# การตรึงและการพิสูจน์ลักษณะของสารไพโอเวอร์ดีน I บนไมเซลล์เท็มเพล็ตซิลิกาที่ดัดแปลงหมู่ฟังก์ชันเป็นสารคีเลต

# Immobilization and Characterization of Pyoverdin I onto Modified Micelle-Templated Silica (MTS) Surface as a Chelating Agent

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## บทคัดย่อ

ในงานวิจัยนี้ได้ผลิตสารไพโดเวอร์ดีน I จากแบคทีเรีย *Pseudomonas aeruginosa* ซึ่งเป็นไซเดอโรฟอร์ประเภทหนึ่งเลี้ยงในอาหารเหลวสูตรเอสเอที่ได้ปรับปรุงแล้ว และสารนี้สามารถเรืองแสงสีฟ้าได้ภายใต้แสงอัลตราไวโอเลต นอกจากนี้ได้แยกสารไพโอเวอร์ดีน I ออกจากสารละลายซุปเปอร์นาแตนท์ไซเดอโรฟอร์โดยใช้คอลัมน์โครมาโทกราฟีที่บรรจุเรซินชนิดแอมเบอร์ไลต์ เอ็กซ์เอดี-16 และนำสารที่แยกได้มาทำให้บริสุทธิ์โดยใช้คอลัมน์ที่บรรจุวัสดุพอลิเมอร์ชนิด ซีเอ็มเซฟพาเด็กซ์ ซี-25 และ ไบโอ-เจล พี-2 ตามลำดับ โดยตรวจสอบความบริสุทธิ์ของไพโอเวอร์ดีน I โดยใช้เทคนิครีเวิร์สเฟสโครมาโทกราฟีของเหลวสมรรถนะสูงด้วยระบบเกรเดียนต์ของอะซิโตรไนไตรจาก 6 ถึง 40 เปอร์เซ็นต์ในสารละลายอะซีเตทบัฟเฟอร์ เข้มข้น 0.01 โมลาร์ ที่พีเอช 6.0 จากนั้นได้ศึกษาสภาวะที่เหมาะสมในการตรึงไพโอเวอร์ดีน I บนผิวของไมเซลล์เท็มเพลทซิลิกาที่ดัดแปลงหมู่ฟังก์ชัน เพื่อใช้เป็นวัสดุดูดซับโลหะหนักในสารละลายหลังจากดัดแปลงพื้นผิวไมเซลล์เท็มเพลทซิลิกาด้วย 3-glycidoxypropyl-trimethoxysilane (GPTMS) ต่อมาทำการตรึงสารไพโอเวอร์ดีน I ลงบนผิวของไมเซลล์เท็มเพลทซิลิกาที่ดัดแปลงโดยสร้างพันธะโควาเลนต์ระหว่างหมู่อะมิโนในโครงสร้างของสารไพโอเวอร์ดีน I กับหมู่อีพอกซีบนผิวของไมเซลล์เท็มเพลทซิลิกา สำหรับการพิสูจน์เอกลักษณ์ของสารไพโอเวอร์ดีน I ที่ตรึงบนผิวของไมเซลล์เท็มเพลทซิลิกาที่ดัดแปลงนั้น โดยอาศัยการพิจารณาจากสเปกตรัมฟลูออเรสเซนต์และอินฟราเรดของสารไพโอเวอร์ดีน I และศึกษาผิวของวัสดุดูดซับนี้ด้วยวิธีการถ่ายภาพ SEM รวมทั้งหาพื้นที่ผิวโดยใช้วิธีของ BET ได้จากข้อมูลทาง nitrogen adsorption isotherm ซึ่งพบว่า สารไพโอเวอร์ดีน I จะส่งผลทำให้พื้นที่ผิวของซิลิกาลดลงจาก 609.2 เป็น 405.4 $m^2.g^{-1}$ จากการศึกษาพบว่าที่ผิวของไมเซลล์เท็มเพลทซิลิกาที่ดัดแปลงมีการเปลี่ยนแปลงเกิดขึ้นหลังจากตรึงไพโอเวอร์ดีน I

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# Abstract

A yellow-greenish biological product, pyoverdin I siderophore was produced from the *Pseudomonas aeruginosa* strain in modified SA medium and appeared as a blue fluorescent pigment under UV light. The isolation of pyoverdin I was performed using an Amberlite XAD-16 resin and subsequently purified by CM Sephadex C-25 and Bio-Gel P-2 column chromatography. The purity of pyoverdin I was investigated by RP-HPLC with a gradient system (6-40% acetonitrile in 0.01 M acetate buffer, pH 6.0). In this work, the optimum conditions for immobilization of pyoverdin I onto modified a micelle-templated silica (MTS) surface were studied as a recyclable adsorbent material for removal of some heavy metals from aqueous solution. The micelle-templated silica was chemically modified with 3-glycidoxypropyl-trimethoxysilane (GPTMS) and then immobilized with pyoverdin I by forming a covalent bond between the amino group on the peptide backbone and an epoxy group. The characteristics of pyoverdin I anchored onto the modified MTS were investigated using fluorescence, infrared spectroscopy and scanning electron microscopy. Additionally, the specific surface area of this material was calculated by the Brunaver, Emmett and Teller (BET) method using nitrogen isotherm adsorption data. The surface area of commercial silica gel decreased from 609.2 to 405.4 $m^2.g^{-1}$, indicating that the pyoverdin I can be immobilized onto the surface of a silica solid support before being used for further analysis.


