

Tailored Adsorption of His₆-tagged Protein onto Nickel(II) Cyclam Grafted Mesoporous Silica

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Supporting Information Available. Synthesis of SBA-15-Ni-cyclam, Synthesis of His₆-Spi and Spi, Enzyme adsorption procedure, **Surface coverage calculations**, **Leaching experiments**, Control experiments Characterization data: **CHN analysis**, Nitrogen adsorption analysis, Thermal gravimetric analysis and **derivation**, Low-angle XRD, **XPS**.

Stepwise synthesis of functionalized SBA-15

Synthesis procedure was adapted from previously published protocols¹⁻³.

Step 1: SBA-15 (350 mg), which was oven-dried at 120 °C overnight, was gently stirred and refluxed with 3-iodopropyl trimethoxysilane (80.5 mg, 0.277 mmol, 118 μL) in 20 mL dry toluene for 12 h under a dry, N₂ atmosphere to prevent hydrolysis of the methoxy functional groups and in the dark to prevent the degradation of the iodo functionality.

Step 2: After 12h of reflux the silicate turned a dark green colour, indicative of the iodo-group on the silicate surface. It was essential that the silicate be kept in dark conditions to retain the iodo-group required for the next step. The toluene was removed and the silicate washed in MeCN (3 x 20 mL) several times. Cyclam (56 mg, 0.277 mmol, 1 eq.) and potassium carbonate (170 mg, 1.227 mmol, 4 eq.) was added and the mixture was refluxed in MeCN (20 mL) for a further 24 h.

Step 3: The silicate was then filtered off to remove any remaining MeCN and washed with H₂O (3 x 20 mL) to remove unreacted potassium carbonate and starting materials. This silicate was then left to air-dry at ambient temperatures overnight.

Step 4: SBA-15-cyclam (100 mg) was refluxed with nickel (II) chloride (13 mg, 0.1 mmol, excess) in H₂O (20 mL) for 1 h. The cyclam to Ni ratio was 0.08/0.1. The silicate was then filtered off and washed with H₂O (3 x 20 mL) to remove any uncomplexed nickel.

Preparation of His₆-tagged Spi

His₆-Spi was purified as previously reported⁴. *E. coli* DH5 α containing the cloned *spi* gene was grown at 37°C until OD₆₀₀ 0.6 was reached. Expression of His₆-Spi was induced by the addition of IPTG to a final concentration of 0.1 mM. His-tagged Spi was purified from the cell lysate by affinity chromatography on chelating resin (GE Healthcare) charged with nickel. His₆-Spi was eluted from the resin using a gradient of imidazole (20 - 500 mM). Peak fractions were pooled and dialyzed against phosphate buffer, pH 7.3. His₆-Spi was further purified by ion exchange chromatography on Q sepharose FF (GE Healthcare) using a NaCl gradient 0 M – 1 M in 10 mM glycine pH 9.0 and dialyzed against 25 mM TRIS-HCl buffer pH7.4.

To generate the non-His₆ tagged protein, the His₆ tag was cleaved off the protein using TEV protease. His₆-Spi was dialyzed against buffer containing 50 mM TRIS-HCl pH8 and 0.5 mM EDTA. To this solution DL-Dithiothreitol (DTT) to a final concentration of 2 mM and TEV protease with a ratio of 40 units/mg protein were added. After cleavage, the protein solution was dialyzed against PBS 500 buffer and Spi was purified from the reaction mixture by affinity chromatography on chelating resin (GE Healthcare) charged with nickel. Peak fractions were collected and dialyzed against 25 mM TRIS buffer.

