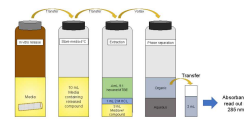


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🌐 In vitro release, extraction, and analysis of PCBs and metabolites from polymeric implants

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We use this protocol and it's working

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Abstract

This protocol describes a method to determine the release of 2,2',5,5'-tetrachlorobiphenyl-4-ol (4-OH-PCB 52), a human-relevant metabolite of PCB 52 (2,2',5,5'-tetrachlorobiphenyl), from polymeric implants *in vitro*. Implants containing 0%, 1%, 5%, or 10% of 4-OH-PCB 52 by weight were incubated in 10% bovine calf serum in PBS (pH = 7.4). The bovine calf serum/PBS mixture was replaced regularly, typically every 24 h, for up to 28 days. The 4-OH-PCB 52 released from the implants was extracted from an aliquot of the bovine calf serum/PBS mixture with hexanes, and levels of 4-OH-PCB 52 were quantified by UV/Vis spectroscopy against a matrix-matched standard calibration curve. This approach demonstrated the continuous release of 4-OH-PCB 52 from the implants over 28 days under physiological conditions.

Materials

Chemicals list

PBS p.H. 7.4 (Gibco, Grand Island, NY, USA, Cat # 14200-075)

Deionized water

Bovine Calf Serum (Hyclone, Logan, UT, USA, Cat # SH30072.03)

Penicillin-streptomycin (Gibco, Grand Island, NY, USA, Cat # 15140-122)

Sodium Chloride (Research Products International)

Hydrochloric acid concentrated (12.5M, Sigma Aldrich)

Hexane, Pesticide grade (Fisher, cat no H300-4)

Methyl tert-butyl ether (MTBE, Fisher, cat no E127-4)

Accessories

500 ml volumetric flasks with stoppers

20 mL amber vials with screw cap

2 × 100 ml volumetric flasks with stoppers

Glass tubes, size 16×125 mm (Biochem Stores, cat no 188950)

PTFE-coated Screw caps, thread 14-415 mm (Fisher, cat no 14-930-15E)

Pasteur pipettes (Fisher, cat no 22-183632)

Instruments

Agitating water bath

Eppendorf centrifuge 5810 R with rotor #A-4-62

UV/vis instrument (Hewlett Packard 8453)

Rotatory inverter for tubes (Fisher Scientific, cat no 15564090)

Accessories and glassware cleaning:

All glassware is rinsed with hot water as soon as possible after use. The glassware is left in soapy water no longer than 24 hrs and rinsed with tap water until soap bubbles are gone followed by 2 rinses with DI water. All glassware is heated in a furnace at 450 °C overnight. After cooling, the glassware is capped with aluminum foil and stored in a clean environment to be protected from dust and other contaminants.

Teflon caps are cleaned by keeping them in a beaker with hexane overnight. Afterward, the solvent decanted and residual solvent is allowed to evaporate in a chemical fume hood. Caps stored in beaker covered with foil.

Polymeric implants

Polycaprolactone implants containing 0%, 1%, 5%, and 10% of 2,2',5,5'-tetrachlorobiphenyl-4-ol (4-OH-PCB 52) were prepared as described in a separate protocol (see references, DOI:

dx.doi.org/10.17504/protocols.io.n2bvj667plk5/v1).



Safety warnings

**Hexane:**

Work under a hood. Do not inhale the substance/mixtures. Avoid the generation of vapors/aerosols.

Hydrochloric Acid:

Do not breathe vapors or aerosols. Avoid substance contact. Ensure adequate ventilation. Evacuate the danger area, observe emergency procedures, and consult an expert.

Methyl *tert*-butyl ether (MTBE):

Do not breathe vapors or aerosols. Avoid substance contact. Ensure adequate ventilation. Keep away from heat and sources of ignition. Evacuate the danger area, observe emergency procedures, and consult an expert.

Penicillin-Streptomycin:

Ensure adequate ventilation. Always wear recommended Personal Protective Equipment.

Polychlorinated biphenyl (PCB):

May cause damage to organs through prolonged or repeated exposure. Very toxic to aquatic life with long-lasting effects. Do not breathe dust/fume/gas/mist/vapors/spray. Avoid release to the environment. This statement does not apply where this is the intended use. Get medical advice/attention if you feel unwell. Collect spillage. Dispose of contents/containers in accordance with relevant regulations.

Before start

Always wear proper Personal Protective Equipment and work in a fume hood when working with hexanes, hydrochloric acid, MTBE, penicillin-streptomycin, and polychlorinated biphenyl (PCBs) derivatives.

In vitro release of 2,2',5,5'-tetrachlorobiphenyl-4-ol (4-OH-PCB 52) from implants

- 1 Add 10 ml of media (10% bovine serum in PBS containing 1% penicillin-streptomycin, v/v) to 20 mL amber vials with screw caps

Note: It is helpful to add penicillin-streptomycin to the media because the samples may be stored for several weeks before extraction and analysis

- 2 Add a 2 cm piece of a polymeric implant containing either 0%, 1%, 5%, or 10% of 2,2',5,5'-tetrachlorobiphenyl-4-ol (4-OH-PCB 52) per vial. Each concentration is analyzed in triplicate. Document the weight of each implant and the time point of media addition and collection

- 3 Place vials in a shaking water bath at 37 °C at a speed of 40 rpm

- 4 Replace the media at designated time points by transferring old media to 10 mL glass tubes and adding 10 mL of fresh media to the vials, leaving the implant in the vials

