Effect of polycaprolactone on *in vitro* release of melatonin encapsulated niosomes in artificial and whole saliva

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In vitro release of melatonin from niosomes with or without polycaprolactone (PCL) into artificial and whole saliva as the receptor media was compared using dissolution-dialysis apparatus. Melatonin niosomes (MN) with high and low PCL incorporation in conjunction with PCL coating were developed. Physicochemical characteristics of MN were investigated. Time to steady state from the release profile (T_{ss}) was observed at 6 h with melatonin solution in both media, while in artificial saliva, that of MN and low-PCL MN at 12 h, and PCL-coated MN 9 h. T_{ss} of all niosome formulations was 12 h using whole saliva. The release kinetics of melatonin from its solution and MN followed first order and Korsmeyer-Peppas models, respectively, indicating that the niosomes modified the diffusion controlled melatonin release. The rates of melatonin release into artificial saliva and whole saliva were reduced by half by niosome encapsulation with or without PCL ($p < 0.05$, both). Retardation of melatonin release into saliva by using niosomes with PCL modifications could be potentially beneficial for topical use of melatonin in oral cavity.

Key words: Melatonin – Niosomes – Saliva – Release study – Polycaprolactone.

Melatonin (N-acetyl-5-hydroxytryptamine) is an endogenous hormone produced primarily by the pineal gland, and found in serum, gingival crevicular fluid, and saliva [1]. Several studies have shown specific functions of melatonin in the oral cavity including antioxidant activity, immunomodulation, and anti-inflammation [2-4]. An *in vitro* study showed that melatonin inhibited inflammatory mediators such as interleukin 1β and 8 [5], cyclooxygenase-2 and nitric oxide synthase, and prostaglandin E2-induced pro-inflammatory cytokine production in human gingival fibroblasts [6]. Intraplantar injection of melatonin (125 μg) also reduced paw edema induced by carrageenan [7-9] or zymosan [10] in rat. Its activity is comparable to 4 mg/kg of indomethacin [11]. Local melatonin (2 mg) placed after tooth extraction reduced products of lipid peroxidation in Beagle dogs [12]. Therefore the local application of melatonin may have benefits for treatment of inflammation in the oral cavity damaged by mechanical processes such as tooth extraction, other oral surgeries, or oral diseases [4, 13]. However, formulations of melatonin specifically for oral cavity use have yet to be developed. The local administration of melatonin in the oral cavity needs careful consideration as it is quite lipophilic (partition coefficient log P = 1.2) [14, 15] so should readily penetrate cell membranes into cellular compartments [13]. The high blood supply in the oral cavity may result in significant undesirable systemic circulation that could cause daytime drowsiness or disrupt normal circadian rhythm, and, furthermore, reduce the efficiency of the local effect.

Melatonin encapsulated in liposome vesicles showed the ability to entrap drug and be retained in tissue for up to 24 h [16]. However liposomes have limitations of instability and high cost. Niosomes are liposome-like bilayer vesicles composed of nonionic surfactants and additives such as cholesterol [17] to improve stability, and with absorption enhancers [18], that can be used to encapsulate drugs. The other advantages of niosomes are that they are inexpensive, essentially nontoxic, biodegradable, and biocompatible [19]. Niosomes can control delivery of drugs in a sustained release manner and can be drug carriers for both local [20] and systemic sites [21]. Shahiwala *et al*. [22] reported drug retention of drug-encapsulated niosomes was higher than plain drug for local application, and thus melatonin entrapped niosomes may provide beneficial local effects in the oral cavity.

The niosomes in this study were modified by coating with the biodegradable polymer polycaprolactone (PCL), a linear aliphatic polyester that is used in medical applications has been approved by the US Food and Drug Administration [23]. Currently, PCL is of interest in the development of controlled drug delivery systems due to its stability, non-toxicity, and low cost [24]. PCL has been demonstrated in many novel drug delivery including microspheres, nanoparticles, scaffold, fibres and films to provide controlled and site specific drug delivery and increase the time of drug retention at the target site [25]. The authors believe this is the first study concerning the effects of niosomes and PCL. Hence, this work investigated the release profile from several different PCL niosomal formulations in two oral models; artificial saliva and whole saliva.

