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Characteristic of Hydroxypropyl Methyl Cellulose Gel Comprising Gallic Acid Dispersed in N-methyl Pyrrolidone

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ABSTRACT

Aqueous system comprising gallic acid dissolved in N- methyl pyrrolidone and dispersed in hydroxypropyl methylcellulose exhibited the pseudoplastic flow behavior with sustainable the release of gallic acid. The viscosity of gel base and gallic acid loaded-gel was decreased as the temperature was increased. The slightly increased release of gallic acid was evident after temperature cycling with the superior antioxidant activity. There was the antimicrobial activity of gels against Staphylococcus aureus, Escherichia coli and Candida albicans. However, the disappearance of this activity against E. coli was found after temperature cycling.

Keywords: Hydroxypropyl methyl cellulose, gel, gallic acid, N-methyl pyrrolidone, characteristic

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INTRODUCTION

Hydroxypropyl methyl cellulose (HPMC) is nonionic cellulose ether polymer widely used in pharmaceutical applications. In aqueous environment, this polymer hydrates rapidly and forms a gelatinous system, therefore it can be employed as gel forming agent [1]. Gallic acid (G) is a phenolic compound exhibiting several biological activities including antioxidant, antityrosinase, antimicrobial, anti-inflammatory and anticancer activities [2-4]. However, the main limitation of gallic acid is its poor water solubility (11.5 mg/mL) [5, 6]. The solubility of gallic acid was increased by dissolving in N-methyl pyrrolidone (NMP). This investigation aimed to study the characteristic of HPMC gel containing gallic acid dispersed in NMP.

MATERIALS AND METHODS

Materials

Gallic acid (G) (Sigma-Aldrich Chemic GmbH, Buchs, Spain), HPMC K 15 M (H) (Dow Chemical, USA) and N-methyl pyrrolidone (NMP; N) (Sigma-Aldrich Chemic GmbH, Switzerland) were used as received. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and (±)-α-tocopherol were purchased from Sigma-Aldrich, Steinheim, Germany. L-ascorbic acid (Fisher Scientific UK Limited, Leics, United Kingdom) was purchased from Ajax Finechem, Australia). Sabouraud Dextrose Agar (SDA), Sabouraud Dextrose Broth (SDB), Tryptic Soy Agar (TSA) and Tryptic Soy Broth (TSB) were purchased from Difco™, USA and used as medium for antimicrobial test. Clotrimazole was kindly supported from T Man Pharma Ltd., Bangkok, Thailand. Ampicillin disc 10 μg (OXOID – Antimicrobial Susceptibility test discs, Oxoid Limited, Hampshire, England) was used as received.

Preparation of Gallic Acid Gel and Rheology Study

Gallic acid gels prepared by dissolving hydroxypropyl methylcellulose (HPMC; H) in distilled water. Then the gallic acid solution using NMP as solvent was added. The prepared system has 3% (w/w) H, 25% (w/w) N and 5% (w/w) G (H3N25G5). The gel base was also prepared as the same method without addition of G (H3N25). The physical stability of prepared gels was also tested after 5 cycles of temperature cycling. For any given cycle, all formulations were kept at 10 ºC for 24 h in the refrigerator and then at 40 ºC for 24 h in the hot air oven (FED 720, Scientific Promotion, Bangkok, Thailand). The rheological behaviors of the gels were investigated by recording their shear stress (F) as a function of shear rate (G) using a Brookfield DV-III Ultra programmable rheometer (Brookfield Engineering Laboratories. Inc., USA) (n=3). The measurements were conducted at three different temperatures (4°C, 28°C and 35°C). Rheological behavior of the gels is expressed as N value (flowing parameter) of the Martin's equation [7].

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Ascorbic Acid Equivalent Antioxidant Capacity (AEAC) Assay

Antioxidant capacities of free gallic acid, gallic gels (H3N25G5), gel bases, ascorbic acid and vitamin E were determined using Ascorbic acid Equivalent Antioxidant Capacity (AEAC) assay. This assay was used to determine the total radical scavenging capacity based on the ability of a compound to scavenge the stable ABTS radical ($ABTS^{\bullet+}$) [8]. IC_{50} value and ascorbic acid Equivalent Antioxidant Capacity (AEAC) were determined.

In Vitro Release Study

The release of gallic acid from H3N25G5 gel and N25G5 mixture (system without gelling agent) were performed using a membrane-less method. Each sample was placed fully in a small porcelain cup and then carefully placed in a glass bottle containing 40 mL citrate-phosphate buffer pH 5.5 at 35°C and stirred at 200 rpm. The amounts of gallic acid release were determined spectrophotometrically at 307 nm. The mean cumulative release was calculated (n=6).

Antimicrobial Activity Test

Antimicrobial activity of gels against Staphylococcus aureus, Escherichia coli and Candida albicans was investigated using an agar-cup diffusion method. The 10 μg ampicillin disc and 40 μg/mL clotrimazole solution were used as positive control for antibacterial test and antifungal test, respectively.

