RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2026.016

ScienceAsia 52 (1): 2026: ID 2026016: **1–6**

Promoting antibacterial performance of natural rubber latex foam using a quinoline derivative

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Received 14 May 2025, Accepted 30 Jan 2026
Available online 21 Feb 2026

ABSTRACT: Natural rubber (NR) latex foam has been used in various products such as pillows, mattresses, and carpet underlays, to name a few. Its cellular structure may lead to an easy growth of microorganisms. This may bring harmful bacteria to the users. Therefore, this work aimed to develop antibacterial NR latex foam and offered the basic formulation for preparing it. An antibacterial NR latex foam was prepared in the presence of quinoline derivative namely 2-hydroxypropyl-3-piperazinyl-quinoline carboxylic acid methacrylate (HPQM). The use of HPQM influences the physical characteristics and antibacterial activities. The clear zones of *Staphylococcus aureus* and *Escherichia coli* increased over the HPQM content where a shorter contact time was used to kill the bacteria. The use of HPQM at only 2 phr is sufficient to gain 100% killing efficiency in less than 2 h of contact time. This study offered resourceful information in fabricating an antibacterial NR latex foam.

KEYWORDS: natural rubber latex, foam, antibacterial performance, quinoline derivative, porous materials

INTRODUCTION

Natural rubber (NR) latex is an interesting biopolymer derived from the *Hevea brasiliensis* tree, commonly known as the rubber tree. In today's modern world, this naturally sourced material is widely used to manufacture various products that enhance convenience and comfort in daily life [1–3]. NR latex foam is one of the NR latex applications that can be converted into various products such as pillows, mattresses, and carpets [4–6]. These products are mostly in contact with the users while using them. This has led consumers to increase their awareness of the hygienic condition [7, 8]. Therefore, the demand for NR latex foam with antimicrobial properties has been steadily rising as consumers become increasingly aware of bacteria and their potential health risks. This trend has been particularly pronounced since the COVID-19 pandemic, prompting manufacturers to address consumer needs by introducing antimicrobial solutions across a wide range of applications.

The porous NR latex foam is one of the materials that is easier for bacteria to grow compared to non-porous materials, which is a significant reason why porous materials become a source of microbes after some period of use. Microbial contamination in the environment can impact human health, especially when they are pathogenic. So, preparing NR latex foam with antibacterial properties is an interesting area of research. Generally, there is a way to control bacterial contamination on surfaces of materials using chemical disinfectants from the groups of alcohols, glutaraldehyde, chlorine-containing compounds, and phenols [9, 10]. These chemicals are commonly used for direct cleaning rather than being incorporated into polymers. The possible route is to find non-toxic chemicals to inhibit microorganisms and incorporate them into polymer materials.

An example of a safe antibacterial agent commonly mixed into polymer materials is a quinoline derivative, namely 2-hydroxypropyl-3-piperazinyl-quinoline carboxylic acid methacrylate (HPQM). HPQM has been widely used in a wide range of applications. Taptim and Sombatsompop [11] used HPQM in silicone rubber, and the results showed an improved ability to inhibit bacteria. Additionally, Jai-eau et al [12] used HPQM in NR with conventional, semi-efficient, and efficient sulfur vulcanization systems, comparing it with silver zeolite. The bacterial inhibition test showed that HPQM had higher antibacterial performance than silver zeolite due to its better ability to spread on the rubber surface. Therefore, this study also outlines a potential formulation strategy to minimize the effect of HPQM on the physical properties of NR latex foam. The findings will serve as a foundation for further development of antibacterial foam. Overall, this work represents a promising step toward the fabrication of antibacterial NR latex foam incorporating HPQM.

MATERIALS AND METHODS

Materials

High Ammonia (HA) - centrifuged latex was purchased from Yala Latex Industry Co. Ltd., Yala, Thailand.

Table 1 Formulation for making NR latex foam.

