ยาฉีดไซโปรเทอโรนอะซีเตทปลดปล่อยนาน ชนิดใช้น้ำมันมะพร้าวผสมตัวทำละลายเป็นน้ำกระสายยา
Cyproterone Acetate Sustained-Release Injection Using Mixed Coconut Oil/Solvent as Vehicle

นิพนธ์ต้นฉบับ

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Original Article

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บทคัดย่อ

วัตถุประสงค์: ไซโปรเทอโรนอะซีเตทมีจำหน่ายทางการค้าในรูปแบบยาฉีดเข้ากล้ามเนื้อ การศึกษานี้เตรียมและประเมินตำรับยาฉีดไซโปรเทอโรนอะซีเตทออกฤทธิ์นานโดยใช้น้ำกระสายยาเป็นน้ำมันมะพร้าวและน้ำมันมะพร้าวผสมตัวทำละลาย **วิธีการศึกษา:** เตรียมยาฉีดไซโปรเทอโรนอะซีเตทในน้ำกระสายยาที่เป็นน้ำมันมะพร้าวที่เติมสารยับยั้งการเกิดไขชนิดสารที่ชอบน้ำ คือ เอ็นเมทิลไพร์โรลิโดน และชนิดไม่ชอบน้ำ คือ เบนซิลเบนโซเอต และ น้ำมันเปเปอร์มินท์ **ผลการศึกษา:** การเติมไซโปรเทอโรนอะซีเตทไม่เปลี่ยนสมบัติกายภาพเคมีของน้ำมันมะพร้าว เช่น อุณหภูมิต่ำสุดที่ยังสามารถตรวจวัดความหนืดได้ อุณหภูมิจุดไหล และอุณหภูมิที่เริ่มมีไขปรากฏ ทั้งนี้การเติมสารยับยั้งไขมีผลให้อุณหภูมิที่เริ่มมีไขปรากฏต่ำกว่า 15 องศาเซลเซียส (°C) การผสมกับน้ำมันเปเปอร์มินท์สามารถลดจุดไหลจากอุณหภูมิ 19 °C เป็น 6 °C และพบว่าอุณหภูมิต่ำสุดที่ยังตรวจวัดความหนืดได้ ซึ่งพิสูจน์การเปลี่ยนจากสถานะน้ำมันเป็นสารกึ่งแข็ง มีค่าอุณหภูมิที่ลดลงจาก 10 °C เป็นน้อยกว่า 4 °C เพราะประสิทธิภาพของสารยับยั้งไข ได้แก่ เอ็นเมทิลไพร์โรลิโดน เบนซิลเบนโซเอต และน้ำมันเปเปอร์มินท์ พบว่าการปลดปล่อยยาไซโปรเทอโรนอะซีเตทเป็นแบบยาวนานถึง 15 วัน ด้วยกลไกการแพร่แบบ Fickian จากน้ำมันมะพร้าว ทั้งนี้การผสมเอ็นเมทิลไพร์โรลิโดนทำให้อัตราการปลดปล่อยยาสูงกว่าการใช้น้ำมันมะพร้าวเดี่ยว ด้วยสมบัติความไม่ชอบน้ำทำให้เบนซิลเบนโซเอตและน้ำมัน เปเปอร์มินท์หน่วงการปลดปล่อยยาด้วยกลไกการแพร่แบบ Fickian **สรุป:** ยาไซโปรเทอโรนอะซีเตทมีการปลดปล่อยยาวนานจากการใช้กระสายยาเป็นน้ำมันมะพร้าวผสมตัวทำละลาย น้ำมันมะพร้าวที่ผสมกับเอ็นเมทิลไพร์โรลิโดน เบนซิลเบนโซเอต และน้ำมันเปเปอร์มินท์ เพราะเหมาะต่อการประยุกต์เป็นน้ำกระสายยาฉีดสำหรับยาไซโปรเทอโรนอะซีเตท และควรศึกษาด้านความปลอดภัยต่อไป

คำสำคัญ: น้ำกระสายยาฉีด, ไซโปรเทอโรนอะซีเตท, การยับยั้งการเกิดไข, น้ำมันมะพร้าว, สารผสมน้ำมันมะพร้าวและตัวทำละลาย

