Development of cordycepin formulations for preclinical and clinical studies

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SUGGESTED RUNNING TITLE:

Formulation development of cordycepin

ABSTRACT

There is extensive literature on *in vivo* studies with cordycepin but these studies were generally conducted without validation of the various formulations, especially in terms of the solubility of cordycepin in the dosing vehicles used. Cordycepin is a promising drug candidate in multiple therapeutic areas and there is a growing interest in studies aimed at assessing the pharmacological activity of this compound in relevant animal disease models. It is likely that many reported *in vivo* studies used formulations in which cordycepin was incompletely soluble. This can potentially confound the interpretation of pharmacokinetics and efficacy results. Furthermore, the presence of particles in intravenously administered suspension can cause adverse effects and should be avoided. Here we present the results from our development of simple and readily applicable formulations of cordycepin based on quantitative solubility assessment. Homogeneous solutions of cordycepin were prepared in phosphate-buffered saline (PBS) at different pH levels, suitable as formulations for both intravenously and oral administration. For the purpose of high-dose oral administration we also developed propylene glycol (PPG)-based vehicles in which cordycepin is completely soluble. The stability of the newly developed formulations was also assessed, as well the feasibility of their sterilisation by filtration. Additionally, an HPLC-UV method for the determination of cordycepin in the formulations, which may also be useful for other purposes, was developed and validated. Our study could provide useful information for improvement of future preclinical and clinical studies involving cordycepin.

KEYWORDS

Cordycepin; formulation; solubility; stability; HPLC-UV.

INTRODUCTION

Cordycepin (3'-deoxyadenosine) is a nucleoside that differs from adenosine by the absence of the 3'-hydroxyl group. It is the main therapeutically active component in extracts of the insect fungus *Cordyceps militaris*, which is a widely used in traditional medicines of the Far East (1-3). The therapeutic potential of cordycepin has been recognised for a wide range of applications and was demonstrated in numerous studies (4). Proposed pharmacological activities of cordycepin include anti-microbial (4), anti-tumour (5-8), anti-mutagenic (9), anti-metastatic (1, 10), anti-angiogenesis (11), anti-fungal (12), anti-diabetic (8, 13), anti-inflammatory (8, 13-17), anti-platelet aggregation (18), immunomodulatory (19, 20), hypoglycaemic (21) and anti-herpes (22) effects. The molecular mechanisms of action of cordycepin are not completely **understood. However, like** many natural products, cordycepin interacts with multiple biological processes, many of which remain to be investigated in detail (2).

Based on diverse *in vitro* studies, *in vivo* preclinical research have been conducted for various indications including cancer (23, 24), cancer metastasis (25), vascular disorders (26), neurodegeneration (27, 28), fungal infection (12), osteoporosis (29, 30), osteogenesis (31), hyperlipidaemia (32), hyperglycaemia (21), viral infections (33), steroidogenesis (34), allergy (35), central nervous system disorders (36, 37), amongst others (38-40) **(Table 1).** Moreover, further studies are anticipated on the pharmacological modes of action and clinical efficacy of cordycepin.

From the many *in vivo* experiments reported that involved cordycepin, it is noted that a substantial range of doses, and therefore a wide range of concentrations of cordycepin in different dosing vehicles, were used in these studies (Table 1). However, the solubility of cordycepin in water or other dosing vehicles used in these studies has not been reported as far

as we can ascertain. Moreover, reports of such studies often do not indicate whether cordycepin formulations at the time of administration were actual homogeneous solutions or merely suspensions. If a formulation is administered orally or intraperitoneally as a suspension, factors such as dissolution and solubilisation could complicate interpretation of the results (41). Moreover, if a formulation for intravenous administration is a suspension rather than a solution, acute adverse effects can occur, especially if the suspension contains large particles. Such adverse effects can include embolism of blood vessels and inflammation (42-45).

Therefore, in this study, simple and readily applicable formulations of cordycepin were developed based on quantitative solubility assessment. Homogeneous solutions of cordycepin were prepared in phosphate-buffered saline (PBS) at different pH levels, yielding formulations that can be administered both intravenously and orally. A propylene glycol (PPG)-based formulation for higher concentration oral cordycepin administration was also developed. Stability and sterilisation studies for the formulations developed were also carried out.

