analysis of the prepared Eudragit RL microspheres showed an increase in the mean microspheres diameter from 235.54 to 292.58 μm with the increase in the amount of polymer from 1:1 to 1:4.

Sustained release casein-chitosan microspheres containing phenobarbitone were prepared using the coacervation technique in completely aqueous conditions. The interaction between chitosan solution in dilute acetic acid (5% v/v) and casein solution in 0.5 M sodium hydroxide was the basis for the microspheres formation. Formaldehyde was used for the surface hardening of the droplets by cross-linking and thus fixing the shape and surface morphology of the formed microspheres.

Studying the physical characteristics of casein-chitosan microspheres containing phenobarbitone were also carried out and the results are shown in Table 2. The obtained results revealed that the incorporation efficiencies of the microspheres increased from 83.20, 87.40, to 90.60% when the casein concentration increased from 10, 20 & 30% (w/v), respectively. The results also show that the mean microspheres diameters increased from 620.5 to 650 and 675 μm, respectively, with the increase of the casein concentration. The angle of repose also increased from 32.0

to 36.7 (θ) with this increase in the polymer concentration while the compressibility index decreased from 22.5 to 15.6.

Increasing the chitosan concentration from 0.5-1.5% w/v increased the yield percent from 85.60-88.5%; on the other hand, it has no regular effect on the actual drug content and incorporation efficiency of phenobarbitone microspheres.

Table 2. The physical properties of casein-chitosan microspheres containing phenobarbitone.

Microspheres properties (Mean ±SD)	Casein conc. (w/v)			Chitosan conc. (w/v)		
	10	20	30	0.5	1.0	1.5
Yield %	85.50	82.50	85.60	85.60	87.0	88.50
Actual drug content (%)	41.60	43.70	45.80	45.80	46.0	45.50
Incorporation efficiency (%)	83.20	87.40	90.60	90.60	92.0	91.0
Angle of repose (θ)	32.0	33.50	36.70	36.70	34.50	33.10
Compressibility (%)	22.50	19.10	15.60	15.60	17.20	18.50
Mean microspheres Diameter (μm)	620.5	650.0	675.0	675.0	653.50	610.0

The dissolution profiles of phenobarbitone from Eudragit RL microspheres prepared from different drug: polymer ratios into distilled water are shown in Fig. 2 and the dissolution results were compared to that obtained from phenobarbitone powder which was used as a control. The obtained data showed that the dissolution of the drug (control) was faster in comparison to that released from Eudragit RL microspheres.

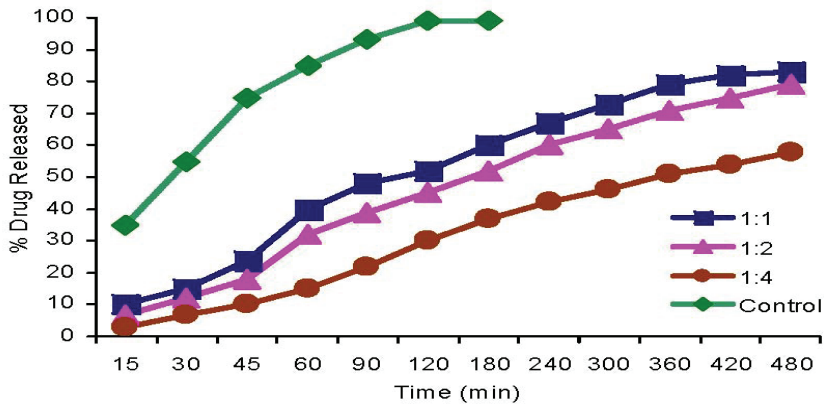

Fig. 2. Effect of core: wall ratios on phenobarbitone release from Eudragit RL microspheres.

The results revealed that the rate of drug release depends mainly on core: polymer ratios and its effect on the diffusion through the acrylic polymer pores and /or dissolution of drug from the surface of microspheres as previously obtained by Lee *et al.*[22].