**คำสำคัญ:** แบคทีเรีย *Pseudomonas aeruginosa* ไพโอเวอร์ดิน I ไมเซลล์เท็มเพลทซิลิกา

Keywords: *Pseudomonas aeruginosa*, Pyoverdin I, modified micelle-templated silica (MTS)

## Introduction

Bacteria produce a vast number of secondary metabolites that have a wide spectrum of biological activities. The presence of Pseudomonas spp. (i.e., P. putida, P. chlororaphis, P. fluorescens and P. aeruginosa) leads to the suppression of plant deleterious microorganism by the excretion production of effective siderophores under low-iron stress growth conditions (Gadd, 2004; Fuch, et al., 2001). Siderophores are low-molecular-weight compounds (350-1500 Daltons) with high affinity and specificity for $Fe^{3+}$ (stability constants, $K_f$ $10^{20}$-$10^{50}$ at neutral pH). The natural siderophores are generally categorized by the main functional groups involved in chelating iron, and may be catecholate, hydroxamate and hydroxycarboxylate types (Boukhalfa et al., 2002; Yvonne et al., 1987). Siderophores have been shown to have a high affinity for a variety of metal ions concerned such as $Cd^{2+}$, $Cu^{2+}$, $Pb^{2+}$, $Zn^{2+}$, $In^{3+}$, $Al^{3+}$ and $Ga^{3+}$ but their stability constants are lower than that of with $Fe^{3+}$. For these reasons, siderophore seemed to be an attractive chelating agent, which can be used to reduce toxicity of heavy metals from contaminated environments. (Gledhill, et al., 2004).

At present, various solid supports have extensive commercial applications as adsorbents, catalysts and catalyst supports due to high surface areas. Modification of the surface of solid support can be worked by many methods. The modified surface is immobilized with chelating agent for removal of heavy metals by adsorption and forming

complexes methods. This material can be synthesized by a number of techniques: (a) adsorption of organic species into the pores of solid support, (b) construction of organic molecule by piece within the confines of solid support cavities, (c) attachment of desired functionality to solid support by covalent bond formation, and (d) direct synthesis into a final composite material. In the previous study, the surface of silica gel was used to modify using organosilane reagent and then immobilized with biological chelating agent. The organosilane reagent was immobilized onto the silica surface, the silanol group of which was converted to a new organofunctional surface that acquires the organophilic properties (Jal et al., 2004). The biological chelating was trapped at the free end of the linking group facilitating better adsorption properties. These modified solid supports were used for preconcentration and separation of a complex mixture, single element and series of similar elements (Ying et al., 1999). The discovery of mesoporous silicates has greatly expanded the possibilities for the design of new nanostructured materials; micelle-templated silicas (MTS) constitute excellent mineral supports for the preparation of inorganic-organic hybrid materials by grafting organic chains onto their surface through silylation. Hence, the surface properties should become more hydrophobic because the silanol groups are involved in sol-gel condensation. The MTS have indicated the involvement of the hydrophobic sites during the silanation procedure through preservation of the hydrophilic patches of the MTS (Pierre et al., 1999). Therefore, according to this hypothesis, the environment of the grafted organic moieties would mainly consist in residual interacting silanol groups. Further end-capping of the epoxy-Si surface with cetyltrimethylammonium bromide (CTAB) minimizes the possible interactions between the anchored catalytic sites and the uncovered surface and, as a consequence, increases their catalytic activity. The methods used for the activation of silica involve the formation of an intermediate silane-coupling agent, which is chosen according to the connecting group of the organic ligand. The ligand has an aminoalkyl group attached to the chelating moiety. The activation of the silica surface with oxirane groups is the most adequate for bonding the ligand by nucleophylic attack and epoxy ring opening (Esteves et al., 2005). Therefore, the study of the purified siderophore immobilized onto a solid support surface to improve the selectivity of heavy metals is interesting. This adsorbent should have a good sorption capacity, chemical stability under experimental conditions and especially be highly selective to some heavy metals. Hence, a highly fluorescent sensor was prepared by entrapping a pyoverdin II in sol-gel glass. This material was used as a reactive solid phase to determine trace amounts of $Fe^{3+}$ by continuous flow injection (FI). This selective sensor was successfully used to determine $Fe^{3+}$ in tap water and human serum (Barrero et al., 1995). Moreover, the preparation and characterization of the hydrophobic and hydrophilic behavior of functionalized MTS were described in Brunel et al. (2000). Then, the encapsulation of pyoverdin II onto the modified MTS surface was studied for the selective uptake of the $Fe^{3+}$ from a multi-metallic solution. However, the amount of encapsulation of pyoverdin II was too low to be practical in a large-scale operation (Mureseanu et al., 2003). In addition, the covalent immobilization of pyoverdin II onto a glycidoxy-grafted MTS and a commercial high grade glycidoxy-grafted silica gel were applied

to uptake and release $Fe^{3+}$ from artificial multi-metallic solution and waste water (Renard et al., 2005).

Geometry of the surface defines the accessibility of adsorption sites. Mainly, surface hydroxyl groups are responsible for adsorption properties. A significant decrease of surface area is due to chemical modification of silica gel. A corresponding decrease in adsorbent surface area and average pore diameter can also be assumed. A decrease in pore volume, surface area and pore diameter were due to increase in alkyl chain length (Renard et al., 2005). A decrease of surface area was determined by the BET method with increase in surface modification. The nitrogen molecules adsorbed preferentially to silanols and a weak adsorption was observed on organic surfaces compared with silica surfaces.

The aims of the present work were to study the pyoverdin I production from *P. aeruginosa* strain under the optimum conditions. The pyoverdin I was isolated and purified from siderophore supernatant by various types of column chromatography and its purity then investigated by using RP-HPLC. The characteristics of the pyoverdin I were elucidated using optical spectroscopy (UV-Vis, Fluorescence and IR). Moreover, this pyoverdin I was selected and used as an alternative biological chelating agent, which immobilized onto the modified micelle-templated silica surface for improving the selectivity to some heavy metals. Finally, the feature characteristic of this synthesized material was investigated by various techniques, including spectrofluorophotometry, IR, SEM and nitrogen adsorption isotherm.