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Abstract

Objective: Cyproterone acetate (CPA) has commercially been formulated as an intramuscular injection. The preparation and evaluations were performed for CPA sustained-release injection using coconut oil (CO) and mixed coconut oil/solvent as vehicle. **Method:** CPA-loaded injectable CO vehicles were prepared with the addition of different wax inhibitors including hydrophilic substances such as *N*-methyl pyrrolidone (NMP), and hydrophobic substances including benzyl benzoate (BB) and peppermint oil (PO). **Results:** CPA did not apparently change the physicochemical properties of CO such as the lowest temperature with the remaining oil viscosity detected (LVD), pour point (PP) and wax appearance temperature (WAT). WAT was reduced to less than 15 °C when wax inhibitors were added, and the incorporation of PP was apparently reduced from 19 °C to 6 °C. LVD value proved that transformation of oil into semisolid matter was also reduced from 10 °C to less than 4 °C because of efficient wax inhibition of NMP, BB and PO. Sustained CPA release 15 days with Fickian diffusion was achieved with CO, in which NMP addition exhibited a higher CPA release rate than plain CO. Owing to hydrophobicity, BB and PO retarded CPA release with Fickian diffusion. **Conclusion:** The CPA release prolongation was attained using mixed coconut oil/solvent. CO incorporated with NMP, BB and PO is suitable for application as an injectable vehicle for cyproterone acetate; nevertheless, the safety of these CPA-loaded mixed CO/solvents should be further investigated.

Keywords: injectable vehicle, cyproterone acetate, wax inhibition, coconut oil, mixed coconut oil/solvent

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Introduction

Many vegetable oils, including fractionated coconut oil (CO), have been used as vehicles for the sustained release of drugs in injectable dosage forms.[1] However, many steps of the extraction process required for fractionated CO contributed to the high costs.[2] Thus, CO produced by sedimentation followed by filtration has been investigated as an injectable vehicle. However, 90% of saturated fatty acids presented in CO induces it to solidify at room or low temperatures.[3,4] This prominent wax crystallization behavior of CO at room temperature should be inhibited because oil as a vehicle

should be liquid at room temperature.[5] The formation of wax crystals can be prevented using wax inhibitors, which are also known as pour point (PP) depressants, flow improvers and wax crystal modifiers.[6]

N-methyl pyrrolidone (NMP) is an organic compound consisting of a 5-membered lactam.[7,8] NMP is a liquid with high boiling (204 °C), low melting point (-24.4 °C), polar aprotic, and low viscosity (1.89 cPs at 25 ºC).[8] NMP has a good solvency for a wide range of organic and inorganic compounds. It is a colorless liquid and it is miscible with water and with most common organic solvents and is used as a vehicle in injectable preparations.[7] NMP is an attractive solubilizer and injectable vehicle in pharmaceutical dosage forms.[8] Moreover, the injectable solution can be prepared using NMP as a water-soluble solvent combined with non-aqueous solution.[9]

Benzyl benzoate (BB) ($C_{14}H_{12}O_2$) has boiling point of 323.5 °C, melting point of 21 °C and a viscosity of 8.292 cPs at 25 °C.[10] It is used as a solubilizing agent and non-aqueous solvent in injectable preparations.[10] Peppermint oil (PO) contains the major compounds including limonene, cineole, menthone, menthofuran, menthyl acetate and menthol which the main constituents were menthol (40.7%) and menthone (23.4%).[11] Its boiling point is 209.00 °C while the freezing point is lower than 10.5 °C whereas its viscosity depends on menthol and other compounds content.[11] It has been used as flavoring agent and cleaning solvent to sanitize and deodorize the environment.[11] The pharmaceutical compositions can be formulated by combining essential oils from plants of the Labiatae family for subcutaneous, intradermal, intramuscular or intravenous injection of mammals, including humans.[12] Moreover, PO has been used to modify the drug release from Eudragit RS injectable *in situ* forming gel systems.[13]

Cyproterone acetate (CPA) is commercially formulated as an intramuscular injection using BB and castor oil as the vehicle.[14] CPA, a practically water insoluble steroidal anti-androgen, has anti-androgenic, anti-gonadotropic and progestin-like activity. CPA can block androgen receptors leading to a decrease in the production of luteinising hormone and testosterone in the plasma. CPA injectable solution has been used in the palliative treatment of advanced prostate carcinoma by preventing testosterone from reaching the prostate cancer cells.[14,15] Moreover, CPA injectable solution has been used in the treatment of acne, hirsutism and female alopecia which are the result of hormonal imbalance.[16]

