

Original Article

PREPARATION AND EVALUATION OF ANTHRALIN BIODEGRADABLE NANOPARTICLES AS A POTENTIAL DELIVERY SYSTEM FOR THE TREATMENT OF PSORIASIS

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ABSTRACT

Objective: Anthralin is one of the most effective drugs in psoriasis management. However, its side effects and unfavourable physicochemical properties limit its clinical use. Therefore, the objective of this study was to prepare and evaluate poly (ethylene glycol)-*block*-poly (ϵ -caprolactone) (PEG-*b*-PCL) nanoparticles as a potential delivery system for anthralin.

Methods: PEG-*b*-PCL nanoparticles were prepared by the co-solvent evaporation method and evaluated using a variety of techniques. The effect of drug/polymer weight feed ratio on the nanoparticle size, drug loading capacity and encapsulation efficiency were studied. Drug release kinetics was studied using the dialysis bag method. Nanoparticle size was measured using dynamic light scattering and confirmed by transmission electron microscopy measurements.

Results: PEG-*b*-PCL formed spherical nanoparticles having a diameter of 40 to 80 nm based on the polymer and level of drug loading. The size observed by TEM measurements was slightly smaller than that obtained by DLS due to nanoparticle dryness during measurement. Drug loading capacity increased with increasing the drug/polymer ratio and a maximum loading of ~25% was obtained. Anthralin encapsulation in the nanoparticles resulted in ~120-fold increase in its aqueous solubility. Anthralin was released from the nanoparticles over a prolonged period of time where ~45% was released in 48 h.

Conclusion: These results confirm the utility of PEG-*b*-PCL nanoparticles in enhancing the aqueous solubility and sustaining the release of anthralin. Therefore, they might be used as a potential delivery system for the treatment of psoriasis.

Keywords: Anthralin, Nanoparticles, Psoriasis, Poly (caprolactone), Drug delivery, Solubility.

INTRODUCTION

Psoriasis is a common, incurable and chronic inflammatory skin disease that is associated with a number of significant comorbidities, such as cardiovascular diseases and seronegative arthritis known as psoriatic arthritis [1]. It is now recognized as one of the most common immune-mediated disorders where tumor necrosis factor alpha, dendritic cells, and T-cells considerably contribute to its pathogenesis [2]. It occurs in 2-3% of the population of the United Kingdom (UK) and in 3.2% of US adults ages 20 y and older [2, 3]. Early onset disease that affects patients at age <40 y accounts for more than 75% of psoriasis cases [2]. Genetic predisposition, in addition to environmental triggers, such as beta-haemolytic streptococcal infections or stress are major determinants of early-onset disease prognosis [1, 2]. Psoriasis can negatively impact patient quality of life and lead to substantial morbidity and mortality [4]. Reduction of the quality of life in psoriasis patients is comparable to that in patients with diabetes, heart diseases and cancer [5]. Depression is significantly higher in psoriasis patients than in the general population and leads to an increased incidence of suicidal attempts [6]. Therefore, a safe and effective therapy of psoriasis is highly desirable.

Anthralin (1,8-dihydroxy-9-anthrone) which was introduced over 80 y ago has shown excellent efficacy in the management of psoriasis [7, 8]. However, anthralin administration is associated with numerous side effects that discourage its wide-spread use. For instance, topical application of anthralin results in skin irritation, stinging, burning, redness and staining of the skin and clothes. Further, anthralin is chemically unstable and is readily degraded by photo-oxidation [9, 10]. The literature shows several attempts to overcome these shortcomings while maintaining or increasing drug efficacy. For instance, shorter application time has been recommended followed by washing the skin with special cleansing agents [11, 12]. Aqueous cream and wax-ester-based preparations of

anthralin have also been recommended [13, 14]. However, anthralin clinical utility remains limited in spite of these modifications. Therefore, efficient drug delivery systems able to minimize anthralin side effects and maximize its efficacy remain yet to be developed.

Pharmaceutical nanotechnology has emerged as an effective tool in improving drug efficacy while minimizing its side effects [15]. Because of their nanometric size and unique core-shell structure, nanoparticles have enhanced penetration through the tissue barriers, in addition to maximum drug protection against harsh conditions, *in vitro* and *in vivo* [16]. Thus, anthralin encapsulated into liposomes and niosomes showed significantly enhanced permeation through mouse abdominal skin [9]. Further, anthralin liposomes showed minimum skin irritation and staining of skin and clothes. Markedly low incidence and severity of perilesional erythema and skin staining were seen with anthralin liposomes in comparison with anthralin conventional cream [8]. However, liposomes suffer from many disadvantages such as chemical instability of phospholipids and liposome tendency to degrade, aggregate and fuse. This can cause premature drug release during storage and after administration [17].