Ingredient	Amount (phr)
60% HA latex	100
20% potassium oleate	2.0
50% ZDEC	1.0
50% ZMBT	1.0
50% sulfur	2.5
50% Wingstay L	1.0
10% HPQM	0–10
15% DPG	1.2
50% ZnO	2.0
20% SSF	1.2

HPQM was obtained from Koventure Co. Ltd., Samut Prakan, Thailand. HPQM is a water-based solution composed of 10 ± 1 wt% HPQM, 2 ± 1 wt% sodium hydroxide, less than 1 wt% polyoxyethylene nonyl phenyl ether, and 87 ± 1 wt% deionized water. Other additives such as 50% sodium silicofluoride (SSF), 15% diphenylguanidine (DPG), 50% sulfur, 50% Wingstay L, 50% zinc 2-mercaptobenzothiazole (ZMBT), 50% zinc diethyl dithiocarbamate (ZDEC), and 20% potassium oleate were supplied by Siamnavakam Co. Ltd., Samut Prakan, Thailand.

Preparation of antibacterial NR latex foam

Table 1 shows the compounding formulation for making antibacterial NR latex foam. All the ingredients mixed with centrifuged HA latex are prepared in dispersion and emulsion forms. The foam processing is referred to the Dunlop process, where the SSF was used as the primary gelling agent. First, HA was added to the cake beater, and stirred for 3 min to evaporate the NH3 available in the latex. Next, 20% potassium oleate, 50% ZDEC, 50% ZMBT, 50% Wingstay L, and 50% sulfur were slowly added. The speed was then increased until reaching the required volume, which was approximately 5 min. Afterward, the 10% HPQM was added depending on their respective amounts. The mixing time depended on the amount of HPQM, ranging from 2–4 min. Next, the 50% DPG and 50% ZnO were subsequently added. Finally, the gelling agent (20% SSF) was promptly added, and the mixture was beaten for an additional 1 min. The resulting un-gelled foam was then immediately poured into an aluminum mold and allowed to gel at ambient temperature for less than 2 min. The gelled foam was subsequently cured in a hot air oven at 100 °C for 45 min. After curing, the foam was removed from the mold and thoroughly washed with water to eliminate residual soap and unreacted substances. Finally, the washed NR latex foam was dried in a hot air oven at 60 °C for 24 h.

Measurement of physical properties

The density of the foam was determined based on mass-to-volume ratio. The foam samples were cut into a cubic shape with dimensions of $3.0 \times 3.0 \times 3.0$ cm^3, weighed, and calculated accordingly.

The cellular structure of the samples was screened using a light microscope at $40\times$ magnification. The image was then captured and imported into the ImageJ software to measure the cell size.

Compression-deflection testing was conducted in accordance with ASTM D575. For this test, specimens measuring $10.0 \times 10.0 \times 2.5$ cm^3 were prepared and compressed using a universal testing machine (Tinius Olsen, H10KS, Pennsylvania, USA) until the thickness was reduced by 25%. The load was recorded immediately. The process was repeated with the same specimen until the load values varied by less than 5%, and the final result was reported in terms of force per unit area.

Compression set was measured according to ASTM D395. A specimen with dimensions of $5.0 \times 5.0 \times 2.5$ cm^3 was compressed to 50% of its original thickness at 100 °C. After 70 h, the load was removed, and the sample thickness was measured after allowing it to recover for 30 min. The compression set was then calculated using the standard formula as follows;

$$\text{Compression set} = \frac{(t_0 - t_1)}{(t_0 - t_s)} \times 100 \qquad (1)$$

where t_0 is the original thickness, t_1 is the recovered thickness, and t_s is the thickness of the spacer.

Antibacterial study

Both qualitative and quantitative antibacterial activities were tested in this study. For the first method, the samples were placed at separate positions on Mueller-Hinton Agar (MHA) plates under hygienic conditions. A second thin layer of MHA (10 ml) was applied on sample. A 15 ml of MHA was also added to sterilised Petri dishes for the bottom layer. The optical density (OD) at 600 nm was used to measure the bacterial concentration where an OD of 0.3 indicated a bacterial concentration of 1×10^8 CFU/ml. Then, 100 µl of solutions containing *S. aureus* and *E. coli* were evenly distributed on the MHA plate surface. The inoculated plates were incubated at 37 °C for 24 h to observe the zone of inhibition.

