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Dual-Functional Beeswaxes on Enhancing Antimicrobial Activity and Water Vapor Barrier Property of Paper

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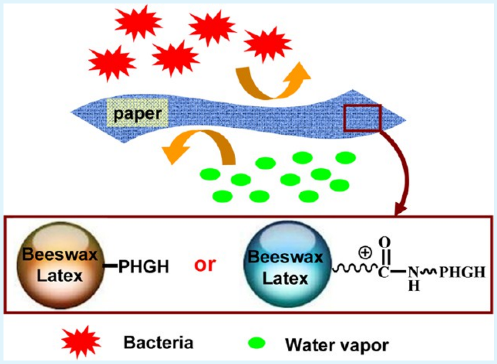
ABSTRACT: The guanidine-based antimicrobial polymers were grafted onto the surface of beeswax latex particles stabilized with or without amphoteric surfactant, *n*-dodecyl-*N*,*N*-dimethylglycine. *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) was used as a coupling reagent to introduce the covalent bonding or ensure sufficient binding force. The resulting novel beeswax latexes were utilized as a dual-functional paper additive, which improved both water-vapor resistance and antimicrobial activities of the paper. Compared with control sample, the water vapor transmission rate of the paper was reduced significantly. The antimicrobial activity of paper against *E. coli* was also improved substantially even at a low dosage of the beeswax latex derivatives (20 mg/g fiber).



KEYWORDS: *antibacterial activity, water vapor transmission rate, guanidine, beeswax, coupling reaction, paper*

1. INTRODUCTION

Water vapor barrier property is a desirable attribute of food packaging materials. Packing materials with capability of water vapor resistance can inhibit water vapor released from food transport through the paper, thus decreasing the water loss and prolonging fresh time of food within them. In the past decades, petroleum-based plastics hold a dominant position in the market owning to their high strength and excellent performance of water vapor resistance. However, the increasingly severe environmental issues and food safety concerns prompted the birth and relevant applications of green packaging materials. Paper-based packing materials, fabricated from natural resources, offer us an important alternative due to its renewable, nontoxic, flexible, and low-cost features.

Typically, paper additives may be applied to improve the characteristics of the paper for packaging. A number of natural polymers, including chitosan,[1] corn zein,[2] whey protein isolates,[3] gluten,[4] starch and its derivatives,[5] have been reported for applications of water-vapor resistance. In addition, a variety of biowaxes were also utilized as paper additives. Beeswax is a type of green biowax formed from a mixture of several compounds, mainly consisting of various long chain alcohols, fatty acids and their esters.[6] Beeswax has a wide range of uses in cosmetics manufacturing, candle processing, pharmaceutical industry, agriculture, food industry, and animal husbandry.

Beeswax is a favorable candidate for the exploitation of water vapor barrier of paper due to its excellent barrier property.[7−9] However, it is vulnerable to bacteria in the application process. In recent years, paper-based hygiene products treated with antimicrobial agents such as chitosan and its derivatives,[10] modified starch,[11−13] active paraffin,[14] and natural essential oils[15] have been well developed, but paper additives with both water vapor resistance and antimicrobial properties are scant.

In this work, we developed a novel approach of grafting antimicrobial polymers, guanidine-based polymers in particular, onto beeswax via a condensation reaction with a water-soluble coupling agent, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC). According to the mechanism of amide formation by carbodiimide for bioconjugation reported by Nakajima and Ikada,[16] it is generally accepted that EDC is able to form amide between carboxylic acid and amine in aqueous media at a low pH.[17,18] Based on the same mechanism, the reaction scheme for the current system is illustrated in Figure 1. A crucial advantage of this method is that the antimicrobial chains are able to be attached covalently to the substrates, thus providing new dual-functional beeswax latexes with improved antimicrobial activity and meanwhile maintaining high water vapor barrier property, which could improve the properties of paper products simultaneously. To our knowledge, this is the first time that the dual-functional beeswax latexes derivatived with PHGH are investigated on their antibacterial and water vapor resistance properties. The antimicrobial performance of the resulting beeswax particles were revealed by shaking flask method; whereas the hydrophobic and water vapor barrier properties were investigated via contact angle and water vapor transmission rate (WVTR) measurements. The key objective of this work was to develop environmentally friendly coating additives with synergistic