Enzyme Immobilisation

SBA-15 and SBA-15-Ni-cyclam (final conc. 5 mg/ml) were exposed to solutions of His₆-Spi of varying concentrations, contained in buffer solutions of varying salt concentrations (25 – 1000 mM) and PEG₄₀₀ content (0 – 2%). These were left to shake at 160 rpm for 20 h and 25 °C. The silicate/protein dispersions and a control protein solution were then centrifuged for 1 minute at 10392 x g, generating a pellet of silicate. The supernatant was removed and tested for the presence of protein by UV-Vis spectrometer at a wavelength of 280 nm. For leaching tests, the silicate pellets were washed with buffer and vigorously shaken for 1 min. This was then

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centrifuged at the above settings and the supernatant tested for protein content. The pellet was then re-dispersed in buffer and left to shake at 160rpm for 20 h at 25°C, centrifuged and the supernatant tested for protein content.

Surface coverage calculations

Nitrogen concentration is used to calculate the number of cyclam rings attached to the mesoporous silicate. Nickel concentration cannot be used as there is not 100 % uptake into the cyclam rings. CHN analysis shows 1.11 mmoles of nitrogen (from cyclam) per 1g silicate and SBA-15 displays a surface area of 742 m²/g.

1.11 mmoles/1g = 0.277 mmoles of cyclam per gram silicate = 1.67×10^{20} cyclam molecules per gram.

The area of cyclam was calculated by measuring the diameter across the widest point of the molecule. A circular disc shape was used for calculation purposes. It was also assumed that cyclam lay flat on the surface of the silicate. The widest diameter was found to be 9.03 Å and the area calculated to be 6.401×10^{-19} m² per molecule. The total surface area of cyclam molecules was found to be 106.9 m²/g or 14.4 % surface coverage.

Leaching experiments

After removing and testing the supernatant for protein concentration, the mesoporous silica pellet was re-dispersed in buffer (25 mM, phosphate buffer, pH 7.4) and vigorously shaken for 1 minute. The sample was centrifuged and the supernatant tested for protein by UV spectroscopy. This was repeated 3 times. The re-dispersed silicate was then frozen and thawed and the supernatant tested for protein presence. This was repeated 3 times.

Control experiments

It should be noted that SBA-15 exposed to NiCl₂ and Ni-cyclam showed no colour change.

Exposure of SBA-15 and SBA-15-Ni-cyclam to PEG₄₀₀.

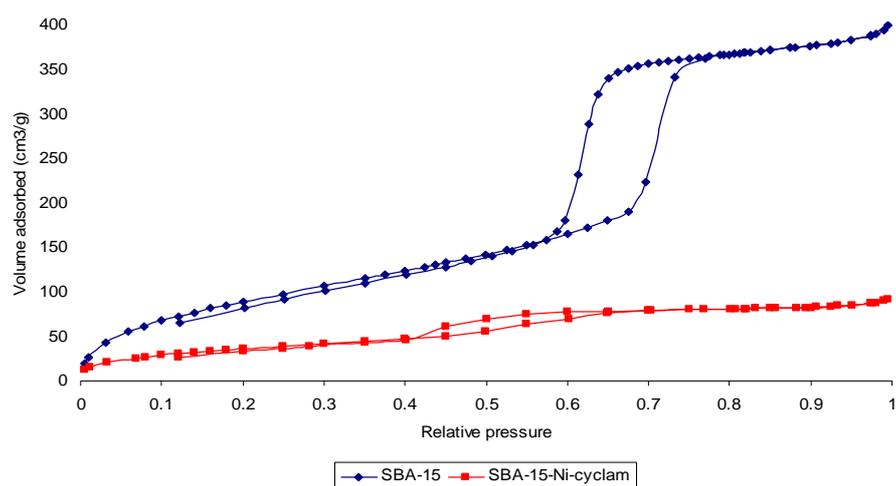
Previous reports have demonstrated that the attachment of PEG to the surface of silicate material, requires alcoholic media and the presence of ammonia⁵. To confirm this, both silicates were incubated in the presence of 2% PEG₄₀₀ without the presence of protein and TGA analysis was performed. PEG₄₀₀ attachment did not occur, as equivalent decreases in mass were obtained for SBA-15 and SBA-15-Ni-cyclam which were not exposed to PEG.