## Materials and Methods

**1. Production, isolation and purification of pyoverdin I**

The strain used in this study was for P. aeruginosa. In the following protocol, one liter of the medium containing di-potassium hydrogen phosphate, 1.0 g; sucrose, 10.0 g and L-Asparagine anhydrous, 4.0 g was used. The medium was adjusted to be pH 6.5 and after autoclaving, 10% (w/v) of steriled magnesium sulfate was added into the medium. This medium was inoculated with the previous subculture of P. aeruginosa and then shaken by magnetic stirring for 20 hours at ambient temperature. After precipitation of proteins, neutralization with 1.0 M NaOH or 1.0 HCl solution and centrifugation for 20 min at 3500 rpm, a yellow-green supernatant was obtained. The isolation of pyoverdin I was performed on column chromatography with an Amberlite XAD-16 resin and further purified using CM Sephadex C-25 and Bio-Gel P-2 column. The separated fraction of the purified siderophore was eluted, concentrated and lyophilized (Ruangviriyachai, 2000). After that, the structural elucidation of the purified siderophore was identical to pyoverdin I by Budzikiewicz and co-workers using spectroscopic methods. The structural elucidation of the purified siderophore was studied by spectroscopic methods. It consisted of amino acids in a peptide chain (L-ornithine, L-threonine, L-lysine, D-serine, L-arginine), a fluorescent multifunctional chromophore (pyoverdins) and side chain (succinic acid). The pyoverdin I structure is shown in Figure 1 which corresponds with that reported by Budzikiewicz et al., 2002.

**2. Investigation of the purity of pyoverdin I**

The purity of an isolated pyoverdin I was investigated using the Waters HPLC system, which consisted of a Nucleosil $C_{18}$ column, 150 x 4.6 mm i.d., 5.0 µm (Phenomenax, Australia) under linear gradient from 6 to 40 % acetonitrile (HPLC grade, Lab Scan, Ireland) in 0.01 M acetate buffer solution (pH 6.0) at a flow rate of 1.0 $mLmin^{-1}$ as mobile phase. The detection of pyoverdin I was carried out using a photodiode array (PDA) at 254, 400 nm and fluorescence detection (Waters) at 460 nm ($\lambda_{ex}$ 400 nm), respectively.

**3. Immobilization of pyoverdin I onto the surface of modified micelle-templated silica (MTS-3)**

3.1 Synthesis of micelle-templated silica (MTS)

The synthesis of the MTS was carried out according to the procedure described by *Ottaviani et al. (2004)*. Briefly, the reactants were added under stirring at room temperature in the following order: deionized $H_2O$, NaOH (Carlo Erba, Italy), cetyltrimethylammonium bromide (CTAB) (Merck, Germany), trimethylbenzene (TMB) (Carlo Erba, Italy) and $SiO_2$ (Carlo Erba, Italy) with a molar ratio of 20 : 0.25 : 0.1 : 1.3 : 1. Hence, the swollen micelles were formed using a TMB/CTAB molar ratio of 13 : 1. After the addition of silica, the mixture was allowed to equilibrate by stirring for 30 min at room temperature. The mixture was then refluxed for 3 h at 115 °C. The MTS obtained from this process was filtered off, washed with distilled water until neutralization and dried at 115 °C. Finally, the MTS was calcinated by thermal treatment at 550 °C for 8 h to eliminate the surfactant organic matter. The proposed structure of the MTS is shown in Figure 2 (a) (Ottaviani et al., 2004).

3.2 Synthesis of the modified micelle-templated silica (MTS-2)

The grafting of the MTS with 3-glycidoxypropyl-trimethoxysilane (GPTMS) from Sigma-Aldrich, USA, was carried out according to the procedure of Brunel et al. (1993). 1.0 g of the MTS was freshly activated overnight at 180 °C. After that, 1.25 mL of GPTMS and 20 mL of toluene (J.T. Baker Chemical, Philippines) were added. The mixture solution was then blended under stirring for 90 min at 70 °C. The mixture was kept further under the reflux for at least 1 h 30 min at 130 °C for completion. The MTS-2 was filtered and washed with toluene and then diethyl ether (VWR International, England). The MTS-2 was then dried overnight at 160 °C. Proposed structure of the MTS-2 is shown in Figure 2 (b) (Jal et al., 2004).

3.3 Immobilization of $Fe^{3+}$-pyoverdin I complex onto the surface of the MTS-2 (MTS-3)

The practical immobilization of $Fe^{3+}$-pyoverdin I complex onto the surface of the MTS-2 was described by Renard et al. (2005). 3.0 g of MTS-2 was activated for 1 h at 130 °C and was then dispersed in 40 mL of dimethylformamide (DMF; Asia Pacific Specially Chemical, Australia) before adding 25 mg of lyophilized $Fe^{3+}$-pyoverdin I complex. The mixture was refluxed under stirring for 48 h at 60 °C. The MTS-3 was collected by filtration and washed with deionized water and mixture solution of water/ethanol (1:1, v/v) followed by drying overnight at 60 °C. The $Fe^{3+}$ ions were firstly decomplexed from MTS-3 by using a 20% w/v $NaHSO_3$ solution before being used for the removal of heavy metals from aqueous solution. The proposed structure of the MTS-3 is shown in Figure 2 (c) (Renard et al., 2005).

3.4 The characteristics of the pyoverdin I-anchored onto the surface of the MTS-2 (MTS-3)

Textural properties of the MTS-3 can be used to identify using spectroscopic techniques. For comparison of the emission spectra of the sorbents, the commercial silica gel and the MTS-3 were measured at 460 nm ($\lambda_{ex}$ 400 nm) with solid sample holder, model RF510 by a Shimadzu Spectrofluorophotometer model RF-5000 (Japan). The IR spectra of pyoverdin I, commercial silica gel and the MTS-3 in KBr disk were then performed with a Perkin Elmer Spectrum One FT-IR spectrometer recording from 450 to 4,000 $cm^{-1}$. Moreover, the surface of the synthetic sorbent was determined by nitrogen adsorption isotherm at 77 K on an Autosorb-1-C TCD controller, Quantachrom Instruments for samples previously out gassed at 300 K under vacuum. The specific surface of the synthetic sorbent was calculated by BET method using the isotherm adsorption data. Photographs of commercial silica gel and the MTS-3 surface were taken by scanning electron microscope (SEM) with LEO model 1450 VP (Variable Pressure) instrument, demonstrating the surface of the synthetic material (Esteves et al., 2005 and Renard et al., 2005).