The aim of the present work was to prepare and evaluate PEG-*b*-PCL nanoparticles and test their potential as a delivery system for anthralin. The nanoparticle size, drug content and drug release were evaluated using different techniques.

MATERIALS AND METHODS

Materials

Anthralin was purchased from Professional Compounding Centers of America (Houston, TX, USA). Dialysis membranes (Spectra/por, MWCO: 3.5-5 kDa, unless otherwise indicated) were purchased from Fisher Scientific (Rancho Dominguez, CA, USA). Poly (ethylene glycol)-*block*-poly (ϵ -caprolactone) (PEG-*b*-PCL) copolymers were

obtained from Polymer Source (Dorval, QC, Canada). Two polymers were purchased, namely PEG2-*b*-PCL5 and PEG2-*b*-PCL10 where PEG molecular weight was 2 kDa, and the PCL molecular weight was either 5 or 10 kDa, respectively. All other chemicals were reagent grade and used as received.

Preparation of empty and anthralin-loaded nanoparticles

Drug-loaded nanoparticles were prepared by the co-solvent evaporation method [18-20]. Empty nanoparticles were prepared by the same method and used as a control. Specific weights of the polymer and drug (drug/polymer ratio of 0–50 wt. %) were dissolved in 1.5 ml of acetone [21, 22]. This solution was added dropwise (1 drop/10 s) to 3 ml of magnetically stirred water adjusted to pH 3.3. The mixtures were stirred in open vials for 24 h to remove acetone and trigger nanoparticle formation. Subsequently, the mixtures were filtered through a 0.45 µm PVDF filter to remove the free (non-incorporated) drug and the nanoparticle solution was characterized by different techniques. Final polymer concentration in water was 10% w/v.

Determination of nanoparticle size by dynamic light scattering

The dynamic light scattering measurements were performed on a Malvern ZetaSizer (Nano-ZS, Malvern Instruments, Worcestershire, UK). The instrument was equipped with a He-Ne laser operating at 633 nm and an avalanche photodiode detector. Samples were filtered through a 0.45 µm Millex Millipore PVDF membrane prior to measurements to remove dust particles. The mean hydrodynamic diameter and polydispersity index of the nanoparticles were determined. Measurements were performed in triplicates at room temperature. A cumulant analysis was applied to obtain the hydrodynamic diameter and polydispersity index of the nanoparticles. The constrained regularized CONTIN method was used to obtain the particle size distribution.

Measurement of nanoparticle size by transmission electron microscopy

Transmission electron microscopy (TEM) images of the nanoparticles were taken using a Phillips CM200 electron microscope equipped with an AMT 2k × 2k CCD camera at an acceleration voltage of 80 kV. TEM samples were prepared by adding 15 µl of the aqueous nanoparticle solutions onto a Formvar-coated 400 mesh grid stabilized with evaporated carbon film. The nanoparticles were negatively stained by adding 15 µl of 1% aqueous uranyl acetate solution. The samples were allowed to dry overnight at room temperature.

Determination of drug encapsulation efficiency and loading capacity

Aliquots of the anthralin-loaded nanoparticle solution were diluted 10 times by acetonitrile and used to determine drug content of the particles by measuring the UV absorbance at 349 nm and using a calibration curve. Empty nanoparticle samples were treated similarly and used as a control. Anthralin encapsulation efficiency and loading capacity were calculated from the following equations:

$$\text{Anthralin loading capacity (weight \%)} = \frac{\text{weight of anthralin in nanoparticles}}{\text{Total weight of nanoparticles tested}} \times 100 \quad (1)$$

$$\text{Anthralin encapsulation efficiency (weight \%)} = \frac{\text{weight of anthralin in nanoparticles}}{\text{Total weight of anthralin used initially}} \times 100 \quad (2)$$

Drug release studies

In vitro release of anthralin from PEG-*b*-PCL nanoparticles was studied by the dialysis bag method in citrate buffer pH 3.3. The buffer contained 2% sodium lauryl sulfate and 20% methanol due to the very limited solubility of anthralin in aqueous media [9]. Aliquots of anthralin nanoparticle solutions in water pH 3.3 (1 ml, [anthralin] = 1.0–1.50 mg/ml) were introduced in a dialysis tube (MWCO = 3.5–5 kDa). The solution was dialyzed against 25 ml of the release medium maintained at 37 °C. At predetermined time intervals, aliquots were taken and replaced by fresh release medium maintained at 37 °C. The concentration of the drug in the release samples was determined from its UV absorbance at 349 nm and using a previously constructed calibration curve. The cumulative percent of drug released was plotted as a function of dialysis time.

