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DEVELOPMENT AND CHARACTERIZATION OF POLY (ε-CAPROLACTONE) MICROSPHERES CONTAINING ACETAZOLAMIDE

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ABSTRACT

The aim of this study was to prepare and evaluate poly(ε-caprolactone) (PCL) microspheres containing acetazolamide. Microspheres were prepared by solvent evaporation method. The influence of formulation factors (polymer: drug ratio, aqueous: oil phase ratio, concentration of aqueous phase, stirring speed and stirring time) on morphology, particle size, encapsulation and recovery efficiency were investigated. The microspheres were spherical with a mean diameter of 99.09±1.71µm, the encapsulation efficiency was 75.28±0.97% (w/w) and the recovery efficiency was 88.23±1.06% (w/w). *In vitro* release studies revealed a controlled release of acetazolamide from PCL microspheres suitable for peroral administration. In conclusion, PCL microspheres containing acetazolamide were successfully prepared by using solvent evaporation method with the selection of appropriate experimental conditions.

INTRODUCTION: Glaucoma is a serious eye disease that can lead to irreversible blindness. Glaucoma is a group of ophthalmic disorders characterized by an increase in intraocular pressure (IOP), which results in damage to the optic nerve and visual field disturbances. Agents used to treat glaucoma are designed to decrease intraocular pressure.

Nevertheless, these drugs present severe side effects; therefore, there is an urgent need to develop new drug delivery systems. Various classes of drugs used in the treatment of glaucoma include, among others, carbonic anhydrase inhibitors (CAIs). Acetazolamide (*N*-5-sulfamoyl-1, 3, 4-thiadiazol-2-yl)-acetamide is a CAI and has been an integral part of anti-glaucoma treatment for more than 40 years [1].

Acetazolamide is used orally for the reduction of IOP in patients suffering from glaucoma. It is used in the pre-operative management of closed-angle glaucoma, or as an adjunct therapy in the treatment of open-angle glaucoma. It is also used in the treatment of various forms of epilepsy and to prevent or ameliorate the symptoms of acute high altitude sickness [2].

To obtain the desired lowering in IOP, large oral doses of acetazolamide are used, and this usually leads to systemic side effects, the most common of which are diuresis, gastric difficulties and metabolic acidosis [2-3]. Several topically applied formulations were developed in order to minimize its side effects. These include surfactant-gel preparations, contact lenses, aqueous solutions containing cyclodextrins and liposomes [2].

It was reported that the incidence of side effects were much lower and the tolerance was much greater with acetazolamide sustained release capsule when compared with the conventional acetazolamide tablet [4].

Controlled drug delivery occurs when a polymer/drug system is designed to release the drug in a predetermined manner. The main purpose of these release systems is to achieve a more effective therapy, i.e., a delivery profile that would yield a high blood level of the drug over a long period of time, avoiding the large fluctuations in drug concentration and reducing the need of several administrations [5].

Microspheres are one of the multiparticulate delivery systems and are prepared to obtain prolonged or controlled drug delivery, to improve bioavailability or stability and to target drug to specific sites. Microspheres can also offer advantages like limiting fluctuation within therapeutic range, reducing side effects, decreasing dosing frequency and improving patient compliance [6-7].

One of the popular methods for the encapsulation of drugs within water-insoluble polymers is the emulsion solvent evaporation method. The emulsion solvent evaporation method was fully developed at the end of the 1970s and has been used successfully in the preparation of microspheres made from several biocompatible polymers such as poly (D-L-lactide-coglycolide) (PLGA) [8-11], poly(ε-caprolactone) (PCL) [12-18] and Eudragits [19-23]. The technique of emulsion solvent evaporation offers several advantages. It is preferred to other preparation methods such as spray-drying, sonication and homogenization, etc., because it requires only mild conditions such as ambient temperature and constant stirring. Thus, a stable emulsion can be formed without compromising the activity of the core materials.

Poly(ε-caprolactone) (PCL) is one of the biocompatible and biodegradable aliphatic polyester polymers that degrades slowly and does not generate an acid environment unlike the polylactide (PLA) or polyglycolide (PLG) polymers. Although the permeability of macromolecules in PCL is low, such low permeability may be sufficient for drug delivery [12, 24]. Other advantages of PCL include hydrophobicity, *in vitro* stability and low cost. Therefore, many investigations have focused on the application of PCL microspheres to drugs in recent years.

However, there have been few studies about experimental parameters using solvent evaporation method with PCL as wall materials. Therefore, the aim of this study was to prepare PCL microspheres containing acetazolamide by emulsion solvent evaporation method to achieve a controlled drug release profile suitable for peroral administration. Firstly, some formulation variables were investigated (polymer: drug ratio (1:1, 2:1, 3:1, 4:1 and 5:1, w/w), aqueous: oil phase ratio (5:1, 10:1 and 15:1, v/v), concentration of aqueous phase (0.05, 0.1, 0.25 and 1.0%, w/v), stirring speed (500, 750 and 1000 rpm) and stirring time (1, 2 and 4 h) to obtain spherical particles. Further, recovery efficiency, particle size distribution, encapsulation efficiency, surface properties and acetazolamide release rate from microspheres were investigated. The influences of formulation variables on the properties of microspheres were examined and the microspheres formulations suitable to achieve goal were determined.