- 5 Store old media capped at 4°C until extraction

Note: The media solutions must be stored in glass vials and PTFE-coated caps because PCB compounds can partition into plastic ware over time

- 6 Record time of media transfers (1 hour, 4 hours, 12 hours, 24 hours, and every 24 hours)

Extraction

- 7 Transfer 3 mL of each media sample from the *in vitro* release experiment described above into clean, well-labeled glass vials for extraction (Vial_A)

- 7.1 TIP: A seven-point calibration curve of the study compound is prepared and extracted concurrently. The calibration curve can then be used to check the instrument for drift. If you are running many samples, consider running the calibration curve before, during, and after all of the samples

- 8 Acidify all 3 mL media samples by adding 1 mL of 2 M HCl and vortex the sample vials (Vial_A)

- 9 Add 4 mL of hexane:MTBE (9:1) to the vials (Vial_A)



- 10 Cap the vials (Vial_A) and shake vigorously
- 11 If an emulsion forms, add 0.5 g of NaCl to break the emulsion. Otherwise, skip this step
- 12 Invert vials (Vial_A) for 5 min and centrifuge vials at 1811 g (3,000 rpm) for 5 min
- 13 Transfer the organic phase (top layer) to new vials (Vial_B)
- 14 Cap the vials containing to organic extract (Vial_B) and store them at -20°C until analysis

UV/Vis samples

- 15 Transfer 2 mL of hexane:MTBE (9:1), calibration standards, or organic sample extract (vial_B) to a clean quartz cuvette
- 16 Analyze samples on the UV/Vis spectrometer at 285 nm. Keep the instrument/sample temperature at 25°C if a circulating water bath is available. Use the sample order shown in steps 16.1 to 16.4

Note: Dilute samples if the reading is over 1 in absorbance
- 16.1 Use 2 mL of hexane:MTBE (9:1, v/v) to zero the instrument as the first sample
- 16.2 Analyze 2 mL of the extract from all calibration standards to generate a calibration curve for quantification purposes

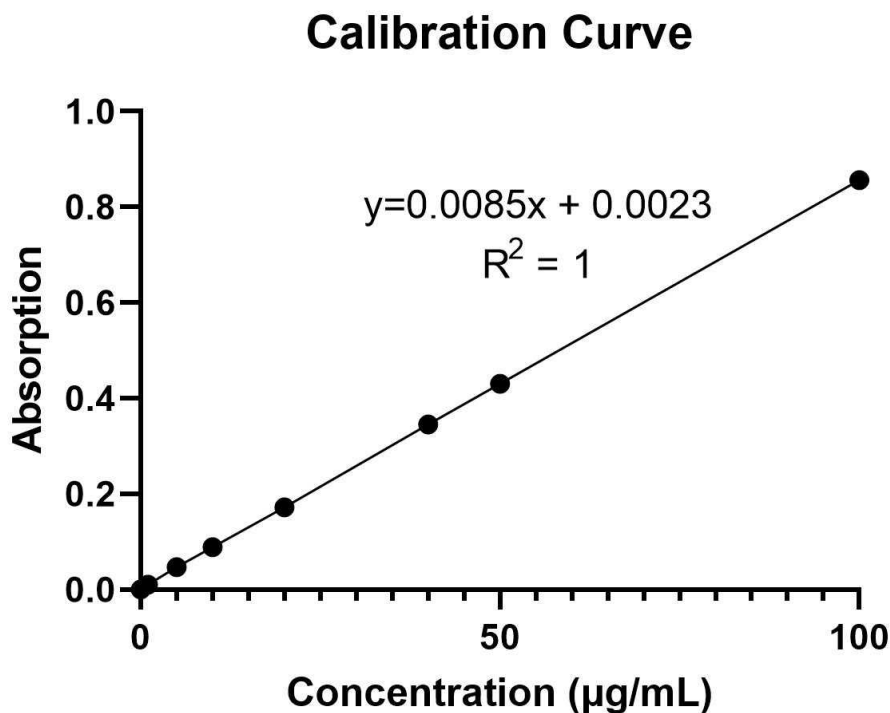


Figure 1. Calibration curve of 4-OH-PCB 52. This matrix-matched calibration curve was prepared by adding known amounts of 4-OH-PCB 52 into the fresh medium. As described in the protocol, aliquots of these standard samples underwent the same extraction process as samples from the implant release study. The absorption was then measured spectrophotometrically using UV/Vis at 285 nm. The file "Calibration_curve_data.xlsx" contains the corresponding data.

 Calibration_curve_data.xlsx

- 16.3 Analyze 2 mL of all sample extracts
- 16.4 Analyze 2 mL of the extract from all calibration standards for a second time after all samples have been analyzed
- 17 Rinse and use the same cuvette (minimize differences between cuvette walls = refraction differences) for each sample.
- 18 Repeat steps 15 to 17 two more times. Analyzing all extracts in triplicate will provide more robust and reproducible results
- 19 Calculate the concentrations of 4-OH-PCB 52 in the hexane:MTBE (9:1, v/v) extracts from the calibration curve.



EXAMPLE: The file "Example_release_data.xlsx" contains release data from four implants containing 5% (sample 1 and 2) and 10% (sample 3 and 4) of 4-OH-PCB 52 from an *in vitro* release study. As outlined in this protocol, media samples were collected at various time points and extracted using hexanes:MTBE (9:1, v/v). The absorption was then measured spectrophotometrically using UV/Vis at 285 nm. The concentration of the test compound in the extract was calculated based on the equation from the calibration curve: concentration = (Absorption-0.0023)/0.0085; see Figure 1.

 Example_release_data.xlsx

Protocol references

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