The test culture was first cultivated on nutrient agar (NA) medium and then incubated for 18 to 24 h at 37 °C to perform quantitative analysis. After that, each colony was moved to Mueller Hinton Broth (MHB) and incubated for 3 h at 37 ± 0.5 °C. The turbidity of the bacterial suspension was brought into compliance with the 0.5 McFarland standard. Subsequently, 100 µl of the prepared bacterial suspension was added to 9 µl of MHB medium containing the test samples. The mixture was incubated at 37 ± 0.5 °C for various time intervals: 0, 2, 4, 6, 8, and 24 h. After incubation, the samples were serially diluted 10-fold using 0.85% normal saline, plated onto NA medium, and incubated

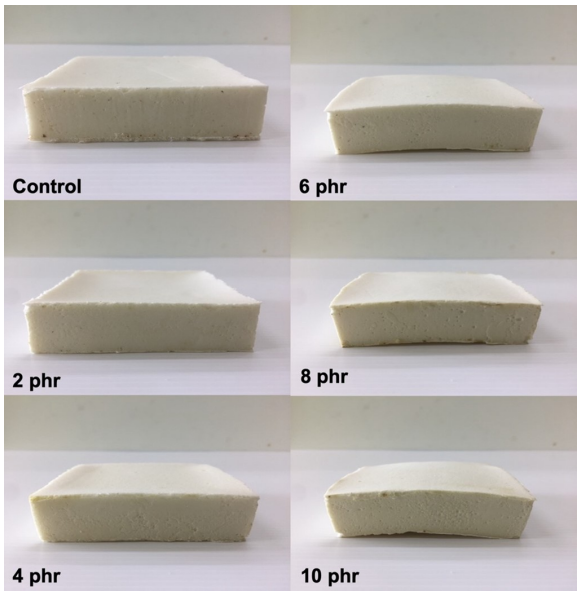

Fig. 1 Physical appearance of the NR latex foam samples prepared at various HPQM content.

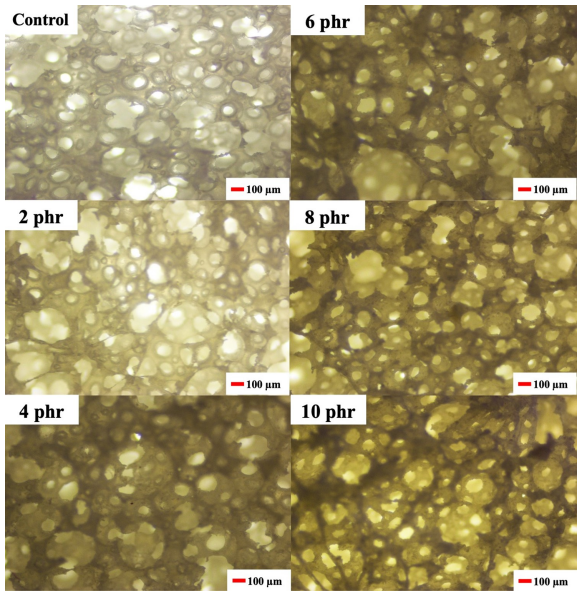

Fig. 2 Optical images at 40 × magnification NR latex foam samples prepared at various HPQM content.

overnight at 37 ± 0.5 °C. The number of surviving bacteria was quantified by counting colony-forming units (CFU/ml). Control trials were conducted in parallel, and results were compared. The percent reduction of bacterial count was based on the following equation.

$$\text{Percent of bacterial count} = \frac{A-B}{A} \times 100 \qquad (2)$$

where A is the bacterial count after a certain contact time, and B is the bacterial count before the test. The negative value indicates the reduction of bacteria.