MATERIALS AND METHODS:

Materials: Acetazolamide was supplied as a gift by Nakoda Chemicals Ltd., Hyderabad, India. Poly(ε-caprolactone) (PCL) with number-average molecular weight (Mn) 44,000 Daltons was supplied by Sigma-Aldrich. Various chemicals including Polyvinylalcohol (PVA) (Mw = 49,000 Daltons) and Dichloromethane (DCM) were obtained from S.D. Fine Chem Limited, Mumbai, India. All other chemical reagents were of analytical grade and were used without any further purification. Deionized water was used for all the experiments.

Methods:

Preparation of Microspheres: The preparation of drug free and drug-loaded PCL microspheres was based on o/w emulsion solvent evaporation technique, which was adapted from the process described by Cuňa et al. (2000) [21]. Different amounts of polymer (0.5, 1.0, 1.5, 2.0, 2.5 or 3.0 g of PCL) were dissolved in 10 ml of dichloromethane, respectively (DCM; internal oil phase) by using a magnetic stirrer. Powdered acetazolamide (0.5 g) was dispersed in the polymer

solution using ultrasonic bath (Sartorius, Labsonic P). The resulting dispersion was then poured into a vessel of 250 ml containing 100 ml of 0.1% polyvinylalcohol solution (external aqueous phase) while stirring. A cylindrical vessel (6 cm inside diameter and 9 cm height) and a mechanical stirrer with a blade (4.8 cm) (United Electrical Industries, Varanasi, India) were used for stirring which was continued for 2 h, until dichloromethane evaporated completely. Polymer: drug ratio (1:1, 2:1, 3:1, 4:1 or 5:1, w/w) and stirring rate (500, 750 and 1000 rpm) of the system were optimized obtain spherical particles. After evaporation of dichloromethane, the microspheres formed were collected by filtration in vacuum, washed 4-5 times with 50 ml deionized water each and dried at room temperature for 24 h. All microspheres formulations were prepared in triplicate.

Scanning Electron Microscopic Analysis: The shape and surface characteristics of microspheres were analyzed by scanning electron microscopy (SEM). Samples were dusted on a double-sided adhesive tape applied previously to an aluminium stub. Excess samples were removed and stub sputter coated (Polaron Sputter 7040) with 30 nm layer of gold-palladium. Samples were then observed with a scanning electron microscope (Leo 0430, Leica Cambridge Ltd., Cambridge, UK).

Particle Size Analysis: The particle size and size distribution of the prepared microspheres were measured by laser diffraction in a particle size analyzer (Mastersizer, Malvern Instruments, UK). The dried powder samples were suspended in deionized water and sonicated for 1 min with an ultra-sound probe before measurement. The obtained homogeneous suspension was determined for the equivalent volume diameter and measurements were made in triplicate for each batch of microspheres.

Determination of Encapsulation Efficiency: A sample of 100 mg of dried microspheres was dissolved in 10 ml of dichloromethane. Then, 45 ml of pH 7.2 phosphate buffer was added to it and vortexed for 5 minutes for extraction of the drug. Upper aqueous layer containing the drug was separated out and assayed spectrophotometrically (Shimadzu UV 1700, Japan) at 265 nm against a suitable blank of dummy microspheres. All the determinations were done in triplicate.

The encapsulation efficiency was calculated using the following formula:

$$\text{Encapsulation efficiency}(\%) = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \times 100 \qquad \text{(Eq.1)}$$

Microsphere Recovery: Microsphere recovery efficiencies were calculated as percentage of weight of the obtained microspheres, taking as reference the total amount of polymer used for the preparation. The percentage of recovery does not take into account the residual water and oil contents in the particles, these parameters have been disregarded because microspheres appear very dry and non-greasy.

Differential Scanning Calorimetric Studies: Differential scanning Calorimetry (DSC) measurements were carried out on a scanning calorimeter (Perkin Elmer, Pyris Diamond). The instrument was calibrated using indium as standard. Samples (10-15 mg) were placed in sealed aluminium pans and heated from 27^{o}C to 300^{o}C at a rate of 10^{o}C/min under nitrogen atmosphere (100 ml/min), with alumina powder as reference.

***In vitro* Release Studies:** *In vitro* dissolution studies were carried out on the microspheres at 37^{o}C (± 0.5^{o}C) at 100 rpm with USP Dissolution Apparatus II (Type II, Veego DA, 6DR Japan).

For the acid stage, an accurately weighed sample of microspheres was suspended in the dissolution media consisting of 500 ml of 0.1 N (pH 1.2) hydrochloric acid without enzymes and dissolution was done for 2 h. At the end of the 2 h, 400 ml of 0.1M tribasic sodium phosphate was added to all dissolution vessels, the pH was adjusted to 7.2 (± 0.2) and the dissolution was continued until the microspheres were depleted of drug or for 24 h.

Aliquots of dissolution fluid were withdrawn at specified time intervals to assay the released drug spectrophotometrically at 265 nm in both stages of dissolution. Each graphical data point was an average of dissolution data from three samples. Corrections were made for the removal of samples.

Drug release pattern from Microspheres: In order to understand the mechanism and kinetics of drug release, the results of the *in vitro* drug release study was fitted with various kinetic equations like zero order (% release *vs* t), first order (log% release *vs* t) and Higuchi model (Mt/M∞ *vs* t). In order to define a model which will represent a better fit for the formulation, drug release data was further analyzed by Peppas equation, $Mt/M\infty = kt^n$, where Mt is the amount of drug released at time t and M∞ is the amount released at time ∞, thus the Mt/M∞ is the fraction of drug released at time t, k is the kinetic constant and n is the diffusional exponent, a measure of the primary mechanism of drug release. R^2 values were calculated for the linear curves obtained by regression analysis of the above plots.

