

1 **Development of cordycepin formulations for preclinical and clinical studies**

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18 **SUGGESTED RUNNING TITLE:**

19 Formulation development of cordycepin

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23 **ABSTRACT**

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

43

44 **KEYWORDS**

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

46

47 **INTRODUCTION**

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

59

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

67

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

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

79

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

86

87 **MATERIALS AND METHODS**

88 **Materials**

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

96

97 **Solubility assay**

98 To assess the solubility of cordycepin in water for injection, normal saline, pH 4.0 PBS, pH
99 4.5 PBS and pH 5.0 PBS, saturated suspensions of cordycepin were prepared (15 mg of
100 cordycepin per mL of solvent). The pH of the formulations in PBS were readjusted using HCl
101 after dissolving cordycepin powder, to readjust the effect of solubilised cordycepin itself on
102 the pH of the formulation. The test suspensions were contained in glass vials and were mixed
103 using a vial roller, overnight at room temperature. The test suspensions were then filtered using
104 Costar Spin-X Centrifuge Tubes (cellulose acetate, 0.22 μm pore size) by centrifugation at
105 2400 g for 5 min at room temperature (Heraeus Fresco 17 Centrifuge, Thermo Electron, MA,
106 USA). The filtrate was analysed for the concentration of cordycepin. Solubility of cordycepin
107 in PPG was assessed by preparing saturated suspensions at 20 mg of cordycepin per mL of
108 solvent and sonicating for 15 min. The test suspensions were filtered using the method
109 described above and the filtrates were analysed for cordycepin concentrations. The assay was
110 conducted in quadruplicate.

111

112 **Formulation preparation**

113 Formulations of cordycepin were prepared based on PBS or PPG. For PBS-based formulations,
114 the pH of PBS was adjusted to 4.0, 4.5 and 5.0 using NaOH or HCl prior to addition of
115 cordycepin. PBS-based formulations were prepared by dissolving cordycepin in PBS at pH 4.0,
116 4.5 and 5.0 at concentrations of 5.5, 4.5 and 3.0 mg/mL, respectively. The formulations were
117 vortex-mixed and the pH of each formulation was then adjusted to its initial pH using HCl to
118 account for the effect of solubilised cordycepin on the pH of the solution. The vortex-mix and
119 pH re-adjustment steps were repeated until they resulted in clear solutions. For the PPG-based
120 formulation, cordycepin was dissolved in PPG at a concentration of 13.0 mg/mL and the
121 formulation was then sonicated in a water bath for 15 min. After confirming that the

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

125

126 **Formulation stability test**

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

132

133 **Filter sterilisation**

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

139

140 **Analytical method**

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

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 μ L was withdrawn and added to 980 μ L of DDW
156 with 10 μ L of internal standard stock solution (1.0 mg/mL adenosine in DDW). The samples
157 were then vortex-mixed and 100 μ L 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 μ g/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 μ L of the cordycepin working standard solutions to 970 μ L of the DDW-based
164 diluents with 10 μ L 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 μ g/mL. The samples
166 were then vortex-mixed and 100 μ L 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

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

178

179 **Statistical analysis**

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

184

185 **RESULTS**

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

195

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

200

201 Stability of the formulations was assessed under four different storage conditions (-80 °C, -
202 20 °C, 4 °C and room temperature) up to 3 months for PBS-based formulations and up to 3
203 weeks for PPG-based formulations. The results in Table 4 show that all PBS-based
204 formulations were stable at -80 °C, -20 °C and at room temperature for up to 3 months.
205 Interestingly, crystallisation of cordycepin was observed in samples stored at 4 °C in PBS-
206 based formulations. The rate of crystallisation differed with pH of the formulations, as
207 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
208 after 11 days. The PPG-based formulation was stable under all four storage conditions up to 3
209 months and crystallisation was not observed in any of the samples.

210

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

216

217 **DISCUSSION**

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

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

226

227 PBS is an isotonic vehicle that causes minimal discomfort when administered intravenously or
228 orally. The solubility of cordycepin in PBS was highest at pH 4.0, probably due to the fact that
229 cordycepin is a weak base ($pK_a = 3.6$, predicted by ACD/Labs, Toronto, Canada). Decreasing
230 the pH further would likely result in even higher solubility. However, the recommended pH
231 range for intravenous formulations is 4-9, and therefore the lowest pH tested in this work was
232 4.0 (42).

233

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

243

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

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

252

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

259

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

269

270

271 **CONCLUSION**

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

280

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283

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

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

RT, room temperature.

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

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

LEGENDS TO FIGURES

Figure 1. Solubility of cordycepin in different media (mean \pm 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 $\mu\text{g/mL}$ 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.