## Results and Discussion

**1. The investigation of the pyoverdin I purity**

The RP-HPLC is used for purity testing of the purified siderophore. Chromatograms of the purified siderophore (Figure 3) showed a single peak at the retention time ($t_r$) of 9.541, 9.642 and 9.646 min when detected with fluorescence detector ($\lambda_{ex}$ 400, $\lambda_{em}$ 460 nm) and PDA detector (254 and 400 nm), respectively.

**2. Characteristics of the pyoverdin I-anchored onto the surface of the MTS-2 (MTS-3)**

After the pyoverdin I was immobilized onto the surface of the MTS-2, it was called the MTS-3. The MTS-3 gave a yellow-green powder and blue fluorescence pigment under UV light as shown in Figure 4. Various techniques were used to investigate the MTS-3 surface including spectrofluorophotometry, Fourier transform infrared spectrometry, scanning electron microscopy and nitrogen adsorption isotherm.

2.1 Spectrofluorophotometry

The maximum fluorescence intensity of the MTS-3 surface (Figure 5) was at 460 nm when excited at 400 nm, indicating that the pyoverdin I can be immobilized onto the MTS-2 surface.

2.2 Fourier transform infrared (FT-IR) spectrometry

Infrared spectra of a pyoverdin I commercial silica gel and the MTS-3 are shown in Figure 6. For analysis of the functional groups of the pyoverdin I, the IR spectrum shows major absorption bands [see Figure 6 (a)]. The IR spectrum of the pyoverdin I appeared as a broadened band at 3284 $cm^{-1}$ in the region of hydroxyl OH stretching. This may include absorption due to the presence of N-H stretching group. However, the overlapping of C-H stretching bands that is characteristic of alkyl functional groups occurs between 2850 and 2980 $cm^{-1}$. Therefore, the absorption band near 2933 $cm^{-1}$ may indicate the presence of alkyl moiety in the pyoverdin I structure. The intensity bands near 1661 and 1543 $cm^{-1}$ relate to C=O and C=C stretching (cyclic or conjugated). Moreover, the peak near 1456 $cm^{-1}$ is assigned to C-O stretching. A weak absorption peak at 1410 $cm^{-1}$ may be the $CH_3$ and the absorption peak found near 1287 $cm^{-1}$ may correspond to C-N

stretching (aromatic). Finally, the weak intensity peak near 1071 $cm^{-1}$ is due to the stretching of C-$NH_2$ and the broad band occurs in the range of 900 to 500 $cm^{-1}$ indicating a typical aromatic ring. From these results, the functional groups of the *P. aeruginosa* pyoverdin I obtained consist of OH, NH, C=C, C-O, C=O, $CH_3$, C-N (aromatic) and C-$NH_2$ groups.

From the infrared spectrum of commercial silica gel, the major chemical groups appear as a broad band near 3460 $cm^{-1}$, which is the silanol group (OH group) and the adsorbed water bound to the silica surface by hydrogen bonding with other silanols. The bending vibrational peak for H-O-H is shown at 1633 $cm^{-1}$. The predominant transmittance peak at 1088 $cm^{-1}$ is due to the siloxane bond (Si-O-Si). The absorption peak between 1000 and 700 $cm^{-1}$ could be attributed to vibration modes of the gel network (Kongmanklang 2004 and Esparza et al., 2005) [Figure 6 (b)].

The infrared spectrum of the MTS-3 surface shows an absorption broad band at 3468 $cm^{-1}$ indicating an OH group on the surface and may also include absorption due to the presence of an N-H group. The absorption band in the range of 3000 to 2800 $cm^{-1}$ may indicate an alkyl moiety in the structure of pyoverdin I. Some additional peaks including sharp peaks at 1655 and 1536 $cm^{-1}$ correspond to C=O and C=C stretching. The predominant transmittance peak at 1087 $cm^{-1}$ is due to the siloxane bond (Si-O-Si). A band assigned to Si-O stretching of silanol groups shifts from 800 to 796 $cm^{-1}$. A small peak at 2950 $cm^{-1}$ corresponds to $CH_2$ stretching due to aliphatic groups of pyoverdin I chains as shown in the FT-IR spectra [Figure 6 (c)]. The results confirm that pyoverdin I can be immobilized onto the MTS-2 surface. The major chemical groups and wavenumbers in the structure of pyoverdin I, commercial silica gel and MTS-3 are summarized in Table 1.

2.3 Scanning electron microscopy (SEM)

The scanning electron microscopic (SEM) photographs of the surface of commercial silica gel, the MTS and the MTS-2 are illustrated in Figures 7 (a), (b) and (c), respectively. After the pyoverdin I is formed by agglomeration onto the surface of the MTS-2, the presence of pyoverdin I does not seem to play a very crucial role in the final structure of commercial silica gel, but some changes are observed in the surface areas of commercial silica gel. As the result, the surface of the MTS-3 is as shown in Figure 8.

