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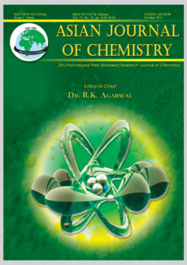

Preparation of Silica Gel Grafting Bisquarternary Phosphonium and its Antibacterial Activities

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Using the activate silica gel as a carrier reacted with (3-chloropropyl)trimethoxy silane, the product was early or late reacted with 1,3-*bis*(diphenylphosphino)propane, 1-chlorotetradecane to give the bisquarternary phosphonium salt water-insoluble antibacterial material. The effect of the reaction temperature, the reaction time and the reaction solvent were studied by means of the orthogonal design method and the single-factor experiment. The optimum conditions are: reaction temperature 150 ºC, reaction time 48 h and the reaction solvent is *o*-dichlorobenzene. The product has grafted with 0.68 mmol/g phosphonium group and 0.15 mmol/g long alkyl. 1,3-*Bis*(diphenylphosphino)propane and 1-chlorotetradecane have been early or late grafted to silica gel, characterized by FTIR and TG. The bactericidal rate of the bactericide material was up to 99.9 % using amount of 20 mg/mL in 45 min. The antimicrobial activity of the bactericidal material is still up to 98.7 %, after 5 times repeated usage.

Key Words: Silica gel, Bisquarternary phosphonium, Water-insoluble, Antibacterial material.

INTRODUCTION

As the requirements of environmental protection and sustainable development, more and more people are trying to develop many novel kinds of sterilizations to improve the industrial water reusable rate. These novel solid water insoluble bactericides have better bactericidal effects and can be reused after bactericidal regeneration and do not make the environment secondary polluted after usage. For example, many authors have developed the water insoluble bactericides, which are made by grafting the small and strong bactericidal groups on the solid carrier. The advantages of the water insoluble sterilization are: no secondary pollutants, easily recovery and easily recycled[1]. Water insoluble quarternary phosphonium salt is one of the bactericides. It is made by chemical bonding reaction between carrier and quarternary phosphonium salt. The quarternary phosphonium group has many advantages, such as, high bactericidal efficient, broad bactericidal range, low toxicity, low dose and the wide range of pH adaptability[2]. It has been a hotspot during these years.

The reports on water insoluble quarternary phosphonium salt area are scanty. Akihiko *et al.*[3-6] have reported poly quarternary phosphonium cationic water insoluble sterilization and have concluded that quarternary phosphonium salt has more bactericidal effect than quaternary ammonium salt with the same structure through comparison experiments. Adriana[7] has reported the bactericidal of chloromethylated bead carrying different tri-displaced phosphine and concluded tri-ethyl phosphine is the best. Chen *et al.*[8-11] have prepared high bactericidal water insoluble sterilizations by using alkylamine to bond chloromethyl resin and quarternary phosphonium salt. Li *et al.*[12] have synthesized novel sterilizations by using chloromethyl resin to graft with different tri-displaced phosphine. The bactericidal ratio is still up 95 % after 5 times reused. Mamoru *et al.*[13] have synthesized a new catalytic agent by using the silica gel to graft triphenylphosphine. Jin *et al.*[14] have made a novel water insoluble sterilization, the bactericidal ratio is 97 % with 50 mg/mL dose and 20 min bactericidal time. Li *et al.*[15] reported a water insoluble bisquarternary ammonium and concluded the dication active center could increase hydrophilic nature and more bactericidal capacity than the single one.

After reviewing the previous works, it is find that they were trying to upgrade the concentration of quarternary phosphonium groups on carrier's specific surface in order to improve bactericidal efficiency. But they were all single quarternary phosphonium, the report of water insoluble bisquarternary phosphonium is very few. In this paper, a kind of water insoluble bisquarternary phosphonium was synthesized by three steps. First, the activated silica gel reacted with (3-chloropropyl)-tri-methoxy-silane to give the chloropropyl silica gel; then, the chloropropyl silica gel was reacted with 1,3-*bis*(diphenylphosphino)propane to give the intermediate product; finally, the intermediate product reacted with long-chain alkyl haloalkane to give the final product. The structure of the product was characterized by FTIR and TG, the bactericidal activity was measured by plate count method.