MATERIALS AND METHODS

Materials

Cordycepin was purchased from Carbosynth Ltd (Berkshire, UK). Adenosine and Dulbecco's phosphate buffered saline (PBS) were obtained from Sigma (Gillingham, UK). Water for injection was purchased from Gibco (Paisley, UK) and normal saline was from Baxter (Berkshire, UK). Costar Spin-X Centrifuge Tubes and propylene glycol were purchased from Fisher Scientific (Loughborough, UK). Sartorius Minisart syringe filters were obtained from Scientific Laboratory Supplies Ltd (Nottingham, UK). All solvents used in the study were HPLC grade or higher.

Solubility assay

To assess the solubility of cordycepin in water for injection, normal saline, pH 4.0 PBS, pH 4.5 PBS and pH 5.0 PBS, saturated suspensions of cordycepin were prepared (15 mg of cordycepin per mL of solvent). The pH of the formulations in PBS were readjusted using HCl after dissolving cordycepin powder, to readjust the effect of solubilised cordycepin itself on the pH of the formulation. The test suspensions were contained in glass vials and were mixed using a vial roller, overnight at room temperature. The test suspensions were then filtered using Costar Spin-X Centrifuge Tubes (cellulose acetate, 0.22 μm pore size) by centrifugation at 2400 *g* for 5 min at room temperature (Heraeus Fresco 17 Centrifuge, Thermo Electron, MA, USA). The filtrate was analysed for the concentration of cordycepin. Solubility of cordyepin in PPG was assessed by preparing saturated suspensions at 20 mg of cordycepin per mL of solvent and sonicating for 15 min. The test suspensions were filtered using the method described above and the filtrates were analysed for cordycepin concentrations. The assay was conducted in quadruplicate.

Formulation preparation

Formulations of cordycepin were prepared based on PBS or PPG. For PBS-based formulations, the pH of PBS was adjusted to 4.0, 4.5 and 5.0 using NaOH or HCl prior to addition of cordycepin. PBS-based formulations were prepared by dissolving cordycepin in PBS at pH 4.0, 4.5 and 5.0 at concentrations of 5.5, 4.5 and 3.0 mg/mL, respectively. The formulations were vortex-mixed and the pH of each formulation was then adjusted to its initial pH using HCl to account for the effect of solubilised cordycepin on the pH of the solution. The vortex-mix and pH re-adjustment steps were repeated until they resulted in clear solutions. For the PPG-based formulation, cordycepin was dissolved in PPG at a concentration of 13.0 mg/mL and the formulation was then sonicated in a water bath for 15 min. After confirming that the

formulation was a clear solution, deionised-distilled water (DDW) was added to the formulation to give a final concentration of 10.0 mg/mL. This resulted in a PPG-based formulation with a composition of PPG:DDW = 77:23.

Formulation stability test

Formulations of cordycepin prepared as described above were tested for their stability at four different storage conditions: -80 °C, -20 °C, 4 °C and room temperature. Prepared formulations were aliquoted into 1.5 mL centrifuge tubes and were stored under different conditions. Three samples of each storage condition were withdrawn after 4 and 11 days, or 3, 8 and 12 weeks after preparation to assess the stability of the formulations.

Filter sterilisation

PBS-based formulations of cordycepin were filter-sterilised using Sartorius Minisart syringe filters (polyethersulfone, 0.2 μm pore size). Aliquots (700 μL) of the formulations prepared as described above were filtered through the devices and air was purged using a 1 mL syringe. Concentrations of cordycepin in the formulations were measured before and after the filter sterilisation procedure. The evaluation was performed in quadruplicate.