Statistical analysis: Experimental results were expressed as mean ± SD. Student's *t*-test and one-way analysis of variance (ANOVA) were applied to check significant differences in drug release from different formulations. Differences were considered to be statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION: Microencapsulation by the solvent evaporation method is, in principal, quite simple and involves two major steps, the formation of stable droplets of the drug-containing polymer solution and the subsequent removal of solvent from the droplets. In practice, however, the reproducible manufacturing of microspheres with the desired properties (good encapsulation efficiency, suitable release profile and particle distribution, acceptable solvent residuals), can be difficult, due to the large number of factors influencing the outcome, such as solvent composition, total volume and phase volume ratio of the phases, polymer concentration, stirring speed, stirring time etc. The effect of each of these parameters has to be determined empirically, predictions and scale up remain a problem. Therefore, more information is needed in order to identify the relevant parameters and save development resources.

Preparation of microspheres by Solvent Evaporation: Solvent evaporation method was used to prepare acetazolamide microspheres. First, trials were made to prepare microspheres by using a solvent evaporation technique in the oil phase, using acetone/liquid paraffin, chloroform/liquid paraffin or dichloro methane/liquid paraffin. Although many formulations were investigated, no spherical particles could be obtained. Then dichloromethane/PVA aqueous solution was used and various formulations with different polymer: drug ratios were tried, stirring speed was also optimized to obtain spherical particles.

The choice and adjustment of the manufacturing parameters for the production of microspheres of defined size were preformed in agreement with the following equation:

$$d \alpha K \frac{D_v R \upsilon_a \gamma}{D_s N \upsilon_o C_s} \qquad \text{(Eq.2)}$$

where *d* is the average particle size, K a variable depending on the apparatus geometry (e.g. type and dimension of stirrer), D_v and D_s are respectively the diameter of the vessel and of the stirrer, R the volume ratio between aqueous and oil phases, υ_a and υ_o their respective viscosities, N the stirring speed, γ the surface tension between the two immiscible phases and C_s the stabilizer concentration [25-26].

The influence of some parameters such as polymer: drug ratio, aqueous: oil phase ratio, viscosity of aqueous phase, stirring speed and stirring time was studied on morphology, mean diameter, encapsulation and recovery efficiency of microspheres.

Polymer: drug ratio: In particular when polymer: drug ratio was too low (1:1, w/w) no spherical particles were obtained independent of stirring speed of the system (500, 750, 1000 rpm). These results show that the amount of solid taken and ultimately the viscosity of the inner phase is an important factor for the preparation of microspheres. Keeping the drug amount and the solvent volume constant, spherical particles with a slightly irregular surface, a mean diameter of 83.41±2.01µm, a recovery of 63.42±1.66% (w/w) with respect to the weight of polymer utilized and an encapsulation efficiency of 62.18±1.74% (w/w) were obtained when the amount of polymer was increased to give a polymer: drug ratio of 2:1.

A further increase in polymer: drug ratio, i.e., 3:1 and 4:1 led to production of spherical particles with a mean diameters of 98.34±1.76µm and 106.10±1.98µm, the recovery of 82.67±2.11% (w/w) and 64.56±1.61% (w/w) and the encapsulation efficiencies of

72.54±1.10% (w/w) and 65.33±2.01% (w/w) respectively. Finally the highest polymer: drug ratio, i.e., 5:1 prevented microsphere isolation, resulting only in several polymer aggregates. PCL solutions with higher concentration are characterized by a viscosity too high to be employed as disperse phase in the solvent evaporation method. **Table 1** summarizes the effect of polymer: drug ratio on some microspheres characteristics.

TABLE: 1. EFFECT OF POLYMER: DRUG RATIO ON MICROSPHERES CHARACTERISTICS

Polymer: drug ratio (w/w)	Mean diameter [a] (µm) ± S.D.	Mean recovery [a,b] (%) ± S.D.	Mean encapsulation yield [a, c] (%) ± S.D.	Notes
1:1	n.d.	n.d.	n.d.	No spherical particle
2:1	83.41 ± 2.01	63.42 ± 1.66	62.18 ± 1.74	Spherical shape with irregular surface
3:1	98.34 ± 1.76	82.67 ± 2.11	72.54 ± 1.10	Spherical shape
4:1	106.10 ±1.98	64.56 ± 1.61	65.33 ± 2.01	Spherical shape
5:1	n.d.	n.d.	n.d.	Several aggregates

N. D.: Not determined. Microspheres were produced by the solvent evaporation method using as disperse phase: 0.1% (w/v) PVA, aqueous: oil phase ratio of 10:1 (v/v), stirring speed: 750 rpm and stirring time: 2 h.

[a] Data represent the mean of three independent experiments. [b] Percentage of weight of microspheres recovered with respect to weight of polymer utilized. [c] Percentage of encapsulated drug with respect to the total amount used.

