# Supplementary Information

Covalent organic nanospheres: facile preparation and application in high-resolution gas chromatographic separation

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## Experimental Section

### Materials and reagents

Tris(4-aminophenyl)amine (TAPA, 98%), terephthalaldehyde (TPA), 4, 4'-biphenyldicarboxaldehyde (BPDA), benzidine, 1,4-benzenediamine, anacetic acid (AcOH, 99.5%) and tris(4-formylphenyl)amine (TFPA, 96%) were purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). Benzene, 2-pentanone, pyridine, 1-butanol, 1-nitropropane, naphthalene, n-hexane, n-pentane, n-heptane, n-nonane, n-octane, n-undecane, n-pentadecane, n-dodecane, n-hexadecane, o-/m-/p-nitroaniline, o-/m-/p-nitrotoluene, ethyl propionate, ethyl acetate, ethyl valerate, ethyl butyrate, ethyl caproate, ethyl lactate, ethyl oenanthate, ethyl caprylate, ethyl nonanoate, ethanol, methanol, 1-butanol, 1-propanol, 1-pentanol, 1-heptanol, 1-hexanol, 1-octanol, acetone, 2-pentanone, cyclopentanone, 2-hexanone, cyclohexanone, 2-heptanone, acetophenone, 2-nonanone, phenylacetone, butyrophenone, acetaldehyde, n-propanol, isobutanol, isoamyl alcohol, 2-pentanol, 2-methyl-2-butanol, 3-methyl-1-butanol and 1-pentanol were purchased from Aladdin Chemistry Co. Ltd. (Shanghai, China). All chemicals and reagents were at least of analytical grade and the mixture solutions were prepared in ethanol. Untreated fused-silica capillary tubing (20 m long × 0.25 mm, i.d.) and CB-5 capillary column were obtained from Lanzhou Donglilong Information Technology Co., Ltd. (Lanzhou, China).

### Apparatus and Characterization

Scanning electron microscope (SEM) images of the covalent organic nanospheres (CONs) and cross section of CONs-based capillary column were obtained on a JSM-6701F field emission scanning electron microscope (JEOL, Japan). Thermal gravimetric analyses (TGA) were recorded with a STA 449F3 Simultaneous Thermal Analyzer (NETZSCH, Germany). Transmission electron microscopy (TEM) images of the CONs were acquired by means of Tecnai G2 TF20 transmission electron microscope (FEI, USA). Chromatographic separation was performed out on an Echrom GC-A90E gas chromatograph equipped with a flame ionization detector. The

chromatographic conditions are as follows: ultrapure nitrogen (>99.999%) was used as a carrier gas at a flow rate of 1 mL min$^{-1}$ and it is also used as make-up gas at 25 mL min$^{-1}$. Purified hydrogen (99.999%) and air (99.995%) were acted as detector gases at flow rates of 30 and 300 mL min$^{-1}$, respectively. Both injector and detector temperatures were maintained at 300 °C temperature. The oven temperature programs were illustrated in their figure captions, respectively.

**Synthesis of CONs**

CONs with different sizes were synthesized from readily available C$_{3v}$-symmetric tris(4-aminophenyl)amine (TFPA) and tris(4-formylphenyl)amine (TFPA) by using the classic Schiff base reaction.

(1) Synthesis of 200 nm sized CONs

1.5 mL of DMF, 0.30 mM of TAPA and 0.30 mM of TFPA were added into 20 mL methanol, and then stirring 10 min at room temperature. Subsequently, a certain amount of acetic acid (0.01 mL) was injected into the above-mentioned mixture, and the mixed solution was left to react for 0.5 h. A deep yellow precipitate was obtained, which was collected by centrifugation at 12000 rpm for 5 min and then respectively washed with anhydrous ethanol, anhydrous acetone, anhydrous dichloromethane, and anhydrous ethanol to remove unreacted residual raw materials. Finally, the obtained sediment was dried at 60 °C in vacuum for 12 h to remove the ethanol and sealed for further use.

(2) Synthesis of 300 nm sized CONs

3.0 mL of DMF, 0.30 mM of TAPA and 0.30 mM of TFPA were added into 20 mL methanol, and then stirring 10 min at room temperature. Subsequently, a certain amount of acetic acid (0.01 mL) was injected into the above-mentioned mixture, and the mixed solution was left at 80℃ react for 0.5 h. Other procedures were consistent with the synthesis process of covalent organic nanospheres (200 nm) mentioned above.

