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Improved Manufacturing Process for Pyronaridine Tetraphosphate

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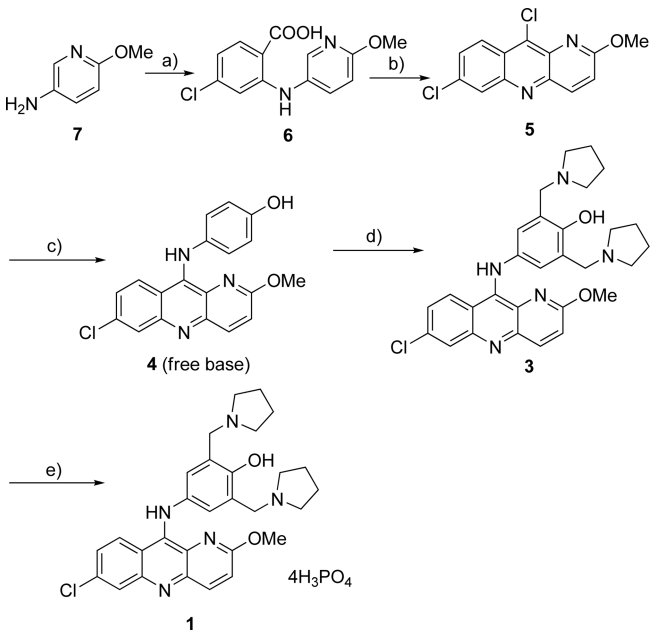
Pyronaridine tetraphosphate (**1**) is a well-known antimalarial drug. However, it required a carefully optimized production process for the manufacture of pyronaridine tetraphosphate. Each step of its manufacturing process was reinvestigated. For the cyclization of 4-chloro-2-(6-methoxy-pyridin-3-yl-amino)-benzoic acid **6** to 7,10-dichloro-2-methoxybenzo[*b*]-1,5-naphthyridine **5**, an improved process was developed to eliminated critical process impurity (BIA). By the redesign of the formation of triphosphate salt, the purity as API grade was increased. Thus, a robust manufacturing process with an acceptable process performance has been developed to produce high quality pyronaridine tetraphosphate.

Key Words : Antimalarial agent, Pyronaridine tetraphosphate, Manufacturing process

Introduction

The classical manufacturing process for pyronaridine tetraphosphate[1-5] (**1**) is outlined in Scheme **1**. It started with a step that 5-amino-2-methoxypyridine **7** was condensed with 2,4-dichlorobenzoic acid to afford **6**.

Subsequently, **6** was cyclized in the presence of phosphorus oxychloride and then condensed with 4-aminophenol to 4-[(7-chloro-2-methoxybenzo[*b*]-[1,5]-naphthyridin-10-yl)amino]-phenol **4** (free base), which underwent a Mannich reaction to give pyronaridine **3**. Finally, **3** was treated with phosphoric acid to yield pyronaridine tetraphosphate **1**.



Scheme 1. a) 2,4-dichlrobenzoic acid, K_2CO_3, CuO, 1-Pentanol, 12 h, reflux; b) $POCl_3$, 1h, reflux; c) 4-aminophenol, H_2SO_4, water, 17 h, reflux; d) 37% formaldehyde, pyrrolidine, EtOH, 12 h, 40-45 °C; e) phosphoric acid, EtOH, 1 h, reflux.

However, when reviewing the whole process, it was not suitable for industrial production because the reaction condition was too violent and produced many impurities which have influences on the quality of pyronaridine tetraphosphate **1** as an active pharmaceutical ingredient (API). To overcome the drawbacks, there is a need to develop new pharmaceutically viable and safe manufacturing process for the preparation of pyronaridine tetraphosphate **1**.

