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Rapid Communication

Sustained-Release Hydromorphone Microparticles Produced by Supercritical Fluid Polymer Encapsulation

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Abstract

Chronic cancer pain remains prevalent and severe for many patients, particularly in those with advanced disease. The effectiveness of analgesic/adjuvant drug treatments in routine practice has changed little in the last 30 years. To address these issues herein, we have developed sustained-release poly(lactic-co-glycolic acid) (PLGA) microparticles of hydromorphone for intrathecal injection aimed at producing prolonged periods of satisfactory analgesia in patients, as a novel strategy for alleviation of intractable cancer-related pain. These hydromorphone-loaded microparticles were produced successfully using organic solvent free supercritical fluid polymer encapsulation. Drug loading at 9.2 % and encapsulation efficacy at 92 % were achieved for particles in the desired size range (20-45µm) with sustained release over a 5 week period *in vitro*.

Keywords

Chronic cancer pain; Intrathecal injection; Poly(lactic-co-glycolic acid) (PLGA); microparticles; Satisfactory analgesia, Supercritical CO_2.

Introduction

One of the most common symptoms caused by cancer is chronic pain, which is the most feared symptom by patients throughout the course of the disease [1]. The pain may be exacerbated by treatment with chemotherapy drugs or by radiation resulting in poorly alleviated pain complicated by a neuropathic component [2,3]. Despite administration of escalating doses of strong opioid analgesics such as morphine, up to 30% of patients do not achieve satisfactory pain relief [4]. The effectiveness of treatment in the clinical setting has changed little in the last 30 years [1,5]. Hence, new strategies are needed to address this issue of intractable cancer-related pain. One such strategy is the development of sustained-release biodegradable analgesic-containing microparticles for intrathecal injection as a means to produce continuous analgesia for several weeks in patients suffering from severe chronic cancer-related pain [6]. Poly(lactic-co-glycolic acid) (PLGA) based hydromorphone-loaded microparticles were prepared by a water-in-oil-in-water (w/o/w) double-emulsion method because of the safety, biodegradability, and successful application of PLGA in the clinical setting [7]. However, micro-encapsulation of drug payloads using conventional w/o/w emulsion methods resulted in particles with poor hydromorphone loading at 1.6% [6]. Additionally, large quantities of toxic organic solvents and surfactants/emulsifiers were also used, potentially

leading to unacceptable levels of residual impurities in the microparticles necessitating further purification steps [8]. However, the use of supercritical fluids such as supercritical CO_2 ($scCO_2$) provides a 'clean' and effective alternative to traditional methods of drug and polymer processing. In particular, $scCO_2$ has a number of unique properties that make it possible to produce sustained-release drug-loaded microparticles without using toxic organic solvents or elevated temperatures [9,10].

In the present study, we produced sustained-release microparticles containing the strong opioid analgesic, hydromorphone, for the first time using organic solvent free supercritical fluid polymer encapsulation (CriticalMix) technology. Hydromorphone drug loading of these microparticles was 9.2% which is very close to our target loading of 10% for intrathecal injection. The particle sizes were the designed size range (<45µm). Importantly, hydromorphone release was sustained over a 5 week period *in vitro*.

1 Materials and Methods

1.1 Preparation of hydromorphone-loaded polymeric microparticles

Hydromorphone HCl (Sigma Aldrich) was converted to the corresponding free base form by adjusting the pH to ~10 using 2 M NaOH added dropwise [6]. The $scCO_2$ microparticle manufacturing (CriticalMix) process was optimised according to Whitaker et al. [11]. Briefly, the PLGA50:50DLG1.5E (Poly(lactic-co-glycolic acid) (PLGA) biodegradable polymer (50:50); ester end-capped, inherent viscosity (0.1-0.2 dl/g) and ~Tg 28°C measured by Differential scanning calorimetry (DSC), custom made by Evonik Health care) and hydromorphone at a ratio of 90 to 10 were loaded into a pressure vessel, pressurised with CO_2 (pharmaceutical grade CO_2 was from BOC Special Gasses, UK) and heated to 40°C at a pressure of 14 MPa for reaching the supercritical point and ensures that the polymer is fluidised [12]. Once temperature was reached the mixture of CO_2/polymer/drug inside the vessel was mixed using mechanical stirring. Liquefaction of the polymer by the $scCO_2$ facilitated drug incorporation into the polymer. About half to one hour later heating was stopped and the polymer-hydromorphone-$scCO_2$ allowed to cool to below 25°C before the CO_2 was slowly vented, depressurised through a nozzle with a 0.6 mm orifice. The polymer solidified to entrap hydromorphone and form microparticles containing hydromorphone. The temperature was reduced to 25°C before unloading to ensure that the drug remained in the polymer and that drug loss was minimized during CO_2 venting. Microparticles were

collected and grounded with a pestle and mortar, and sieved through a 45 micron sieve. The final product was stored refrigerated at a mean (±SD) temperature of 5(±3)°C and protected from light in a desiccator.