RESULTS AND DISCUSSION

N values (flowing parameter) of the Martin's equation of H3N25 and H3N25G5 systems at different temperature were in the range of 1.6508-1.7427 indicating the pseudoplastic flow behavior (data not shown). The freshly prepared gel and gel after temperature cycling showed the thermosensitive property as presented in Fig. 1. The viscosity was decreased as the temperature was increased. After temperature cycling the gel viscosity was slightly increased.

Fig 1: Viscosity of H3N25G5 gel at different temperature (n=3); freshly prepared (A) and after temperature cycling (B).

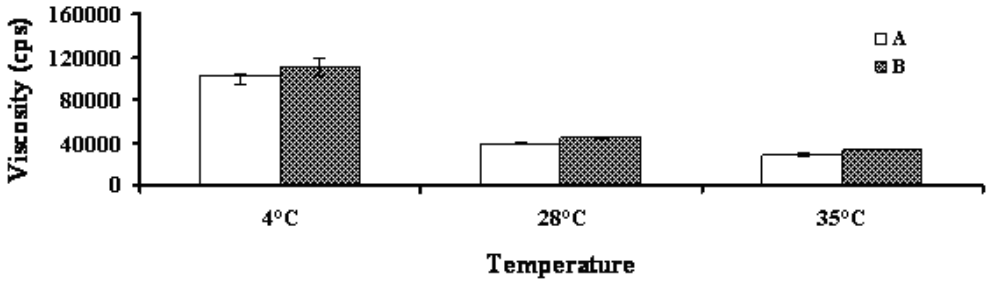

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Table 1: IC_{50} and AEAC of ascorbic acid, vitamin E, gallic acid and gallic acid gels

Substance	IC_{50}(µg/mL)	AEAC	Equation	r^2
ascorbic acid	132.62	1.0000	y = 0.3695x + 0.9981	0.9970
vitamin E	358.38	1.0409	y = 0.1295x + 3.5899	0.9977
gallic acid	43.54	4.5183	y = 0.8585x + 12.62	0.9950
H3N25G5 (A)*	80.22	2.3523	y = 0.5548x + 5.4914	0.9971
H3N25G5 (B)*	49.90	3.2908	y = 0.8661x + 6.7831	0.9945

*(A: freshly prepared; B: after temperature cycling)

The antioxidant activity of gallic acid was apparently better than ascorbic acid and vitamin E, respectively, whereas that of the gallic acid in the gel (A) was slightly lower (Table 1). However its activity was increased after the temperature cycling (B) with the result was corresponding with the release result (Fig. 2). After temperature cycling the gallic acid release was enhanced therefore this treatment promoted gallic acid antioxidant activity.

Fig 2: Release profile of gallic acid from various formula in citrate-phosphate buffer pH 5.5 at 35° (n=6).

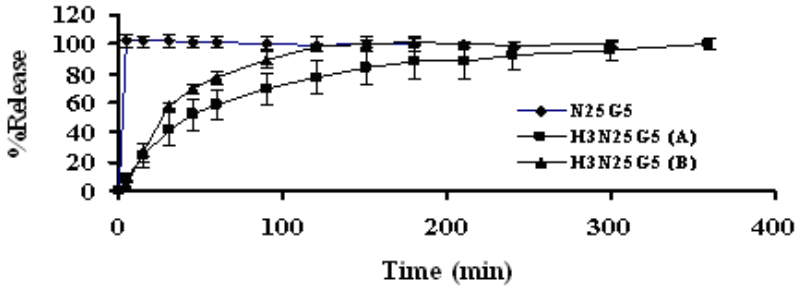

Table 2: Inhibition zones of ampicillin disc, gel bases and gallic acid gels against three microbes obtained from the agar diffusion method (n=3).

Microorganism	Test samples	A		B	
		mean	S.D.	mean	S.D.
E. coli	H3N25	0.70	0.50	0.00	0.00
	H3N25G5	0.73	0.52	0.00	0.00
	Ampicillin (10 µg)	1.97	0.05	1.63	0.08
S. aureus	H3N25	0.00	0.00	1.4	0.35
	H3N25G5	1.17	0.06	1.43	0.30
	Ampicillin (10 µg)	3.75	0.23	3.87	0.09
C. albican	H3N25	1.80	0.00	2.00	0.00
	H3N25G5	2.00	0.00	1.95	0.07
	Clotrimazole (40 µg/mL)	2.50	0.10	2.40	0.10

Both H3N25 and H3N25G5 gels exhibited the antimicrobial activity against Staphylococcus aureus, Escherichia coli and Candida albicans (Table 2). NMP has the antimicrobial activity; therefore the gel base could also inhibit the tested microorganism's

growth. However, the disappearance of this activity against E. coli was found after the temperature cycling hence the treatment might minimize the antimicrobial activity for some microorganisms.

CONCLUSION

HPMC gel comprising N-methyl pyrrolidone could sustain the gallic acid release and inhibit microbial growth. The temperature cycling influenced its antioxidant and antimicrobial activities.

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