Research Progress of Common Organic Guanidine Antibacterial Agents

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Abstract

This study aims to investigate the antimicrobial properties of organic guanidine because of their strong alkalinity, high stability, low toxicity, and useful biological activity. By referring to the relevant domestic and foreign literature, the physical and chemical properties, applications, preparation methods, germicidal efficacy, and germicidal mechanism of guanidine compounds commonly used in alkyl guanidine, aryl guanidine, and polymerized guanidine with antibacterial properties were briefly analyzed and summarized. It has been found that dodecylguanidine acetate in long-chain alkyl aminoguanidine, iminoctadine in long-chain alkyl biguanide, chlorhexidine in aryl guanidine, polyhexamethylene biguanide hydrochloride(PHMB) and polyhexamethylene guanidine hydrochloride(PHMG) in polymerized guanidine all had killing or inhibiting effects on bacteria, fungi, and viruses. Guanidine compounds with broad-spectrum and high-efficiency bactericidal effect can not only be used as antibacterial agents alone, but also can be introduced into polymers to prepare materials with antibacterial properties, and the development prospect of organic guanidine antibacterial coatings has prospected.

Keywords: Organic Guanidine, Alkyl Guanidine, Aryl Guanidine, Polymerized Guanidine, Physical And Chemical Properties, Applications, Preparation Method, Germicidal Mechanism, Germicidal Efficacy.

1. Introduction

With the increasing awareness of health care, people pay more and more attention to low toxicity and high efficiency bactericides. The commonly used antimicrobial agents are mainly divided into three categories: natural antimicrobial agents, inorganic antimicrobial agents and organic antimicrobial agents. Natural antibacterial agents are mainly extracts from natural plants, which are difficult to be popularized due to the limitation of resources; The bactericidal action of inorganic antibacterial agents is slow; Organic antibacterial agents mainly include aldehydes, phenols, alcohols, peroxides, guanidine, ethers, etc., but except guanidine, most of them have toxic side effects, are harmful to human body, have poor heat resistance, are easy to decompose and have odour, while guanidine antibacterial agents have no carcinogenicity and teratogenicity, tasteless, non-irritant, high temperature resistance, good antibacterial performance and long-lasting antibacterial effect [1]. Organic guanidine antibacterial agents have been widely valued at home and abroad because of their good bactericidal effect, easy preparation and low toxicity, and have broad prospects for development.

As early as 1861, Streeker discovered guanidine(CH_5N_3), a nitrogen-containing organic compound, with the structural formula shown in Fig. 1. From the structural point of view, guanidine is also called "imine (ammonia) urea". Guanidine is mainly found in natural products such as protein, nucleic acid, streptomycin and many other plants such as beet, rice husk, mushroom and beans. There are also traces of guanidine in human and animals bodies and some diseases cause the increase of guanidine in blood or urine [2]. Guanidine is a colorless and hygroscopic crystal, dissolving easily in water and alcohol. Its melting point is about 50 ℃, and when it is heated to 160 ℃, it will decompose and release nitrogen to form melamine. Its hydrate is a monobasic organic base

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equivalent to NaOH. This remarkable alkalinity is due to the formation of stable cations after the acceptance of protons by guanidine, and guanidine ions can be combined with acid ions to form guanidine salt [3]. It is difficult to separate free guanidine because it is easy to absorb carbon dioxide in the air and generate guanidine carbonate. So the general commodity is its salt, such as guanidine hydrochloride, guanidine nitrate, guanidine carbonate, guanidine phosphate, guanidine sulfate, guanidine stearate, etc. [4].

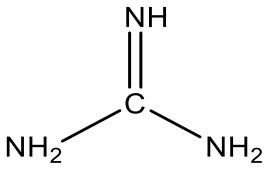


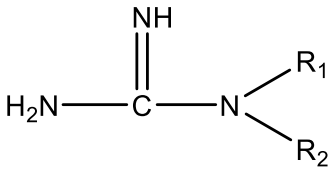
Fig. 1 Structural formula of guanidine.