Aqueous phase volume: As external dispersing phase different volumes of PVA aqueous solution (50, 100, 150 ml) were employed, resulting in different ratios between aqueous external and oil internal phases (w/o ratio), namely 5:1, 10:1, 15:1. **Table 2** summarizes the obtained results. The polymer: drug ratio was 3:1. The use of the lower w/o ratio (5:1, i.e., 50 ml) led to formation of irregularly collapsed microspheres with a mean diameter of 125.64±2.27 µm, a recovery efficiency of 65.11 ± 1.82% (w/w) and an encapsulation efficiency of 65.18±1.34% (w/w). The highest w/o ratio (15:1, i.e., 150 ml) led to a collapse of particles after isolation. Conversely particles produced by a 10:1 w/o ratio (100 ml) enabled the production of spherical microspheres with a mean diameter of 102.17±1.48 µm, a recovery efficiency of 87.48±1.07% (w/w) and the encapsulation efficiency of 73.07±1.18% (w/w). The decrease of w/o ratio resulted in an increase of particles diameter, in agreement with the findings of Arshady (Eq. 2).

TABLE: 2. EFFECT OF AQUEOUS: OIL PHASE RATIO ON MICROSPHERES CHARACTERISTICS

Aqueous: oil phase ratio (w/w)	Mean diameter [a] (µm) ± S.D.	Mean recovery [a, b] (%) ± S.D.	Mean encapsulation yield [a,c] (%) ± S.D.	Notes
5:1	125.64 ± 2.27	65.11 ± 1.82	65.18 ± 1.34	Irregular shape
10:1	102.17 ± 1.48	87.48 ± 1.07	73.07 ± 1.18	Spherical shape
15:1	n.d.	n.d.	n.d.	Collapsed particles

n.d.: Not determined. Microspheres were produced by the solvent evaporation method using as disperse phase: 0.1% (w/v) PVA, polymer:drug ratio: 3:1 (w/w), stirring speed: 750 rpm and stirring time: 2 h.

[a] Data represent the mean of three independent experiments. [b] Percentage of weight of microspheres recovered with respect to weight of polymer utilized. [c] Percentage of encapsulated drug with respect to the total amount used.

Concentration of Aqueous Phase: Increasing concentration of the external phase by addition of increasing concentration of PVA (0.05, 0.1, 0.25 and 1%, w/v) led to an increase in the particle size (from 95.25±1.46 µm with 0.1% PVA to 176.82±2.45µm with 1% PVA) without alteration on the uni-model size distribution.

However, no spherical particles were obtained with 0.05% PVA. In fact using 3:1 polymer: drug ratio and 10:1 w/o ratio, it was found that 0.25% and 1% PVA concentration led to particles with large mean diameters of 118.76±0.82µm and 176.82±2.45µm, recovery of 59.12±1.19 % (w/w) and 59.62±2.09% (w/w) and encapsulation efficiencies of 68.42±2.45% (w/w) and 56.92±1.79% (w/w) respectively, which could be due to a reduction of the stirring efficiency. The use of 0.1% PVA led to the formation of spherical particles with a mean diameter of 95.25±1.46µm, a recovery of 83.42±0.48% (w/w) and an encapsulation efficiency of 74.16±1.37% (w/w). **Table 3** summarizes, for comparison, the results thus obtained.

TABLE: 3. EFFECT OF CONCENTRATION OF AQUEOUS PHASE ON MICROSPHERES CHARACTERISTICS

Concentration of aqueous phase (%, w/v)	Mean diameter [a] (µm) ± S.D.	Mean recovery [a,b] (%) ± S.D.	Mean encapsulation yield [a,c] (%) ± S.D.	Notes
0.05	N.D.	N.D.	N.D.	No spherical particle
0.1	95.25 ± 1.46	83.42 ± 0.48	74.16 ±1.37	Spherical shape
0.25	118.76 ± 0.82	59.12 ± 1.19	68.42 ± 2.45	Spherical shape
1.0	176.82 ± 2.45	59.62 ± 2.09	56.92 ± 1.79	Spherical shape with irregular surface

N.D.: Not determined. Microspheres were produced by the solvent evaporation method using polymer: drug ratio: 3:1 (w/w), aqueous: oil phase ratio of 10:1 (v/v), stirring speed: 750 rpm and stirring time: 2 h.
[a] Data represent the mean of three independent experiments. [b] Percentage of weight of microspheres recovered with respect to weight of polymer utilized. [c] Percentage of encapsulated drug with respect to the total amount used.

Stirring Speed: Variation of stirring speed had a strong influence on PCL microspheres production. In fact using 3:1 polymer: drug ratio, 10:1 w/o ratio and 0.1% viscosity of aqueous phase, it was found that a 500 rpm stirring speed was too low to obtain structured microspheres. During solvent evaporation microspheres appeared very big when observed through optical microscope (tentative diameter 200-300µm) only resulting in collapsed beads after isolation. On the contrary, a double stirring speed, namely 1000 rpm, led to the production of spherical microspheres, characterized by 82.45±2.46µm mean diameter, 62.87±1.71% (w/w) recovery and 59.87±2.41% (w/w) encapsulation efficiency. These findings are in agreement with results published by Arshady (1990) and Esposito *et al*. (1996) [25, 27]. Nevertheless, the vorticose motion caused by the high stirring speed led to a loss of polymer droplets from the beaker during microspheres production, finally resulting in a decrease of recovery.

The best results in term of recovery were obtained by the use of 750 rpm stirring speed (86.45±2.29%, w/w), microspheres in this condition were spherical, with a 99.67±1.23µm mean diameter and 74.57±1.24% (w/w) encapsulation efficiency. **Table 4** summarizes data for comparison of the obtained results.

