Towards Systematic Hazard Analysis of Nanomaterials to the Environmental Safety

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Purpose

Factors of the adverse effect of the nanomaterials into the organisms

- Properties/conditions of the surface of the particle itself
- Because of the large surface area, adsorption amount of the toxic substance is large.
- Smaller particle is more easy to be incorporated = more toxic
- Biological reaction is changed when the particle size becomes (less than) a certain size.

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The environmental hazard assessment methodology for nanomaterials is still very limited.

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Acute toxicity and bioconcentration test of fullerene C_{60}

Test item

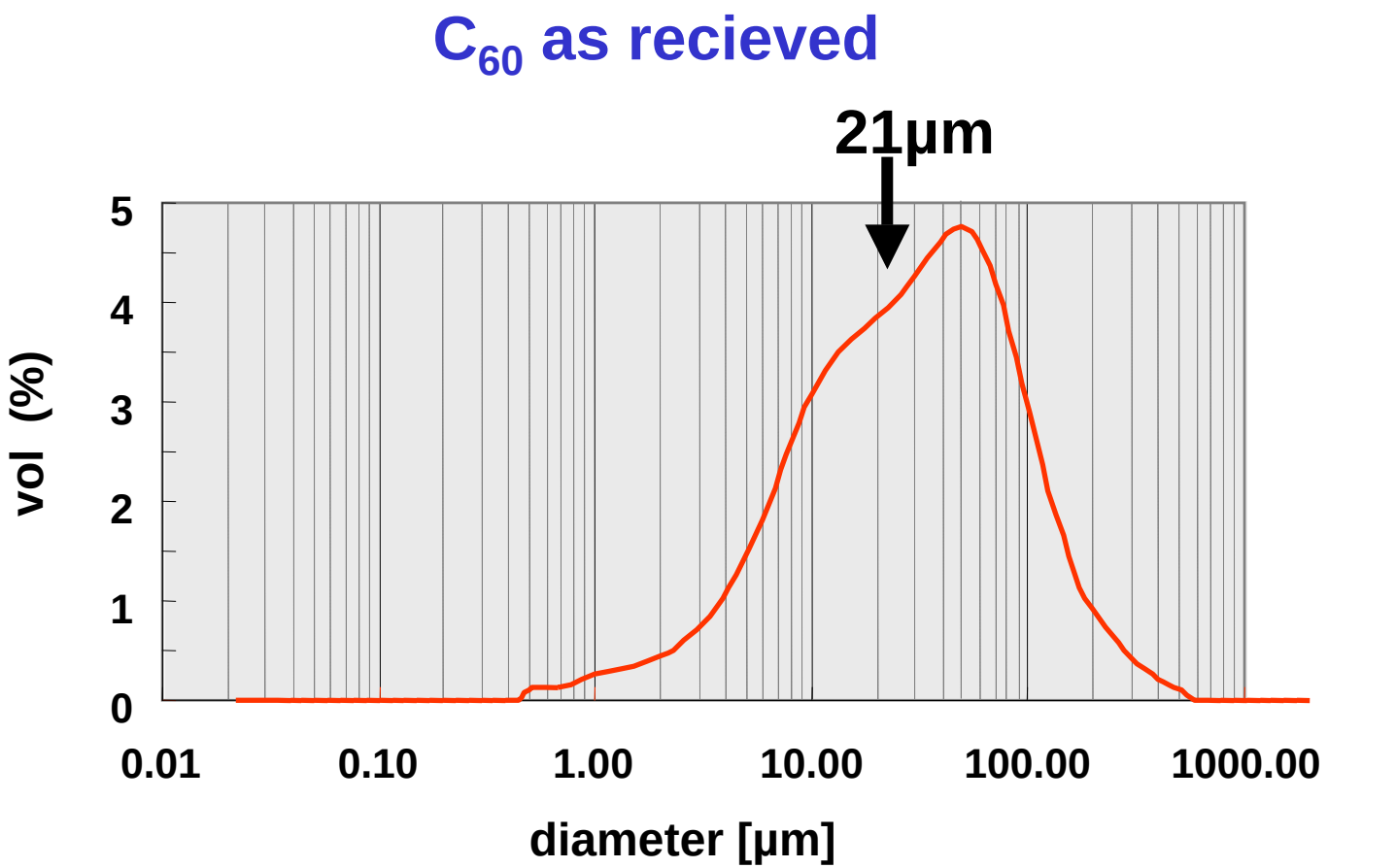
Supplier: Frontier Carbon Corporation

Lot number: 4A0314-A

Purity: 99%

Appearance: Black powder

Average aggregate size: 21μm



Data supplied by Frontier Carbon Corporation

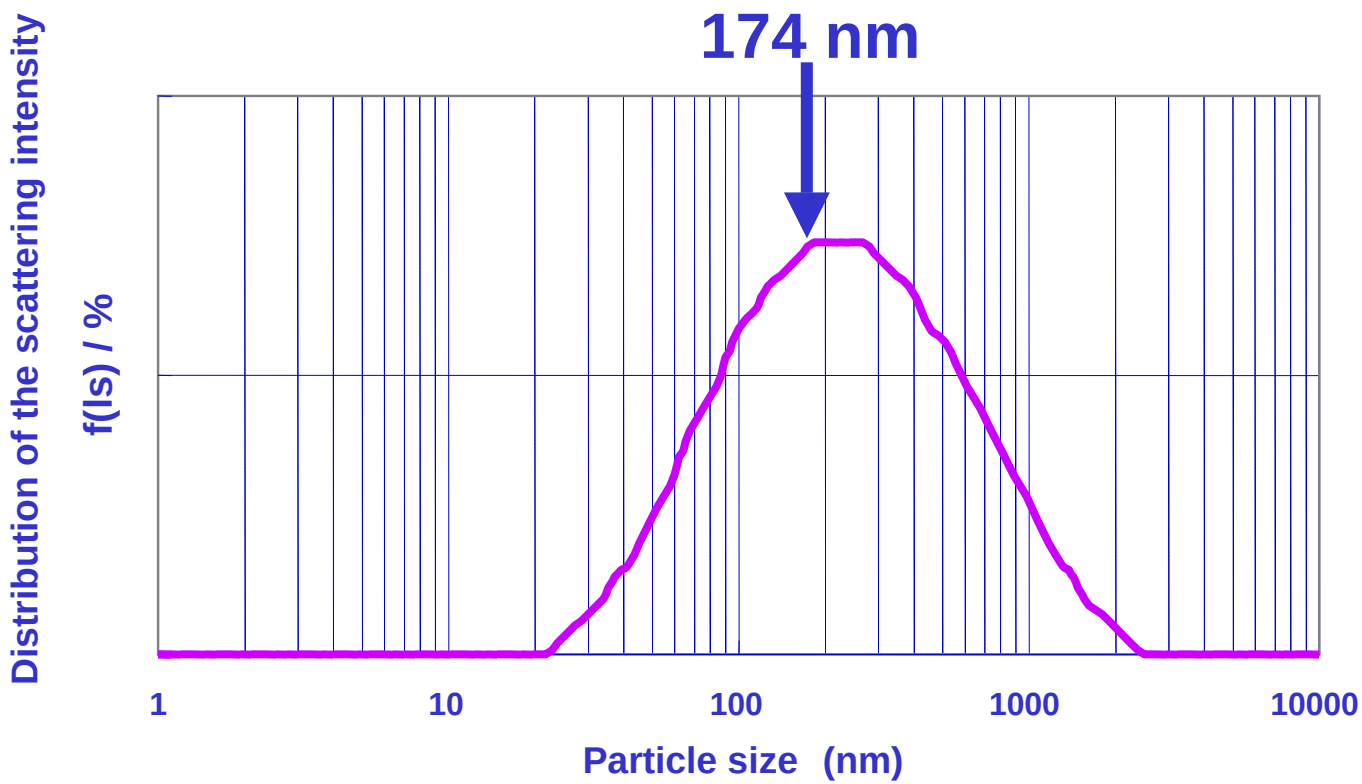
Fig. 1 Average diameter of the particle and the distribution of the particle size of as received C_{60}.

Preparation of the water dispersion

Dispersants

Sugar candy, HCO- 40 (hydrogenated castor oil)

500 mg/L stock solution



Data supplied by Frontier Carbon Corporation

Average diameter of the particle: the cumulant method
Distribution of the particle size : the CONTIN method

Fig. 2 Average diameter of the particle and the distribution of the particle size of the stock solution.