The removal of a hydrogen atom from a guanidine molecule is called guanidine group. Guanidine salt is an important component of guanidino compounds and can be derived into a wide range of guanidine derivatives. These derivatives have good antibacterial properties, and some of them have gradually attracted people's attention as a non-toxic and efficient broad-spectrum antibacterial agent. In recent years, with the in-depth research of these compounds, organic guanidine antibacterial agents have been widely used in medical disinfection, agricultural product protection, food and daily necessities preservatives and other aspects. It can be seen from many literatures that there are mainly three kinds of antibacterial guanidine compounds in common use: alkyl guanidine, aryl guanidine and polymerized guanidine. At present, dodecylguanidine acetate and iminoctadine in long-chain alkyl guanidine, chlorhexidine in aryl guanidine, polyhexamethylene guanidine hydrochloride and polyhexamethylene biguanide hydrochloride in polymerized guanidine are widely used as antimicrobial agents. However, most of the literature only studied one of the antibacterial properties or synthesis methods. In this paper, the commonly used guanidine antibacterial agents are classified and summarized, and their physical and chemical properties, applications, preparation methods, sterilization mechanism are briefly summed. Finally, the development prospect of organic guanidine antibacterial coatings is prospected.

2. Common organic guanidine antibacterial agents

2.1. Long chain alkyl guanidine

Alkyl monoguanidine and biguanide have killing or inhibiting effects on microorganisms, such as bacteria, fungi and viruses, and have certain biological activities. Horst [5] et al. found that the alkyl guanidine derivatives with molecular formula as shown in Fig. 2 are good antibacterial agents, especially for controlling white stalk disease of rape. Resnick [6] research shows that the alkyl guanidine derivatives with molecular formula shown in Fig. 3 are all good antibacterial agents, which are widely used in crops, especially for controlling black rust of black and white spotted legumes.



R_1 represents H, alkyl, R_2 represents H, alkyl, C (NH) NH_2, $CONH_2$, C (S) NH_2

Fig.2 General formula 1 of alkyl guanidine with antibacterial activity.

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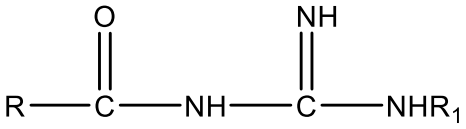

R represents C_{4-22} alkyl, R_1 represents H, C_{1-4} alkyl

Fig. 3 General formula 2 of alkyl guanidine with antibacterial activity.

Alkyl guanidine with biological activity is often used as agricultural bactericide and disinfectant. Because of being made into the form of salt, alkyl guanidine bactericide is easy to dissolve in water and easy to use. At the same time, due to its broad-spectrum antibacterial properties, low toxicity, and its more and more extensive range of sterilization, so the biologically active alkyl guanidine is not only used for surface disinfection of hospitals, food and alcohol beverage industry disinfection, but also for sterilization of fiber paper, daily necessities such as towels, sweaters, masks, etc. [7].

2.1.1. Dodecylguanidine acetate (alkyl monoguanidine)

2.1.1.1. Physical and chemical properties and applications

Dodecylguanidine acetate, also known as Dodine, has a molecular formula of $C_{15}H_{33}O_2N_3$. The pure product is a colorless crystal with a melting point of 136 ℃ and slightly soluble in water. It was initially used as a pesticide in some countries, and it was largely used in fruit trees and crops to prevent leaf curling, fruit scabbing and insects, etc. With the in-depth study of this compound, its use in daily life is more and more extensive. It can also be used as a disinfectant, which can effectively kill bacteria, fungi and algae. For example, it can inhibit the growth of *Staphylococcus aureus* by adding pulp, and also be used for industrial water treatment and disinfection and sterilization of women's and children's products and food packaging paper [8].

2.1.1.2. Preparation method

Dodecylguanidine acetate is usually synthesized from dodecylamine, cyanamide and acetic acid. Dodecylamine undergoes nucleophilic addition reaction with cyanamide under acid catalysis, and then neutralizes with acid to form corresponding guanidine salt [8]. The reaction principle is shown in Fig. 4.

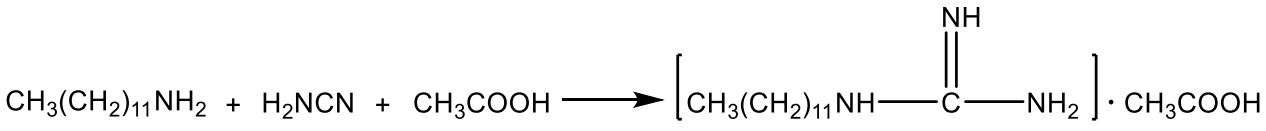

Fig. 4 Reaction principle of dodecylguanidine acetate.