Owing to its apparent hydrophobicity, the commercial CPA product contains castor oil and BB as a mixed vehicle. This research aimed to use CPA as the model drug in the developed vehicle comprising mixed CO/solvents. A previous study showed that BB and NMP decreased the wax deposition temperature of ibuprofen injection.[17] Thus, in the present study, BB, PO and NMP were selected as the additives to prevent the wax crystallization of CPA-loaded CO and CPA-loaded mixed CO/solvents. The physicochemical properties, such as the lowest temperature with the remaining oil viscosity detected (LVD), PP and wax appearance temperature (WAT), were determined. In addition, the wax crystallization behavior, such as melting point (MP) was determined using a differential scanning calorimeter and the *in vitro* CPA release behavior of CO and mixed CO/solvent was investigated.

Methods

Materials

CPA (lot no. NP0060M, V&S Chemical, Bangkok, Thailand) was utilised as the model drug. Acetonitrile (high-performance liquid chromatography [HPLC] grade, batch no. 10080273, V. S. Chem House, Bangkok, Thailand) was used as the mobile phase. BB (Pharmaceutical Traders Co., Ltd., Bangkok, Thailand), NMP (lot no. A0277037, Acros Organic, Morris, NJ, USA) and PO (PC. Drug Center Co., Ltd., Bangkok, Thailand) were used as the wax inhibitors. Cold-pressed CO (Tropicana Oil Co., Ltd., Nakhon Pathom, Thailand) was supplied and utilised as the injectable vehicle.

Preparation of CO and mixed CO/solvents with CPA loading

This study used CO obtained from sedimentation of cold-pressed CO followed by three-step filtration. Then, 100 mL NMP, BB and PO were individually incorporated into 100 mL CO at the ratio of 1:1 by volume and mixed together using a magnetic stirrer. CPA (0.5% w/v) was added to both CO and mixed CO/solvents. The dose of CPA used in this study was lower than the commercial product (300 mg in 3 ml) since it was used as the model drug and this concentration was suitable for determining its amount in release medium.

Evaluations

Pour point (PP) measurement

PP is defined as the lowest temperature at which oil can still be poured. PP was determined using the American

Society for Testing and Materials (ASTM) D97 method.[18] CO and CPA-loaded CO and mixed CO/solvents were heated to 45 °C in a water bath to dissolve the precipitated wax. These oils were cooled down to enable the formation of wax crystals. Every 3 °C decrement, the container was tilted to check the movement of the oils until it stopped moving. The temperature 3 °C above the point at which the oils stopped moving, i.e. when the container was held in a horizontal position for 5 sec, was recorded as PP (n = 3).

Wax appearance temperature (WAT) determination

WAT is the temperature at which visible wax crystals occur depending on the concentration and molecular weight of waxes and the chemical nature of the non-waxy part of the oil. This temperature is also called the cloud point (CP). Many methods, such as viscometry, ASTM standard and differential scanning calorimetry (DSC), have been used to determine WAT.[19,20]

Viscometry method

The viscosity of CO and CPA-loaded CO and mixed CO/solvents was measured using a Brookfield DV-III ultra programmable cone plate rheometer with cone spindle CPE-40 (Brookfield Engineering Laboratories, Inc., Middleborough, MA, USA) connected to the thermostatic bath. The viscosity was investigated in the temperature range of 40 °C to 0 °C at 25 sec^{-1} shear rates. Readings of viscosity were taken every decrement of 2 °C (n = 3). LVD was recorded as the temperature at which this equipment was limited in reading the viscosity. The WAT of the oils was determined from the plots of viscosity versus temperature at the point which the curve diverged.

ASTM standard method

The cloud point (CP) is the temperature at which agglomerates of wax crystals first appeared in a liquid and defined as the temperature at which wax precipitation begins. The ASTM standard method is the most routinely used to determine WAT. In this study, CP was determined using the ASTM D2500 method.[18] The CO and CPA-loaded CO and mixed CO/solvents were heated to 45 °C in a water bath to dissolve the precipitated wax. Then, they were cooled to enable the formation of wax crystals. Every 1 °C decrement, the container was raised to check the appearance of cloud wax crystals. When cloudiness was observed at the bottom of the test jar, the temperature was recorded as the CP (n = 3).