(3) Synthesis of 400 nm sized CONs

1.5 mL of DMF, 0.15 mM of TAPA and 0.15 mM of TFPA were added into 20 mL methanol, and then stirring 10 min at room temperature. Other procedures were consistent with the synthesis process of covalent organic nanospheres (300 nm) mentioned above.

(4) Synthesis of 600 nm sized CONs.

The synthesis of 600 nm sized CONs is similar to that of 200 nm sized CONs except that the reaction solvent is ethanol.

**Synthesis of another six imine-linked covalent organic nanomaterials**

Another six imine-linked covalent organic nanomaterials were synthesized. The difference is the ratio of the reagent pairs (0.30 mM of TAPA and 0.45 mM of terephthalaldehyde (TPA), 0.30 mM of TAPA and 0.45 mM of 4, 4'-biphenyldicarboxaldehyde (BPDA), 0.30 mM of TFPA and 0.45 mM of 1, 4-benzenediamine, 0.30 mM of TFPA and 0.45 mM of benzidine, 0.30 mM of benzidine and 0.30 mM of BPDA, 0.30 mM of benzidine and 0.30 mM of TPA), other procedures were consistent with the synthesis process of covalent organic nanospheres (200 nm) mentioned above.

**Preparation of CONs-based capillary column**

The pre-treatment of the capillary column before static coating was as follows: an untreated fused silica capillary column was washed with 0.1 M HF for 30 min, and ultrapure water until the outflow reached neutral. The capillary was then purged with nitrogen at 40 °C initially, and then raised at a rate of 10 °C min$^{-1}$ to 230 °C and held for 6 h.

CONs-based capillary column was fabricated by a static coating approach: firstly, 1 mg CONs was dispersed in 2 mL acetone and sonicated for 5 min. Subsequently, the above-mentioned CONs stationary liquid was pumped into the whole pre-treated capillary column using nitrogen at a pressure of 0.02 MPa. During the coating process, one end of the filled capillary column was sealed with neutral silicone sealant and the other end was connected to a vacuum system to gradually evaporate solvent in water

bath at 30 °C. The coating process was finished when the solvent was completely evaporated. Finally, the resulting CONs-coated capillary column was treated at 40 °C for 30 min and then conditioned by increasing its temperature at a rate of 5 °C min⁻¹ from 40 to 280 °C (held at the high-end temperature for 6 h), it is worth noting that the whole process was carried out at a constant pressure of nitrogen (0.02 MPa).

**Thermo gravimetric analysis**

The fabricated CONs were conducted by TGA, and the result confirmed its high thermal stability plotted in Fig. 1D. The initial degradation stage of our prepared CONs (the weight loss was about 3.4%) was due to the loss of the moisture absorbed on the CONs surface. However, when the temperature was above 500 °C, a major mass loss was emerged due to the total disintegration of the structure of CONs. Furthermore, increasing the temperature also caused other organic solvent/organic group to decompose.[1-3] The above result is favorable for its subsequent high-resolution gas chromatographic application.

**Analysis of the thermodynamic parameter**

The value of thermodynamic parameter, such as enthalpy change △H, entropy change △S, which could illuminate the energy effect on the interaction between the proposed CONs separation medium and studied target analyte, was calculated according to the Van't Hoff equation (Eq. 1).[4,5]

$$lnk = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} + ln\varphi \qquad (1)$$

Where k is retention factor, T stands for absolute temperature, R represents gas constant and the value is 8.314 J mol⁻¹·K⁻¹, φ denotes phase ratio (which is defined as the volume of the stationary phase divided by the volume of the mobile phase). For a capillary column, the phase ratio is related to the column diameter ($d_c$) and the film thickness ($d_f$) via the following formula:[6]

$$\varphi = \frac{r_c}{2d_f} \qquad (2)$$

Thus, in this work, the φ value was calculated to be 189.40 according to the thickness of the coating thickness (about 0.33 μm).

**Kinetic evaluation**

Golay equation[7] describes the band broadening effect in the open tubular gas chromatographic. The CONs is coated to the inner wall of the column, so there is no eddying diffusion effect. Thus, the theoretical plate height is only related to longitudinal diffusion and mass transfer resistance.

$$H = \frac{2D_m}{u} + \frac{(1 + 6k' + 11k'^2)r^2}{24(1 + k')^2 D_m}u + \frac{r^2 k'^3}{6(1 + k')^2 K^2 D_s}u \qquad (3)$$