2.1.1.3. Sterilization effect

Dodecylguanidine salt is a fungicide, which is very effective in killing pathogenic bacteria of crops and animals, and also an effective algal growth inhibitor [9]. The studies of Song [10] showed that dodecylguanidine salt has a strong antibacterial activity, with the antibacterial rate of 100% against *Staphylococcus aureus and Escherichia coli* at 25 mg/L and 99.5% against *Candida albicans* at 100 mg/L.

2.1.2. iminoctadine (alkyl biguanide)

2.1.2.1. Physical and chemical properties and applications

The chemical name of iminoctadine is bis-(8-guanidino-octyl) amine acetate, the molecular formula is

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$C_{24}H_{53}O_6N_7$, the pure product is white crystal powder, and the melting point is 138~141℃. It is easily soluble in water with a solubility of 76.4 g/100 ml, slightly soluble in ethanol with a solubility of 11.7 g/100 ml, and stable in neutral or acidic medium [11,12]. Because of its non-toxic, odorless, non-irritating, high-temperature resistant and good antibacterial properties, people pay more and more attention to this compound. It can be used as a new broad-spectrum bactericide for agriculture and horticulture [13], and it has a high growth inhibition activity to the main fungi for agriculture and horticulture. So it can be used not only to control various diseases of fruit trees and vegetables, but also to control diseases of citrus, apple, grape and other crops during the storage period, especially to inhibit Penicillium. It is the promising and promotional value pesticide variety in the prevention and control of fruit diseases during the storage period in China [14].

2.1.2.2. Preparation method

At present, iminoctadine is mainly prepared from O-methylisourea acetate and dioctyl triamine in China. This method is easy to operate, with high product purity and good yield [15]. The reaction principle is shown in Fig. 5.

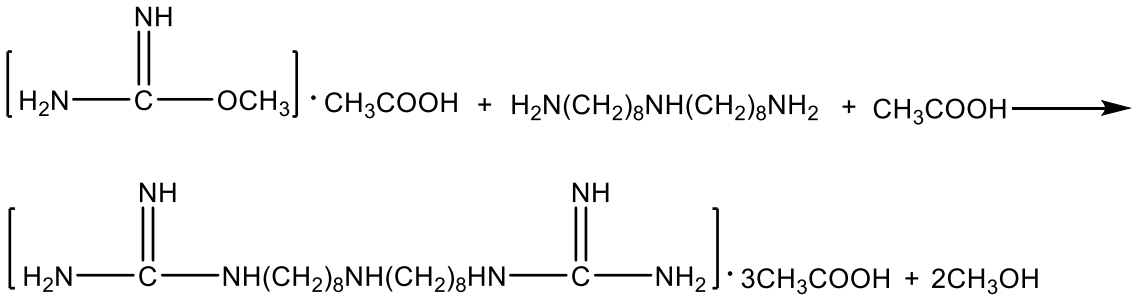

Fig. 5 Reaction principle of iminoctadine.

2.1.2.3. Sterilization effect

Zou[15] research showed that iminoctadine had strong inhibitory effect on a variety of plant pathogens, including *Deuteromycotina* (Citrus penicillium), *Phoma* (Phomopsis asparagi), *Coniothyrium* (Coniothyrium diplodiella), *Colletotrichum* (Banana colletotrichum, Cucumber colletotrichum), *Alternaria* (Alternaria mali Roberts, Alternaria kiknchiana Tanaka), and *Sclerotinia* (Sclerotinia sclerotiorum) in ascomycotina, etc. Under the low concentration of 6.25 μg/ml, iminoctadine could inhibit the Phomopsis asparagi, Coniothyrium diplodiella and Banana colletotrichum by 100%. At this concentration, the inhibition rates of Citrus penicillium, Alternaria mali Roberts and Alternaria kiknchiana Tanaka were 84.0%, 95.0% and 98.0%, respectively. This drug also had moderate virulence to Alternaria solani, Gibberella zeae, Aspergillus oryzae and Physalospora piricola. The inhibition rate of iminoctadine to these pathogens was similar with the decrease of the concentration, which indicated that it was possible to achieve satisfactory effect by increasing the concentration properly in the field application.