EXPERIMENTAL

(3-Chloropropyl)-tri-methoxy-silane (AR), 1,3-*bis*(diphenylphosphino)propane (AR), 1-chloro-tetradecane (AR), silica gel (CP, 100-200 mesh), dimethylbenzene (AR), chlorobenzene (AR), *n*-octane (AR), N,N-dimethylformamide (AR), *n*-pentanol (AR), 1,4-dioxane (AR), toluene (AR), *o*-dichlorobenzene (AR).

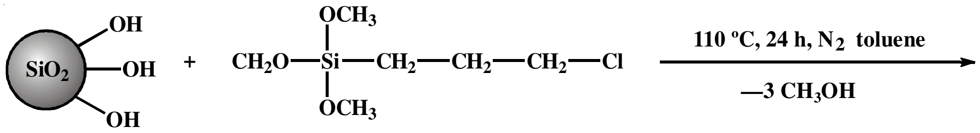
Nicolet 380 FT-IR spectrometer (KBr tablet), thermogravimetry (TG), ZK-82BB Vacuum Drying Oven, XMTA-3001 temperature control meter, electronic balance.

Synthetic routes: (1) The preparation of activated silica gel was performed by the reported method of Jing *et al.*[14]. (2) The preparation of chloropropyl silica gel was prepared by the method of Li *et al.*[15].

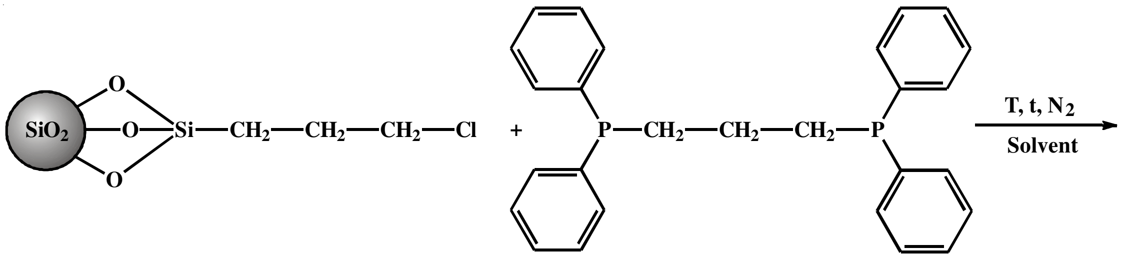
(3) First quarternary phosphonium reaction: A 3-g sample of chloropropyl silica gel weighed accurately by electronic balance, 30 mL solvent and 3-g of 1,3-*bis*(diphenylphosphino)propane were added to a 250 mL round-bottom flask fitted with a reflux condenser, mechanical stirrer, thermometer and previously purged with nitrogen. The mixture was heated in an oil bath at the special temperature and maintained stirring for special time. When the reaction time was running out, stopped heating and began cooling the round-bottom flask to the room temperature. After cooling, the intermediate product were separated by filtration, washed with 1,2-dichloromethane (3 × 10 mL), ethyl ether (3 × 10 mL), ethanol (3 × 10 mL) and dried in vacuum at room temperature for 24 h.

(4) Second quarternary phosphonium reaction: The required amount of 1-chloro-tetradecane mixed in solvent (V: V = 1:1), was added to the intermediate product weighed accurately by electronic balance. The reaction process was at the same condition as above.