DSC method

The DSC thermogram of CO and CPA-loaded CO and mixed CO/solvents was investigated using a differential scanning calorimeter (Model DSC7, Perkin Elmer, Waltham, MA, USA) operated under an atmosphere of nitrogen gas. The samples were weighed accurately at 10 mg in an open aluminium pan and placed in the DSC module with a similar empty pan as reference. Both cooling and heating experiments were conducted. In the cooling experiment, the oils were heated to 50 °C at a rate of 10 °C/min to dissolve and homogenize any precipitated wax in the oil. Then the oils were cooled to -50 °C at a steady rate of 5 °C/min using liquid nitrogen. In the heating experiment, after the oils were cooled to -50 °C in the cooling experiment, the oils were again heated to 50 °C at a steady rate of 5 °C/min.

HPLC analysis

To apply the HPLC method to the drug release study, HPLC method validation was conducted. The mobile phase composed of acetonitrile and reversed osmosis water at the ratio of 3: 2 was filtered through a nylon membrane and degassed before use. Chromatography was performed at room temperature using a 1.0 mL/min flow rate and a 15 min run time. The test was run using a C18 bonded HPLC column with the extra selectivity of a pentafluorophenyl (PFP) phase (ACE C18-PFP, ACE, Chadds Ford, PA, USA) with HPLC (Agilent, Waldbronn, Germany). The injection volume was fixed at 20 µL. CPA retention time was approximately 9 min. UV detection was conducted at 282 nm. The linearity, range, accuracy, precision and system suitability were assessed in the same manner as HPLC method validation.

Chromatographic condition for HPLC analysis was developed in this study for application to the drug release study. CPA in the prepared systems was analyzed by HPLC at 282 nm. The concentrations of standard CPA were in the range of 40 - 160 µg/mL and the equation for the standard curve was y = 92.9361x - 112.0881 (r^2 = 1.0000). The validated analysis results were obtained and acceptable.

Distribution coefficient

The distribution coefficient (D) of the model drug (CPA) between phosphate buffered saline (PBS) pH 7.4 and CO was determined at 37 ± 0.5 °C. The oil phase was 0.5 %w/v of CPA incorporation into CO. First, 50 ml of PBS 7.4 was poured into a glass bottle. Then, 10 ml of oil phase was added. Subsequently, the bottle was placed in a shaking incubator

with a shaking rate of 200 rpm for 48 h. The CPA concentration in the aqueous phase before and after distribution was measured by HPLC with the previously mentioned conditions (n = 3). *D* was calculated using the following equation.[21]

$$D = \left(\frac{Ci - Cw}{Cw}\right)\left(\frac{Vw}{Vo}\right)$$

where C_i is the concentration of the model drug in the oil phase before distribution, C_w is the concentration of the model drug in the aqueous phase after distribution, V_w is the volume of the aqueous buffer solution and V_o is the volume of the oil phase.

In vitro drug release and analysis of drug release data

The release study was conducted using the dialysis method, as previously reported.[22] First, 1 g of each sample was poured into a dialysis tube (MWCO 6000 - 8000 Spectra/Por®). Then, the dialysis tube was placed in a glass bottle containing 100 mL PBS pH 7.4 as the release medium. Subsequently, the bottle was placed in a shaking incubator with a shaking rate of 50 rpm at 37 ± 0.5 °C. At appropriate time intervals, a 5 mL of a test release medium was withdrawn and replaced with fresh PBS pH 7.4 with an equal volume to maintain the sink conditions. The concentration of CPA was determined by HPLC method as the above mentioned condition. The mean cumulative drug releases ± SD were calculated (n = 3). To predict the mechanism of drug release, the cumulative percentage of drug release profiles was fitted with different mathematical release equations. Least square fitting of the experimental data (cumulative drug release > 10% and up to 80%) to the mathematical equations (i.e. power law, first-order, Higuchi's and zero–order equations) was conducted using the Scientist™ for Windows, version 2.1 programme. The r^2 was used to indicate the degree of curve fitting and the goodness-of-fit was also obtained using model selection criteria (msc).

Statistical data analysis

For the experimental measurements that were collected in triplicate, the values were expressed as the mean ± SD. The statistical significance of viscosity and PP was examined using a one-way analysis of variance. The statistical significance level was set at *P*-value < 0.05. The analysis was performed using SPSS for Windows.

Results and Discussions

CO was miscible with BB, NMP and PO and the obtained mixed coconut oil/solvent systems were clear solutions. CPA-loaded CO and CPA-loaded mixed coconut oil/solvent were clear solutions owing to complete dissolution of this drug in these vehicles.