2.2. Aryl guanidine

2.2.1. Chlorhexidine

The chemical name of chlorhexidine is 1,6-(dichlorobenzene-biguanide) hexane, and the molecular formula is $C_{22}H_{30}N_{10}Cl_2$. Because of the existence of imino group in chlorhexidine molecule, chlorhexidine itself is an alkaline substance, and because it is difficult to ionize, it is difficult to dissolve in water. But it can form chlorhexidine salt with inorganic acid such as acetic acid, hydrochloric acid, or organic acid such as gluconic acid

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, which dissolved in water and exist stably. Chlorhexidine is usually combined with hydrochloric acid, acetic acid and gluconic acid to form salts which are Chlorhexidine hydrochloride, Chlorhexidine acetate(CHA) and Chlorhexidine gluconate(CHG) respectively. The solubility of Chlorhexidine hydrochloride in water is only 0.06%, so its preparation is mainly ointment which has been eliminated in real life [16]. In China, CHA is commonly used [17] in clinical practice, but in Europe and America, CHG is mainly used [18,19.20,21]. In 1954, chlorhexidine was synthesized and applied to the disinfection of hands, skin and mucous membrane. Due to its advantages of good bactericidal effect, stable performance, small toxicity to human body and convenient use, chlorhexidine has been occupying a place in the field of disinfection [22].

2.2.1.1. Physical and chemical properties and applications

The chemical name of Chlorhexidine acetate (CHA) [23] is 1,6-bis-(p-chlorophenyl-biguanide) n-hexane diacetate. It is white crystal, tasteless and the decomposition temperature is 260℃. At 20℃, the solubility of CHA in water is 1.8% and it is also soluble in ethanol. It has small irritation, wide range of sterilization and stable performance. In the early 1970s, CHA began to be used for skin disinfection before injection, hand soaking disinfection of the surgical staff and immersion disinfection of surgical instruments [16] in China. Some research scholars (Ling et al. [24], killoy WJ et al. [25]) have shown that CHA could not only kill *Staphylococcus aureus*, *Gram positive bacteria*, *Escherichia coli* and molds, but also inhibit some fungi and bacterial spores. It is applicable to disinfection of hospitals, families, hotels, offices, skin, etc., and it also has antibacterial and mildewproof effect on plastic rubber products and food.

The chemical name of Chlorhexidine gluconate (CHG) [23] is 1,6-bis-(p-chlorophenyl-biguanide) n-hexane digluconate. It is a white or light yellow crystal which may decompose under light, so it must be kept away from light. Its solubility in water is 20%, and it can also dissolve in glycerin, alcohol, etc. Zeng [26] et al. analyzed that CHG has the largest solubility in water, mainly because gluconic acid roots can increase the critical micelle solubility in water compared with acetic acid roots, thus greatly increasing its solubility in water. CHG has a broad-spectrum of inhibition on bacteria [23], and it is safe and easy to use. It has a strong killing effect on *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. CHG has been used as a safety disinfectant and a bactericide for Chlamydia for a long time, and it can be used in food, medical equipment, plastics, rubber, paint and other fields.

2.2.1.2. Preparation method

Taking chlorhexidine acetate as an example, the preparation method of chlorhexidine is introduced. It can be seen from the literature that the better method to prepare chlorhexidine is p-chloroaniline condensation [27]. In this method, p-chlorophenylcyanguanidine is synthesized from p-chloroaniline acetate by condensation with sodium dicyandiamide, then with hexanediamine acetate to CHA . The reaction principle is shown in Fig. 6.

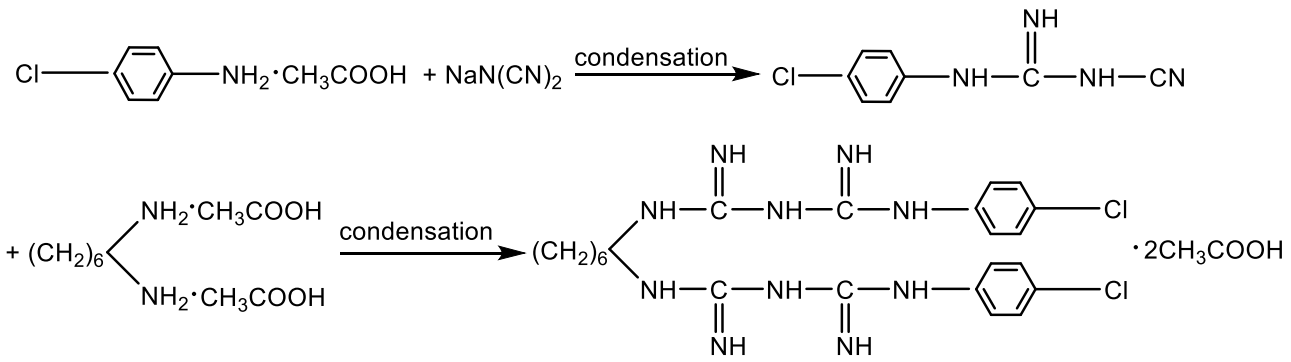

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Fig. 6 Reaction principle of CHA.