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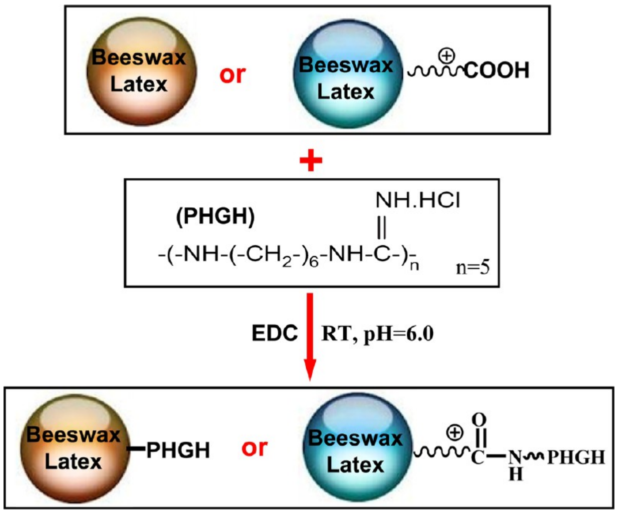

Figure 1. Coupling reaction of PHGH with carboxyl groups on the surfaces of beeswax latex particles with and without the amphoteric surfactant, *n*-dodecyl-*N*,*N*-dimethylglycine.

antimicrobial and water vapor barrier properties for high-value-added paper products.

2. MATERIALS AND METHODS

2.1. Materials. Bleached beeswax, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), Luria Bertani (LB) agar, and phosphate-buffered saline (PBS) were obtained from Aldrich. *N*-Dodecyl-*N*,*N*-dimethylglycine was purchased from Affymetrix company. Guanidine-based polymer, specifically poly(hexamethylene guanidine hydrochloride) (PHGH), was synthesized according to the procedures described in our previous work, and the average molecular weight of polyhexamethylene guanidine measured by mass spectroscopy is approximately 770 Da.[11,19]

2.2. Preparation of Surfactant-Free Beeswax Latexes and Beeswax Latexes Stabilized with *n*-Dodecyl-*N*,*N*-dimethylglycine. Amounts of 2.5 g of beeswax, 50 mg of *n*-dodecyl-*N*,*N*-dimethylglycine (Gly), and 97.5 g of deionized water were added into a 250 mL beaker. The mixture was heated to 90 °C in an oil bath and was sequentially stirred with a high-speed agitator at 28k rpm for 3 min. The product BW-Gly was then cooled in an ice water bath. The surfactant-free beeswax latex was prepared via similar described above.

2.3. Preparation of Beeswax Latex Particles Derivatized with PHGH. Volumes of 100 mL of 2.5 wt % beeswax latexes (BW and BW-Gly) were prepared and used as coupling substrates. Carboxyl groups on the surface of the substrates were derivatized with 0.24 g PHGH using 1.2 g of EDC, with all reagents being dispersed in a 250 mL beaker at pH 6.0. The pH of the solution was adjusted with 0.1 M HCl. The mixtures were stirred at 25 °C for 3 h and then dialyzed against deionized water for 5 days.

2.4. Analysis of Beeswax Latexes and Their Derivatives. Zeta potential and particle size measurements were performed using a Brookhaven ZetaPlus zeta potential analyzer. All data were averaged of 6 cycles with 10 scans for each.

The contact angle measurements were conducted using an Attension Theta Optical tensiometer. All pictures of the drops were taken at the seventh second. Scanning electron microscopy (SEM) images of dried, gold-coated samples were obtained with a JEOL JSM-6400 SEM instrument (Japan).

2.5. Characterization of Antimicrobial Activities. A shaking flask method was employed to quantify the antibacterial activities of hand-sheets made of eucalyptus wood fibers treated with beeswax derivatives against Gram-negative bacteria *Escherichia coli* (*E. coli*, ATCC 11229). In this method, 1.0 g of eucalyptus wood fibers and 20 mL of 0.1 wt % beeswax latex derivatives were sufficiently mixed and the resulting suspension was dried overnight in an oven. Subsequently, 0.01 g of product (20 mg latexes/1g fibers) was added into 5 mL of 0.01 M PBS solution. The sample bottle was then immersed in a water bath shaker and shaken (200 rpm) at 37 °C for 30 min.