The results in Fig. 2 also show that phenobarbitone was released from the microspheres having 1:1 drug: polymer ratio at a higher release rate than those having 1:2 and 1:4 where after 4 h the percentage drug released were 67.56, 60.57 and 42.85 and after 8 h the amount of drug release was 83.56, 79.0 and 58.50% respectively. These results revealed that phenobarbitone release from Eudragit RL microspheres was greatly extended and delayed as the drug: polymer ratio was decreased. These results might be attributed to the increase in the wall thickness of the microspheres arising due to the increase in the polymer: drug ratio leading to increase the length of the diffusional pathway through the polymer membrane. Similar finding were reported by Vanichtanunkul *et al.*[20] and Al-Omeran *et al.*[23].

The release profiles of phenobarbitone from different formulae of casein-chitosan microspheres are shown in Fig. 3 & 4. An initial fast release of the drug is exhibited in all the prepared microspheres where 15-30% of the drug was released in the first 20 min in all the cases depending on the preparation conditions. However, the retarded release pattern of the drug into distilled water was demonstrated.

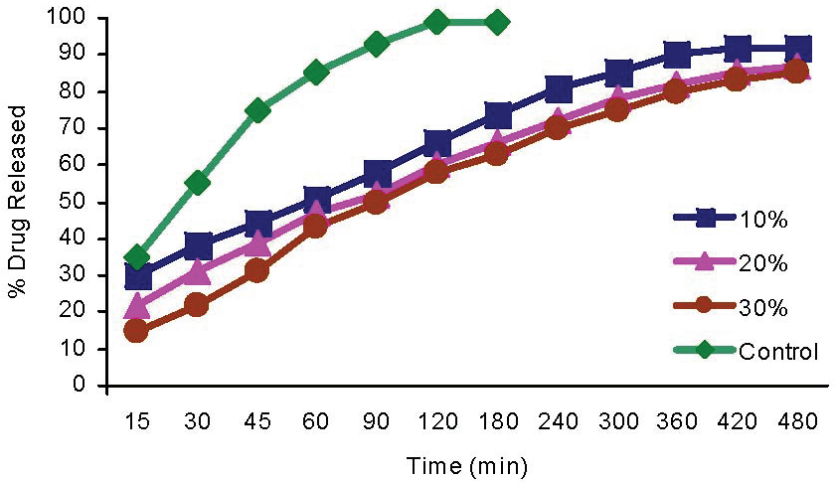

Fig 3. Effect of casein concentration (% w/v) on the release of phenobarbitone from casein-chitosan microspheres.

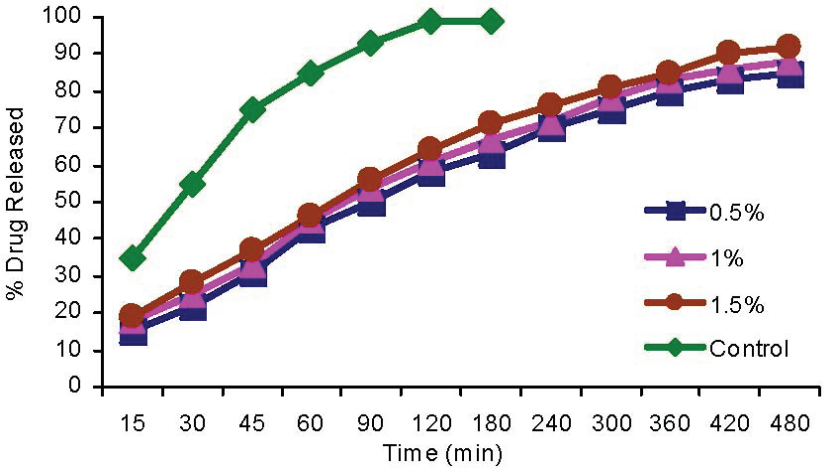

Fig 4. Effect of chitosan concentration (% w/v) on the release of phenobarbitone from casein-chitosan microspheres.

The results in Figure 3 show that increasing casein concentration significantly decreases the rate of phenobarbitone release from the microspheres ($p < 0.05$). This retardation in drug release could be attributed to the increase of the particle size of the microspheres as well as increasing the matrix density. This relation was confirmed by the results obtained by Grander *et al.*[24] and Bayomi *et al.*[14].