2.2.1.3. Sterilization effect

Foreign researchers [28] compared CHG with CHA in clinical practice and found that CHG was more effective, milder and less adverse reactions in skin disinfection. Lin [16] of chlorhexidine rapid sterilization experiments showed that after the new formulation of chlorhexidine killed *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* for 30 seconds, their logarithm of bacterial colony number decreased more than 5 Lg CFU/ml. Therefore the bactericidal ability of chlorhexidine was the same as that of Anerdian Ⅰ type, Anerdian Ⅲ type, Dot Er Kang and 10% iodine, while its bactericidal ability to *Staphylococcus aureus* was better than that of Anerdian Ⅰ type and Dot Er Kang. As for the clinical long-term antimicrobial property of chlorhexidine is not only related to the concentration of chlorhexidine, but also related to the use-pattern of it. Charles [29] reported that after using 4% CHG hand sanitizer and running water to wash the arm, the bacteriostatic effect was still obvious after 3 hours. Zeng [30] et al. used 1% anhydrous CHG hand sanitizer for surgical hand washing and found that the bacteriostatic effect of CHG could be maintained for more than 6 hours. Liu [31] et al. used 2% CHG as oral root canal flushing agent and found that the bactericidal effect could be maintained for more than 72 hours.

2.3. Polymeric guanidine

Among the polymerized guanidine, polyhexamethylene guanidine has better germicidal efficacy. Polyhexamethylene guanidine has biguanide salt and monoguanidine salt, but polyhexamethylene biguanide hydrochloride(PHMB) and polyhexamethylene guanidine hydrochloride(PHMG) are commonly used. In recent years, it has been reported as a new generation of guanidine disinfectant at home and abroad. Its high polymer structure makes it less toxic and and more effective in sterilization, so it has been widely used in health care, family life, food industry, animal husbandry and other fields. Through more and more scholars' research on the properties and efficacy of guanidine disinfectants, their application has been continuously developed [22].

2.3.1. Polyhexamethylene biguanide hydrochloride (PHMB)

2.3.1.1. Physical and chemical properties and applications

The molecular formula of PHMB is $(C_8H_{17}N_5)_n \cdot xHCl$,n=12~16, and the average molecular weight is 1100~1800. It has no unpleasant odour and it is a colorless and transparent liquid with the content of 19%~21%, pH4.0~6.0, relative density of 1.05 (25℃), and boiling point of 102 ℃. PHMB is a kind of guanidine disinfectant which has become a widely used cationic disinfectant in the world because of its excellent antibacterial activity, stable chemical properties, low toxicity and reasonable price [32]. PHMB can kill *Gram-positive bacteria*, *Gram-negative bacteria*, fungi, yeast etc., so it has been widely used for sterilization and antisepsis in medicine. As a medical product, it is used for disinfection of contact lens fluid, eye drops and surgical operations. Due to the strong tolerance of eyes to PHMB, PHMB can be used to treat Acanthamoeba keratitis, prevent and treat other eye diseases. At the same time, PHMB is also widely used in cosmetics, personal care products, textiles, food industry, etc. With the development of science and technology, PHMB also has many new applications. For example, it can be used as flocculant, bactericide and algaecide in swimming pool and industrial water treatment, bactericide in oil extraction and water injection , and can clean and remove harmful substances on glassware and other hard surfaces [33].

2.3.1.2. Preparation method

PHMB is synthesized from 1,6-bis-(cyano-guanidino) hexane and hexanediamine hydrochloride as raw materials by solution polymerization [34]. Compared with other methods, this method has no production of small

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molecular products that are difficult to remove, and has a lower cost, so it has a broad prospect of industrial preparation [35]. The reaction principle is shown in Fig. 7.