I. MATERIALS AND METHODS

1. Materials

Melatonin (GMP, 99.68 % purity) was purchased from Huang-Gang SaiKang Pharmaceutical Co. (PR China). Sorbitan monostearate (Span 60), sodium deoxycholate (SDC) and polycaprolactone (PCL) were purchased from Sigma-Aldrich (United States), cholesterol from Fluka Chemie (GmbH, Japan), chloroform from RCI Labscan (Thailand), methanol from VWR International (France), sodium bicarbonate from Tata Chemicals Europe (United Kingdom), and sodium dihydrogen orthophosphate and calcium chloride from Carlo Erba (Italy). All chemicals were of analytical grade and used as received. Cellulose tubular membrane (Cellu•Sep T4, 12,000 Dalton MW cutoff, 0.0025 μm) from Membrane Filtration Products Inc (United States) was chosen to restrict macromolecular transport, i.e. only small molecules e.g. melatonin with a molecular weight of 232.3 g/mol could passively diffuse through.

2. Formula and preparation of melatonin niosomes

Melatonin niosomes (MN) were prepared by the thin film hydration technique [26]. In brief, sorbitan monostearate 0.021 g and cholesterol 0.019 g (molar ratio of 1:1, respectively) were dissolved in organic solvents, chloroform (1 mL) and methanol (8 mL) and mixed, followed by rotary evaporation at about 60 °C (EYEL4, Eyela Tokyo Rikaki-

kai, Japan) to form a thin film, then hydrated by an aqueous mixture (20 mL) of sodium deoxycholate 0.040 g and melatonin 0.020 g (molar ratio 2:1, respectively) with gentle agitation for 1 h and sonication for 5 min (Kudos SK6210HP, Kudos, China) to form a liquid dispersion. Melatonin niosomes were modified by adding PCL into the organic solvent mix during thin film formation at the final concentration of 2.5 and 0.5 mg/mL (high-PCL MN and low-PCL MN), respectively. The final formulation (PCL-coated MN) was obtained by adding 200 μL of PCL stock solution in chloroform (0.1 mg/mL) into an MN dispersion of 20 mL (PCL was equal to 0.001 mg/mL of final concentration), then sonicating the dispersion for 10 min at about 30 °C. The residual of chloroform was removed by evaporation at 58 °C until chloroform was no longer seen on the surface of the dispersion. The dispersion was weighed before and after evaporation. Melatonin aqueous solution (1 mg/mL) in this study was prepared using deionized (DI) water.

3. Release test

Release studies of melatonin were carried out using artificial and whole saliva as the receptor media [27]. Artificial saliva containing 0.17 % sodium bicarbonate, 0.05 % sodium dihydrogen orthophosphate, and 0.02 % calcium chloride in water was prepared. Whole or human saliva collection and preparation methods were derived from WHO/IARC guidelines [28]. Volunteers were asked to refrain from eating, drinking or using oral hygiene products for at least one hour prior to collection. Then volunteers were asked to rinse their mouths with water. Five minutes after oral rinsing, they were asked to salivate into a 15 mL tube kept on ice. The time of collection was 4-5 p.m. Five millilitres of saliva was collected within 30 min. Whole saliva was centrifuged at 2,600 g for 15 min at 4 °C and stored at -20 °C until use.

Melatonin niosomes and aqueous solution were subjected to release study using a dissolution-dialysis apparatus developed in our laboratory and modified from Devaraj *et al*. [29]. A 0.8 cm diameter donor compartment was covered with a piece of Cellu•Sep tubular cellulose membrane, presoaked in deionized water for 30 min prior to use. The effective permeation area was 0.5 cm^2. The pre-packed donor compartment with the preparation and the membrane was submerged in 0.5 mL of receptor media, i.e. artificial or whole saliva, which was constantly stirred at 100 rpm by magnetic stirrer. At predetermined intervals (1, 3, 6, 9, 12, 18, and 24 h), samples of 0.3 mL were drawn from 0.5 mL of the receptor and replaced with 0.3 mL of fresh receptor media. The samples were diluted 100 fold using 10 % methanol in DI water (to release any melatonin bound to the saliva) and melatonin content released measured by spectrofluorometry (LS 50B, Perkin Elmer, United States) at λ_{exc} 285 and λ_{ems} 357 nm and estimated with standard curves of melatonin in 10 % methanol. Care was taken to prevent photodegradation of melatonin.