TABLE: 4. EFFECT OF STIRRING SPEED ON MICROSPHERES CHARACTERISTICS.

Stirring speed (rpm)	Mean diameter [a] (µm) ± S.D.	Mean recovery [a, b] (%) ± S.D.	Mean encapsulation yield [a,c] (%) ± S.D.	Notes
500	N.D.	N.D.	N.D.	Collapsed particles
750	99.67 ± 1.23	86.45 ± 2.29	74.57 ± 1.24	Spherical shape
1000	82.45 ± 2.46	62.87 ± 1.71	59.87 ± 2.41	Spherical shape

N.D.: Not determined. Microspheres were produced by the solvent evaporation method using as disperse phase: 0.1% (w/v) PVA, polymer: drug ratio: 3:1 (w/w), aqueous: oil phase ratio of 10:1 (v/v) and stirring time: 2 h.
[a] Data represent the mean of three independent experiments. [b] Percentage of weight of microspheres recovered with respect to weight of polymer utilized. [c] Percentage of encapsulated drug with respect to the total amount used.

Stirring Time: For a constant speed of 750 rpm, a polymer: drug ratio of 3:1, a w/o ratio of 10:1 and a 0.1% viscosity of aqueous phase, an increase of the stirring time from 1 to 4 h resulted in a 48.71% reduction in microspheres size (from 120.52±2.42 to 58.71±2.13µm). These observations could be explained by the increased shear stress generated in the emulsions associated to the increase in the duration of agitation at high homogenization rates tending to divide the droplets of the emulsions and finally inducing a decrease in the mean particle size. A 2 h stirring time was chosen because the entrapment efficiency was higher (75.28±0.97%, w/w) than after 4 h (67.22±2.01%, w/w). **Table 5** summarizes data for comparison of the obtained results. At last the "standard conditions" for microspheres production by solvent evaporation have been assessed: (a) a polymer: drug ratio of 3:1 (w/w), (b) a dispersing phase constituted of 100 ml of PVA aqueous solution (w/o ratio, 10:1 (v/v)), (c) a viscosity of 0.1% w/v of aqueous phase, (d) a stirring speed of 750 rpm, (e) a stirring time of 2 h. In these conditions the obtained microspheres were characterized by spherical shape, absence of aggregates, a mean diameter of 99.09±1.71µm, a recovery of 88.23±1.06% (w/w) and an encapsulation efficiency of 75.28±0.97% (w/w).

TABLE: 5. EFFECT OF STIRRING TIME ON MICROSPHERES CHARACTERISTICS.

Stirring time (h)	Mean diameter [a] (µm) ± S.D.	Mean recovery [a, b] (%) ± S.D.	Mean encapsulation yield [a,c] (%) ± S.D.	Notes
1	120.52 ± 2.42	64.34 ± 1.66	69.14 ± 0.91	Spherical shape with irregular surface
2	94.56 ± 1.72	83.42 ± 1.26	75.28 ± 0.97	Spherical shape
4	58.71 ± 2.13	62.27 ± 1.89	67.22 ± 2.01	Spherical shape

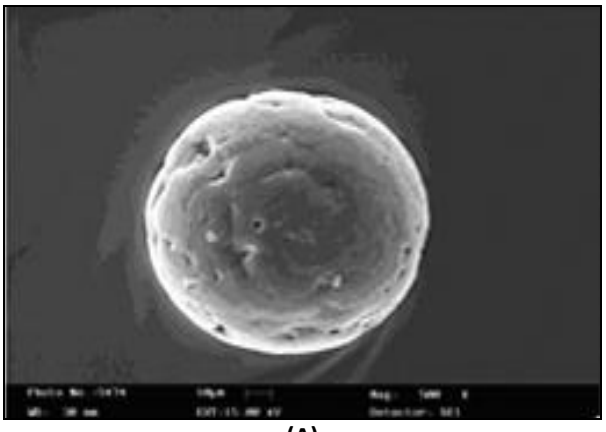
Microspheres were produced by the solvent evaporation method using as disperse phase: 0.1% (w/v) PVA, polymer: drug ratio: 3:1 (w/w), aqueous: oil phase ratio of 10:1 (v/v) and stirring speed: 750 rpm.

[a] Data represent the mean of three independent experiments. [b] Percentage of weight of microspheres recovered with respect to weight of polymer utilized. [c] Percentage of encapsulated drug with respect to the total amount used.

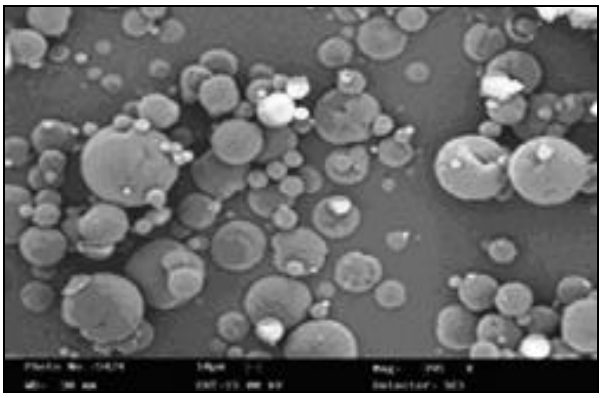
Scanning Electron Microscopic Studies: Surface morphology of the microspheres was examined by SEM. As shown in **Figure 1**, microspheres are spherical in nature without agglomerations. In addition, micropores were observed on the surface of microspheres at higher magnification (micrograph A of figure 1).