Acute toxicity test of fullerene C_{60}

Test conditions (OECD TG 202)

- Test organism : *Daphnia magna*
- Test concentration: 2.50 mg/L, a vehicle control and a control
- Vol. of test water : 100 mL (4 vessels)
- No. of organisms : 5 daphnids / vessel (4 vessels)
- Duration of exposure : 48 h
- Exposure method : Semi-static system (renewal of test water, at 24 h)
- Temp. of test water : 19.8 - 20.4 ºC
- Light : 16-h light / 8-h dark photoperiod daily with room lamp
- Feeding : No feeding
- Aeration : No aeration

Acute toxicity test of fullerene C_{60}

Test conditions (OECD TG 203)

- Test fish : Medaka (*Oryzias latipes*)
- Test concentration: 2.50 mg/L, a vehicle control and a control
- Vol. of test water : 2.5 L (2 vessels)
- No. of fish : 5 fishes / vessel (2 vessels)
- Duration of exposure : 96 h
- Exposure method : Semi-static system (renewal of test water, at every 24 h)
- Temp. of test water : 23.3 - 24.8 ºC
- Light : 16-h light / 8-h dark photoperiod daily with room lamp
- Feeding : No feeding
- Aeration : Aeration was gently employed for test solutions after 24-h exposure

Quantitative analysis for test item

Analytical conditions

HPLC : LC-10A (Shimadzu Corporation)

Column : L-column ODS (CERI) 15 cm × 4.6 mm I.D.

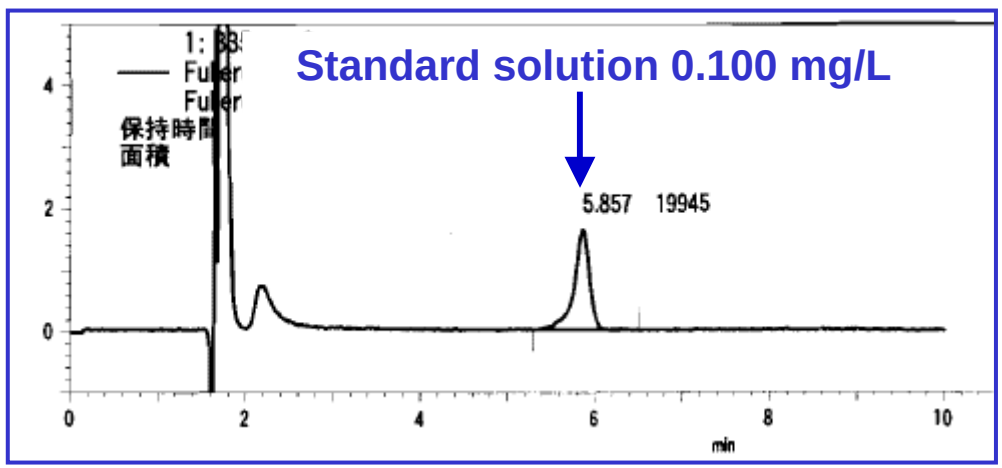
Eluent : Toluene / Methanol (45/55, v/v)

Measurement wavelength : 335 nm

Temperature : 40 ºC

Flow rate : 1.0 mL/min

Sample size : 50 µL



Pretreatment for analysis of test water

Test water 50 mL

← Sodium chloride 15 g
← Toluene10 mL
- Shaking for 10 min.

Lower layer | Upper layer

- Filtration and dehydration (1PS filter paper)
- Filling up to 25 mL (toluene)
- Taking out 0.5 mL

← Methanol 0.5 mL

- Filling up to 25 mL (toluene / methanol 50/50, v/v)

Sample for HPLC analysis

Results of recovery and blank test (125 µg test item added)

Recovery rate: Ave.98 %(n=2)
Blank : N.D.

Result of acute toxicity test of fullerene C_{60}

- ***Daphnia magna***

  48-h EC50 (Median Effective Concentration)

  > 2.25 mg/L*

- **Medaka (*Oryzias latipes*)**

  96-h LC50 (Median Lethal Concentration)

  > 2.15 mg/L*

*** The geometric mean of the measured concentration**

The 96-h LC50 value of the dispersants

Sugar candy	>50,000 mg/L
HCO-40	70,700 mg/L

Bioconcentration test of fullerene C_{60}

Test conditions

- Test fish : Common Carp (*Cyprinus carpio*)
- Test concentration : 0.02 mg/L
- Test tank : 70-L glass tank
- Number of fish : 10 / level
- Temperature of test water : 24.7 - 25.0ºC
- Concentration of DO* : 8.3 mg/L
- pH of test water : 8.1 - 8.2
- Light : 16-h light/8-h dark photoperiod daily
- Feeding : Amount corresponding to about 2 % of total body weight was fed twice a day in halves
- Flow rate of test water:

  2 mL/min for stock solution and 800 mL/min for dilution water, 1,155 L/day of test water, were supplied.