4. Characterization of melatonin niosomes

Particle sizes and distributions of melatonin niosomes were measured by laser beam based mastersizer (Mastersizer 2000, Malvern Instruments, United Kingdom) and are reported as dv_{10}, dv_{50} and dv_{90} values. The morphology of each niosome formulation was observed by scanning electron microscope (SEM, Leo 1450PV, Leo Electron Microscopy, United Kingdom).

5. Statistical analysis

The time to steady state (T_{ss}) release of all formulations was analyzed by paired t-test, and percentages released between formulations were compared by ANOVA. Statistical significance was considered as $p < 0.05$. Various kinetic models were used to investigate the release kinetic of each formulation. The zero order model is used to explain a system which is independent of the concentration (*Equation 1*) and derived from a linear plot between cumulative drug release (%) versus time. The first order model is for release rates depending on its concentration (*Equation 2*) and is plotted as log cumulative drug remaining versus time. The Higuchi model describes the release of a drug from a controlled release system (*Equation 3*) and plotted as cumulative release versus square root of time. The Hixson-Crowell model explains the relationship from the polymer erosion or dissolution with a change in the surface area and diameter of the particle (*Equation 4*). The mechanism of drug release is obtained by the Korsmeyer-Peppas model from the first 60 % of drug release data (*Equation 5*) [30]:

$$Q_t = K_0 t \quad \textbf{Eq. 1}$$

where Q is the amount of drug dissolved at time t, and K_0 is the zero order release constant (concentration/time);

$$\log C = \log C_0 - Kt/2.303 \quad \textbf{Eq. 2}$$

where C_0 is the initial concentration, K is the first order rate constant and t is the time;

$$Q = Kt^{1/2} \quad \textbf{Eq. 3}$$

where Q is the amount of drug released at time t, and K is the Higuchi dissolution constant;

$$Q_0^{1/3} - Q_t^{1/3} = Kt \quad \textbf{Eq. 4}$$

where Q_t is the amount of drug release at time t, Q_0 is the initial amount of drug, and K is the Hixson-Crowell rate constant;

$$M_t/M_\infty = Kt^n \quad \textbf{Eq. 5}$$

where M_t/M_∞ is fraction of drug released at time t, K is the rate constant and n is the release exponent.

II. RESULTS AND DISCUSSION

1. Melatonin saturated solution

Freshly-prepared near-saturated solution of melatonin of 1 mg/mL in deionized water was non-viscous and clear. The effect of two receptor media, artificial and whole saliva, on the diffusion of melatonin from its near-saturated solution in 24 h, is compared in *Figure 1* and *Table I*, and shows no obvious differences between the two media at the time before steady state. Using the same method for comparison, time to steady state (T_{ss}) was about 6 h and its cumulative release at steady state of 80.2 and 85.4 % into artificial and whole saliva, respectively ($p > 0.05$) as shown in *Table I*. Analysis of diffusion rates with both media within the first 6 h. show that 80-90 % of melatonin freely diffused through the membrane and reached steady state within 6 h in both receptors.

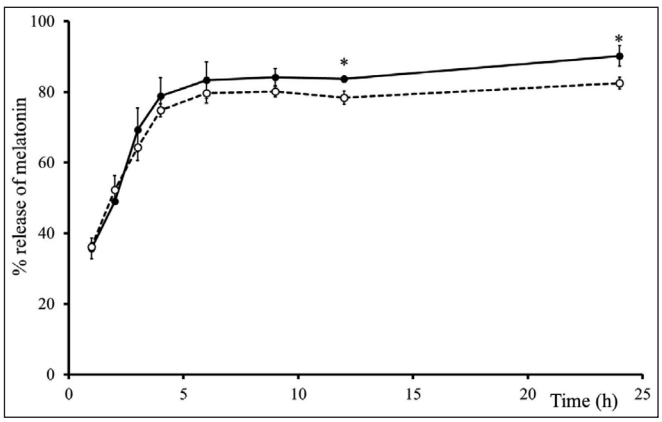

Figure 1 - Release (%) of melatonin from its solution (1 mg/mL) through cellulose tubular membrane into artificial saliva (---) and whole saliva (—) at 37 °C, 100 rpm. *$p < 0.01$ (n = 4).