Experimental

4-Chloro-2-(6-methoxy-pyridin-3-yl-amino)-benzoic acid (6). Purified water (53.0 mL) was mixed potassium carbonate (21.2 g, 0.15 mol) in a suitable reactor. 2,4-dichlorobenzoic acid (21.2 g, 0.11 mol) was added to the reactor. The mixture was heated to 80-85 °C until mixture was cleared. **7** (10.6 g, 0.085 mol) was added to the reactor followed by CuI (2.12 g). The mixture was heated to 80-85 °C for at least 5 h. The reaction mixture was cooled to 25-30 °C. The by-products removed by filtration and washed with acetone (26.5 mL). The filtrate was heated to 40-50 °C and then c-HCl (10.6 mL) was added to the mixture followed by purified water (53.0 mL). The product was washed with purified water (21.2 mL) and dried under vacuum at 50-60 °C for at least 4 h. Yield: 18.0 g (75.7%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 13.25 (s, 1H), 9.46 (s, 1H), 8.13 (s, 1H), 7.84 (b, 1H), 7.69 (d, J = 8.4 Hz, 1H), 6.99 (d, J = 8.4 Hz, 1H), 6.76 (s, 2H), 3.87 (s, 3H).

7,10-Dichloro-2-methoxybenzo[*b*]-1,5-naphthyridine (5). Ethylenedichloride (EDC) (90.0 mL) and $POCl_3$ (44.6 g, 0.29 mol) were mixed with **6** (18.0 g, 0.065 mol) in a suitable reactor. The mixture was heated to reflux temperature for at least 2 h. The mixture was checked for reaction completion. The reaction mixture was cooled to 10 °C and MeOH (135 mL) was added carefully in the mixture followed by adding a solution of sodium hydroxide. The mixture was stirred for 2 h at 20-30 °C. The product was harvested by

filtration and washed with MeOH (54.0 mL). The product was harvested by filtration and washed with purified water (36.0 mL). The product was dried under vacuum at 50-60 °C at least 4 h. Yield: 13.1 g (72.7%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.45 (d, J = 9.2 Hz, 1H), 8.38 (d, J = 9.2 Hz, 1H), 8.29 (d, J = 2.0 Hz, 1H), 7.84 (dd, J = 9.2, 2.0 Hz, 1H), 7.51 (d, J = 9.2 Hz, 1H), 4.19 (s, 3H); ^{13}C NMR δ 162.59, 146.38, 144.38, 140.58, 137.05, 134.87, 134.79, 128.94, 127.85, 126.22, 124.47, 120.77, 54.06; Mass *m/z* 280.9 [M+1].

4-[(7-Chloro-2-methoxybenzo[*b*]-1,5-naphthyridin-10-yl)amino]-phenol hemisulphate (4). Purified water (196.5 mL) was mixed 4-aminophenol (6.7 g, 0.061 mol) in a suitable reactor. Sulfuric acid (12.0 g, 0.122 mol) was added to the reactor followed by **5** (13.1 g, 0.047 mol). The mixture was heated to 75-85 °C for at least 7 h and held at reflux for at least 10 h. The mixture was checked for reaction completion (**5** not more than 3.0% by HPLC). The reaction mixture was cooled to 10-30 °C and a solution of sodium hydroxide is added to the reaction mixture. The mixture was stirred for 1 h at 10-30 °C. The product was harvested by filtration and washed with water (26.2 mL) followed by methanol (20.7 mL). The product was dried under vacuum at 50-60 °C for at least 4 h. Yield: 18.0 g (95.7%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.10 (b, 1H), 9.70 (s, 1H), 8.23 (d, J = 12.0 Hz, 1H), 7.94 (d, J = 4.0 Hz, 1H), 7.76 (d, J = 8.0 Hz, 1H), 7.48 (d, J = 12.0 Hz, 1H), 7.33 (dd, J = 12.0, 4.0 Hz, 1H), 7.21 (d, J = 8.0 Hz, 2H), 6.90 (m, 2H), 4.00 (s, 3H); ^{13}C NMR δ 160.0, 156.4, 148.6, 144.5, 138.1, 136.8, 136.4, 133.2, 127.8, 127.1, 126.7, 124.0, 123.5, 121.2, 116.4, 113.9, 54.6; Mass *m/z* 352.0 [M+1].