A volume of 0.1 mL of each culture was seeded on an agar plate, and then the plate was incubated at 37 °C for 24 h. The number of the colonies was carefully counted, and the inhibition of the cell growth was calculated based on eq 1:

$$\text{growth inhibition of bacteria (\%)} = \left(\frac{A - B}{A}\right) \times 100 \tag{1}$$

where *A* and *B* are the number of the colonies detected from the control and the treated samples, respectively. Three repetitions were conducted to get an average value for each sample.

2.6. Water Vapor Transmission Rate (WVTR) Tests. WVTR measurements were carried out using a sorption apparatus which consists of a high sensitivity microbalance with an accuracy of 0.1 μg and a turbomolecular high vacuum pumping system (IGA-003; Hiden-Isochema, Warrington, U.K.). The IGA machine can be utilized to measure water vapor transmission rate on the basis of ASTME 96/E96M-05 standard (2005). In this method, paper samples were cut into circle discs with 1 cm diameter, and then clamped in the sample cell. All measurements were performed at 38 °C with 90% relative humidity (RH). Humidity was controlled by an automatic humidity generator (HumidSys, InstruQuest Inc.).

When the WVTR curve reaches its steady state, WVTR data can be calculated by eq 2:

$$\text{WVTR} = \frac{m}{A \times T} \tag{2}$$

where *m* is the mass change of the container, *A* is the area of exposed films, and *T* is a specified time interval during the steady state.

3. RESULTS AND DISCUSSION

3.1. Characterization of Beeswaxes and Their Derivatives. *3.1.1. Particle Size Analysis.* The average particle diameters and standard errors of dual-functional beeswax derivatives are listed in Table 1. Beeswax latexes stabilized with *n*-dodecyl-*N*,*N*-dimethylglycine have smaller sizes than the corresponding latexes in the absence of the surfactant, altering from approximately 240 to 599 nm. The surfactant provided extra surface charge for the biowax latexes, thus facilitating the formation of smaller latex particles. As shown in Table 1, the diameter of BW and BW-Gly latexes after grafting with PHGH

Table 1. Particle Sizes, Zeta Potential, WVTR, and Antibacterial Property of Beeswax Latexes and Their Derivatives

latexes	diameter/std error (nm)[a]	zeta potential/std error (mv)[b]	WVTR ($g/m^2/d$)[c]	growth inhibition (%)[d]
BW	599.1/18.1	−9.64/3.98	396.0/10.5	0
BW-PHGH	782.2/38.5	41.47/1.97	440.8/8.4	92.2
BW-Gly	240.3/10.9	6.94/5.31	195.6/5.0	0
BW-Gly-PHGH	493.7/28.0	36.76/1.40	222.0/10.1	93.1

[a] The measurements of latex diameters were performed in 2 mM NaCl at 25 °C. [b] All measurements were conducted in 2 mM NaCl aqueous solutions at pH 7.0 and 25 °C. [c] The coating amount of all latexes on copy paper was controlled at 12 ± $0.5 g/m^2$, and all the measurements were performed at T = 38° and RH = 90%. The WVTR of the copy paper without coating is 2788.8 g/m^2/d. [d] Shaking flask method was employed to measure the growth inhibition of *E. coli* for beeswax latex and its derivatives adsorbed on the copy paper. Eucalyptus wood fiber without adsorption of beeswax latexes was used as a control.

is approximately 200 nm larger than the corresponding latexes before grafting, indicating the formation of latex aggregates in water.

3.1.2. Zeta Potential of the Latexes. A zeta potential analyzer was employed to investigate the surface charges of the beeswax latexes and their derivatives with PHGH. The zeta potential values at pH 7.0 shown in Table 1 demonstrate that the beeswax latex particles were negatively charged at neutral pH, owing to the cerotic acid and other fatty acids on the surface of the beeswax latexes. By contrast, the beeswax latexes stabilized with *n*-dodecyl-*N*,*N*-dimethylglycine showed net positive charge at neutral pH, illustrating the existence of quarternary ammonium groups on the latex surfaces, contributed from the amphoteric surfactant. After the derivation of PHGH on the surface of BW and BW-Gly latex particles, the resulting particles, BW-PHGH and BW-Gly-PHGH, showed strong positive zeta potential at pH 7.0 due to the abundant positive charges on PHGH.