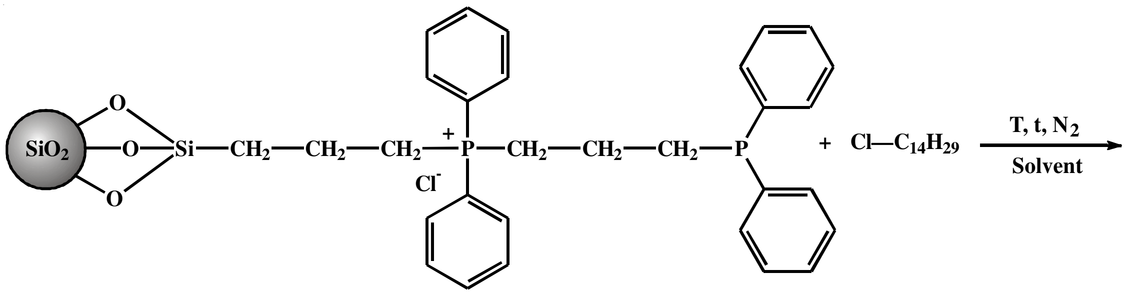
Setp-2: Preparation of chloropropyl silica gel



Setp-3: First quarternary phosphonium reaction



Setp-4: Second quarternary phosphonium reaction



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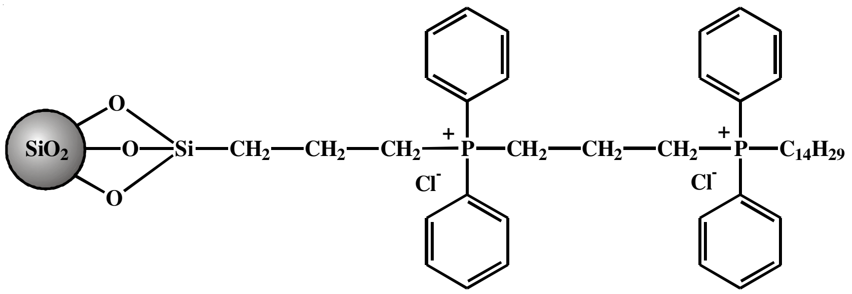

TABLE-1
ORTHOGONAL EXPERIMENT AND THE ITS RESULTS

Exp. No.	Factors			Targets	
	Time (h) A	Temperature (ºC) B	Solvent C	Ratio of P (mmol/g)	Ratio of alkyl (mmol/g)
1	6	90	Dimethylbenzene	0.22	0
2	6	110	Chloro benzene	0.46	0.061
3	6	130	*n*-Octane	0.41	0.033
4	12	90	Chloro benzene	0.22	0
5	12	110	n-Octane	0.39	0.014
6	12	130	Dimethylbenzene	0.53	0.089
7	18	90	*n*-Octane	0.22	0
8	18	110	Dimethylbenzene	0.56	0.103
9	18	130	Chloro benzene	0.64	0.143

Computational formula for grafting ratio: Grafting ratio of quarternary phosphonium groups

$$\text{Ratio of P groups} = \frac{m_1 - m_0}{412.44 \times m_0} \times 1000 \times 2$$

m_1: mass of the first quarternary phosphonium reaction product, m_0: mass of the chloropropyl silica gel.

Grafting ratio of long chain alkyl groups

$$\text{Ratio of } C_nH_{2n+1} = \frac{m_2 - m_1}{232.83 \times m} \times 1000$$

m_2: mass of the final product, m_1: mass of the first quarternary phosphonium reaction product.

RESULTS AND DISCUSSION

Orthogonal experiment: In order to find the best conditions for quarternary phosphonium reaction, the reaction time, the reaction temperature, the reaction solvent were chosen as the most important factors. The experiment was designed by orthogonal experiment to find the basic reaction condition (Table-1).

Table-2 shows the range analysis of the orthogonal experiment.