4-[(7-Chloro-2-methoxybenzo[*b*]-1,5-naphthyridin-10-yl)amino]-2,6-bis-(1-pyrrolidinylmethyl)phenol (3). Paraformaldehyde (30.7 g, 1.022 mol) and ethanol (113.5 mL) were added to a reactor. The mixture was cooled to −5 °C to 5 °C and pyrrolidine (72.8 g, 1.024 mol) is added, maintaining the temperature below 70 °C. The mixture was cooled to 25-35 °C and **4** (18.0 g, 0.051 mol) was added to the mixture. The mixture was stirred for at least 15 h at 45-53 °C. Purified water (144.0 mL) was added and the mixture cooled to 15-25 °C. The mixture was stirred for at least 1 h, filtered and the product was washed with purified water (36.0 mL). Methanol (142.0 mL) was added and the mixture was heated at reflux for at least 1h. The mixture was cooled to 5-15 °C, stirred for at least 2 h, and filtered. The product was washed with methanol (28.5 mL) and dried under vacuum at 50-60 °C for at least 4 h. Yield: 19.0 g (81.67%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.04 (s, 1H), 8.21 (d, J = 9.2 Hz, 1H), 7.93 (d, J = 2.4 Hz, 1H), 7.76 (d, J = 9.2 Hz, 1H), 7.30 (d, J = 9.2 Hz, 1H), 7.17 (dd, J = 9.6, 2.4 Hz, 1H), 6.92 (s, 2H), 3.94 (s, 3H), 3.65 (s, 4H), 2.50 (m, 8H), 1.69 (s, 8H); ^{13}C NMR δ 159.7, 153.1, 148.7, 144.8, 143.2, 140.9, 134.3, 134.0, 128.2(2C), 127.3, 124.7, 123.4, 123.3, 119.5, 115.6, 55.6, 54.2, 53.6, 23.6; Mass *m/z* 518.7 [M+1].

4-[(7-Chloro-2-methoxybenzo[*b*]-1,5-naphthyridin-10-yl)amino]-2,6-bis-(1-pyrrolidinylmethyl)phenol triphosphate (2). 3 (19.0 g, 0.037 mol), purified water (106.4 mL) and phosphoric acid (12.7 g, 0.130 mol) were added to a reactor and stirred for at least 0.5 h at 45-55 °C. The mixture was filtered through a polishing filter at 45-55 °C into a second reactor and washed with purified water (7.6 mL). Acetone (51.0 mL) was added for at least 1 h at 45-55 °C to the reactor contents. The mixture was stirred for at least 2 h. A second acetone charge (60.0 mL) was added for at least 1h at 45-55 °C and the mixture was stirred for an additional at least 2 h. Finally, a third acetone charge (105.1 mL) was added for at least 1 h at 45-55 °C and the mixture was cooled to 20-30 °C and stirred for at least 1 h. The product was filtered, washed with acetone (30.0 mL) and dried under vacuum at 50-60 °C for at least 4 h. Yield: 28.0 g (94.02%).

4-[(7-Chloro-2-methoxybenzo[*b*]-1,5-naphthyridin-10-yl)amino]-2,6-bis-(1-pyrrolidinylmethyl)phenol tetraphosphate (1). 2 (28.0 g, 0.035 mol), purified water (95.2 mL) and phosphoric acid (0.39 g, 0.004 mol) were added to a reactor and heated at 55-65 °C for at least 0.5 h. The mixture was filtered and washed with purified water (2.8 mL). Acetone (31.0 mL) was added for at least 1 h at 45-55 °C and the mixture was stirred for at least 2 h. Acetone (66.3 mL) was added for at least 1 h at 45-55 °C and the mixture was stirred for at least 2 h. Acetone (82.9 mL) was added for at least 1 h at 45-55 °C. The mixture was cooled to 20-30 °C, stirred for at least 1 h and filtered. The product was washed with acetone (44.3 mL). Wet cake **2**, acetone (176.9 mL), phosphoric acid (3.97 g, 0.041 mol) and purified water (11.2 mL) were added to a reactor. The mixture was heated to reflux and stirred for at least 7 h. The mixture was cooled to 40-50 °C, filtered, the product was washed with acetone (44.3 mL) and dried under vacuum at 50-60 °C for at least 4 h. The product was sieved and packed. Yield: 27.5 g (87.6%). ^{1}H-NMR (400 MHz, D_2O) δ 8.05 (dd, J = 9.4, 2.0 Hz, 1H), 7.74 (s, 1H), 7.45 (s, 2H), 7.38 (dd, J = 9.2, 2.0 Hz, 1H), 7.35 (d, J = 9.6 Hz, 1H), 7.13 (d, J = 9.6 Hz, 1H), 4.37 (s, 4H), 3.90 (s, 3H), 3.47 (m, 4H), 3.08 (m, 4H), 1.99 (m, 4H), 1.91 (m, 4H); ^{13}C NMR δ 160.7, 154.4, 151.9, 140.8, 139.4, 132.7, 132.4, 131.1, 130.7, 127.2, 125.2, 123.2, 122.2, 118.5, 111.1, 54.5, 54.0, 53.2, 22.4; Mass *m/z* 518.1 [M+1].