Figure 4 shows that the variation in chitosan concentration affects the drug release from the microspheres, while increasing the concentration of chitosan leads to a significant increase ($p < 0.05$) in drug release. This could be attributed to the formation of a more porous structure in the presence of larger amounts of chitosan[14].

Discussion

The preparation of Eudragit RL microspheres containing phenobarbitone based on the emulsification of Eudragit RL solution into liquid paraffin containing 1% w/v of span which in turn will form a dispersion of drug-polymer solvent droplets. The application of heat will lead to the evaporation of acetone leaving uniform rounded microspheres. The presence of span in the external phase is helpful for increasing the stability of the emulsion by forming a protective sheath

around the polymer droplets to avoid the coalescence of the droplets during the tacky stage of the microspheres formation as mentioned before by Ruiz *et al.*[19]. The fair flow properties of Eudragit RL microspheres could be attributed to the rounded shape and the smooth surface of the microspheres. The formation of casein-chitosan microspheres based on the neutralization reaction between casein and chitosan solutions. That is essential to induce coacervation using formaldehyde as a cross linking agent because casein is known to form a hard and insoluble plastic matrix with formaldehyde as published previously[14,25]. Varshosaz and Alinagari[26] obtained similar results when they used citric acid as cross-linking agent on insulin loaded chitosan microspheres.

The initial release values were higher from casein-chitosan in comparison to Eudragit RL microspheres. This could be attributed to the higher solubility of casein-chitosan combination in the dissolution medium and the larger surface area due to the irregular shape of the microspheres. The obtained results also showed that the release of phenobarbitone from both types of microspheres closely follow the diffusion mechanism.

Conclusion

In conclusion, Eudragit RL microspheres prepared using the emulsification/solvent evaporation technique show improved physical properties and uniform slow drug release for a longer time. On the other hand, the entrapment efficiencies of casein-chitosan microspheres were less than that of the Eudragit RL microspheres due to aqueous conditions used during the preparation. Moreover, Eudragit RL microspheres showed more a prolonged and slower drug release rate than casein-chitosan microspheres, however, the release rates were sufficiently prolonged from both types of microspheres.

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تحضير حويصلات دقيقة تحتوي على عقار الفينو باربيتون باستخدام عديد الجزيئات الطبيعية والتخليقية

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المستخلص. أمكن تحضير حويصلات دقيقة ذات شكل مستدير ومتجانس تحتوي على دواء الفينو باربتون باستخدام عديد الجزئيات (الأيدراجيت RL). ولقد أظهرت النتائج أن النسبة المحضرة، والمحتوى الفعلي من الدواء، ومدى قدرة عديد الجزئيات على احتواء العقار، تزداد بازدياد نسبة العقار إلى عديد الجزئيات، وكذلك فإن تحليل حجم الحويصلات أظهر أن متوسط الحجم يزداد من (٢٣٦) إلى (٢٩٣) ميكروميتر، بازدياد نسبة عديد الجزئيات الى الدواء من ١:١ إلى ٤:١، كما إن دراسة معدل ذوبان العقار قد امتدت إلى فترة أطول نتيجة لهذه الزيادة. ولقد أمكن أيضًا تحضير حويصلات دقيقة من عديد الجزئيات الكازيين – شيتوزان باستخدام تقنية الفصل من الوسط المائي، وأظهرت النتائج أن كفاءة الحويصلات على احتواء العقار تتراوح من ٨٣,٢ – ٩٥,٦٪ وحجم الحويصلات يتراوح بين ٦٢٠,٥ – ٦٧٥ ميكروميتر. كما ان قيمة الإنسانية ومعدل ذوبان العقار تتأثر بظروف تحضير

الحويصلات ، حيث وجد أن تركيز الكازيين وكذلك الشيتوزان لهما التأثير الرئيسي في صفات الحويصلات الدقيقة المتكونة. ولقد أظهرت الحويصلات الدقيقة المحضرة من الأيدراجيت انطلاقًا بطيئاً لدواء الفيوباربتون لمدة أطول من تلك المنطلقة من الكازيين – شيتوزان.