TABLE-2
RANGE ANALYSIS OF ORTHOGONAL EXPERIMENT

Targets	Factors	k1	k2	k3	Range
Ratio of P (mmol/g)	A	0.360	0.382	0.472	0.112
	B	0.217	0.469	0.527	0.310
	C	0.437	0.438	0.339	0.099
Ratio of alkyl (mmol/g)	A	0.032	0.034	0.082	0.051
	B	0	0.060	0.088	0.088
	C	0.064	0.068	0.011	0.053

From the Table-2, the three factors have the same trend to grafting ratio of quarternary phosphonium groups (abbreviate 'ratio of P') and grafting ratio of long chain alkyl groups (abbreviate 'ratio of alkyl'). That is the ratio of alky increases with the ratio of P. The reaction temperature is most influential fact in the three factors; the other two are nearly equal. The optimum combination is A3B3C2 relative to others.

Single-factor experiment

Selection of solvent: In order to select the best solvent, a lot of frequently-used solvent were attempted as the solvent, the other two factors were chosen temporarily. According to the results of orthogonal experiment, the reaction time was 18 h and the reaction temperature was 130 ºC (Table-3).

TABLE-3
EFFECTS OF SOLVENT ON THE GRAFTING RATIO

Exp. No.	Solvent	Ratio of P (mmol/g)	Ratio of alkyl (mmol/g)
1	N,N-Dimethylformamide	0.55	–
2	*n*-Pentanol	0.58	–
3	Toluene (*)	0.40	0.088
4	1,4-Dioxane (*)	0.33	0.041
5	Chloro-benzene	0.64	0.143
6	*o*-Dichlorobenzene	0.65	0.145

Notation: (*) mean their boiling point are lower than 130 ºC. '–' mean the ratio are approximately equal to zero.

The best solvent is *o*-dichlorobenzene by comparing different solvents in Table-3.

Selection of reaction time: A lot of reaction time were tried, while the solvent was *o*-dichlorobenzene and the reaction temperature was 130 ºC (Table-4).

TABLE-4
EFFECTS OF REACTION TIME ON THE GRAFTING RATIO

Exp. No.	Reaction time (h)	Ratio of P (mmol/g)	Ratio of alkyl (mmol/g)
1	18	0.64	0.145
2	24	0.65	0.146
3	36	0.65	0.147
4	48	0.66	0.148

The ratio of P and alkyl increase slowly with the extension of time. The best reaction time is 48 h in present experiment.

Selection of reaction temperature: A lot of reaction temperatures were tried, while the solvent was *o*-dichlorobenzene and the reaction time was 48 h (Table-5).

TABLE-5
EFFECTS OF REACTION TEMPERATURE
ON THE GRAFTING RATIO

Exp. No.	Temperature (ºC)	Ratio of P (mmol/g)	Ratio of alkyl (mmol/g)
1	130	0.65	0.148
2	150	0.67	0.149
3	170	0.66	0.147
4	180 (B.P.)	0.65	0.139

The ratio of P and alkyl decrease slowly, when the temperature exceeds 150 ºC. The best temperature is 150 ºC.

The optimum reaction conditions for silica gel grafting bisquarternary phosphonium has been selected by concluding Tables 3-5. The solvent is *o*-dichlorobenzene, the reaction time is 48 h and reaction temperature is 150 ºC. The experiment can be repeated at the reaction conditions and the ratio of P is up to 0.68 mmol/g and the ratio of alkyl is up to 0.15 mmol/g.

Characterization: The intermediate product and the final product are characterized by IR (Fig. 1).

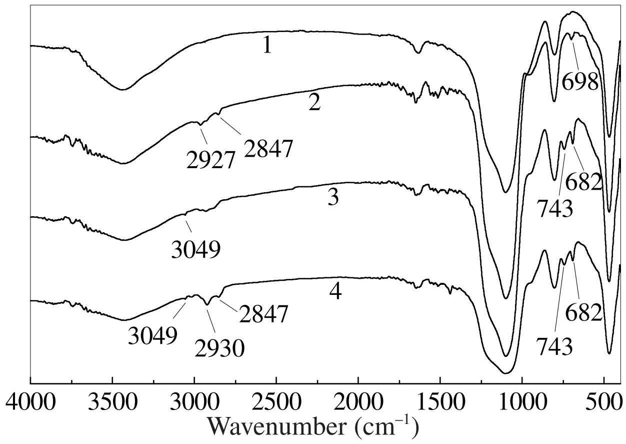

Fig. 1. FTIR spectra of silica gel and intermediate products. (1) activated silica gel, (2) chloropropyl silica gel, (3) the product of the first quarternary phosphonium reaction, (4) final product