3.2. Properties of the Beeswax Latexes and Their Derivatives. *3.2.1. Hydrophobicity.* Contact angle method was used to monitor the hydrophobicity of paper coated with beeswax latexes and their derivatives; the results are summarized in Figure 2. The melted BW-PHGH and BW-Gly-PHGH latexes coated on a copy paper exhibited similar behavior on hydrophobicity. The contact angles of both beeswax latex derivatives increased steadily with increasing coating amount of latexes. When the coating amount is 24 g/m^2, the contact angles of BW-PHGH and BW-Gly-PHGH were 123.78°and 123.21° respectively, compared with the contact angles of BW-PHGH (70.39°) and BW-Gly-PHGH (67.71°) which has the relatively low coating amount (4g/m^2). However, even at such low dosage, there was a notable enhancement on hydrophobicity. We suggested that some melted beeswax latex derivatives may permeate into the pores within cellulose fiber networks, mainly driven by the capillary force. As a result, beeswax derivatives were incapable of forming a smooth and uniform film, which in turn lowered the contact angle. When the coating amount was low, for example, 4g/m^2, this phenomenon became rather apparent. A detailed description of the mechanism will be provided in a future publication.

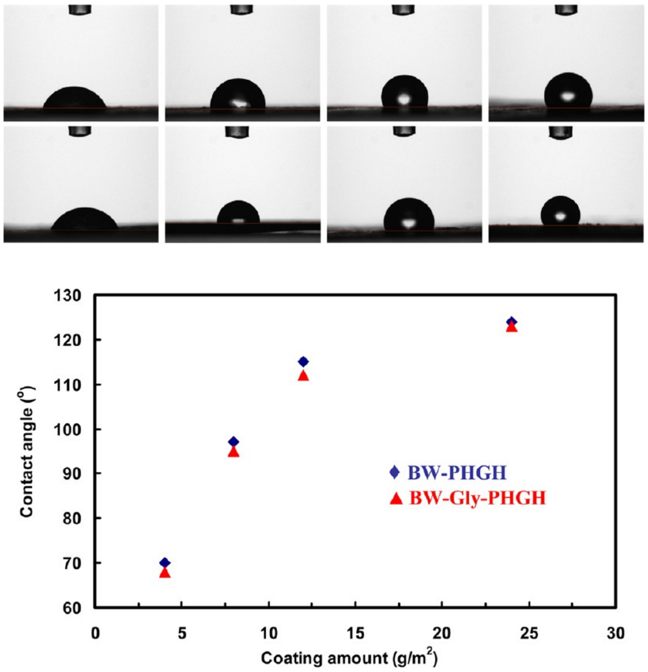

Figure 2. Contact angles of BW-PHGH and BW-Gly-PHGH coated on the copy paper (75g/m^2) as a function of coating amount. The measurements were conducted after melting of the two beeswax latexes on copy papers.

3.2.2. Water Vapor Transmission Rate. Table 1 shows the WVTR values of the copy paper coated with beeswax latexes and their derivatives (BW, BW-Gly, BW-PHGH and BW-Gly-PHGH). The WVTR of the control sample (75g/m^2) without coating is 2788.8 g/m^2/d, but the WVTR of the paper coated with BW decreased by 85.8% to 396.0 g/m^2/d, when the coating amount was controlled at 12 ± 0.5 g/m^2. On the other hand, the WVTR of copy paper coated with 12 g/m^2 BW-Gly latexes was as low as 195.6 g/m^2/d or decreased by 93.0%. We propose that BW-Gly latexes stabilized with *n*-dodecyl-*N*,*N*-dimethylglycine has smaller particle size (240.3 nm) than BW latexes (599.1 nm), thus facilitating the penetration of latex particles into smaller pores within fiber networks. After the melting of the beeswax latexes, BW-Gly is then able to fill smaller voids and block more transit of water vapor, thus further lowering the WVTR. Moreover, the WVTR values of both BW-PHGH (448.8 g/m^2/d) and BW-Gly-PHGH (222.0 g/m^2/d) latexes were higher than those of BW (396.0 g/m^2/d) and BW-Gly (195.6 g/m^2/d) latexes. This result may be explained by the fact that the surfaces of BW and BW-Gly latex particles after grafting with PHGH were more hydrophilic than previous ones. Therefore, BW-PHGH and BW-Gly-PHGH were more approachable to water vapor and then make their WVTR slightly higher.