Here k′ stands for the retention factor; r is the inner radius of the capillary column; $D_s$ and $D_m$ denote the diffusion coefficients of the target analytes in the stationary phase and mobile phase, respectively; if the peak of an unretained substance is taken as an example (k′= 0), formula (4)[8] can be ultimately simplified to formula (5):

$$H = \frac{B}{u} + Cu \qquad (4)$$

$$H = \frac{L}{N} \qquad (5)$$

In which H denotes plate height (here the height equivalent to a theoretical plate), B represents the coefficient of longitudinal diffusion ($cm^2 s^{-1}$), u represents the average linear velocity of the carrier gas ($cm s^{-1}$) and C stands for the coefficient of mass transfer resistance (s), N is theoretical plate number.
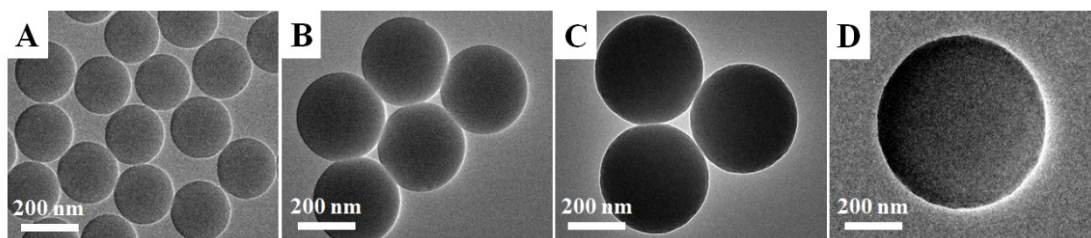


**Fig. S1** TEM images of different sizes of the CONs (A. 200 nm; B. 300 nm; D. 400 nm; D. 600 nm)

**(A)**



**(B)**

**Fig. S2** Photos for pH (A) stability and solvent (B) stability of the dispersed CONs after 24 h.
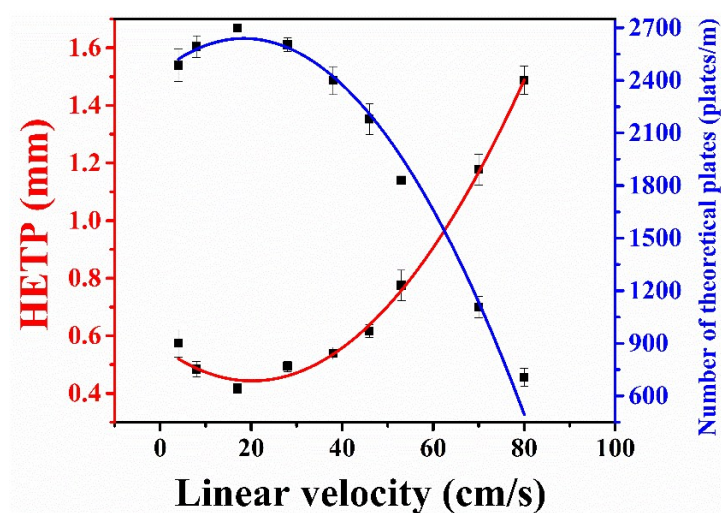


**Fig. S3** The dependence of the numbers of theoretical plates per meter and HETPs on the average linear velocity of the mobile phase for naphthalene.

**Table S1** McReynolds constants of CONs and commercial column.

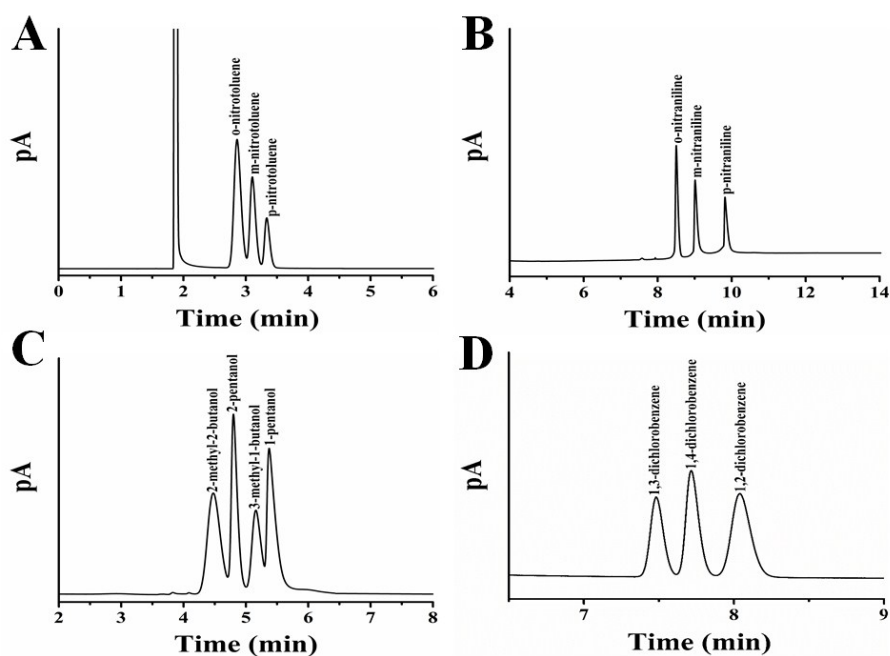| Stationary phase | X′ | Y′ | Z′ | U′ | S′ | Sum | Average |
|---|---|---|---|---|---|---|---|
| I for CONs | 598 | 681 | 791 | 815 | 780 | | |
| I for squalane | 653 | 590 | 627 | 652 | 699 | | |
| ΔI for CONs | -55 | 91 | 164 | 163 | 81 | 444 | 88 |
| ΔI for CB-5 | 28 | 63 | 89 | 90 | 162 | 447 | 89 |