Organic phase droplets are the precursors of pores due to the phase separation occurring in the aqueous phase during the hardening of microspheres. However, microspheres produced with or without drug loading did not exhibit any effect on the surface properties.

Hence, the loading of acetazolamide did not cause any significant change in morphology (micrographs B and C of figure 1). The surface morphology of acetazolamide incorporated blend microspheres was evaluated after the *in vitro* release experiments. SEM photographs of the neat microspheres have the characteristic porous structure on the surface. An increase in the pore size was noticed after the drug release (micrograph D of figure 1).



(A)



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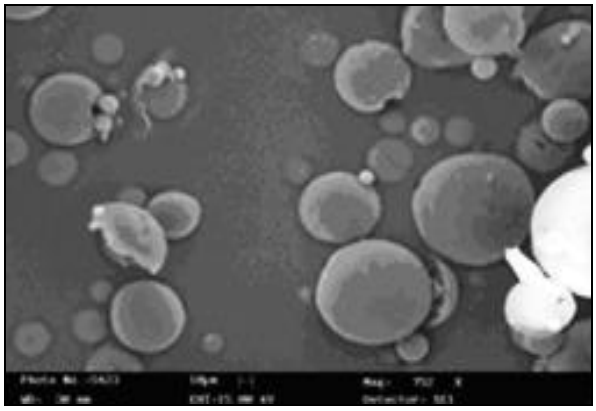

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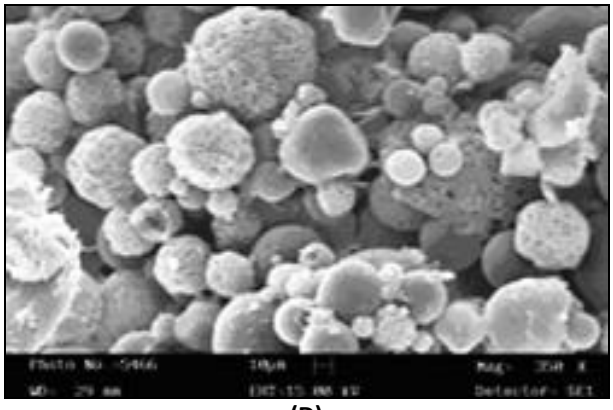
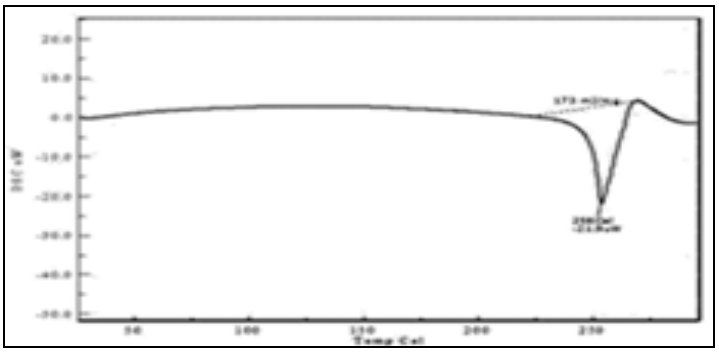


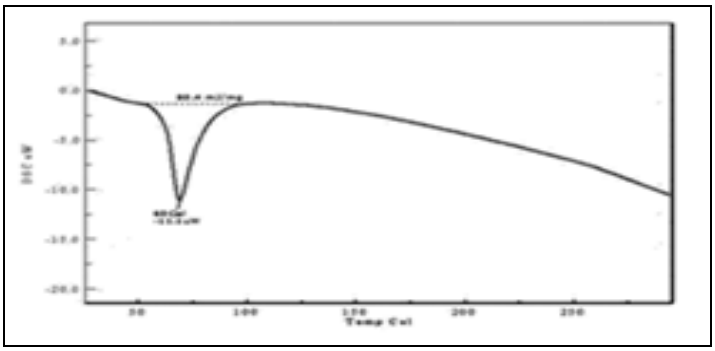
(D)

FIGURE 1: SEM PHOTOGRAPHS OF MICROSPHERES: (A) ACETAZOLAMIDE LOADED SINGLE PARTICLE, (B) PLACEBO GROUP OF PARTICLES, (C) ACETAZOLAMIDE LOADED GROUP OF PARTICLES, (D) ACETAZOLAMIDE LOADED GROUP OF PARTICLES AFTER RELEASE

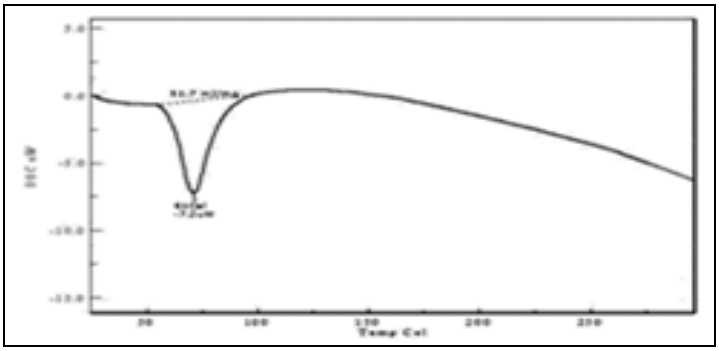
Differential Scanning Calorimetric Studies: The results of DSC studies of pure drug, drug free and drug loaded microspheres of PCL are summarized in **Figure 2**. The DSC thermogram of pure drug showed a peak at 258°C corresponding to its melting point (thermogram of figure 2). In case of drug free microspheres of PCL peak was noted at 60°C (thermogram b of figure 2), while in case of drug loaded microspheres of PCL, thermogram showed a peak at 61°C corresponding to the melting point of PCL (thermogram c of figure 2). This shift may be due to physicochemical interaction of drug and polymer. No drug peak was observed in drug loaded microspheres suggesting that drug was molecularly dispersed throughout the polymer matrix.