RESULTS AND DISCUSSION

Preparation and evaluation of anthralin nanoparticles

Anthralin is an unstable hydrophobic drug with an aqueous solubility ≤ 2 µg/ml [9]. The nanoparticles were prepared in an aqueous solution of pH 3.3 due to the drug instability at higher pH values. PEG-*b*-PCL copolymers are known to form nanoparticles having a hydrophobic PCL core surrounded by a PEG corona [21]. Anthralin incorporation into the hydrophobic cores of PEG-*b*-PCL nanoparticles could enhance its aqueous solubility, sustain its release and protect it against photolytic degradation [9]. Blank and anthralin-loaded nanoparticles were prepared by the co-solvent evaporation method, which is proven useful for the incorporation of many hydrophobic drugs [18, 20]. DLS measurements were used to ascertain the formation of nanoparticles and characterize their size and polydispersity. Fig.1 shows the size distribution of anthralin-loaded PEG2-*b*-PCL5 and PEG2-*b*-PCL10 nanoparticles. The nanoparticles had narrow size distribution indicating uniformity of size and absence of aggregates. This was further confirmed by measuring the polydispersity index (PDI) of the nanoparticles which was ≤ 0.25 confirming the monodispersity of the nanoparticles [23]. The nanoparticle shape and morphology were studied by TEM measurements (fig. 2). A TEM image of the nanoparticles F6 (PEG2-*b*-PCL5 containing ~20% w/w anthralin) shows that the nanoparticles are spherical, well-dispersed and free of aggregates. The average size obtained from this TEM image is 67.8±6.9 nm, which is slightly smaller than that obtained by DLS due to the drying of the nanoparticles during sample preparation. This difference is because TEM gives the size of the dry nanoparticles while DLS measures the hydrodynamic diameter of the particles in solution [24, 25].

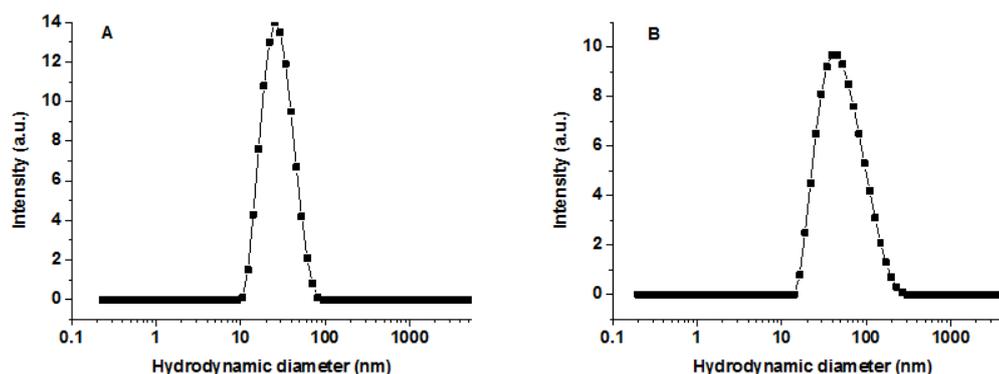


Fig. 1: Distribution of the hydrodynamic diameter of (A): anthralin-loaded PEG2-*b*-PCL5 nanoparticles and (B): anthralin-loaded PEG2-*b*-PCL10 nanoparticles (Solvent: water pH 3.3; polymer concentration: 1.0 g/l; anthralin/polymer ratio: 30 wt.%)

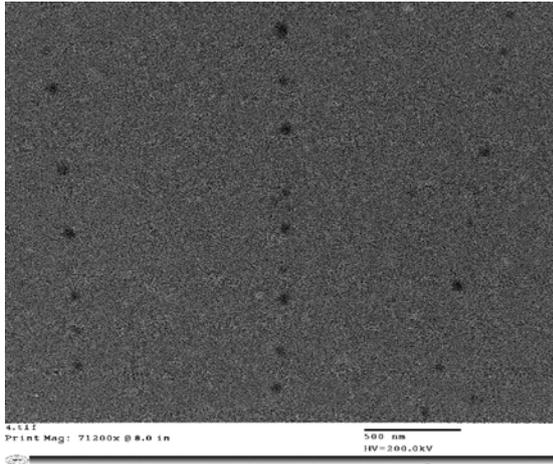


Fig. 2: TEM image of PEG2-*b*-PCL5 nanoparticles prepared by the solvent evaporation method at polymer concentration of 1.0 mg/ml and drug/polymer weight ratio of 20%