Viscosity

The viscosity-temperature profiles of CO, CPA-loaded CO and mixed CO/solvents containing different wax inhibitors are shown in Figure 1. The viscosity of CO at 10 °C was 151.72 cPs, decreased with the increase in temperature. The high temperature promotes the enhancement of kinetic motion but breaks the intermolecular bonds between adjacent layers.[23] Thus, the viscosity of the oil decreased with the increment of temperature. The viscosity of CO could not be measured at less than 10 °C because the formation of wax crystals provoked the viscous environment of CO. The addition of low concentrations of CPA did not affect the viscosity of CO, as shown in Figure 1. However, when additives were added, the viscosity obviously decreased, which proved that the wax crystallization temperature of CO decreased. By comparison, the viscosity of CO+NMP+CPA showed the lowest viscosity followed by CO + BB + CPA and CO + PO + CPA.

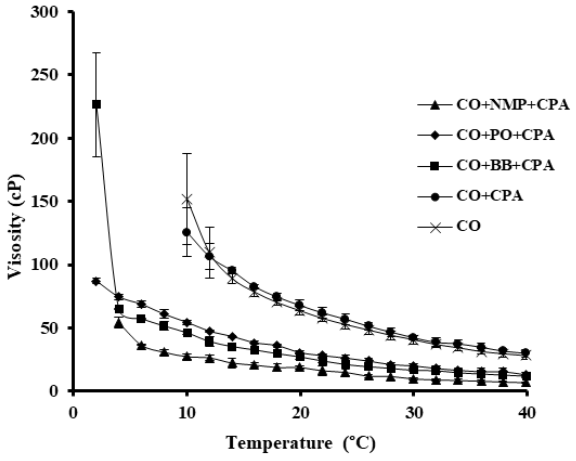

Figure 1 Viscosity temperature profiles of mixing systems between CPA-loaded CO and mixed CO/solvents (n = 3).

Physicochemical properties

The physicochemical properties of CO and mixed CO/solvents loaded with CPA are presented in Table 1. The LVD value of CO showed no significant changes upon the addition of CPA. However, the addition of additives to CO+CPA decreased the LVD value, indicating that the decrease in temperature led to semisolid formation. Typically,

the measurement of PP provides the quality and applicability information of oil. The PP value of CO slightly increased with the addition of CPA (Table 1). Evidently, even at low temperature of approximately 6.0 ± 0.0 °C, the CPA-loaded mixed CO/solvents could still be pourable and were not transformed into semisolid matter. The hydrocarbon chains of wax inhibitors interact with oil and its polar parts improve the oil solubility and decrease the temperature for formation of wax crystals or WAT.[6,24] NMP, an attractive solubiliser[8] interferes with the formation of wax crystals and improves the solubility of CO, which impedes the reduction of the WAT. In addition, this efficient lowering WAT of CO should result from the wax solubilizing effect of NMP because it could promote the dissolution of the main saturated fatty acids of CO owing to their hydrophobicity and solubilizing property.[8,9] Similarly, BB and PO improved the solubility of CO and reduced the transformation of wax crystals into agglomerates.

When using CO as an injectable vehicle it should have a WAT not higher than 15 °C.[25] In this study, the WAT values from viscometry, ASTM standard and DSC methods, are shown in Table 1. The viscometry method showed that the WAT of CO was 21.3 ± 2.3 °C. Meanwhile, the addition of CPA did not decrease the WAT of CO (22.0 ± 2.0 °C). Therefore, a low amount of this drug did not significantly influence WAT of CO. However, upon the addition of additives, the WAT of CO + CPA decreased to less than 15 °C whereas the WAT of CO + PO + CPA was 14.7 ± 1.2 °C. The WAT of CO + BB +C PA was not significantly different from that of CO + NMP + CPA (*P*-value > 0.05) but was significantly lower than that of CO+PO+CPA (*P*-value < 0.05). The WAT mostly depends on the concentration and molecular weight of waxes and the chemical nature of the non-waxy part of the oil.[19]

The ASTM standard method showed that the CP of CO significantly decreased upon the addition of CPA (*P*-value < 0.05) but was still higher than the observed temperature of 18.3 ± 1.2 °C. When the additives were added, the WAT of CO decreased to less than 15 °C. The comparison results indicated that the CPs of CO+BB+CPA (11.7 ± 1.2 °C) and CO + PO + CPA (10.7 ± 0.6 °C) were not significantly different (*P*-value > 0.05), but these values were lower than those of CO + NMP + CPA (14.7 ± 0.6 °C) (*P*-value ≤ 0.05).