**Fig. S4** Separations of isomers on the CONs-based capillary column. Oven temperatures: 240 °C for (A) nitrotoluene isomers, 290 °C for (B) nitroaniline isomers, 190 °C for (C) pentanol isomers and 250 °C for (D) dichlorobenzene isomers.

**Table S2** Values of $\Delta H$, $\Delta S$, $\Delta S/\Delta H$ and $R^2$ for nitroaniline isomers, nitrotoluene isomers and pentanol isomers.

| Analytes | -$\Delta H$ (kJ/mol) | -$\Delta S$ (J/mol K) | $\Delta S/\Delta H$ ($10^{-3}$/K) | $R^2$ |
|---|---|---|---|---|
| o-nitroaniline | 70.71 | 156.46 | 2.21 | 0.987 |
| m-nitroaniline | 77.06 | 168.26 | 2.18 | 0.991 |
| p-nitroaniline | 93.45 | 199.98 | 2.14 | 0.999 |
| o-nitrotoluene | 85.04 | 197.75 | 2.32 | 0.999 |
| m-nitrotoluene | 89.74 | 206.91 | 2.31 | 0.998 |
| p-nitrotoluene | 91.16 | 208.19 | 2.28 | 0.999 |
| 2-methyl-2-butanol | 49.95 | 155.50 | 3.11 | 0.990 |
| 2-pentanol | 52.30 | 159.65 | 3.05 | 0.991 |
| 3-methyl-1-butanol | 60.79 | 173.28 | 2.85 | 0.989 |
| 1-pentanol | 68.48 | 189.39 | 2.77 | 0.989 |

**Table S3** Comparison of the repeatability and reproducibility of retention times for separation of fatty acid ethyl esters mixture on CONs-coated capillary column and CB-5 commercial capillary column.