Characterization Data

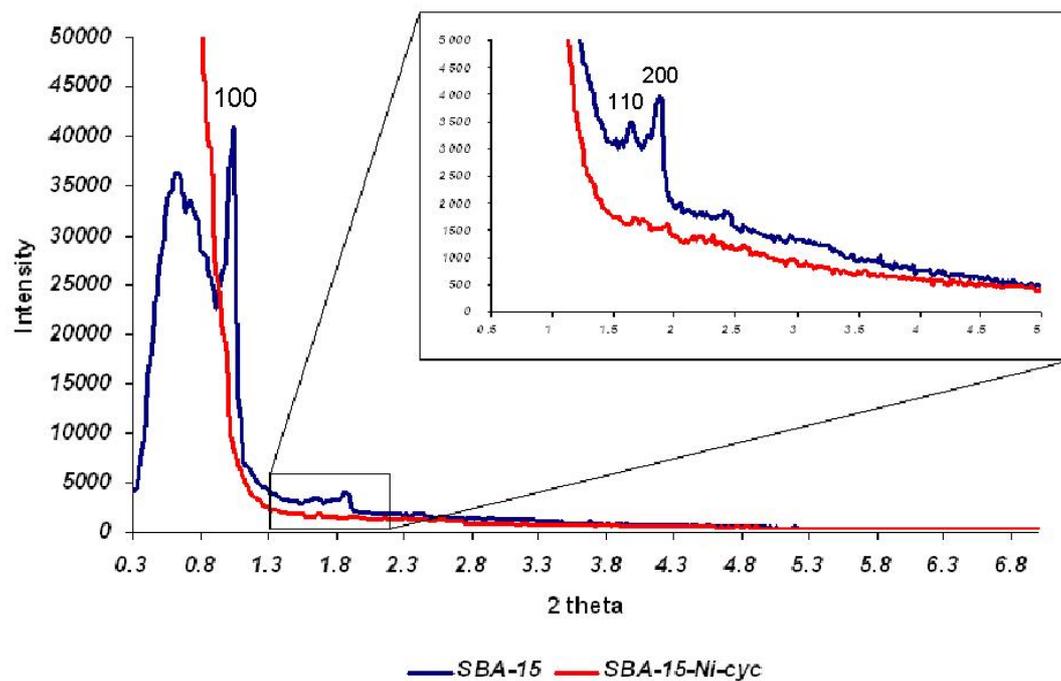
CHN analysis

SBA-15-Ni-cyclam: C = 11.61, H = 2.3, N = 1.56

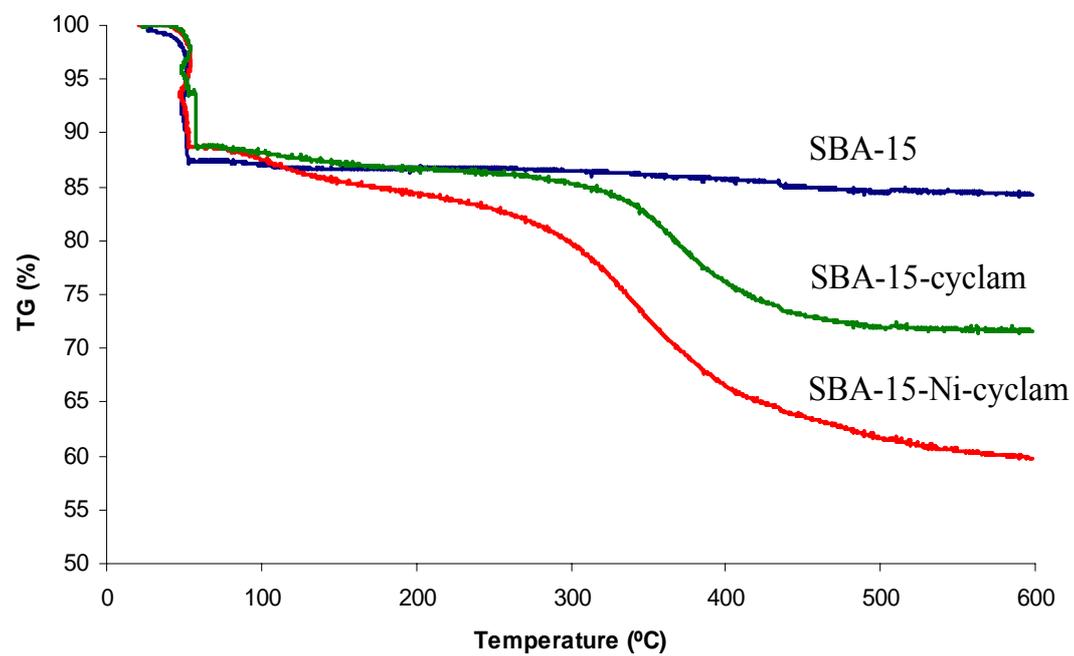