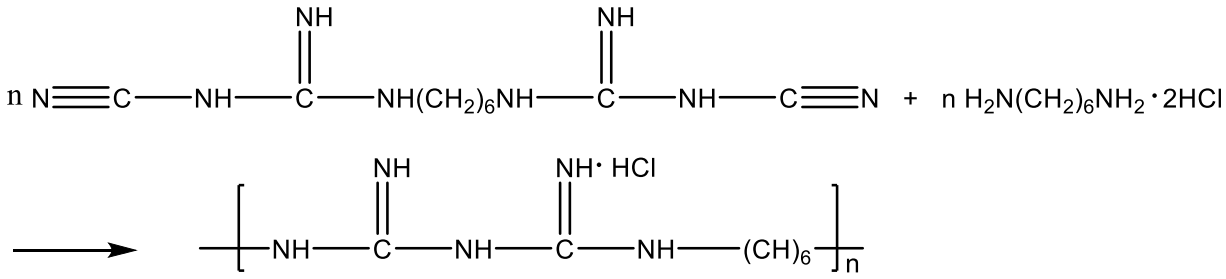

Fig. 7 Reaction principle of PHMB.

2.3.1.3. Sterilization effect

The bactericidal effect of PHMB is related to its molecular weight. The larger the molecular weight is, the better the sterilization effect is and the longer the action time is. At present, the molecular weight of PHMB used by most enterprises is about 2500 [34]. PHMB has a good effect on the *Gram-negative bacteria* which are difficult to be treated by general bactericides, and it can also achieve a good bactericidal effect even at low concentration and will not make bacteria produce drug resistance at the same time; In addition, the bactericidal speed is also very fast; After sterilization, because PHMB cation is adsorbed on the surface of the article, it can inhibit the growth of bacteria for a long time [35]. Cui [22] research showed that the disinfectant containing 100 mg/L PHMB acted on *Staphylococcus aureus* and *Escherichia coli* in the suspension for 3 min, and the average killing logarithm was greater than 5.00; The disinfectant containing 1000 mg/L PHMB acted on *Candida albicans* in the suspension for 20 min, and the average killing logarithm was greater than 4.00; Moreover, its bactericidal effect increased with the increase of disinfectant concentration and action time.

2.3.2. Polyhexamethylene guanidine hydrochloride (PHMG)

Some countries, such as Russia and Eastern Europe, have found that polyhexamethylene monoguanidine has stronger bactericidal activity and better performance than polyhexamethylene biguanide [36]. It is a new generation of bactericidal disinfectant. Compared with chlorhexidine gluconate, polyhexamethylene guanidine disinfectant has more significant bactericidal ability and longer time effect on bacteria and viruses.

2.3.2.1. Physical and chemical properties and applications

The molecular formula of PHMG is $(C_7H_{16}N_3Cl)_n$, which is a white or yellowish amorphous powder with bitter taste and melting point of 181℃~183℃, soluble in water and alcohol easily, and almost insoluble in acetone, benzene and ether. After dissolving in water, it is colorless to light yellow liquid, and the water solution is non combustible, non explosive, no special smell, no corrosion to metal, no bleaching effect on fabric. The decomposition temperature of PHMG is over 400 ℃, so it's very stable [22]. PHMG is a new type of environment-friendly guanidine disinfectant, which has strong bactericidal and bacteriostatic properties, broad spectrum and high efficiency of sterilization, long validity period, non-toxic side effects, non corrosiveness and irritation, and is safe to use, degradable in natural environment, environmentally friendly and pollution-free. Therefore, PHMG has been widely used in many fields. For example, PHMG can be used as disinfectant and bactericide on the surface of objects, mildew inhibitor for daily necessities, wet tissue disinfectant, antiseptic agent for food processing, building materials and oil exploitation, algaecide and bactericide for aquaculture, swimming pool and lake water, disinfectant for livestock farm, fabric surface treatment agent, sewage treatment flocculant,

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polymer antibacterial modifier, etc. [37].

2.3.2.2. Preparation method

PHMG is mostly obtained by high temperature polycondensation of guanidine hydrochloride with hexanediamine [38]. The reaction principle is shown in Fig. 8.