2,7,10-Trichloro-benzo[*b*]-1,5 naphthyridine (BIA). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.63 (d, J = 9.2 Hz, 1H), 8.52 (d, J = 9.2 Hz, 1H), 8.35 (d, J = 2.0 Hz, 1H), 7.99 (d, J = 9.2 Hz, 1H), 7.89 (dd, J = 9.6, 2.0 Hz, 1H); Mass *m/z* 281.95 [M].

4-[(2,7-Dichloro-benzo[*b*]-1,5-naphthyridin-10-yl)-amino]-2,6-bis-(1-pyrrolidinylmethyl)phenol (DIA). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.81 (s, 1H), 8.27 (d, J = 8.8 Hz, 1H), 7.99 (d, J = 2.0 Hz, 1H), 7.64 (d, J = 9.6 Hz, 1H), 7.60 (d, J = 8.8 Hz, 1H), 7.01 (s, 2H), 6.96 (dd, J = 9.6, 2.0 Hz, 1H), 3.74 (s, 4H), 2.61 (s, 8H), 1.83 (s, 8H); ^{13}C NMR δ 155.22, 151.50, 147.35, 146.64, 143.34, 140.54, 136.97, 132.35, 130.70, 128.62, 127.61, 127.50, 125.49, 124.54, 123.75, 114.11, 56.66, 54.22, 23.92.

4-[(7-Chloro-2-methoxybenzo[*b*]-1,5-naphthyridin-10-yl)amino]-2-(1-pyrrolidinylmethyl)phenol (DIN). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.40 (b, 1H), 8.21 (d, J = 9.2 Hz, 1H), 8.01 (d, J = 2.4 Hz, 1H), 7.56 (d, J = 9.6 Hz, 1H), 7.21 (d, J

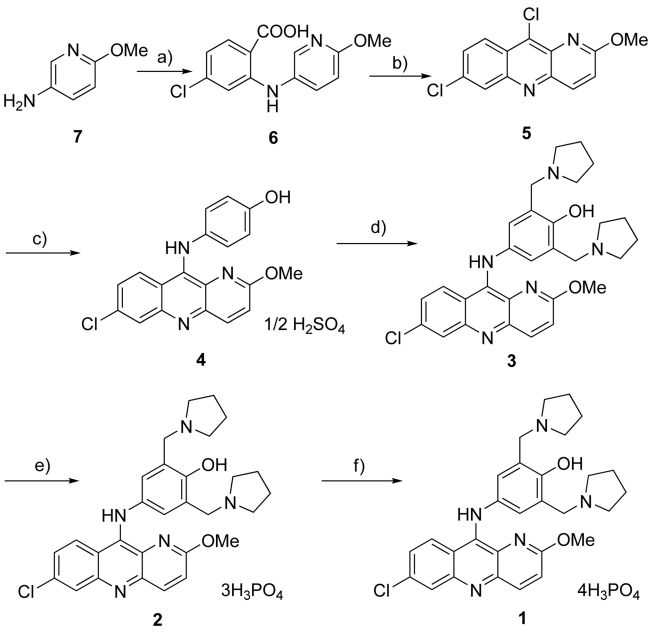
= 9.2 Hz, 1H), 7.09 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.98 (dd, *J* = 9.4, 2.4 Hz, 1H), 6.84 (m, 2H), 4.09 (s, 3H), 3.77 (s, 2H), 2.64 (s, 4H), 1.87 (b, 4H); ^{13}C NMR δ 160.59, 156.39, 149.26, 142.61, 140.66, 135.33, 134.33, 134.05, 128.46, 128.25, 127.09, 124.92, 124.12, 123.76, 119.49, 117.09, 114.76, 58.97, 54.21, 53.82, 24.02.