2.4 Nitrogen adsorption isotherm

To study the nitrogen adsorption isotherm of the synthetic sorbent (Figure 8), the surface area of a commercial silica was calculated through the BET equation (surface area, $S_{BET}$ = 609.7 $m^2.g^{-1}$). However, the $Fe^{3+}$-pyoverdin I complex was selected to anchor onto the MTS-2 surface, which is involved in the binding of $Fe^{3+}$ with epoxy groups or silanols. Hence, the formation of the $Fe^{3+}$-pyoverdin I complex leaves only one free amino group on the peptide backbone. This amino group reacts covalently with the epoxy group of the spacer during the anchoring process. The depression of the surface area of commercial silica gel after incorporating pyoverdin I is due to the fact that the adsorption of nitrogen molecules on the surface is blocked by the presence of pendant groups, resulting in the depression of the surface area of the MTS-3 (surface area, $S_{BET}$ = 405.4 $m^2.g^{-1}$).

## Conclusions

This paper demonstrates high selectively of immobilized *P. aeruginosa* pyoverdin I isolated onto the MTS surface as an alternative solid support for metal remediation. This material was prepared by immobilization of the pyoverdin I onto the MTS-2 surface. In the first step, the silica was epoxy activated using reaction with GPTMS in toluene. Then, this epoxy-silica was subjected to a process of end-capping by reacting with CTAB in order to minimize the number of remaining silanol groups on the surface which could interfere with the chelating ability and selectivity of the functionalized silica. Surfactant templated silica materials were synthesized using cationic CTAB, keeping detergent inside the silica network avoiding material calcinations. Surfactant shape and its polar head charge strongly influence the type of aggregates formed during silica polymerization. Finally, ligand pyoverdin I was immobilized in the solid matrix by chemical coupling through the reaction of the epoxy groups of the support with the aminoalkyl pendent group of the ligand at slightly basic conditions. Moreover, various techniques were used to investigate for immobilization of the purified pyoverdin I onto the MTS-2. Some characteristics of the MTS-3 were confirmed by spectrofluorophotometry, FTIR, SEM and nitrogen adsorption isotherm techniques. Further, the synthesized solid support will be used for reducing the toxicity of some heavy metals in aqueous solution.

## Acknowledgements

Financial support from the Center for Innovation in Chemistry: Postgraduate Education and Research Program in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education is gratefully acknowledged. We also thank Khon Kaen University and the National Research Council of Thailand (NRCT) for facilitating both instruments and chemicals including research grants.

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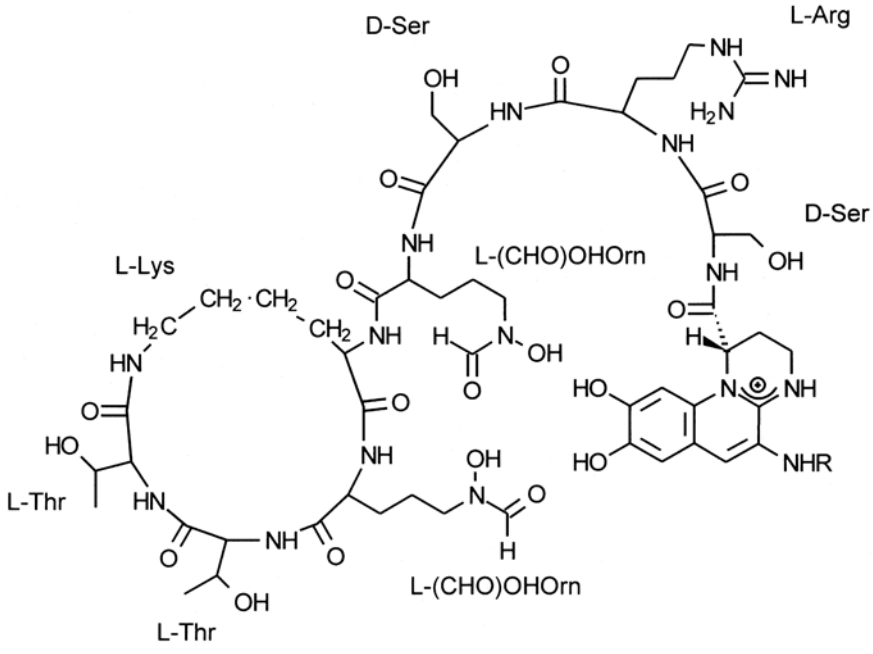


**Figure 1** Structure of the pyoverdin I obtained from *P. aeruginosa* as reported by Budzikiewicz et al. (2002).

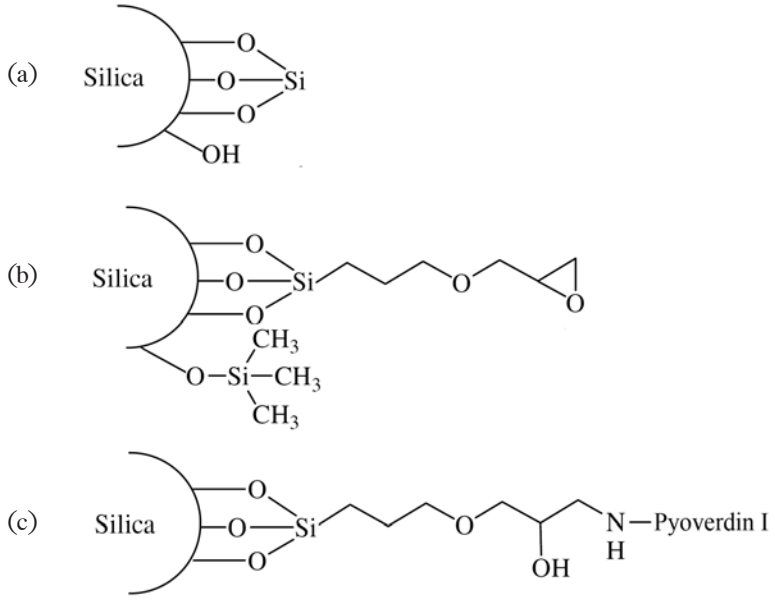


**Figure 2** Proposed structures of; (a) the MTS (Ottaviani et al., 2004), (b) the MTS-2 (Jal et al., 2004) and (c) the MTS-3 (Renard et al., 2005).