Analytical method

Analysis of cordycepin concentrations in the samples was performed using a HPLC-UV system consisting of a Waters Alliance 2695 separations module equipped with a Waters 996 photodiode array detector. The autosampler temperature was maintained at 10 °C and the column temperature was set at 40 °C. The stationary phase was a Capcell Pak C18 4.6 × 150 mm, 3 μm particle size column (Shiseido, Tokyo, Japan), protected by a SecurityGuard 2 × 4 mm, 3 μm particle size column (Phenomenex, Macclesfield, UK). The mobile phase was an

147 isocratic composition of acetonitrile:DDW at a ratio of 6:94 (v/v). The flow rate of the mobile
148 phase was 0.8 mL/min and the chromatograms were monitored at a wavelength of 259 nm. The
149 injection volume was 40 μL.
150
151 **Sample preparation**
152 For HPLC-UV sample preparation, formulation samples were firstly diluted with DDW to yield
153 1.0 mg/mL of cordycepin (dilution ratios were 18.2:81.8, 22.2:77.8, 33.3:66.7 **and** 10:90 for
154 formulations of PBS pH 4.0, PBS pH 4.5, PBS pH 5.0 and **PPG-based formulation**,
155 respectively). From these diluted samples, 10 μL was withdrawn and added to 980 μL of DDW
156 with 10 μL of internal standard stock solution (1.0 mg/mL adenosine in DDW). The samples
157 were then vortex-mixed and 100 μL aliquots were transferred to HPLC vials for analysis.
158
159 Calibration curve samples were prepared with working standard solutions of cordycepin (in
160 DDW) at concentrations of 5, 10, 25, 50, 100, 250, 500 and 1000 μg/mL. DDW-based diluents
161 were prepared to match the composition of each formulation sample medium (0.187% PBS pH
162 4.0, 0.227% PBS pH 4.5, 0.344% PBS pH 5.0 and 0.079% PPG). Samples were prepared by
163 adding 20 μL of the cordycepin working standard solutions to 970 μL of the DDW-based
164 diluents with 10 μL of internal standard stock solution (1.0 mg/mL adenosine in DDW). The
165 resulting calibration curve points were 0.1, 0.2, 0.5, 1, 2, 5, 10 and 20 μg/mL. The samples
166 were then vortex-mixed and 100 μL aliquots were transferred to HPLC vials.
167
168 **Validation of the analytical method**
169 Validation of the analytical method was performed for accuracy and precision. Accuracy was
170 expressed as relative error (RE), which represents the bias from nominal concentrations.
171 Precision was expressed as relative standard deviation (RSD), which represents the coefficient

of variation at each concentration level **(46, 47).** Both accuracy and precision were assessed by intra-day (six replicates in one day) and inter-day (replicates on six different days) variability. The concentration levels of quality control (QC) samples were the lower limit of quantification (LLOQ, 0.1 μg/mL), low QC (LQC, 0.25 μg/mL), medium QC (MQC, 1.5 μg/mL) and high QC (HQC, 15 μg/mL). The QC samples were prepared in the same manner as calibration curve samples in each formulation sample medium.

Statistical analysis

All data were expressed as mean ± standard deviation (SD). Statistically significant differences between measurements in different media were assessed using the unpaired two-tailed t-test. The differences between measurements in one sample before and after sterilisation were assessed using the paired two-tailed t-test. Statistical significance was declared when $p < 0.05$.

RESULTS

Prior to formulation development, the maximal solubility of cordycepin in water for injection, normal saline, PBS at three different pH levels (4.0, 4.5 and 5.5) and PPG was assessed. The results are shown in Figure 1. The use of PBS at pH 4.0 and PPG resulted in significantly higher solubility of cordycepin compared to water or normal saline (both $p < 0.001$). Following the solubility results, formulations were developed in each medium at concentrations lower than the maximal solubility in the respective medium (Table 2). The PBS-based formulations were intended for intravenous and oral administration, while the PPG-based formulation was developed for higher dose oral administration. The achievable dose for each route of administration was calculated based on recommendation from a previous report **(42, 43)**.

The analytical method used for the stability assessment of the formulations was fully validated by intra-day and inter-day validation in order to evaluate the reliability of the method. The results in Table 3 show that the method has acceptable precision and accuracy at all QC levels tested. Representative chromatograms from the analytical method are shown in Figure 2.