The WVTR of copy paper as a function of coating amount of BW-PHGH latex was also studied in this work. As shown in Figure 3, the WVTR of copy paper coated with BW-PHGH latex decreased sharply with increasing coating amount up to 12g/m^2. Subsequently, there was a slow and steady downward trend with increasing coating amount of BW-PHGH. When the coating amount reached 24 g/m^2, the WVTR of BW-PHGH became 236.4 g/m^2/d, which is only 8.5% of the WVTR of blank copy paper (2788.8 g/m^2/d).

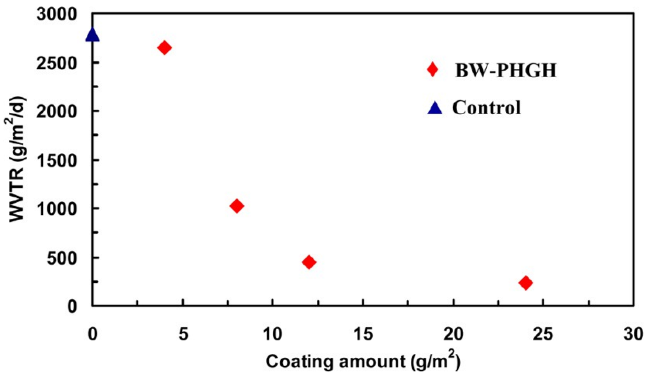

Figure 3. WVTR of paper samples as a function of coating amount of BW-PHGH latex. A copy paper (75g/m^2) was used as the control. The WVTR of the copy paper without coating is 2788.8 g/m^2/d.

3.2.3. Surface Morphology of Paper Coated with BW-PHGH and BW-Gly-PHGH Latexes. SEM was used to reveal the surface morphology of the copy paper coated with latexes. The results are shown in Figure 4, in which the A series of micrographs were taken at low magnifications and the B series were obtained at high magnifications. A-1 and A-2 show