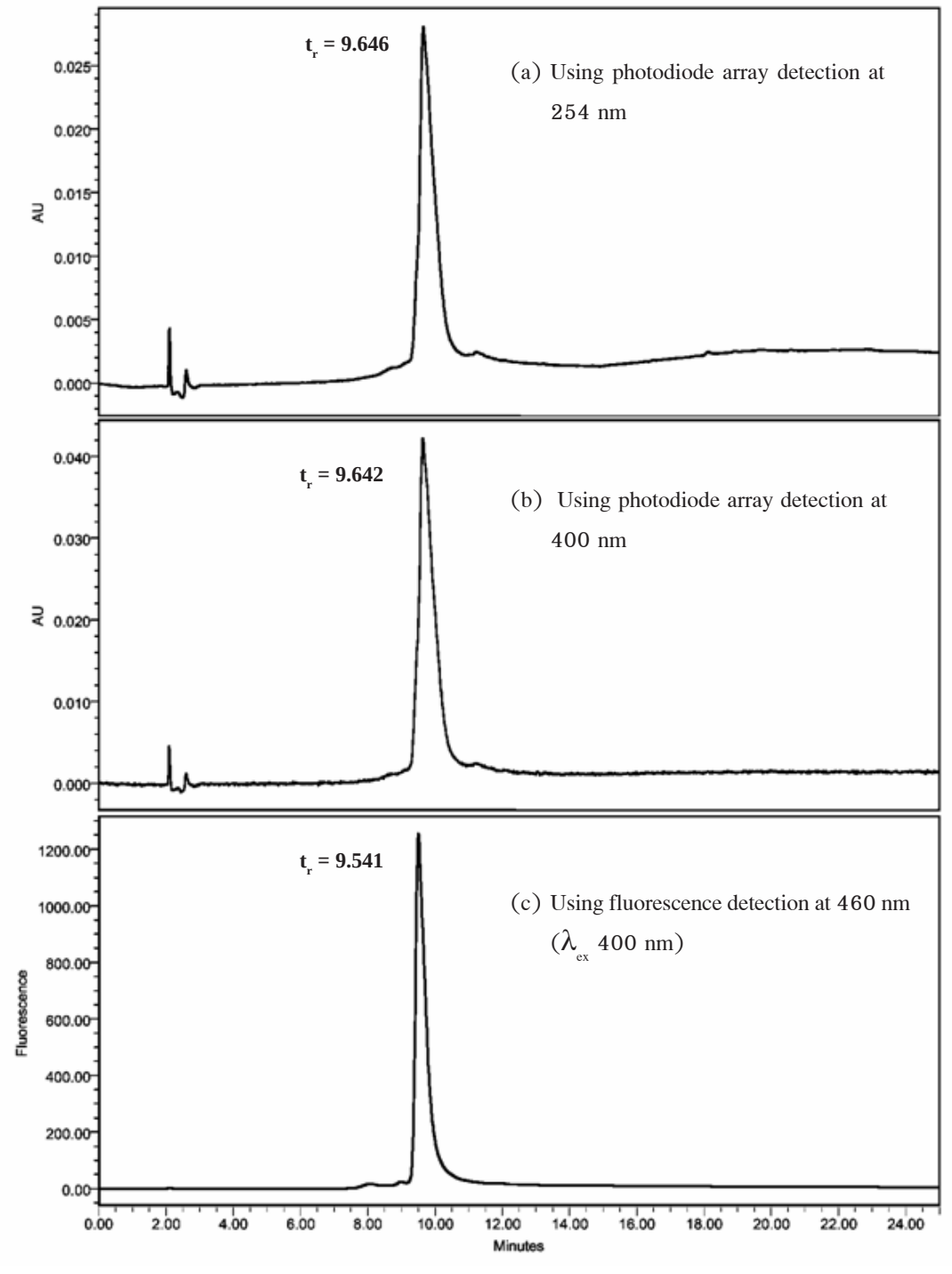


**Figure 3** HPLC chromatograms of the purified siderophore obtained from Bio-Gel P-2 column, photodiode array detection at 254 nm (a), photodiode array detection at 400 nm (b) and fluorescence detection at 460 nm ($\lambda_{ex}$ 400 nm) (c).