The nanoparticles were prepared at different drug/polymer ratios and their drug loading capacity and encapsulation efficiency were

determined. Table 1 shows the drug loading capacity and encapsulation efficiency for anthralin/PEG2-*b*-PCL5 nanoparticles. The drug loading capacity increased with the increase in drug/polymer weight ratio. A similar trend was observed for the anthralin encapsulation efficiency (table 1). Thus, increasing the drug/polymer weight ratio from 10 to 50% resulted in increasing the drug loading capacity from 3.26 ± 0.21 to $19.05 \pm 0.42\%$. This was associated with increasing anthralin aqueous solubility to $\sim 190 \mu\text{g/ml}$. This represents ~ 95 -fold enhancement in anthralin aqueous solubility. Table 2 shows the drug loading capacity and encapsulation efficiency for anthralin/PEG2-*b*-PCL10 nanoparticles. Similar to PEG2-*b*-PCL5 nanoparticles, the drug loading capacity and encapsulation efficiency increased with increasing the drug/polymer ratio. However, the drug loading capacity and drug solubility obtained for the nanoparticles prepared at drug/polymer weight ratio of 50% were higher for PEG2-*b*-PCL10 nanoparticles. Thus, anthralin aqueous solubility obtained under these conditions was $\sim 240 \mu\text{g/ml}$, which represents about 120-fold enhancement in the aqueous drug solubility. It is noteworthy that the limited anthralin solubility in some conventional bases, such as soft paraffin results in the incorporation of extra drug concentrations in its crystalline form to maintain the therapeutic drug effect. However, the insoluble form of the drug causes irritation and staining of normal skin [9]. Therefore, the extraordinary anthralin loading and enhancement of aqueous solubility achieved by PEG-*b*-PCL nanoparticles are expected to be advantageous in overcoming the drug shortcomings.

Table 1: Composition and properties of blank and anthralin-loaded PEG2-*b*-PCL5 nanoparticles

Formulation code	Drug/polymer (wt%)	% LC ^a	% EE ^b
F1	0	-	-
F2	10	3.26 ± 0.21	32.62 ± 2.08
F3	20	6.80 ± 0.25	34.01 ± 1.25
F4	30	13.94 ± 0.25	46.45 ± 0.83
F5	40	17.88 ± 0.32	44.69 ± 0.80
F6	50	19.05 ± 0.42	38.09 ± 0.83

^aPercent drug loading capacity, calculated using equation 1. Mean of three measurements \pm SD, ^bPercent encapsulation efficiency, calculated using equation 2. Mean of three measurements \pm SD.

Table 2: Composition and properties of blank and anthralin-loaded PEG2-*b*-PCL10 nanoparticles

Formulation code	Drug/polymer (wt%)	% LC ^a	% EE ^b
F7	0	-	-
F8	10	3.11 ± 0.27	31.12 ± 2.70
F9	20	7.20 ± 0.26	36.01 ± 1.31
F10	30	11.88 ± 0.29	39.59 ± 0.95
F11	40	11.60 ± 0.46	29.01 ± 1.14
F12	50	24.15 ± 0.82	48.29 ± 1.65

^aPercent drug loading capacity, calculated using equation 1. Mean of three measurements \pm SD, ^bPercent encapsulation efficiency, calculated using equation 2. Mean of three measurements \pm SD.