Stability of the formulations was assessed under four different storage conditions (-80 °C, -20 °C, 4 °C and room temperature) up to 3 months for PBS-based formulations and up to 3 weeks for PPG-based formulations. The results in Table 4 show that all PBS-based formulations were stable at -80 °C, -20 °C and at room temperature for up to 3 months. Interestingly, crystallisation of cordycepin was observed in samples stored at 4 °C in PBS-based formulations. The rate of crystallisation differed with pH of the formulations, as crystallisation at pH 4.0 and at pH 4.5 in PBS was found after 6 days but at pH 5.0 in PBS only after 11 days. The PPG-based formulation was stable under all four storage conditions up to 3 months and crystallisation was not observed in any of the samples.

The feasibility of simple filter sterilisation of the PBS-based formulations was assessed by measuring the concentration of cordycepin before and after filtration. The results in Table 5 show that there was no statistically significant loss during the filter sterilisation ($p > 0.05$). Therefore, filter sterilisation can be applied without compromising the concentration of the PBS-based formulations.

DISCUSSION

Knowledge of the maximal solubility of cordycepin in various dosing vehicles is essential information for the development of solution phase-based formulations. Some preclinical studies listed in Table 1 used concentrations that in fact exceed the maximal solubility of

cordycepin in the respective dosing vehicles. Therefore, it is likely that the formulations used in these studies were in a suspension state. As previously mentioned, suspension-based formulations could complicate the interpretation of the results due to dissolution-related factors involved following oral or intraperitoneal administration. Intravenous administration of heterogeneous suspensions can also a cause adverse effects.

PBS is an isotonic vehicle that causes minimal discomfort when administered intravenously or orally. The solubility of cordycepin in PBS was highest at pH 4.0, probably due to the fact that cordycepin is a weak base (pK_a = 3.6, predicted by ACD/Labs, Toronto, Canada). Decreasing the pH further would likely result in even higher solubility. However, the recommended pH range for intravenous formulations is 4-9, and therefore the lowest pH tested in this work was 4.0 (42).

A PPG-based formulation was developed in order to enable higher doses of cordycepin to be delivered by the oral route. PPG is a widely-used and a well-tolerated dosing vehicle in preclinical studies. A previous study reported that oral administration of as much as 1000 mg/kg/day of PPG for 90 days was tolerable in rats (48). The solubility of cordycepin in PPG was 2.7-fold higher than in pH 4.0 PBS (Figure 1). However, pure PPG as a dosing vehicle can present difficulties in practical use during preclinical experiments due to its high viscosity. Therefore PPG was diluted with DDW to produce the final formulation. As a result, our PPG-based formulation is composed of PPG:DDW = 77:23 at 10 mg/mL cordycepin and addition of water did not cause any precipitation of cordycepin in this formulation.

The analytical method used in this study was developed based on a previous report where cordycepin was analysed in extracts of *Cordyceps* using HPLC-UV methods (49). However,

this previously reported analytical method was limited by the fact that an internal standard was not used and validation results were not provided. Therefore, in the present study, we incorporated an internal standard to improve the precision and accuracy of the method and we also performed a full validation of the method (Table 3). This HPLC-UV method could provide useful information for future studies where concentrations of cordycepin are to be analysed in other formulations.

Formulations intended for intravenous administration should not only be free of particles but should also be aseptic (42). In this study, filter sterilisation was tested for its feasibility, as it is one of the techniques that does not require heating of the formulation. One concern for filter sterilisation can be that the drug might have non-specific binding to the filter or the device. The results in our case showed that the PBS-based cordycepin formulations can be filter-sterilised without affecting the concentration of the formulations (Table 5).

Surprisingly, and rather counterintuitively, we observed that a temperature of 4°C is the only storage condition to be avoided for PBS-based formulations. This is probably because the slow cooling process provided upon storage at 4°C resulted in crystallisation of cordycepin. It is often reasonable to assume that pharmaceutical formulations are more stable at lower temperatures (50). Moreover, when a label instructs to store a drug below a certain temperature (e.g. “store below 25 °C”), this usually means that storage in the fridge would provide stable conditions (51). However, the current study emphasises that storage of the formulations or dosage forms at lower temperature might in fact result in poorer stability **from a physical point of view**.