*Dissolved oxygen

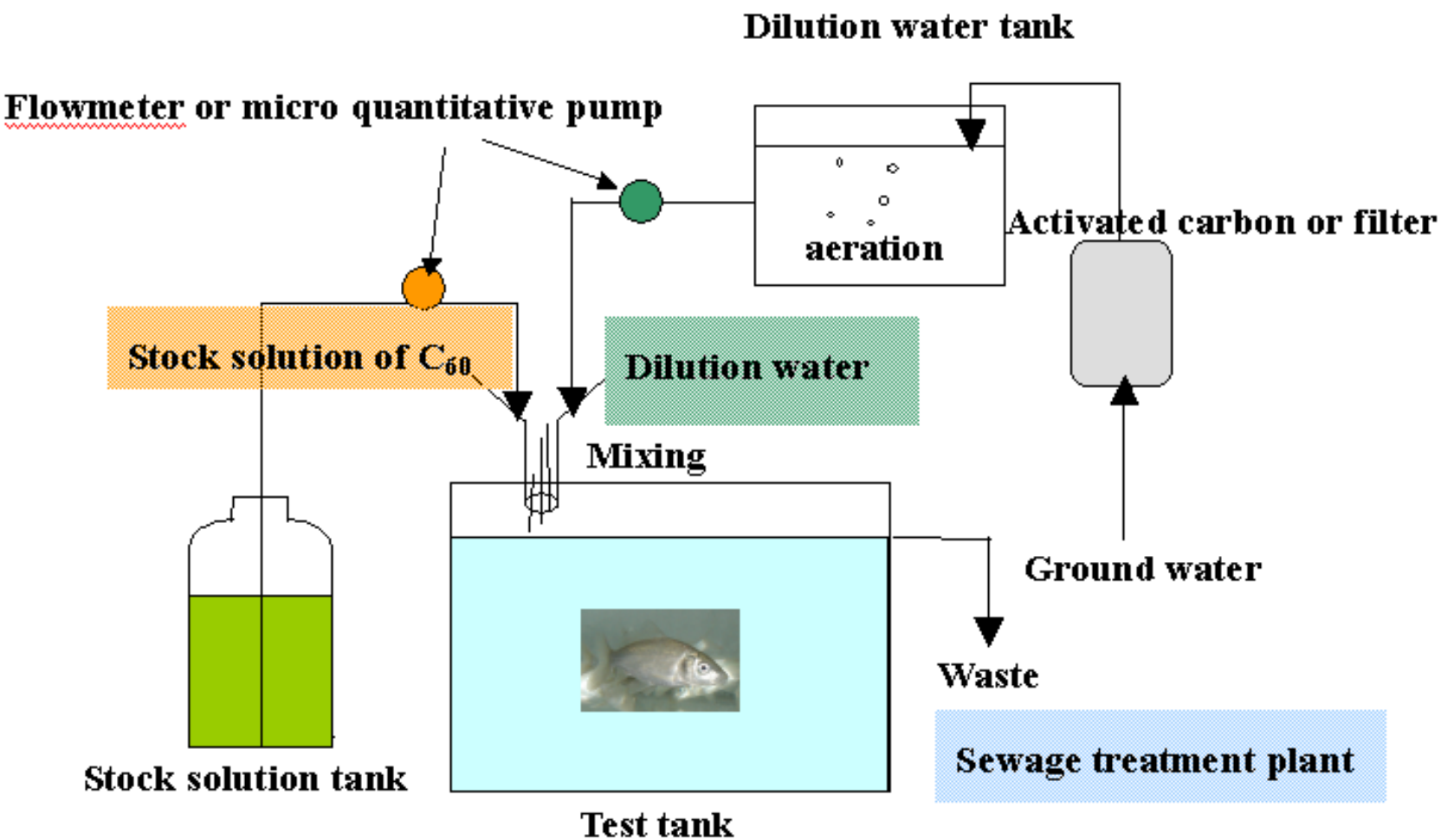


Fig. 3 Diagram of flow-through system for this bioconcentration test.