1.2 Characterizations of drug-loaded PLGA microparticles

Drug incorporation efficiency. Triplicate ~5mg samples were weighed and dissolved in 2ml aliquots of acetonitrile. The hydromorphone concentrations were quantified using HPLC with UV detection at 280nm. Drug incorporation efficiency, expressed as actual drug loading (% w/w) and encapsulation efficiency (EE % w/w) were calculated using equations (1-2) respectively. The individual values for three replicate determinations and their mean (±SD) values are reported.

Eq. (1) Drug loading (%) = 100 × mass of drug in microparticles/ mass of microparticles

Eq. (2) EE (%) = 100 × mass of drug in microparticles/ mass of drug in microparticles theoretically

***In vitro* drug release.** Triplicate samples (~10mg) of hydromorphone-loaded polymeric microparticles (n=3) were suspended in 1 mL of PBS and transferred to dialysis tubes. Each dialysis tube was sealed, placed into a capped container containing 20ml PBS, and then placed into an incubator maintained at 37.5°C and shaken horizontally at an oscillating frequency of 120 min^{-1}. Timepoints were taken at 3 and 24 h, 3, 7, 14, 21 and 28 days. At each timepoint a 1 ml aliquot of buffer was taken for analysis, and replaced with fresh buffer. At each time point, the 1 ml samples (n=3) were analysed by HPLC and the cumulative release determined.

Morphology and Particle Size. The sustained-release hydromorphone-loaded PLGA microparticles were sputter coated with platinum using an Auto Smart Coater (JFC-1300, JEOL Ltd. Tokyo, Japan) and then examined using a scanning electron microscope (Jeol IT300, JEOL Ltd. Tokyo, Japan) to determine particle shape and surface morphology.

2 Results and Discussion

Sustained-release hydromorphone-loaded PLGA microparticles were successfully prepared for the first time using organic solvent free supercritical fluid polymer encapsulation (CriticalMix) technology by incorporation of hydromorphone into PLGA50:50DLG1.5E at a ratio of 10 to 90 respectively, followed by milling. Importantly, hydromorphone was stable under the $scCO_2$ processing conditions. Hydromorphone loading at 9.2(±0.2)% was achieved

with EE at 92% with these parameters being 4-5 times higher than our previous work using a w/o/w emulsion method (drug loading of 1.6% and EE of 26%) [6] (Table 1). Additionally, the microparticle size was mainly in the range of 20-45 µm after Grinding (Figure 1) which was within our designed size range of 20-60 µm [6]. Regarding release, there was an initial 20.7% burst release in the first 24 hours that was followed by a slower zero-order release phase lasting for up to ~35-days (Figure 2). Grinding and sieving should have little effect on burst release as shown by the *in vitro* release profile in Figure 2. There was no clear difference in the morphology of sustained-release hydromorphone-loaded microparticles (Figure 1A) and drug free microparticles (Figure 1B). Both microparticles were random in shape without aggregation even after 9 months in storage at 2-8°C. Our findings demonstrate that $scCO_2$ encapsulation technology has potential as a drug delivery platform for small molecules such as hydromorphone (as the free base) with the polymeric particles being free from organic solvent residues and surfactants after processing [13].

Our data extend work by others whereby large protein molecules such as human growth hormone (hGH, a 22 kDa protein) [14] and vaccines of tetanus toxoid (TT) [15] were successfully encapsulated into PLGA/poly(lactic acid) (PLA) and PLA particles respectively using the $scCO_2$ CriticalMix process. These results together with our data herein demonstrate the potential of the $scCO_2$ CriticalMix process for preparation of clean, organic-solvent free, particles loaded with either large and small molecules.