Results and Discussion

We developed industrially suitable process to produce pyronaridine tetraphosphate with a pharmaceutically acceptable impurity level. (Scheme 2).

Process for 4-Chloro-2-(6-methoxy-pyridin-3-yl-amino)-benzoic acid (6). In the conversion of **7** with 2,4-dichlorobenzoic acid to **6**, **6** was manufactured thorugh Ulmann type reaction with 1-pentanol as a solvent and copper oxide as a catalyst.[6,7] However due to the high reaction temperature and expensive solvent, this process had no advantage from an industrial point of view. We tried to solve these problems by changing the solvent during condensing process. Water was used instead of 1-pentanol because water was the most economical and environment-friendly solvent. Subsequently, a copper catalyst was also considered because it was the most suitable catalyst in a water solvent.[8] As shown in Table **1**, the coupling reactions of 2,4-dichlorobenzoic acid and **7** with various copper catalysts in water were tested. The results showed that a satisfying yield (%) was achieved when CuI as catalyst was used.

Process for 7,10-Dichloro-2-methoxybenzo[*b*]-1,5-naphthyridine (5). The cyclization of **6** to **5** is the most important stage in the synthesis of pyronaridine tetraphosphate **1**. However, because **5** had a poor solubility against a common



Scheme 2. a) 2,4-dichlrobenzoic acid, K_2CO_3, CuI, water, 5 h, 80-85 °C; b) $POCl_3$, ethylenedichloride, 2 h, reflux; c) 4-aminophenol, H_2SO_4, water, 17 h, reflux; d) Paraformaldehyde, pyrrolidine, EtOH, 15 h, 48-53 °C; e) phosphoric acid, acetone, 5 h, 45-55 °C; f) phosphoric acid, acetone, 13 h, 45-55 °C.

Table 1. Various copper catalyzed coupling of 2,4-dichlorobenzoic acid with 5-amino-2-methoxypyridine (AMP): reaction condition[a]

Entry	Catalyst	Yield (%)[b]
1	Cu(I) Oxide	64.4
2	**Cu(I) Iodide**	**75.4**
3	Cu(II) Acetate	62.7
4	Cu(II) Bromide	68.5
5	Cu(II) Chloride dihydrate	59.5
6	Cu(II) Sulfate	45.3
7	Cu(II) Carbonate	38.9
8	Cu(II) Nitrate	41.7

[a]Reaction condition: AMP 1.0 eq, 2,4-dichlorobenzoic acid 2.0 eq, K_2CO_3 2.0 eq, catalyst 20% w/w water 10 vol, reflux. [b]HPLC area %

solvent, it was difficult to perform the purification process with previously known methods such as recrystallization or column chromatography. During the reation, undesired impurities, especially for BIA (Figure 1) was produced and had bad influences on quality of pyronaridine tetraphosphate **1**. In the case of performing neat reaction with phosphorus oxychloride, the higher levels of BIA was produced. They cause low quality of pyronaridine tetraphosphate **1** as API. The control of undesired impurities was the key point to improve the quality of pyronaridine tetraphosphate **1**. In order to reduce the amount of BIA produced during reaction, ethylenedichloride (EDC) as solvent and short reaction time were applied to process and BIA was dramatically reduced. Using this improved manufacturing process, **5** was synthesized to have a high purity, increased yield about 30% and lower cost.

Process for 4-[(7-Chloro-2-methoxybenzo[*b*]-1,5-naphthyridin-10-yl)amino]-phenol hemisulphate (4). In conversion of **5** to **4**, Free base of **4** had bad influences on the properties of hemisulphate **4** and the manufacturing process especially for the step of purification in an industrial scale. To decrease the production of free base of **4**, pH at the end of reaction was adjusted to pH 2-4. The steps of filteration and washing were carried out easily by optimizing the pH range.