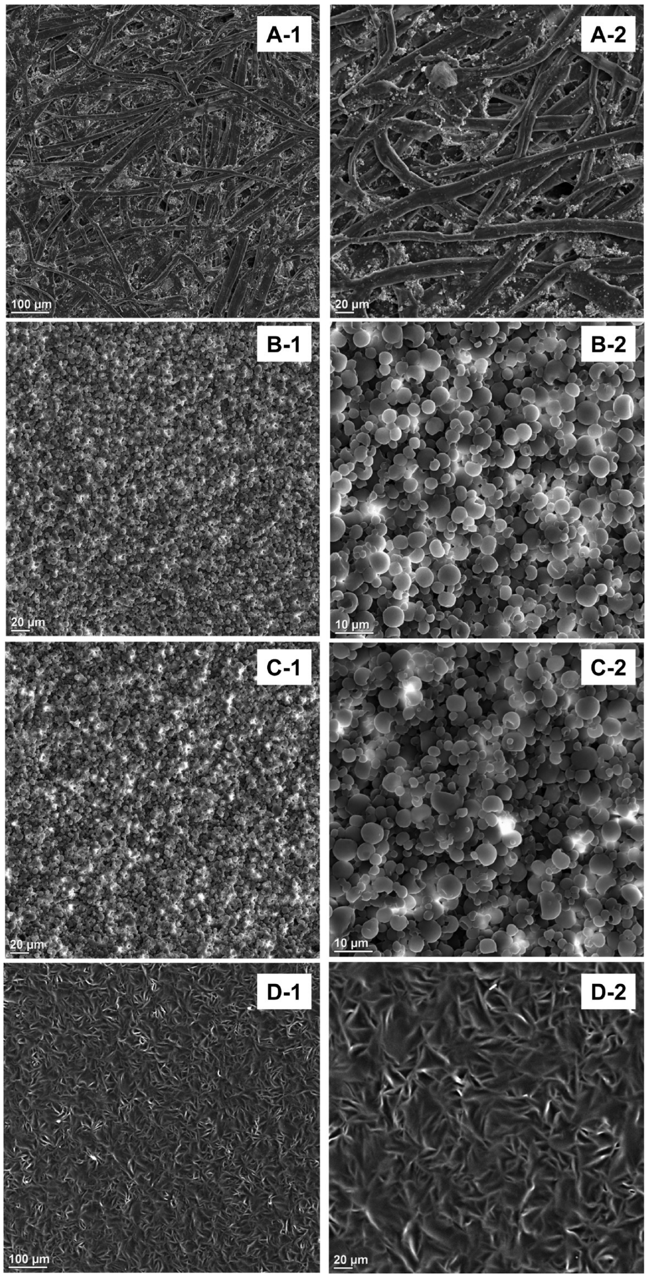

Figure 4. SEM images of BW-PHGH and BW-Gly-PHGH latexes coated on copy paper: (A-1, A-2) control experiment: blank copy paper, (B-1, B-2) coated with BW-PHGH latexes, (C-1, C-2) coated with BW-Gly-PHGH latexes, and (D-1, D-2) coated with BW-PHGH latexes after melting. A-1, B-1, C-1, and D-1 were photographed at low magnifications; A-2, B-2, C-2, and D-2 were photographed at high magnifications.

unmodified copy paper as a control. The layered network structure constituted by the cellulose fibers is clearly visible. A number of pores and some calcium carbonate fillers were also observed in fiber networks. B-1/B-2 and C-1/C-2 show the copy paper coated with BW-PHGH and BW-Gly-PHGH latexes, respectively. It can be seen that the individual latex particles were aggregated but remained spherically during drying at room temperature (B and C series), thus generating large numbers of voids. The voids constituted channels for the pass-through of water vapor. We believe that it is the essential reason for their poor water vapor resistance and high WVTR. Subsequently, beeswax latexes were heated above 70 °C and melted. D-1 and D-2 in Figure 4 show the SEM images of the paper coated with BW-PHGH after heating. Obviously, a layer of uniform beeswax film was formed; and the wrinkle pattern generated during melting process was also observed. Close inspection revealed that the beeswax film covered the surface of the copy paper and filled up the pores of fiber networks. Meanwhile, the surface of paper became smoother than before, which led to the change of their corresponding contact angles.

3.2.4. Antimicrobial Activity. It is well-known that guanidine polymers inhibit the growth of bacteria by decomposing the cell membrane of bacteria and thus giving rise to the leakage of intracellular components.[20−22] Previous work performed in our group demonstrated that PHGH is an efficient antimicrobial polymer.[23,24] In this work, two beeswax latexes were derivatized with PHGH; and the growth inhibition of beeswax derivatives against *E. coli*, determined by shaking flask method, is shown in Table 1. Two derivatives of beeswax latex particles absorbed on the eucalyptus wood fibers (20 mg latexes/1 g fibers) exhibited excellent inhibition of *E. coli*. The growth inhibition values of both derivatives were higher than 92%. By contrast, the beeswax latex without grafting of PHGH had no obvious effect on antimicrobial activity. Moreover, based on the experimental results, we conclude that beeswax latexes stabilized with or without the surfactant, *n*-dodecyl-*N*,*N*-dimethylglycine, were bare of difference as the support of antimicrobial agents on antimicrobial activities.

4. CONCLUSIONS

PHGH derivatized beeswax latex particles were successfully prepared and applied to copy paper, providing the capability of water vapor resistance and also evident antibacterial activities against *E. coli*. As a dual-functional agent, the beeswax-PHGH stabilized with *n*-dodecyl-*N*,*N*-dimethylglycine (BW-Gly-PHGH) has smaller particle size than BW-PHGH and led to the significant reduction of WVTR of the coated paper, thus further improving the barrier property.

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Notes

The authors declare no competing financial interest.

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