| Analytes | Run-to-Run (n=5) | | | | Day-to-Day (n=5) | | | | Column-to-Column (n=3) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CONs | | CB-5 | | CONs | | CB-5 | | CONs | | CB-5 | |
| | $t_R$ (min) | RSD (%) | $t_R$ (min) | RSD (%) | $t_R$ (min) | RSD (%) | $t_R$ (min) | RSD (%) | $t_R$ (min) | RSD (%) | $t_R$ (min) | RSD (%) |
| ethyl acetate | 4.10 | 1.25 | 3.82 | 2.28 | 4.06 | 1.56 | 3.91 | 2.79 | 4.09 | 3.16 | 3.71 | 2.51 |
| ethyl propionate | 5.24 | 1.02 | 4.17 | 2.29 | 5.19 | 1.17 | 4.29 | 2.63 | 5.18 | 1.27 | 4.06 | 2.59 |
| ethyl butyrate | 6.25 | 0.85 | 4.75 | 2.29 | 6.21 | 1.01 | 4.90 | 2.39 | 6.19 | 0.29 | 4.64 | 2.70 |
| ethyl valerate | 6.84 | 0.58 | 4.91 | 2.29 | 6.80 | 1.10 | 5.07 | 2.24 | 6.85 | 1.49 | 4.80 | 2.77 |
| ethyl caproate | 7.23 | 0.74 | 5.73 | 1.81 | 7.19 | 0.84 | 5.89 | 1.68 | 7.25 | 0.48 | 5.64 | 2.25 |
| ethyl lactate | 8.07 | 0.68 | 6.94 | 1.53 | 8.04 | 0.77 | 7.11 | 1.31 | 8.05 | 1.51 | 6.86 | 1.95 |
| ethyl oenanthate | 8.83 | 0.64 | 8.25 | 1.33 | 8.79 | 0.69 | 8.43 | 1.10 | 8.80 | 2.12 | 8.17 | 1.67 |
| ethyl caprylate | 9.54 | 0.65 | 9.49 | 1.25 | 9.49 | 0.70 | 9.68 | 1.16 | 9.46 | 0.78 | 9.40 | 1.41 |
| ethyl nonanoate | 11.47 | 0.79 | 11.70 | 1.24 | 11.39 | 0.70 | 11.88 | 1.16 | 11.38 | 2.43 | 11.57 | 1.15 |
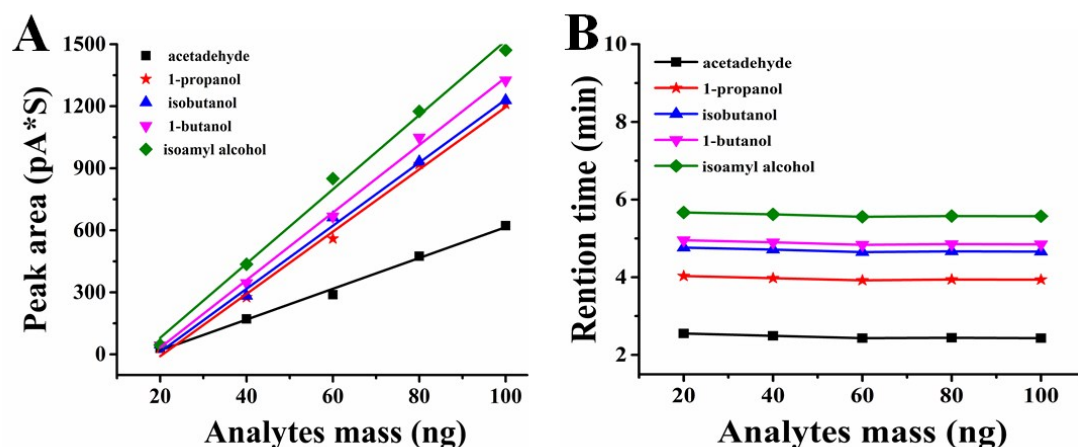
**Fig. S5** (A) Effect of analytes mass on the peak area for the separation of edible alcohol on the CONs-based capillary column (20 m long × 0.25 mm i.d.); (B) Effect of analytes mass on the retention time for the separation of edible alcohol on the CONs-based capillary column (20 m long × 0.25 mm i.d.).
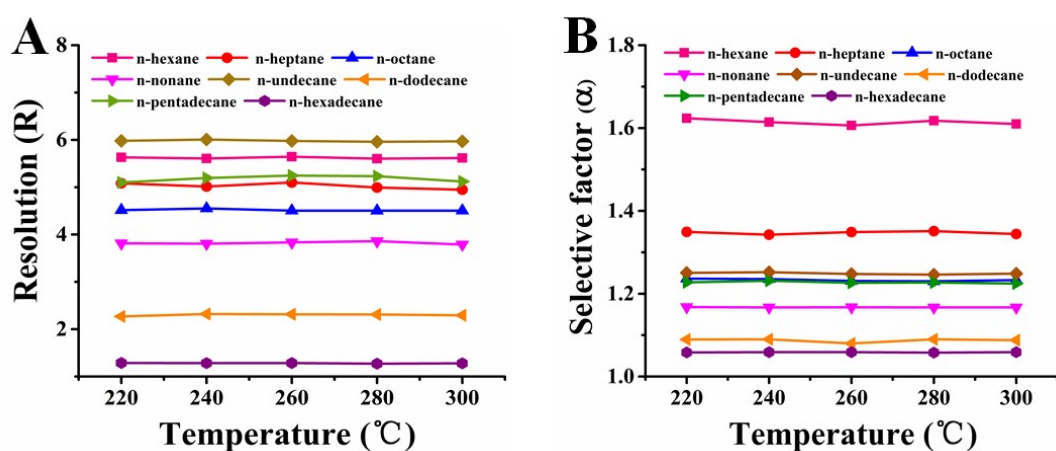


**Fig. S6** (A) Effect of conditioning temperature on resolution (R) of n-alkanes on CONs-based capillary column (20 m long × 0.25 mm i.d.); (B) Effect of conditioning temperature on selectivity factor (α) of n-alkanes on CONs-based capillary column (20 m long × 0.25 mm i.d.) after the column was conditioned up to each of the indicated temperatures for 2 h.

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