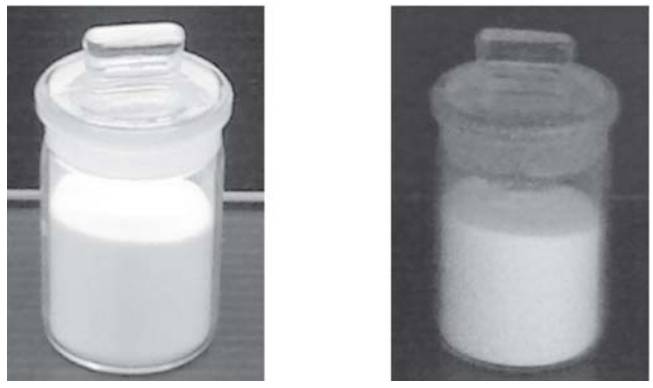

**Figure 4** Photographs of the MTS-3, visual observation (a) and under UV light (b).

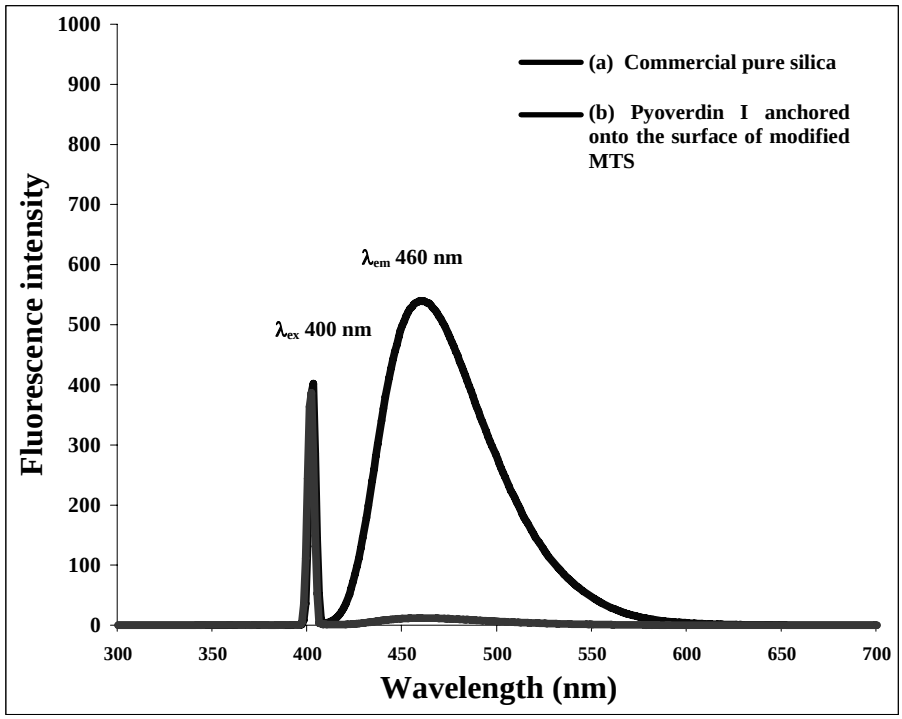


**Figure 5** Fluorescence spectra of the commercial silica gel (a) and the MTS-3 (b).

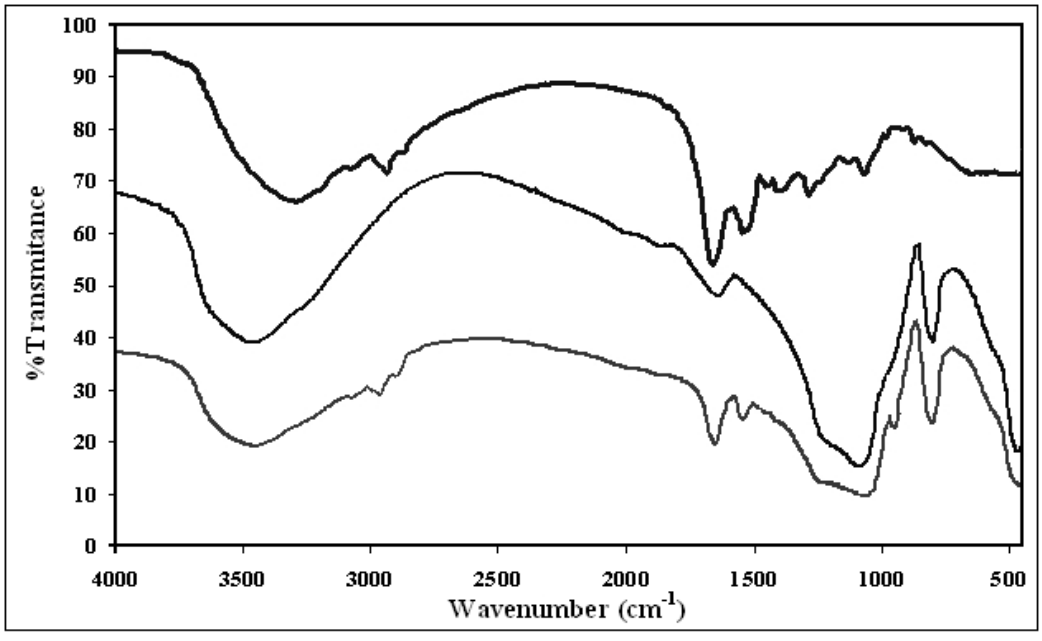


**Figure 6** Fourier transform infrared (FTIR) spectra of; the pyoverdin I (a), the commercial silica gel (b) and the MTS-3 (c).

**Table 1** Major chemical groups and wave numbers present in the structure of the pyoverdin I, the commercial silica gel and the MTS-3 by FT-IR analysis.

| **Functional groups** | **O-H stretching** | **$CH_2$ stretching** | **C=O stretching** | **C=C stretching** | **C-O stretching** | **C-N stretching** | **C-N stretching** | **$C-NH_2$ stretching** | **Aromatic ring** |
|---|---|---|---|---|---|---|---|---|---|
| **Pyoverdin I ($cm^{-1}$)** | **3248** | **2933** | **1661** | **1543** | **1456** | **1410** | **1278** | **1071** | **900-500** |
| **Functional groups** | **O-H stretching** | **H-O-H stretching** | **-** | **-** | **Si-O-Si stretching** | **-** | **-** | **Si-O stretching** | **O-Si-O stretching** |
| **Commercial silica gel ($cm^{-1}$)** | **3460** | **1633** | **-** | **-** | **1088** | **-** | **-** | **801** | **460** |
| **Functional groups** | **O-H stretching** | **$CH_2$ stretching** | **C=O stretching** | **C=C stretching** | **Si-O-Si stretching** | **-** | **$C-NH_2$ stretching** | **Si-O stretching** | **O-Si-O stretching** |
| **MTS-3 ($cm^{-1}$)** | **3468** | **2950** | **1655** | **1536** | **1087** | **-** | **985** | **799** | **469** |