A



B



C

FIG. 2: DSC THERMOGRAM OF: (A) PURE DRUG, ACETAZOLAMIDE, (B) DRUG FREE PCL MICROSPHERES, (C) DRUG LOADED PCL MICROSPHERES

***In vitro* release behavior of PCL microspheres:** *In vitro* release profiles give important informations on the efficiency of the delivery systems for the controlled release of drugs. An *in vitro* drug release study is indeed a prerequisite to obtain correct predictions in order to design and test the *in vivo* activity of controlled drug delivery forms [28].

Since the main goal of this work was to prepare a controlled release system for acetazolamide, the solubility of the pure drug was first evaluated in two biological fluids, which are, pH 1.2 buffer and pH 7.2 phosphate buffer simulating the intestinal environment. In **Figure 3**, the solubilization of acetazolamide as a function of time is presented for the two media.

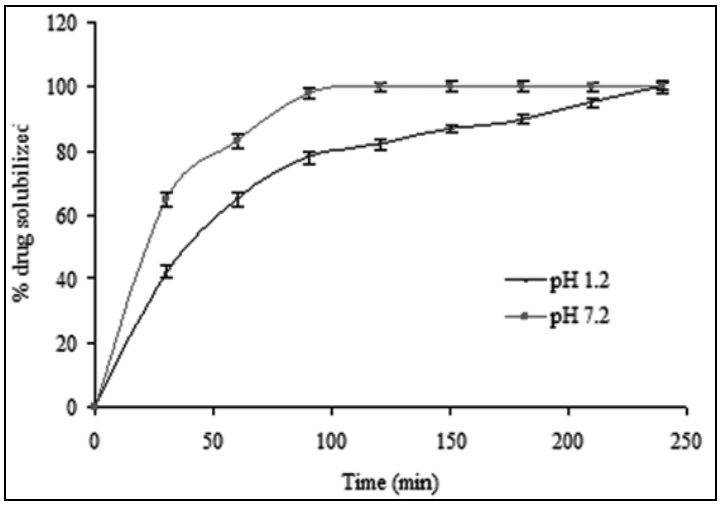


FIG. 3: RELEASE PROFILE OF PURE DRUG, ACETAZOLAMIDE. DATA REPRESENT THE AVERAGE OF THREE INDEPENDENT EXPERIMENTS

It was observed, the pure drug is totally dissolved in the two media in 4 h. However, acetazolamide solubilizes faster in a neutral environment than in the acidic environment and its complete dissolution is thus achieved in 1.5 h instead of 4 h. Acetazolamide is a weak acid drug and its solubility is greater at high pH, as expected. This faster dissolution rate is clinically undesirable as it does not promote the contact of the drug with the mucosal membranes over a time sufficient enough to be absorbed, so the drug will be "washed out" of the body. Therefore a system that enables a sustained release of the drug is of great interest for the delivery of acetazolamide.

In vitro acetazolamide release studies from PCL microspheres were performed in pH 1.2 buffer (first 2 h) and phosphate buffer, pH 7.2 (after 2 h) at 37 ± 0.5°C. The cumulative release of acetazolamide significantly decreased with increasing PCL concentration ($p < 0.05$). The increased density of the polymer matrix at higher concentrations results in an increased diffusional path length. This may decrease the overall drug release from the polymer matrix. Furthermore, smaller microspheres are formed at a lower polymer concentration and have a large surface area exposed to dissolution medium, giving rise to faster drug release. The release behavior of acetazolamide from PCL microspheres (formulation parameters are presented in **Table 6**) is illustrated in **Figure 4**, which indicates the sustained release pattern over 24 h. At the initial stage, the burst effect related to the drug entrapped near the surface of the microparticles was remarkably small. Such a small initial burst is an especially interesting phenomenon, which is probably due to the low permeability of water in PCL. To be released, preferentially, the diffusion path must be filled up by water. In the other words, the hydrophobic property of PCL causes the delay of water penetration, thus the diffusion of the drug through the amorphous region into the release medium was retarded, which results in a small burst effect.

TABLE: 6. FORMULATION PARAMETERS OF THE MICROSPHERES

Batch code	Polymer: drug ratio (w/w)	Aqueous: oil phase ratio (v/v)	Concentration of aqueous phase (%, w/v)	Stirring speed (rpm)	Stirring time (h)
AP-2	2:1	10:1	0.1	750	2
FOAP-3	3:1	10:1	0.1	750	2
AP-4	4:1	10:1	0.1	750	2