Quantitative analysis for test item

Analytical conditions

HPLC : LC-10A (Shimadzu Corporation)

Column : L-column ODS (CERI) 15 cm × 2.1 mm I.D.

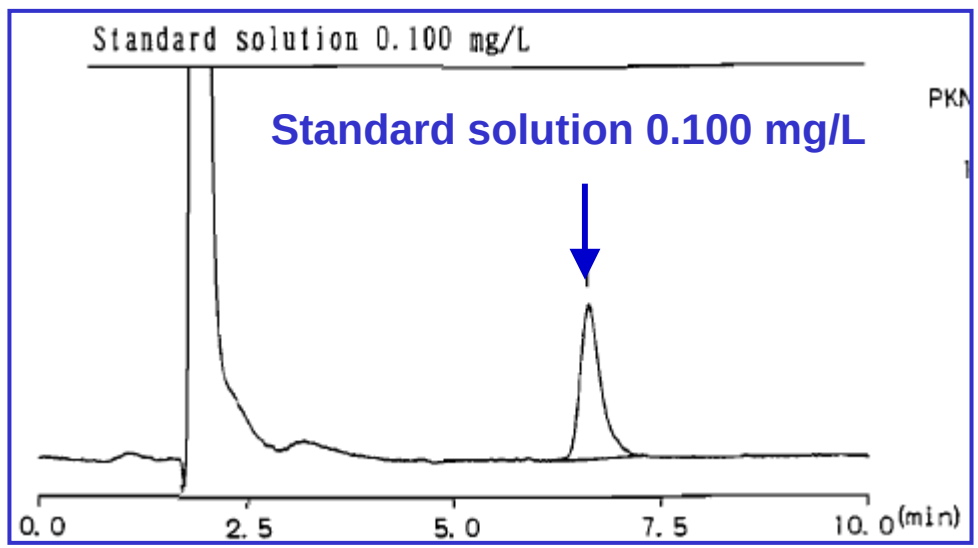
Eluent : Toluene / Methanol (45/55, v/v)

Measurement wavelength : 335 nm

Temperature : 35 ºC

Flow rate : 0.2 mL/min

Sample size : 10 µL



Pretreatment for analysis of test water

Test water 50 mL

← Sodium chloride 15 g
← Toluene10 mL
- Shaking for 10 min.

Lower layer

Upper layer

- Filtration and dehydration (1PS filter paper)
- Concentration
  (rotary evaporator, about 40 ºC)

← Filling up to 10 mL (toluene / methanol 45/55, v/v)

Sample for HPLC analysis

Results of recovery and blank test
(1 µg test item added)

Recovery rate: Ave.85 %(n=2)
Blank : N.D.

Pretreatment for analysis of test fish

Test fish ca. 5 g

- Chopping into pieces (scissors)
- Homogenization (polytron)

Fine sample

- Taking out 1 g
- Dehydration (anhydrous sodium sulfate ca. 8 g)

←Toluene 20 mL

- Homogenization (polytron)
- Centrifugation (7,000 × *g*, 5 min.)

Residue

Supernatant

- Filtration (absorbent cotton)
- Concentration (rotary evaporator, about 40 ºC)
- Filling up to 10 mL (Toluene / methanol 45/55, v/v)

Sample for HPLC analysis

Results of recovery and blank test (20 µg test item added)
Recovery rate: 78 % (n=1)
Blank : N.D.