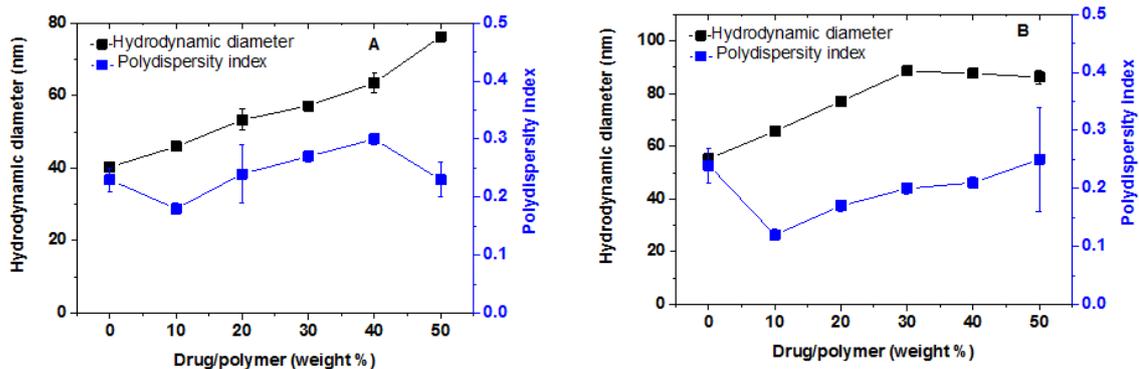


Fig. 3: Hydrodynamic diameter and polydispersity index as a function of drug/polymer weight ratio for (A) anthralin/PEG2-*b*-PCL5 nanoparticles and (B) anthralin/PEG2-*b*-PCL10 nanoparticles. Nanoparticles were prepared by the co-solvent evaporation method at polymer concentration of 1.0 mg/ml

Fig. 3 shows the hydrodynamic diameter and polydispersity index of empty and anthralin-loaded PEG-*b*-PCL nanoparticles. Empty PEG2-*b*-PCL5 nanoparticles had a hydrodynamic diameter of 40.3 ± 0.28 nm. There was a general increase in the nanoparticle size upon drug incorporation. Thus, the PEG2-*b*-PCL5 nanoparticle size increased from 40.3 ± 0.28 to 76.3 ± 1.10 nm upon incorporation of $\sim 20\%$ drug (fig. 3A). Similar trend was observed for PEG2-*b*-PCL10 nanoparticles (fig. 3B). The increase in nanoparticle size upon drug loading might be due the drug encapsulation in the nanoparticle core resulting in their expansion to accommodate the loaded drug [18, 21, 26]. In general, PEG2-*b*-PCL5 nanoparticles were smaller in size compared to those of PEG2-*b*-PCL10 [27]. This might be attributed to the bigger PCL segment molecular weight in PEG2-*b*-PCL10. The PCL segment forms the nanoparticle core and therefore longer PCL chains result in the formation of bigger nanoparticles.

Drug release studies

Anthralin release from the nanoparticles was studied by the dialysis bag method which is widely used for studying drug release from nanoparticles [28-30]. Fig.4 shows the release rate as a function of time of anthralin from PEG2-*b*-PCL5 and PEG2-*b*-PCL10 nanoparticles. The release pattern was similar for both nanoparticle formulations indicating that the drug release rate is affected neither by the polymer molecular weight nor by the level of drug loading. Similar behavior was noticed previously for other drug-loaded nanoparticle systems [20]. Anthralin release was slow in both nanoparticle systems where only $\sim 45\%$ was released in 48 h. The slow release might be attributed to the hydrophobic nature of the drug which facilitates its hydrophobic interactions with the core-forming segments of the copolymers. Sustained release is beneficial in reducing the frequency of drug administration and thus improving patient compliance.

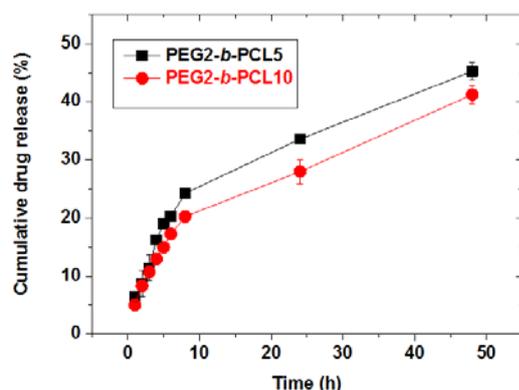


Fig. 4: Cumulative percent anthralin released from PEG2-*b*-PCL5 and PEG2-*b*-PCL10 nanoparticles in citrate buffer pH 3.3 at 37 °C

CONCLUSION

PEG-*b*-PCL block copolymer nanoparticles were used as a potential delivery system for anthralin for the treatment of psoriasis plaques. Anthralin was loaded into the nanoparticles by a simple, yet effective method which resulted in drug loading capacity up to ~ 25 wt.% for PEG2-*b*-PCL10 nanoparticles. This was accompanied by ~ 120 -fold increase in anthralin aqueous solubility. The formed nanoparticles had a spherical shape and were uniform in size and shape. The nanoparticle size was dependent on the polymer used and level of drug loading, where higher drug loading resulted in bigger particle size. Drug release from the nanoparticles was slow confirming the ability of the nanoparticles to sustain the drug release. These results confirm the potential of PEG-*b*-PCL nanoparticles as an effective delivery system for anthralin in the treatment of psoriasis.

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CONFLICT OF INTERESTS

The authors report no conflicts of interest in this work

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