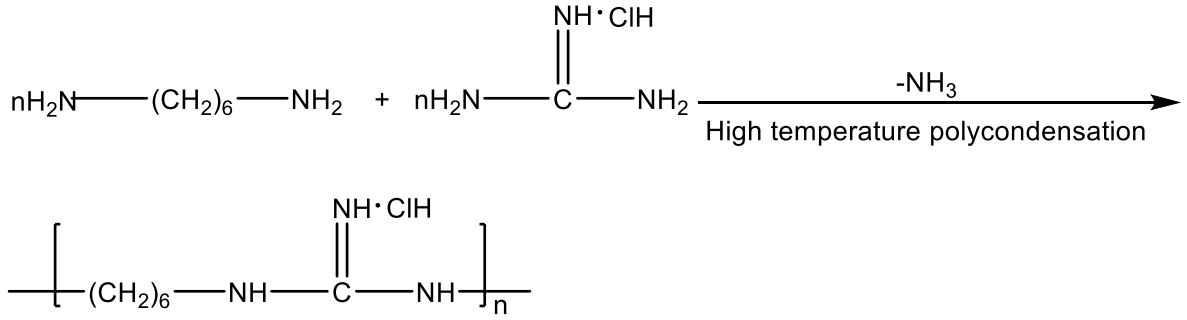

Fig. 8 Reaction principle of PHMG.

2.3.2.3. Sterilization effect

According to the method of the American Association of official analytical chemists and the method recommended by the Canadian general standards committee, some researchers tested the germicidal efficacy of PHMG. PHMG with a concentration of 10~1000 mg/L was applied to act on *Staphylococcus aureus*, *Salmonella cholerae* and *Pseudomonas aeruginosa* for 0.5~10 min and the phenol coefficient values were 7.5, 6.1 and 5, respectively. No matter diluted with distilled water, tap water or hard water, PHMG acted on Methicillin-resistant *Staphylococcus aureus* and *Escherichia coli* for 1.5 min, and the minimum bactericidal concentrations were 400 mg/L and 50 mg/L, respectively.

In addition, it has been proved that the average killing rate is 99.90% for *Staphylococcus aureus*, 99.92% for *Escherichia coli* and 99.92% for *Pseudomonas aeruginosa* with the concentration of 500 mg/L PHMG aqueous solution for 10 min. HIV can be completely killed by the action of PHMG solution with a concentration of 1000 mg/L for 5 min [15].

2.4. The bactericidal mechanism of organic guanidine antibacterial agents

The cell membrane of bacteria is mainly composed of phospholipid bimolecular layer and protein, and the phospholipid functional group in the outer layer of cell membrane is negatively charged. After guanidine salt is dissolved in water [14], guanidine group is positively charged and easily adsorbed on the surface of the negatively charged microorganisms, so as to restrict the free activities of microorganisms, inhibit their splitting function and make them lose the ability of division and reproduction. In addition, polymerized guanidine salt can concentrate multiple guanidine groups on the same molecule, which strengthens this effect. Under the action of electric field gravity, the negative charge distribution on bacterial cell wall and cell membrane is uneven, resulting in bacterial deformation and physical rupture, making low molecular weight cytoplasm flow out, and finally killing the bacteria [16]. In the solution system, guanidine salt can also penetrate into the body of microorganisms, inhibit the enzyme system of bacteria, especially dehydrogenase and oxidase, so as to cause metabolic disorder. At high concentration, guanidine salt can also make the cytoplasm gather into blocks, condense and denature, leading to bacterial death [39]. In addition, the film formed by the polymer will block the respiration channel of the microorganism and make the microorganisms suffocate rapidly and die, thus achieving the bactericidal effect [40].

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3. Conclusion

According to the above analysis and overview, we can see that the dodecylguanidine acetate in long-chain alkyl monoguanidine, the iminoctadine in long-chain alkyl biguanide, the chlorhexidine in aryl guanidine, and the PHMB and the PHMG in the polymerized guanidine can kill or inhibit bacteria, fungi, viruses, etc. At the same time, due to their low toxicity and wide range of sterilization, they have been widely used In agriculture, horticulture, medicine, industry, daily chemicals, food and other aspects of sterilization and antisepsis.

4. Outlook

Guanidine compounds have good antibacterial properties and biological activity. In order to further improve the stability of low molecular weight guanidine, the durability of antibacterial effect, and further expand its application scope, it is a new development direction to introduce guanidine salt and its derivatives into cellulose, chitosan, resin and other polymers to prepare coatings with antibacterial properties. The obtained guanidine salt high molecular antibacterial agent has strong antibacterial activity, safety to human body and high water solubility, which can make it have a broader application prospect.

Abbreviations

CHA: Chlorhexidine acetate; CHG: Chlorhexidine gluconate; CFU: Colony forming unit; PHMB: polyhexamethylene biguanide hydrochloride; PHMG: Polyhexamethylene guanidine hydrochloride.

Conflicts of Interest: The authors declare no conflict of interest.

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