The DSC thermograms indicate the exothermic and endothermic peaks of CO, CO+ CPA and mixed CO/ solvents (Figure 2A and 2B) . The exothermic peaks of mixed CO/ solvent + CPA were present at lower temperature than those of CO and CO + CPA. These apparent lower exothermic peaks observed in thermograms (from cooling experiment) of CPA-loaded mixed CO/solvent should be mainly from the recrystallization of wax from CO in which its crystal formation was disturbed with the presence of these solvents. The same trend was observed for the endothermic peaks of mixed CO/ solvents. The heat flow decreased with the loss of heat energy in the oils, which was regarded as the onset of WAT.[26]

The MP of the systems was derived from the heating curves. The MP of PO has not been reported; however, some literature reported the MP of BB at 19 °C[27] and NMP at -25 °C.[28] The WAT values of CO and CO + CPA obtained from the DSC thermograms were 8.0 °C and 8.1 °C, respectively. The mixed CO/ solvents showed lower WAT values than CO + CPA. The WAT of CO + NMP + CPA was at -3.1 °C, that of CO + PO + CPA was -4.4 °C and that of CO + BB + CPA was -6.9°C. The obtained data proved that the addition of CPA did not have a significant effect on the WAT of CO. Similarly, the addition of CPA did not markedly change the MP of CO. When compared, the MP of CO+ BB+ CPA (12.3 °C) was lower than that of CO + PO + CPA (13.9 °C), CO + NMP + CPA (16.1 °C) and CO + CPA (23.4 ° C).

Table 1 Physicochemical properties of the mixing systems of CO with and without additives with CPA (n = 3).

Sample	LVD ± SD (°C)	PP ± SD (°C)	MP (°C)	WAT ± SD (°C)		
				Viscometry	ASTM	DSC
CO	10.0±2.0^{a}	19.0±1.7^{a}	26.4	21.3±2.3^{a}	23.3±0.6^{a}	8.0
CO+CPA	10.6±1.2^{a}	21.0±0.0^{b}	26.3	22.0±2.0^{a}	18.3±1.2^{b}	8.1
CO+PO+CPA	2.7±1.2^{b}	6.0±0.0^{c}	17.4	14.7±1.2^{b}	10.7±0.6^{c}	-4.4
CO+BB+CPA	2.7±1.2^{b}	6.0±0.0^{c}	16.4	10.7±1.2^{c}	11.7±1.2^{c}	-6.9
CO+NMP+CPA	4.7±1.2^{b}	6.0±0.0^{c}	19.0	10.0±0.0^{c}	14.7±0.6^{d}	-3.1

Note: The superscripted letters represent the statistical significance (p < 0.05) of the data, of which the same letter means 'not different by statistical significance' whereas different letters mean 'different by statistical significance'.

This finding is consistent with the results of viscosity and other WAT data mentioned previously. These results proved that the solvents decreased the WAT, MP and PP. The additives could also modify the wax crystalization behavior by incorporating themselves into the wax network of CO or decreasing the wax crystallization of CO.[6,24] Thus the agglomeration and deposition of wax crystal from CO were prevented. Therefore, NMP, BB and PO are suitable wax inhibitor for CO used as an injectable vehicle for the preparation of CPA- loaded sustained - release parenteral dosage forms.

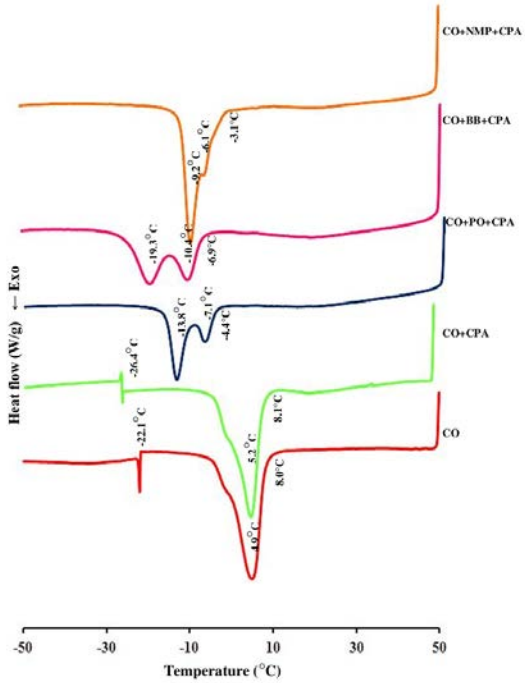

Figure 2 DSC thermograms of the mixing systems between CPA and mixed CO/solvents in cooling experiment.