CONCLUSION

Herein, we report a study where simple and readily applicable formulations of cordycepin were developed and tested for their stability. An HPLC-UV method for quantification of cordycepin in the formulations was developed and validated. Additionally, the PBS-based formulations could be sterilised by filtration without loss of material. The PBS-based formulations were stable for up to 3 months at room temperature**, -20°C and -80°C** but not at 4°C, which suggests, rather counterintuitively, that storage of the formulations or dosage forms at lower temperature might in fact result in poorer **physical** stability. This study could provide useful information for future preclinical and clinical studies with cordycepin.

ACKNOWLEDGEMENT

The research was funded by Arthritis Research UK grant 20795 awarded to CHdM.

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Table 1. List of preclinical studies conducted with cordycepin

Literature (Reference)	Dose	Dosing volume	Concentration	Vehicle	Test species	ROA	Indication
Won *et al.,* 2009 (26)	10 mg/kg	2 mL/kg	5 mg/mL	Water	Rat	p.o.	Vascular disorder
Yoshikawa *et al.,* 2004 (23)	5, 15 mg/kg	N.S.	N.S.	Water	Mouse	p.o.	Cancer
Cheng *et al.,* 2011 (27)	10, 20 mg/kg	N.S.	N.S.	Water	Mouse	p.o.	Neuroprotective activity
Rottenberg *et al.,* 2005 (38)	2 mg/kg	N.S.	N.S.	N.S.	Mouse	i.p.	African Trypanosomiasis
Sugar *et al.,* 1998 (12)	1.5 mg/kg	N.S.	N.S.	N.S.	Mouse	i.p.	Antifungal activity
Wei *et al.,* 2009 (39)	500 μmol/kg	N.S.	N.S.	2% Tween 80	Mouse	p.o.	Prodrug studies
Leu *et al.,* 2011 (34)	20, 40 mg/kg	1 mL/mouse	0.6, 1.2 mg/mL	Saline	Mouse	i.p.	Steroidogenesis
Tsai *et al.,* 2010 (40)	10 mg/kg	1 mL/kg	10 mg/mL	Saline	Rat	i.v.	Pharmacokinetic studies
Sato *et al.,* 2013 (25)	0.5, 5 mg/kg	N.S.	N.S.	DPBS	Mouse	i.p.	Cancer metastasis
Zhang *et al.,* 2015 (30)	22.2, 44.4, 88.9 mg/kg	10 mL	0.5, 1, 2 mg/mL	Water	Rat	p.o.	Osteoporosis
Sun *et al.,* 2011 (32)	140 mg/kg	N.S.	N.S.	2.5% carboxymethyl cellulose sodium	Hamster	p.o.	Hyperlipidaemia
Du *et al.,* 2016 (33)	50 mg/kg	N.S.	N.S.	N.S.	Mouse	i.p.	Epstein-Barr virus
Tianzhu *et al.,* 2015 (35)	20, 40 mg/kg	N.S.	N.S.	N.S.	Mouse	p.o.	Allergic asthma
Ma *et al.,* 2015 (21)	8, 24, 72 mg/kg	N.S.	N.S.	Saline	Mouse	i.p.	Hyperglycaemia
Cai *et al.,* 2013 (36)	5, 10, 20 mg/kg	N.S.	N.S.	Water	Mouse	p.o.	Cognitive function
Hu *et al.,* 2013 (37)	2, 4 mg/kg	N.S.	N.S.	Saline	Rat	N.S.	Sleep regulation
Pan *et al.,* 2015 (24)	20 mg/kg	N.S.	0.1 % cordycepin	PBS	Mouse	N.S.	Cancer
Wang *et al.,* 2015 (31)	1, 5, 10, 20 mg/kg	N.S.	N.S.	N.S.	Mouse	i.p.	Osteogenesis
Dou *et al.,* 2016 (29)	10 mg/kg	N.S.	N.S.	Water	Mouse	p.o.	Osteoporosis
Yuan *et al.,* 2016 (28)	1, 5, 10, 20 mg/kg	N.S.	N.S.	Saline	Rat	i.v.	Traumatic brain injury

N.S., not specified; p.o., oral; i.p., intraperitoneal; i.v., intravenous.