Process for 4-[(7-Chloro-2-methoxybenzo[*b*]-1,5-naphthyridin-10-yl)amino]-2,6-bis-(1-pyrrolidinylmethyl)-phenol (3). Paraformaldehyde was used[9] instead of 37% formaldehyde to convert **4** to **3** in order to reduce the level of the remained intermediate DIN (Figure 2. The level of DIN was increased when formaldehyde was used to convert **4** to **3**. It was also found that reaction temperature had an influence on the level of DIN and the yield of **3**. The reaction temperature was optimized to 48-53 °C. At the higher temperature, unknown impurities were increased and the yield of **3** was lowered. Below the current optimized temperature, the level of DIN was increased with a low reaction rate. By using

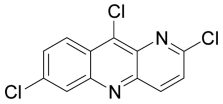


Figure 1. Structure of BIA.

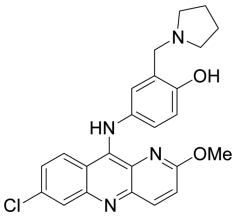


Figure 2. Structure of intermediate (DIN).

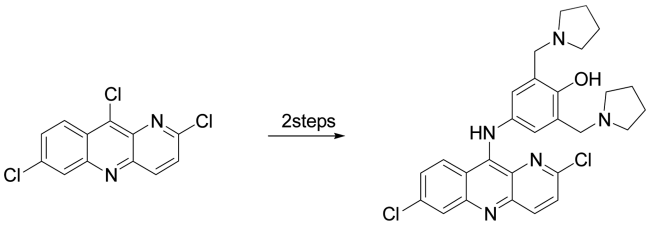


Figure 3. Structure of DIA.

paraformaldehyde and optimizing the reaction temperature, the quality of **3** was improved. Finally, production yield with paraformaldehyde increased about 20%.

Process for 4-[(7-Chloro-2-methoxybenzo[*b*]-1,5-naphthyridin-10-yl)amino]-2,6-bis-(1-pyrrolidinylmethyl)-phenol triphosphate (2). Pyronaridine acid salt form was generally known as tetraphosphate. But we found that pyronaridine triphosphate **2** was formed when three equivalents of phosphoric acid were added to one equivalent of **3** in acetone solvent. The potential impurity (DIA) coming from BIA (Figure 3) was removed by synthesizing pyronaridine triphosphate **2** before the final step to manufacture pyronaridine tetraphosphate **1**.

Process for 4-[(7-Chloro-2-methoxybenzo[*b*]-1,5-naphthyridin-10-yl)amino]-2,6-bis-(1-pyrrolidinylmethyl)phenol tetraphosphate (1). In the conversion of pyronaridine triphosphate **2** to pyronaridine tetraphosphate **1**, one equivalent of phosphoric acid was supplemented. It is inferred that pyronaridine tetraphosphate **1** has two crystal habits of a plate type(a) and a needle type(b) (Figure 4) during the conversion of **1** from **2**. There was only the needle type of **1** in early studies. The needle type had some trouble in filtering, washing and drying process in the industrial batch scale. The plate type of **1** was obtained by manufacturing **1** from the conversion of **2**. The plate type was more stable and easy to handle comparing with the needle type of **1**. The plate type of **1** was more suitable for the formulation development for finished production.

Conclusion

The improved manufacturing process for pyronaridine tetraphosphate **1** was described. For the conversion of **7** to **5** (coupling through copper catalyst and cyclization under suitable condition) was developed with low undesirable impurity (BIA) profiles. The manufacturing stage for **4** was improved by optimizing pH at the end of reaction to get rid of free base of **4**. In manufacturing stage for **3**, the quality and yield of **3** were improved by using paraformaldehyde and optimizing the reaction temperature. The manufacturing process to synthesize **2** from **3** was developed and the level of impurity DIA was decreased sufficiently. Plate type of **1** was obtained by the optimized manufacturing process. Plate type of **1** was more stable, easier to handle and more suitable for the formulation development of drug product.[10]

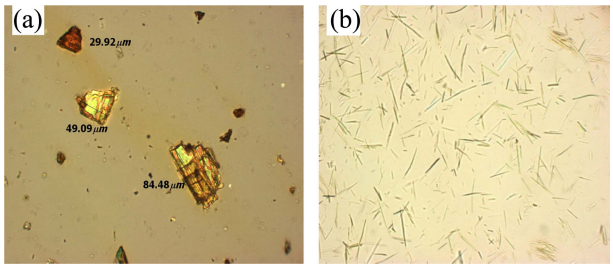


Figure 4. Optical microscopic images of (a) Plate type; (b) Needle type.

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