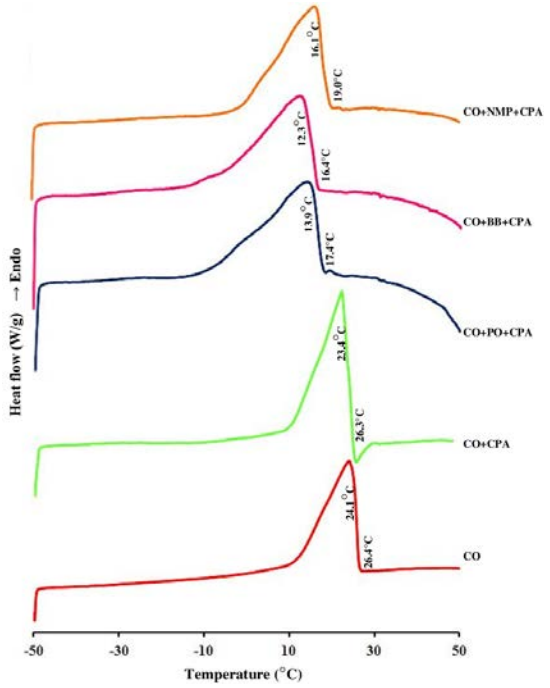

Figure 3 DSC thermograms of the mixing systems between CPA and mixed CO/solvents in heating experiment.

Distribution coefficient (D)

The high *D* value of CPA, i.e. 1,297.9 ± 46.8, indicates the high solubility of CPA in CO.[29] Typically, the *D* value is influenced by the chain length and degree of unsaturation of fatty acids in CO.[21] The octanol/water partition coefficient (log $K_{o/w}$) of CPA is 3.10.[30] The log P value of CPA in the presence of hydroxypropyl-β-cyclodextrin in phosphate buffer, pH 7.4 also has been reported as 3.40 with a maximum solubility of 1854.3 µg/ml.[31] The solubility of this compound in phosphate buffer pH 7.4 was 0.9 ± 0.3 µg/ml.[32] Therefore, CPA is the hydrophobic compound which exhibits high solubility in oil and very low solubility in aqueous medium. The difference of hydrophobicity of octanol and CO could influence directly on partition coefficient. A previous study stated that D values of bupivacaine and ropivacaine between PBS 7.4 and CO were 161 and 58.8, respectively.[33] Thus, the steroid drugs exhibit notably higher solubility in oil than in aqueous phase. Thereafter, this property could affect the drug release in aqueous medium.

In vitro drug release and analysis

The comparison results indicate that the release rate of CPA from CO+NMP was higher than that from CO, CO + PO and CO + BB (Figure 4). The release rates of CPA from CO + BB and CO + PO were lower than that from CO because of the high hydrophobic nature of BB. Moreover, PO prevented the drug from penetrating the release medium. The initial burst release of CPA occurred before 8 h. Furthermore, the initial burst release from CO+NMP was higher than from CO, CO + PO and CO + BB. NMP has high water affinity[34] that promotes drug diffusion to the release medium and initiates the burst release of CPA when compared with the system containing only CO. The addition of PO to the doxycline hyclate-loaded Eudragit RS *in situ* forming gel minimized the burst drug release.[13] BB could also minimize the burst drug release of implant systems.[35-37] Drug release profiles of granisetron hydrochloride from injectable *in situ* forming implants showed that initial burst decreased by using hydrophobic solvents in rank of BB > tetraglycol, propylene carbonate.[35] The low burst release of CPA from CO + BB and CO + PO was evident, with a notably sustained release. The effective prolonged release of steroid drugs with BB has been reported previously.[39] There was the rather low CPA release from formula in Figure 4. The high distribution coefficient (D) value of CPA indicates the high solubility of CPA in CO; thus, its release with diffusion/partition into aqueous medium was limited. This D value is important for a drug dissolution step in the oil phase prior to release into the aqueous phase.[33] The initial fast release of ropivacaine in the acceptor phase was reported followed by a constant flux of comparable magnitude to release amount obtained for both aqueous and oil suspensions of the ropivacaine base.[33]