Table 2. Composition, concentration of developed formulations of cordycepin and their achievable dose in various routes of administration

	Composition	Cordycepin concentration (mg/mL)	Achievable dose (mg/kg)[a]	
			Intravenous (5 mL/kg)	Oral (20 mL/kg)
PBS-based	pH 4.0 PBS	5.5	27.5	110.0
	pH 4.5 PBS	4.5	22.5	90.0
	pH 5.0 PBS	3.0	15.0	60.0
PPG-based	PPG:DDW = 77:23	10.0	-	200.0

[a] Achievable dose in each route of administration calculated according to recommendation from reference (42, 43).

Table 3. Intra-day and inter-day validation of the analytical method in each formulation sample medium (n = 6)

	Intra-day		**Inter-day**	
Concentration levels	**Accuracy (RE, %)**	**Precision (RSD, %)**	**Accuracy (RE, %)**	**Precision (RSD, %)**
Samples of pH 4.0 PBS				
LLOQ (0.1 μg/mL)	10.45	3.45	1.76	6.89
LQC (0.25 μg/mL)	1.98	2.77	-2.29	4.40
MQC (1.5 μg/mL)	4.00	0.71	-1.11	6.15
HQC (15 μg/mL)	0.80	0.76	1.44	3.64
Samples of pH 4.5 PBS				
LLOQ (0.1 μg/mL)	8.95	2.85	6.54	4.01
LQC (0.25 μg/mL)	2.83	0.76	-0.53	3.48
MQC (1.5 μg/mL)	2.56	1.50	-2.69	3.43
HQC (15 μg/mL)	2.28	0.57	-1.46	2.44
Samples of pH 5.0 PBS				
LLOQ (0.1 μg/mL)	7.37	6.26	4.76	9.16
LQC (0.25 μg/mL)	4.36	2.49	0.55	5.65
MQC (1.5 μg/mL)	6.73	5.56	-1.13	6.34
HQC (15 μg/mL)	1.08	0.93	-2.42	4.70
Samples of PPG:DDW = 77:23				
LLOQ (0.1 μg/mL)	1.48	2.96	1.07	4.87
LQC (0.25 μg/mL)	2.43	2.98	2.22	3.14
MQC (1.5 μg/mL)	-2.63	2.05	-1.81	2.03
HQC (15 μg/mL)	2.70	2.66	4.06	1.99

Table 4. Stability of cordycepin formulation samples (mean ± SD, n = 3)

Formulation	Storage condition	4 days	11 days	3 weeks	2 months	3 months
pH 4.0 PBS (5.5 mg/mL)	**RT**	98.8 ± 0.2	99.9 ± 1.8	97.7 ± 2.5	102.8 ± 3.7	104.8 ± 2.7
	4°C	97.2 ± 2.0	88.8 ± 2.2	67.3 ± 3.8	72.2 ± 9.8	62.0 ± 8.0
	-20°C	98.0 ± 0.4	97.5 ± 1.7	100.5 ± 4.7	99.4 ± 1.2	101.3 ± 3.4
	-80°C	98.4 ± 0.8	98.2 ± 0.5	99.1 ± 9.2	99.6 ± 1.4	98.8 ± 8.4
pH 4.5 PBS (4.5 mg/mL)	**RT**	93.9 ± 0.9	99.7 ± 1.2	101.9 ± 1.2	101.3 ± 0.4	97.4 ± 5.2
	4°C	96.0 ± 3.8	72.0 ± 9.5	76.8 ± 11.1	41.7 ± 26.0	70.4 ± 18.4
	-20°C	101.3 ± 4.8	98.7 ± 1.1	99.6 ± 1.2	94.0 ± 3.1	96.3 ± 4.5
	-80°C	100.2 ± 7.8	99.9 ± 2.9	100.8 ± 1.1	109.8 ± 11.5	99.0 ± 6.2
pH 5.0 PBS (3.0 mg/mL)	**RT**	96.9 ± 0.7	100.5 ± 2.7	102.8 ± 3.8	106.2 ± 2.5	106.8 ± 1.2
	4°C	96.6 ± 1.4	87.4 ± 10.6	97.2 ± 11.8	85.2 ± 17.7	66.5 ± 4.2
	-20°C	98.0 ± 2.8	99.2 ± 3.0	102.7 ± 5.3	110.4 ± 10.5	99.7 ± 2.2
	-80°C	97.4 ± 0.9	99.5 ± 1.1	104.6 ± 0.3	108.3 ± 10.1	99.4 ± 7.0
PPG:DDW = 77:23 (10.0 mg/mL)	**RT**	102.1 ± 2.6	104.7 ± 1.6	106.0 ± 3.7	109.9 ± 1.0	104.0 ± 2.4
	4°C	100.6 ± 0.2	104.1 ± 3.4	106.2 ± 0.5	105.5 ± 3.9	107.3 ± 0.8
	-20°C	99.9 ± 1.5	104.9 ± 1.0	106.3 ± 0.7	102.4 ± 3.6	106.8 ± 2.0
	-80°C	98.8 ± 1.3	103.8 ± 1.0	105.4 ± 2.4	104.9 ± 3.5	105.3 ± 0.6