RESULTS AND DISCUSSION

Physical appearance and morphology

The physical appearance of NR latex foams containing various HPQM content is shown in Fig. 1. From the images, it was observed that the prepared foams did not show significant changes with increasing HPQM content. However, using a high amount of HPQM, particularly at 10 phr, resulted in shrinkage and collapse of the foam. This may be due to the fact that HPQM was in the form of 10 wt% (A water-based liquid form), and was used at a high concentration, such as 10 phr. This could also increase the diluent, such as water, in the latex compound. After pouring the un-gelled foam to a certain volume of the mold, it would reduce the ratio of rubber phase in the foam. This increased the bubble rupture and ultimately led to the shrinkage and collapse of the foam.

Microscopic images at 40 × magnification of NR latex foams containing various HPQM content are also shown in Fig. 2. It revealed that cell size decreased as the HPQM content increased. The cell sizes, measured using ImageJ software, were 133.56 ± 20.68, 139.62 ± 12.32, 132.47 ± 8.70, 126.61 ± 30.12, 116.47 ± 20.72, and 109.72 ± 7.93 µm, corresponding to HPQM content ranging from 0 to 10 phr. It decreased from 133.56 to 109.72 µm, showing approximately 24 µm in average. The shrinkage and collapse also occurred after vulcanization. This is because HPQM is a solid phase when dried, which made the bubble formation more difficult. This may have resulted in smaller cell sizes as the HPQM content increased. A similar observation was found in the case of using solid particles in the NR latex foam [13].

Antibacterial performance

The qualitative test of antibacterial performance was assessed in this study. This test is a preliminary test to observe whether antibacterial activity exists in the sample. The images captured after the disk diffusion test are shown in Fig. 3, where the measurement of the inhibition area is summarized in Table 2. The experiment revealed that incorporating HPQM into the NR latex foam enabled inhibition of both *S. aureus* and *E. coli*, with the inhibition zone remaining more or less the same over the increment of HPQM. Specifically, the inhibition zone for *E. coli* ranged from 7.95 to 29.17 mm, while for *S. aureus*, it ranged from 0 to 33.74 mm. The clear zone was even greater than the positive control from Chloramphenicol (C30) which was 26.93 and 27.21 mm for *E. coli* and *S. aureus*, respectively. At a similar content of HPQM, the sample showed more or less the same inhibition against

S. aureus rather than *E. coli*.

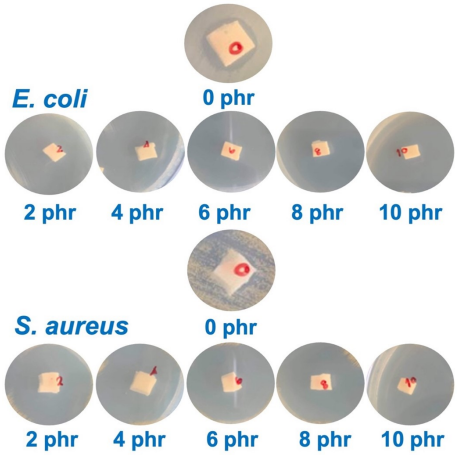

Fig. 3 Photos captured after antibacterial test against *E. coli* and *S. aureus* of the NR latex foam samples prepared at various HPQM content.

Table 2 Inhibition zone measured after antibacterial test against *E. coli* and *S. aureus* of the NR latex foam samples prepared at various HPQM content.

HPQM content (phr)	Inhibition zone (mm)	
	E. coli	*S. aureus*
Chloramphenicol (C30)	26.93 ± 0.18	27.21 ± 0.24
Control	7.95 ± 0.25	0
2	32.61 ± 1.03	29.90 ± 0.54
4	30.40 ± 1.41	31.45 ± 3.37
6	33.38 ± 0.93	33.59 ± 4.62
8	28.77 ± 0.89	33.85 ± 4.60
10	29.17 ± 0.32	33.74 ± 3.59

The antibacterial mechanism of HPQM can be explained similarly to compounds with a similar structure, specifically quinolone derivatives. According to research by Chen et al [14], a comparative study on the antibacterial properties of quinolone derivatives including HPQM was performed and demonstrated its strong antibacterial activity. In addition, Kumar et al [15] investigated the antibacterial effects of quinolone derivatives and found that they inhibit bacterial growth by disrupting deoxyribonucleic acid (DNA) replication in each bacterial chromosome. Mistgcher [16] further reported that quinolone compounds inhibit bacterial growth by interfering with DNA replication at the DNA gyrase stage, where tightly wound DNA strands are unwound during replication. During DNA replication, the DNA is unwound by an enzyme called DNA gyrase. The quinolone derivative works by inhibiting the action of DNA gyrase, preventing the bacterial cells from dividing and multiplying. This causes stress within the bacterial cell, ultimately leading to bacterial cell death.

