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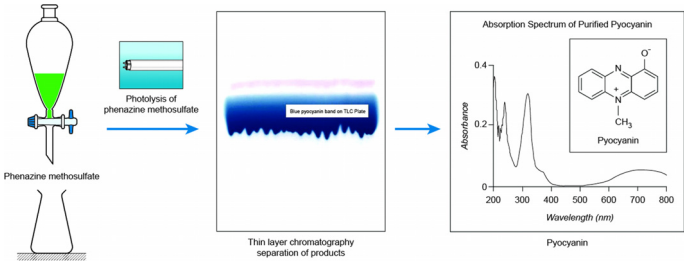
# Standardized chemical synthesis of *Pseudomonas aeruginosa* pyocyanin

Rajkumar Cheluvappa *

*Department of Medicine, St. George Clinical School, University of New South Wales, Sydney, NSW, Australia*

## GRAPHICAL ABSTRACT




## ABSTRACT

Preparation of the toxin pyocyanin from the bacterium *Pseudomonas aeruginosa* is an exacting procedure. Pyocyanin is expensive to commercially purchase. The sellers do not give out the extraction procedure. Classically, pyocyanin preparation involves complicated multi-step *P. aeruginosa* culturing and solvent transfer extractions. The chemical synthesis first used (1979) has not been adequately described. We devised an easily reproducible protocol which consistently decreases the time taken for synthesis, extraction and purification of pyocyanin, and increases the pure pyocyanin proportion produced.

Our procedure:

- Involves more purification steps (chloroform/methanol/acidification/alkalinization).
- Starts with a different pH (7.4 instead of 7), and lesser concentration of phenazine methosulfate; and retrenches a rotary evaporation step.
- Removes 2 lyophilization steps, and entails different solvent proportions for thin layer chromatography.

As we have extracted pyocyanin both from *P. aeruginosa* cultures, and via chemical synthesis; we know the procedural and product-quality differences. We endorse the relative ease, safety, and convenience of using the chemical synthesis described here. Crucially, our "naturally endotoxin-free" pyocyanin can be extracted easily without using infectious bacteria.



* Correspondence to: Inflammation and Infection Research Centre, School of Medical Sciences, Wallace Wurth Building, University of New South Wales, Gate 9 High Street, Sydney, NSW 2052, Australia. Tel.: +61 0406 0406 20; fax: +61 02 9385 1389.
*E-mail address:* rajkumarchel@gmail.com.

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## Materials

*Reagents*

1. Phenazine methosulfate or PMS (Laboratory reagent grade) – P9625 (Sigma-Aldrich Pty. Ltd, Sydney, Australia)
2. Nitrogen (Industrial grade) – 032 (BOC gases, Sydney, Australia)
3. Chloroform (Laboratory reagent grade) – 132950 (Sigma-Aldrich Pty. Ltd, Sydney, Australia)
4. Hexane (Laboratory reagent grade) – 296090 (Sigma-Aldrich Pty. Ltd, Sydney, Australia)
5. Methanol (Laboratory reagent grade) – 322415 (Sigma-Aldrich Pty. Ltd, Sydney, Australia)
6. Millipore water
7. Stock HCl (Laboratory reagent grade) – 320331 (Sigma-Aldrich Pty. Ltd, Sydney, Australia)
8. TRIS–HCl (Laboratory reagent grade) – T3253 (Sigma-Aldrich Pty. Ltd, Sydney, Australia)
9. NaOH pellets (Laboratory reagent grade) – S8045 (Sigma-Aldrich Pty. Ltd, Sydney, Australia)

*Equipment*

1. Nitrogen tank, pressure gauge with outlet, gas tubes
2. pH meter/probe
3. Fluorescent tube light – Phillips TLD 18 W/54 (TIS.958-2533 and TIS.236-2533)
4. Separation funnels
5. Pear-shaped glass flasks
6. Fume hood
7. Pasteur pipettes
8. Tape and fasteners
9. Centrifuge (5000 rpm – capable with 50 ml polypropylene tube-holders)
10. 50 ml polypropylene tubes
11. Polypropylene syringe
12. Type EH 0.5 m filters
13. Thin Layer Chromatography (TLC) Plates – TLC plates from Merck (HPTLC Pre-coated Silica Gel 60 Plates)
14. Glass gas cage without paper lining
15. A4 size paper
16. Microwave oven
17. Desiccator
18. Sample applicator (Camag Nanomat), applicator syringe, 1 ml and 5 ml applicator glass tips
19. Computer scanner
20. Sterile scalpel blades
21. Small glass tubes, and refrigerated centrifuge with small slots to hold the small glass tubes
22. Shimadzu Spectrophotometer with deuterium lamp emitting UV light (wavelength ranging from 200 to 350 nm) and a halogen lamp (wavelength ranging from 350 to 800 nm – visible light)
23. Quartz cuvettes

## Method details

Our "customization" of the original method [1] is succinctly posited in Table 1. Most of the steps are different, and hence the preference of the Table 1 summary over stepwise annotation in this section.