RT, room temperature.

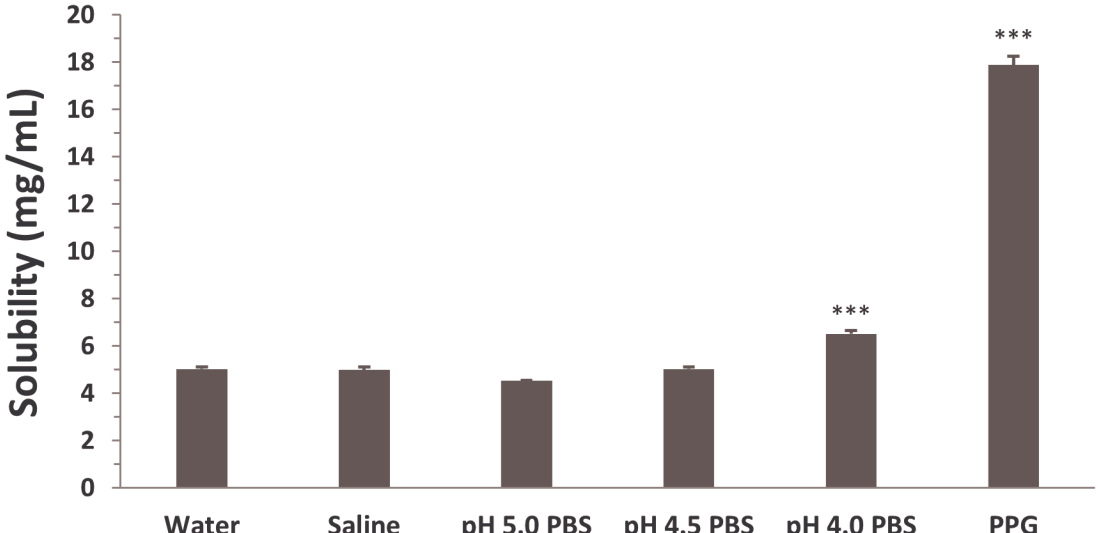
Table 5. Effects of filter sterilisation on concentrations of PBS-based cordycepin formulations (mean ± SD, n = 4)