Importantly, our simple CriticalMix process to produce hydromorphone-loaded microparticles extends previous work by Cabezas et al. [16-18] who used a one-step $scCO_2$ procedure to produce either indomethacin or 5-fluorouracil impregnated PLGA porous scaffolds. In the present study, the temperature of 40°C was needed to be above the critical point of 31.1°C and it ensured that the polymer was fluidised[12]. Temperature also has some effect on drug loading and encapsulation. If the drug is soluble in the CO_2 then raising the temperature would lead to higher solubility in CO_2 and lower the loading. On the other hand, raising the temperature will also reduce the viscosity of the CO_2 plasticised polymer. This can enhance mixing and hence could increase loading. So a sensible balance point has to be achieved. The $scCO_2$ mixing process yielded a monolithic material that was of the order of centimetres cubed – it filled the high pressure vessel. This material was then controllably milled to yield the particles for release analysis. It is clear from our collective findings that payload solubility in $scCO_2$ as well as CO_2 sorption and swelling of the polymer vary with temperature, pressure, contact time, stirring rate of the $scCO_2$/polymer/drug solution as well

as depressurization conditions including CO_2 venting rate and venting temperature. By systematically varying these parameters, the desired loading and release profile can be optimised for each drug of interest incorporated into PLGA-particles or PLGA porous scaffolds.

3 Conclusion

Hydromorphone encapsulated PLGA microparticles were successfully prepared using organic solvent free supercritical fluid polymer encapsulation technology followed by milling. The ~35-day sustained-release profile was achieved using PLGA50:50DLG1.5E with hydromorphone loading at 9.2% and EE of 92% *in vitro* in the absence of organic solvents or surfactants. Our data show that supercritical fluid polymer encapsulation technology has potential for producing microparticles containing analgesic drugs for sustained release over several weeks for potential administration by the intrathecal route, as a means to improve the management of otherwise poorly-alleviated severe cancer-related pain.

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Conflicts of Interest

The authors have no conflicts of interest to declare in association with this work.

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Figure Legends:

Figure 1. Microparticle size and morphology.

There is no clear difference between hydromorphone-loaded PLGA microparticles (**A**) and drug free PLGA microparticles (**B**) after 9 months of storage at 2-8°C. The particles are similar in shape and size as freshly prepared particles.

Figure 2. Mean (±SEM) *in vitro* release profile of hydromorphone microencapsulated in PLGA5050-1.5E (n=3) with particles prepared using a $scCO_2$ method. Sustained-release PLGA5050-1.5E based hydromorphone-loaded microparticles released their payload over an approximately 35-day period *in vitro*.

Table 1. Physical characteristics of hydromorphone-loaded PLGA microparticles

Parameters	$scCO_2$ method	Emulsion method
Polymers IV (dL/g)	PLGA5050 ester end-capped IV (0.1-0.2)	PLGA5050 acid end-capped IV (0.55-0.75)
Theoretical drug loading (%)	10	6-12
Drug loading (%)	9.2	1.6
EE (%)	92	26
Size (μm)	20-45	20-60
Period of *in vitro* release	~35days	~28days
Solvent & surfactants	None	DCM, PVA
References	Present data	[6]

DCM, dichloromethane; EE, encapsulation efficiency; IV, inherent viscosity; PLGA, poly(lactic-co-glycolic acid); PVA, poly (vinyl alcohol); $scCO_2$, supercritical CO_2

Figure 2

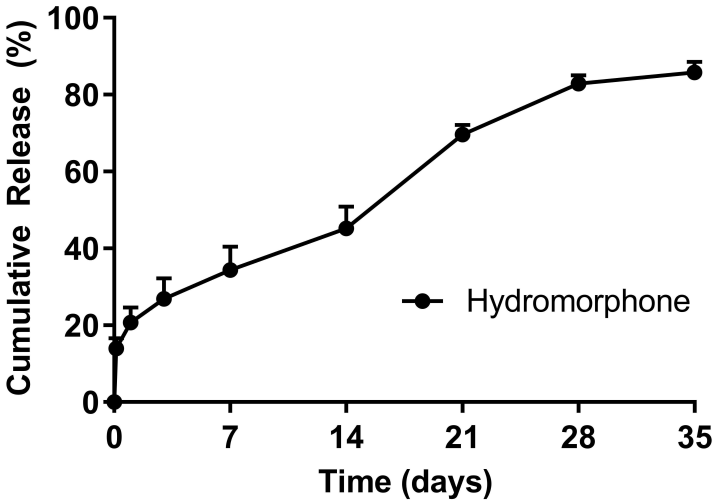

Figure 1