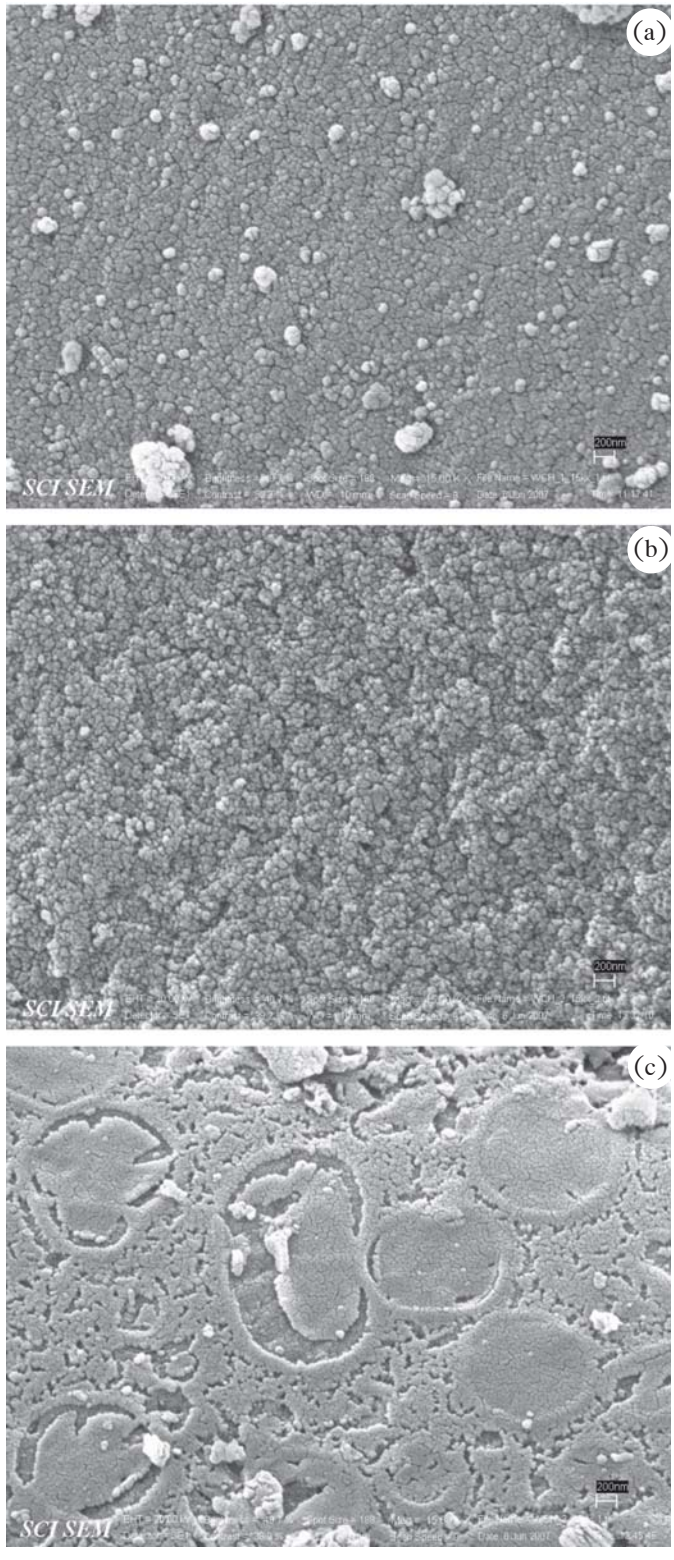


**Figure 7** SEM photographs of the commercial silica gel (a), the MTS (b) and the MTS-2 surface (c), x 1.5k.

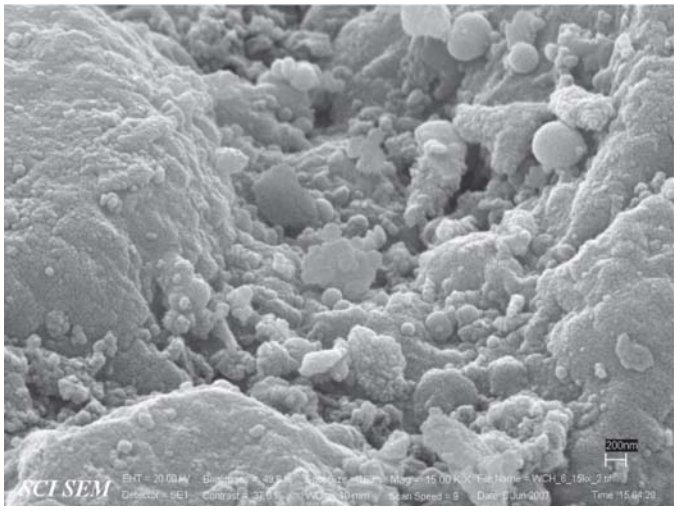


**Figure 8** SEM photograph of the MTS-3 surface, x 1.5k.

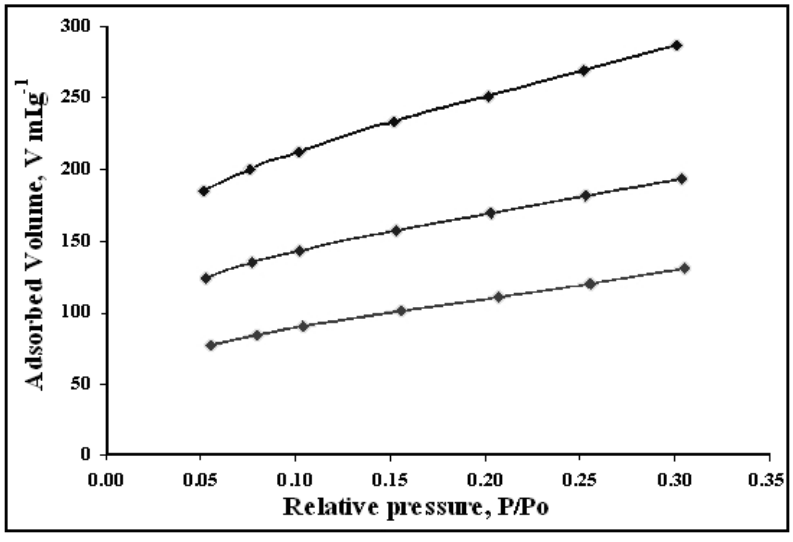


**Figure 9** Nitrogen adsorption isotherms of the commercial silica gel (a), the MTS-2 (b) and the MTS-3 surface (c)