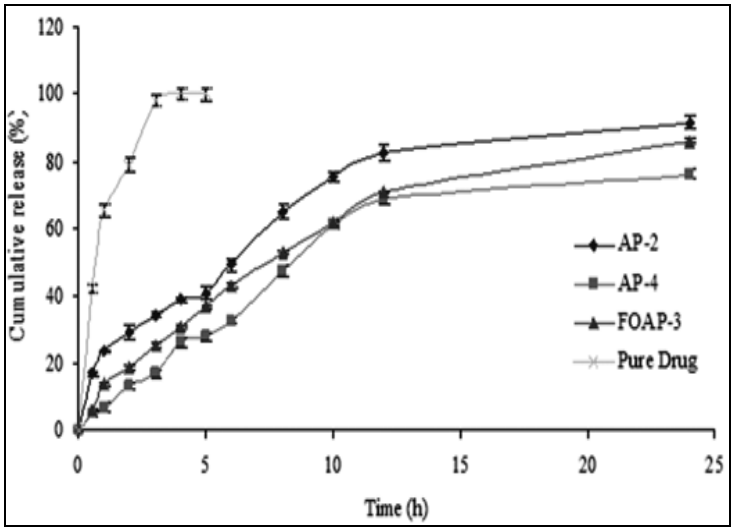


FIG. 4: RELEASE PROFILE OF ACETAZOLAMIDE FROM PCL MICROSPHERES OBTAINED BY SOLVENT EVAPORATION METHOD AND THE STANDARD CONDITIONS DEFINED IN TABLE 6. DATA REPRESENT THE AVERAGE OF THREE INDEPENDENT EXPERIMENTS

Kinetics of drug release: In order to investigate the release mechanism of present drug delivery system, the data obtained from *in vitro* release of final optimized batch (FOAP-3) were fitted into equations for the zero-order, first-order, Higuchi release model and Peppas equation. The interpretation of data was based on the values of the resulting regression coefficients.

The in vitro drug release showed the regression coefficient values for Higuchi's model (**Figure 5**) (R^2 = 0.9758) and Peppas model (R^2 = 0.9799) and a value of n = 0.6994 (**Figure 6**) indicating anomalous transport. This fact can be explained as follows. Release from biodegradable polymer is frequently governed by a combination of erosion and diffusion, which depends on the relative rates of erosion and diffusion.

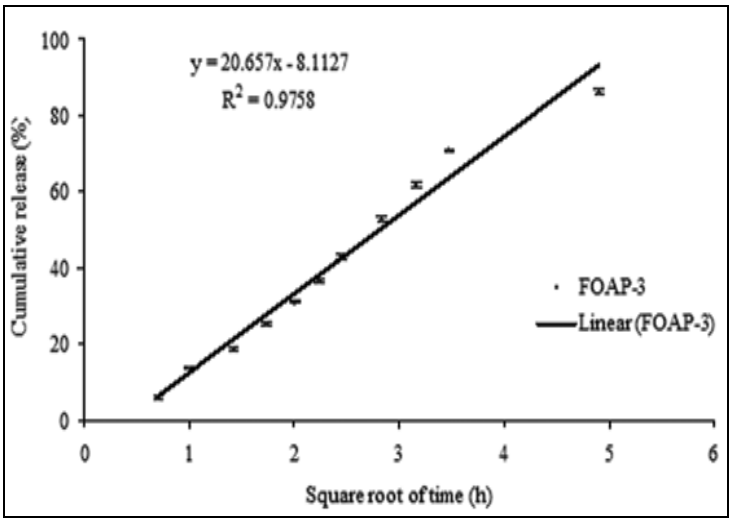


FIG. 5; HIGUCHI PLOT OF FINAL OPTIMIZED BATCH (FOAP-3) OF PCL MICROSPHERES

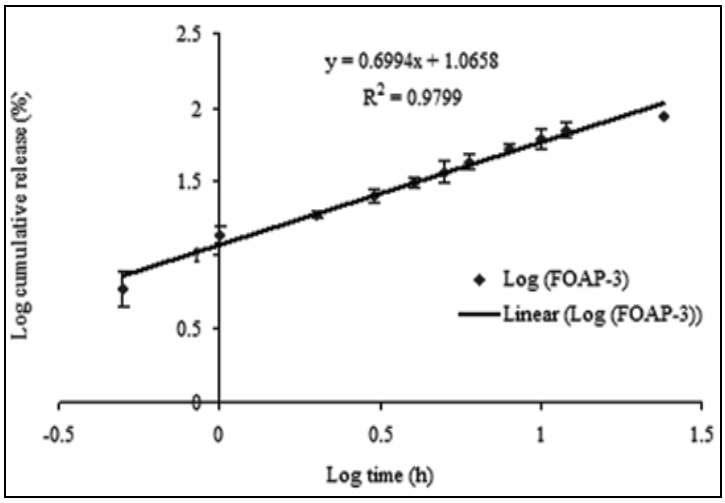


FIG. 6: PEPPAS PLOT OF FINAL OPTIMIZED BATCH (FOAP-3) OF PCL MICROSPHERES

CONCLUSION: A preformulation study aimed to produce PCL microspheres by a solvent evaporation method has been presented. These investigations have also provided an understanding of the effects of some process parameters on particle size and shape, recovery and encapsulation efficiency. Selection of the appropriate experimental conditions result in the production of PCL based microspheres characterized by spherical shape, absence of aggregates, a mean diameter of 99.09±1.71µm, high encapsulation efficiency of 75.28±0.97% (w/w) and an almost quantitative recovery of 88.23±1.06% (w/w).

By the use of PCL as an excipient for the drug it should be possible to eliminate or minimize the gastrointestinal side effects of the drug. Controlled release without initial peak levels achieved with these microspheres formulations can reduce dosing frequency, decrease side effects and improve patient compliance.

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