Result of Bioconcentration test of fullerene C_{60}

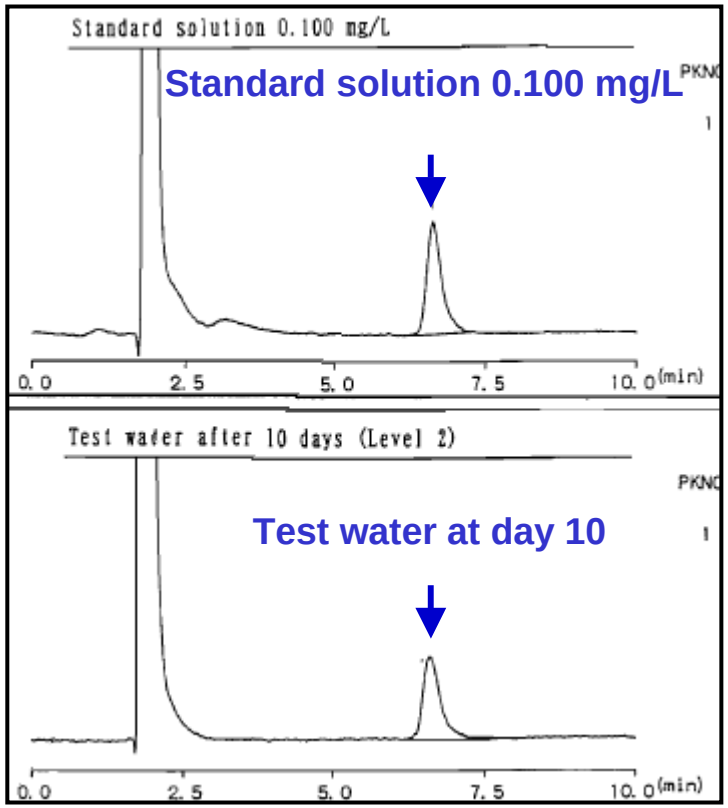


Fig.4 Chromatograms of test water

Test water :0.0212 mg/L

Test fish :N.D. (BCF ≤ 5.8)

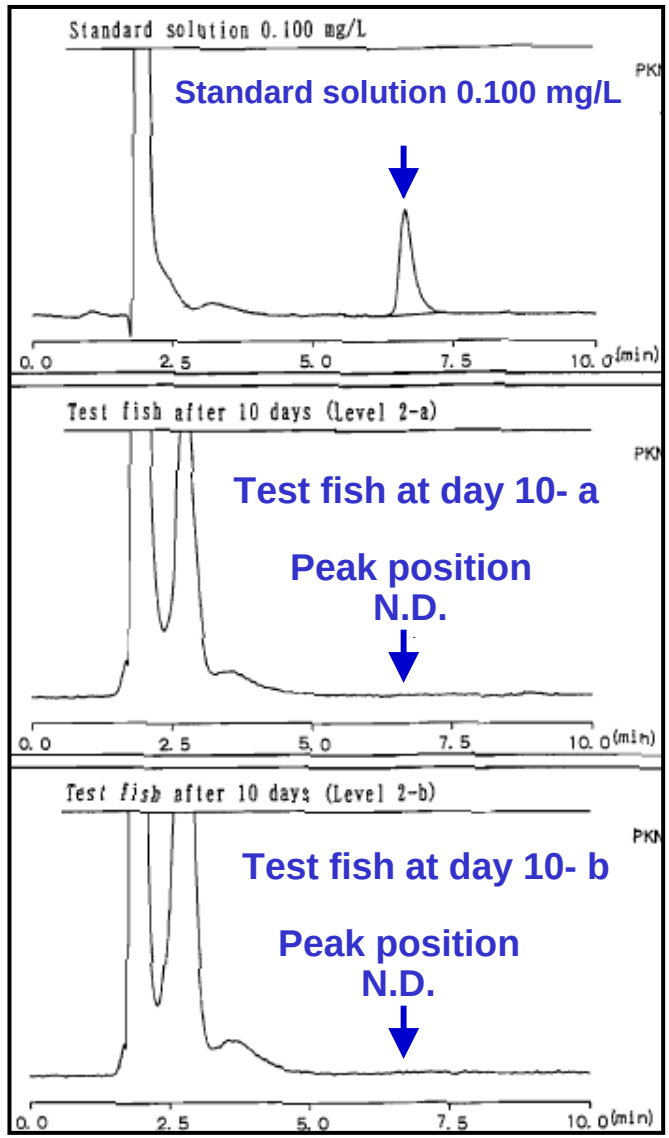


Fig.5 Chromatograms of test fish

Conclusion

- Good water dispersion of fullerene C_{60} is obtained by using sugar candy and HCO-40, resulting in the nano-size particle.

- Fullerene C_{60} (the nominal concentration: 2.50 mg/L) had no effect on the acute toxicity in daphnids and medaka using the water dispersion.

- Bioconcentration potential of fullerene C_{60} in common carp was low using the water dispersion.