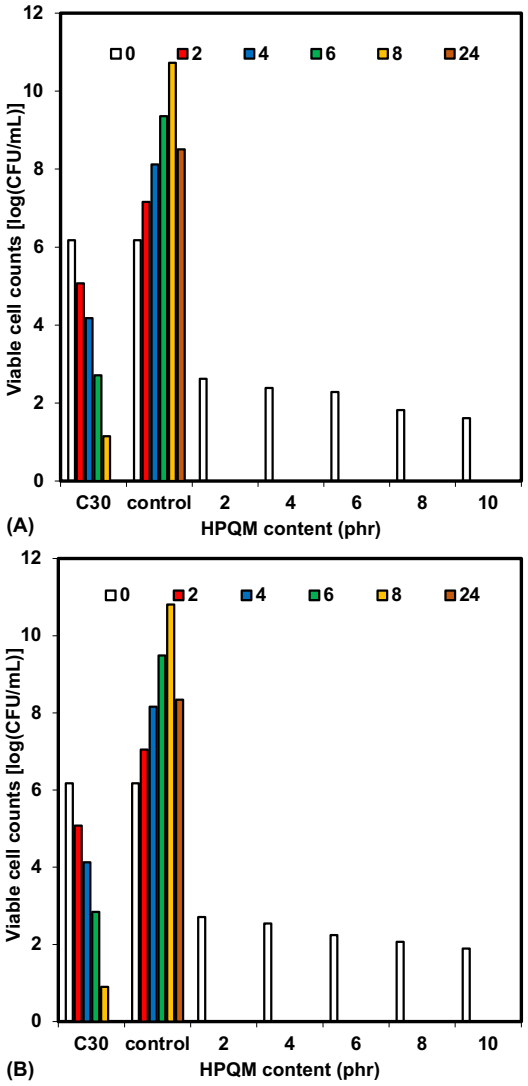

Fig. 4 Viable cell counts against *E. coli* (A) and *S. aureus* (B) of Chloramphenicol and the NR latex foam samples prepared at various HPQM content.

The quantitative antibacterial efficiency test of NR foams containing HPQM was observed. It was reported in terms of viable cell counts and percent survival over the contact times. Fig. 4 shows the viable cell counts of both *E. coli* (Fig. 4A) and *S. aureus* (Fig. 4B) over the contact times at 0–24 h. The results indicate a significant reduction in bacterial count in the NR latex foams containing HPQM. Notably, no surviving bacteria were detected from the 2nd hour of contact time where only 2 phr of HPQM is sufficient to gain 100% reduction. This can be clearly seen from the calculated percent reduction over the contact times summarized in Table 3. Comparing *E. coli* and *S. aureus*, *S. aureus* exhibited the faster reduction in bacterial count. Moreover, the NR latex containing HPQM can kill the bacteria earlier

Table 3 Percent reduction of *E. coli* and *S. aureus* against the Chloramphenicol and the NR latex foam samples prepared at various HPQM content.

HPQM content (phr)	Percent reduction of *E. coli* (%)					
	0 h	2 h	4 h	6 h	8 h	24 h
Chloramphenical (C30)	0.00	17.80	33.17	54.05	85.44	100.00
Control	0.00	+14.08	+32.04	+53.56	+74.92	+34.95
2	56.15	100.00	100.00	100.00	100.00	100.00
4	58.90	100.00	100.00	100.00	100.00	100.00
6	63.75	100.00	100.00	100.00	100.00	100.00
8	66.50	100.00	100.00	100.00	100.00	100.00
10	69.42	100.00	100.00	100.00	100.00	100.00
HPQM content (phr)	Percent reduction of *S. aureus* (%)					
	0 h	2 h	4 h	6 h	8 h	24 h
Chloramphenical (C30)	0.00	17.96	32.36	56.15	81.39	100.00
Control	0.00	+15.86	+31.39	+51.46	+73.62	+37.70
2	57.61	100.00	100.00	100.00	100.00	100.00
4	61.33	100.00	100.00	100.00	100.00	100.00
6	63.11	100.00	100.00	100.00	100.00	100.00
8	70.55	100.00	100.00	100.00	100.00	100.00
10	73.95	100.00	100.00	100.00	100.00	100.00

+ symbol shows an increase in bacterial count.