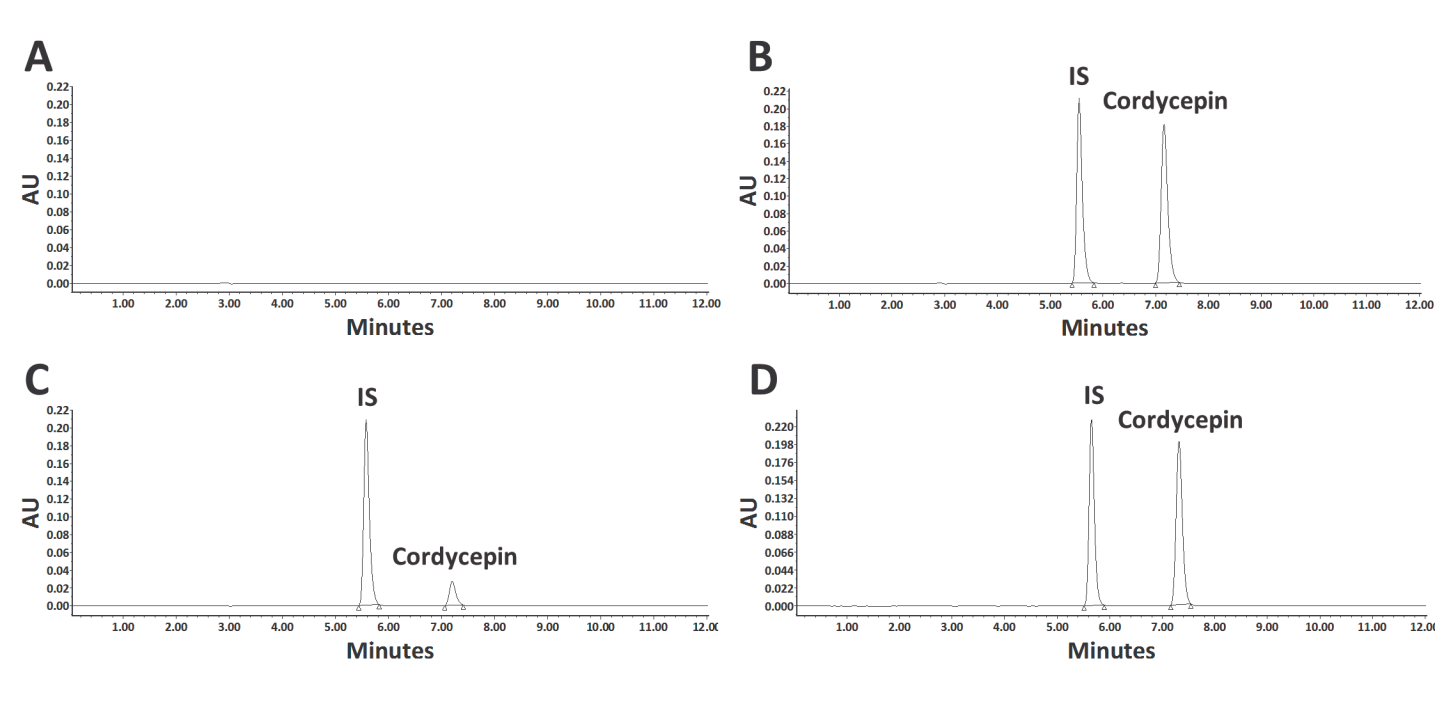
Formulation	Cordycepin concentration (mg/mL)		% remaining after sterilisation
	Before sterilisation	After sterilisation	
pH 4.0 PBS	5.52 ± 0.03	5.41 ± 0.20	98.09 ± 3.35
pH 4.5 PBS	4.45 ± 0.02	4.35 ± 0.11	97.91 ± 2.96
pH 5.0 PBS	3.02 ± 0.05	2.95 ± 0.06	97.77 ± 1.85

LEGENDS TO FIGURES

Figure 1. Solubility of cordycepin in different media (mean ± SD, n = 4). ***, $p<0.001$ compared to water and saline by unpaired two-tailed t-test.

Figure 2. Representative chromatograms of cordycepin and internal standard (IS, adenosine) from HPLC-UV analysis. **A**, baseline chromatogram observed after injection of 100% DDW; **B**, calibration curve sample spiked with 10 ug/mL cordycepin and IS into pH 4.5 PBS-based medium; **C**, MQC sample in PPG-based medium; **D**, stability test sample of pH 4.0 PBS formulation after 3 weeks of storage at 4°C.


Solubility (mg/mL)
20
18
16
14
12
10
8
6
4
2
0
***
***
Water
Saline
pH 5.0 PBS
pH 4.5 PBS
pH 4.0 PBS
PPG


A
B
IS
Cordycepin
C
IS
Cordycepin
D
IS
Cordycepin
AU
Minutes