The formation of the chloropropyl groups grafted on the activated silica gel is confirmed by 698 cm^{-1} assigned to stretching vibration (C-Cl), 2927 cm^{-1}, 2847 cm^{-1}, assigned to stretching vibration (C-H) and 1650 cm^{-1}-1350 cm^{-1} assigned to bending vibration (C-H) by comparing (2) to (1).

The formation of the 1,3-*bis*(diphenylphosphino)propane grafted on the chloropropyl silica gel is confirmed by 743 cm^{-1}, 682 cm^{-1}, assigned to bending vibration of (C-H) on single replaced benzene, 3049 cm^{-1} assigned to stretching vibration of unsaturated bond on benzene. The weak spectrum of (C-P) is in 1440-1430 and 1120-1100 cm^{-1}, which is flooded with fingerprint region and the vibration of silica gel's skeleton (Si-O-Si).

The formations of the long chain alkyl groups is proved by strong absorption at 2930 and 2847 cm^{-1}, curve 4th, which is the symmetrical and asymmetrical stretching vibration of (C-H).

The intermediate product and the final product are inspected by TG. The results are showed in Figs. 2 and 3.

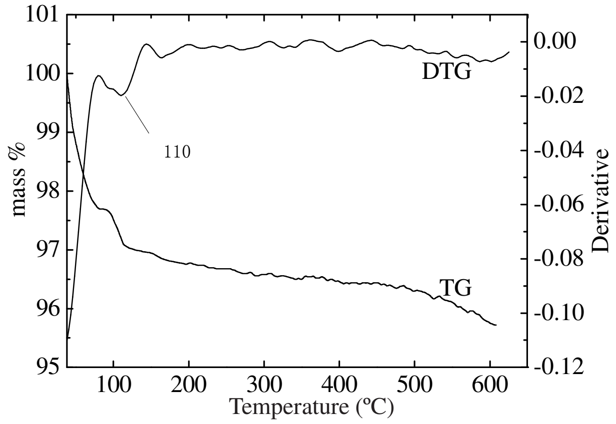

Fig. 2. TG-DTG curves of silica gel

In Fig. 2, a weight loss between 50-150 ºC is due to the desorption of CO_2 and H_2O in the hole of silica gel. Another weight loss above 500 ºC is the decomposition of silica gel's skeleton.

In Fig. 3, four clearly and respectively weight loss at 170, 220, 360 and 500 ºC, are distinguished from Fig. 2. They are assigned respectively to decomposition of long chain alkyl groups, 1,3-*bis*(diphenylphosphino)propane groups, chloropropyl groups and silica gel's skeleton.

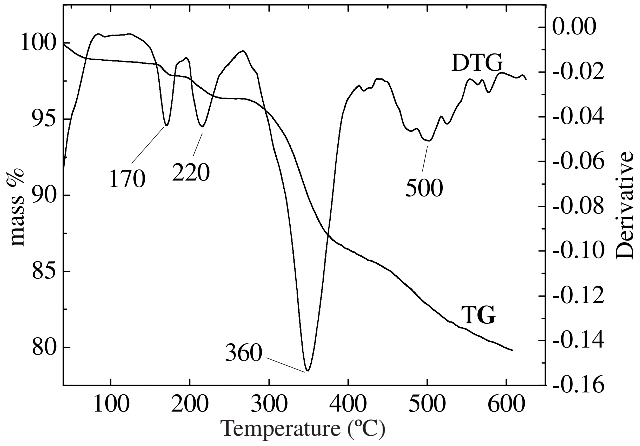

Fig. 3. TG-DTG curves of silica gel grafting bisquaternary phosphonium salt

A conclusion can be made based on Figs. 1-3 that the (3-chloropropyl)-tri-methoxy-silane, the 1,3-*bis*(diphenylphosphino)propane and the 1-chloro-tetradecane are grafted on the silica gel successively.