Table 4 Physical characteristics of the NR latex foam samples prepared at various HPQM content

HPQM content (phr)	Density (g/cm^3)	Compression set (%)	Compressive stress at 25% deformation (kPa)
0	0.15 ± 0.00	14.75 ± 0.61	9.85 ± 0.18
2	0.16 ± 0.00	13.90 ± 0.93	11.93 ± 0.08
4	0.17 ± 0.00	14.24 ± 0.54	12.92 ± 0.28
6	0.17 ± 0.00	26.67 ± 0.58	13.00 ± 0.22
8	0.19 ± 0.01	32.95 ± 0.27	15.53 ± 0.85
10	0.19 ± 0.00	44.32 ± 1.40	19.97 ± 1.24

than that of Chloramphenicol (C30), which kills the bacteria completely after 24 h of contact time. This is a good indication that HPQM effectively offers a great option to be used in NR latex foam. Considering from both qualitative and quantitative analyses, it can be concluded that the use of HPQM at only 2 phr is sufficient to gain effective antibacterial activities in the NR latex foams.

Physical properties

Table 4 presents the foam density, compression set, and compressive stress at 25% deformation. It was observed that the foam density of the NR latex foams increased with the HPQM content. This is because HPQM exists in solid form after being dried through vulcanization. When HPQM reverts to its solid state, similar to the principle of adding fillers in conventional rubber foam formulations. The addition of solid particles increases the mass of the foam while maintaining the same foaming volume [17, 18]. As a result, the foam density, which is the mass per unit volume, increases as the HPQM content increases. As for the compression set, HPQM did affect the set property of the NR latex foam. Compression set indicates the ability of the sample to recover after compression [19, 20]. A lower compression set exhibits a higher ability for the sample to recover. Higher compression set was found over the HPQM content. HPQM is solid after vulcanization. It reduced the rubber phase when added to the foam. The set property relates to the crosslink density in the rubber phase. Reducing the rubber phase may reduce the crosslinking of the specimen. This may influence the ability of the rubber to recover. When the sponge is compressed for an extended period, the HPQM particles move closer together, preventing full recovery. Notably, at HPQM concentrations between 4–10 phr, the compression set increases rapidly due to increased stiffness, making it more prone to permanent deformation after compression. Furthermore, the compressive stress also increased with the addition of HPQM. As the HPQM is stiff after vulcanization, it can help to resist the compression force when applied to the sample. This leads to an increase in hardness and its ability to withstand compression [21, 22].

CONCLUSION

The idea of this study was to prepare the antibacterial NR latex foam. The antibacterial agent used in this work was based on the quinoline derivative, namely HPQM. The bacteria used in the test were divided into Gram-positive bacteria, *S. aureus*, and Gram-negative bacteria, *E. coli*. Adding the HPQM content increased the inhibition zone of bacterial growth against *S. aureus* and *E. coli*. HPQM greatly influenced the physical properties of the NR latex foam, increasing foam density, compression set, and compression-deflection. This

is simply due to its hard phase after vulcanization. The incorporation of HPQM at just 2 phr, with a minimum contact time of 2 h, is sufficient to achieve a strong antibacterial effect while causing only minimal changes to the physical properties of the NR latex foam. The use of HPQM in NR foam can be a piece of resourceful information for preparing the antibacterial NR latex foam.

Acknowledgements: The authors would like to thank the Rubber Innovation Research Institute, Prince of Songkla University, for the Invention Development Fund through Grant No. SAT6201085S.

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