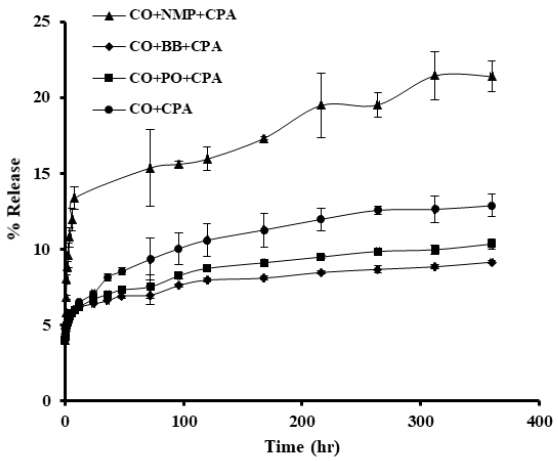

Figure 4 *In vitro* drug release profiles of the mixing systems between CPA and selected mixed CO/solvents (n = 3).

Table 2 Comparison of the degree of goodness-of-fit from curve fitting of the *in vitro* drug release profiles of the prepared systems of CO and mixed CO/solvents containing CPA.

Sample	Power law			First order		Higuchi's		Zero order	
	r^2	msc	n	r^2	msc	r^2	msc	r^2	msc
CO+CPA	0.9968	5.49	0.21	0.8487	1.71	0.9129	2.27	0.8411	1.67
CO+PO+CPA	0.9917	4.54	0.15	0.7194	1.10	0.8776	1.93	0.8246	1.57
CO+BB+CPA	0.9891	4.26	0.10	0.4271	0.38	0.8281	1.59	0.7837	1.36
CO+NMP+CPA	0.9655	2.62	0.45	0.4579	0.11	0.9653	2.86	0.9609	2.74

Table 3 Estimated parameters from curve fitting of the *in vitro* drug release profiles of the prepared systems of CO and mixed CO/solvents containing CPA.

Sample	$k \pm SD*10^{-3}$
CO + CPA	7.16 ± 0.28
CO + PO + CPA	5.29 ± 0.24
CO + BB + CPA	4.43 ± 0.24
CO + NMP +CPA	9.33 ± 0.36

Note: The values of k (rate constant) was obtained from Higuchi's equation for the prepared systems of CO and mixed CO/solvents containing CPA.

The higher values of r^2 and msc indicated the high quality of the release profile fitted with the mathematical equations.[40] According to the data shown in Table 2, the degree of curve fitting to the Higuchi's equation was better than that to the zero-order and first-order equations. Thus, the CPA release profiles of CO and mixed CO/solvents fitted well with Higuchi's equation. The n value of the power law kinetic signified that CPA release followed the Fickian diffusion mechanism. The comparison results indicated that k of CO + BB + CPA obtained from the profile fitted with Higuchi's equation was lower than those of CO + PO + CPA, CO + CPA and CO + NMP + CPA (Table 3), corresponding to the CPA release behavior as mentioned previously.

Conclusion

CO has been used in tropical countries for thousands of years because of its health benefits. However, the major problem of the use of CO as an injectable vehicle is the formation of wax crystals at temperature less than 25 °C. NMP, BB and PO could improve the physicochemical properties of CO by decreasing the values of LVD, PP and WAT. The addition of 0.5% CPA did not apparently change the LVD, PP and WAT of CO. The addition of these additives also achieved efficiently a wax inhibition on CO with WAT less than 15 °C. The PP was apparently reduced from 19 °C to 6 °C and the LVD was reduced from 10 °C to less than 4 °C because of the wax inhibition effect of NMP, BB and PO. The release rates of CPA from CO+BB and CO + PO were lower than that from CO. NMP promoted the higher release rate of CPA from CO + NMP than those from CO, CO + BB and CO + PO. Meanwhile, the hydrophobic nature of BB and PO retarded CPA release. Thus, the apparent prolongation of drug liberation was attained using mixed coconut oil/ solvent. CPA release followed Higuchi' s drug release kinetics with Fickian diffusion. COs incorporated with NMP, BB and PO are suitable for application as the injectable vehicles; nevertheless, the safety of these CAP- loaded mixed CO/solvents should be further investigated.

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Conflict of Interest

The authors report no conflicts of interest in this research.

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