Bactericidal inspection: In order to inspect the sterilizing rate of the antiseptic, two factors, the adding amount and the sterilizing time were examined. After experiment, the bactericidal activity was regenerated by soaking in 10 % sodium hypochlorite solution.

Study of the adding amount: Add different amount of the antiseptic into 100 mL water, which was enriched previously. The concentration of bacterium was measured after sterilization for 0.5 h with stirring. The original concentration is 1.84 × 10^{7} mL (Table-6).

TABLE-6
EFFECT OF ADDING AMOUNT ON STERILIZING RATE

Adding amount (mg/mL)	Average of bacteria colonies (mL)	Sterilizing rate (%)
1	4.3×10^6	76.6
5	6.0×10^5	96.7
10	7.3×10^4	99.6
15	2.8×10^4	99.8

The more adding amount, the better is sterilizing rate. The antimicrobial rate will exceed 99.9 %, if the adding amount is more than 20 mg/mL. After sterilizing, the bacteria colony concentration will decrease 3 orders of magnitude.

Study of sterilizing time: A sample of 1.5 g antiseptic was added into 100 mL water, measured the concentration of bacterium every 15 min (Table-7). The original concentration is 1.33 × 10^{8} mL.

TABLE-7
EFFECT OF STERILIZING TIME STERILIZING RATE

Sterilizing time (min)	Average of bacteria colonies (mL)	Sterilizing rate (%)
15	7.8×10^6	94.1
30	3.1×10^5	99.8
45	8.0×10^4	99.94
60	1.5×10^5	99.9
75	9.0×10^5	99.3

The sterilizing rate is not better and better as the sterilizing time extension, especially, when it is more than 45 min, the sterilizing rate goes down.

Study of reusability: A sample of 1.5 g antiseptic was added into 100 mL water, measured the concentration of bacterium after 45 min. Regenerate the bactericidal activity by soaking in 10 % sodium hypochlorite solution for 2 h. The solid sterilization were separated by filtration, washed with ethanol (3 × 10 mL) and water until pH = 7, then dried in vacuum at room temperature for 24 h (Table-8).

TABLE-8
ANTIBACTERIAL RATE AFTER REGENERATION

Number of reusability	Original bacteria colonies (mL)	Residue bacteria colonies (mL)	Sterilizing rate (%)
1	1.33×10^8	1.5×10^5	99.9
2	1.88×10^8	3.7×10^5	99.8
3	5.0×10^8	1.3×10^6	99.7
4	5.9×10^8	4.4×10^6	99.3
5	1.4×10^9	1.8×10^7	98.7

Sterilizing rate of the bisquarternary phosphonium sterilization is still 98 % after 5 times reused, according to Table-8.

Conclusion

(1) The preparation of silica gel grafting bisquarternary phosphonium water-insoluble sterilization is successful. The process has three steps.

(2) The best condition for quarternary phosphonium reaction is 150 ºC and 48 h in *o*-dichlorobenzene.

(3) The grafting ratio of quarternary phosphonium groups can achieve 0.68 mmol/g and the grafting ratio of long chain alkyl groups can achieve 0.15 mmol/g.

(4) The sterilizing rate is not lower than 99.9 % with 20 mg/mL adding amount and sterilizing 45 min. After sterilizing, the concentration of bacterium will decrease 3 orders of magnitude.

(5) The bactericidal activity can be regenerated, and the sterilizing rate is not lower than 98.7 % after 5 times used.

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