Nitrogen Adsorption Analysis

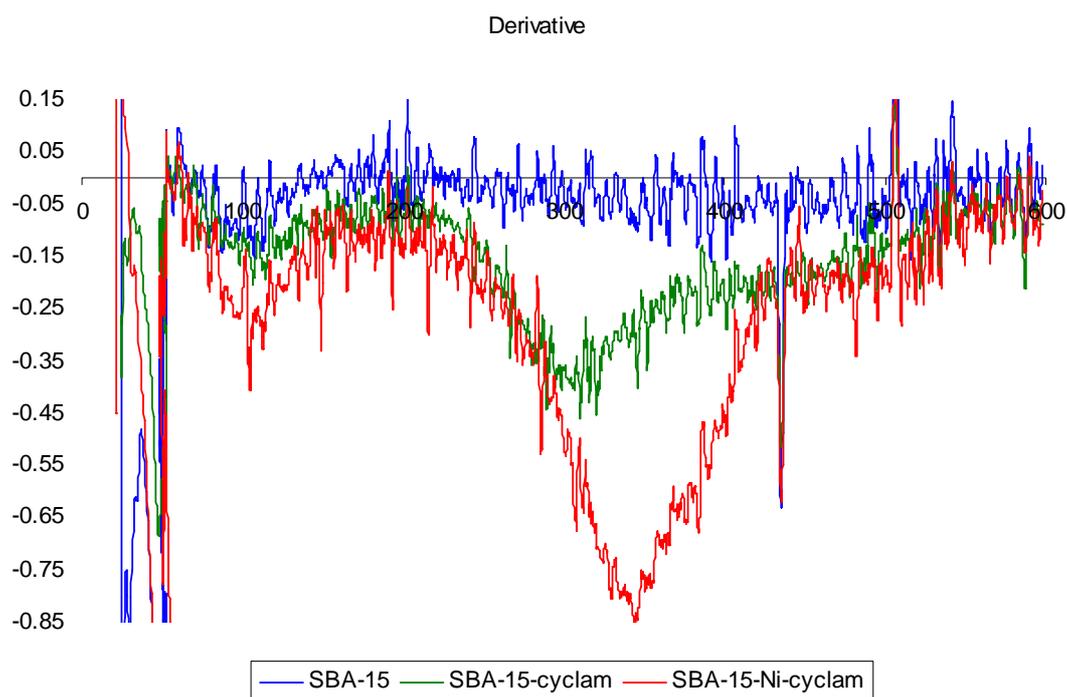


Low-angle X-Ray Diffraction



Thermal Gravimetric Analysis

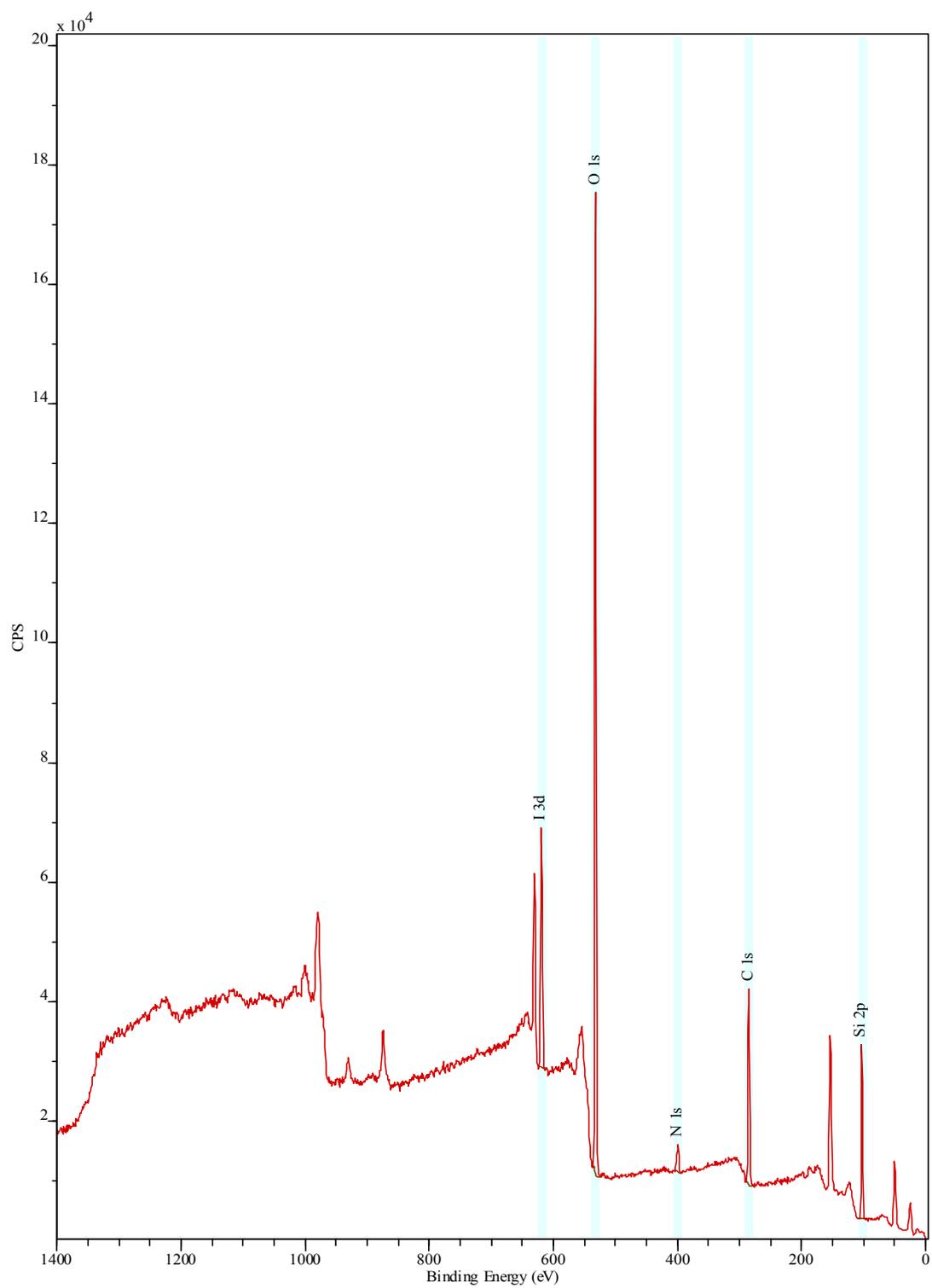




The results obtained are in good agreement with previous reports that have detailed the decomposition of cyclam⁶ and $\text{CoCl}_2(\text{cyclam})$ ⁷.

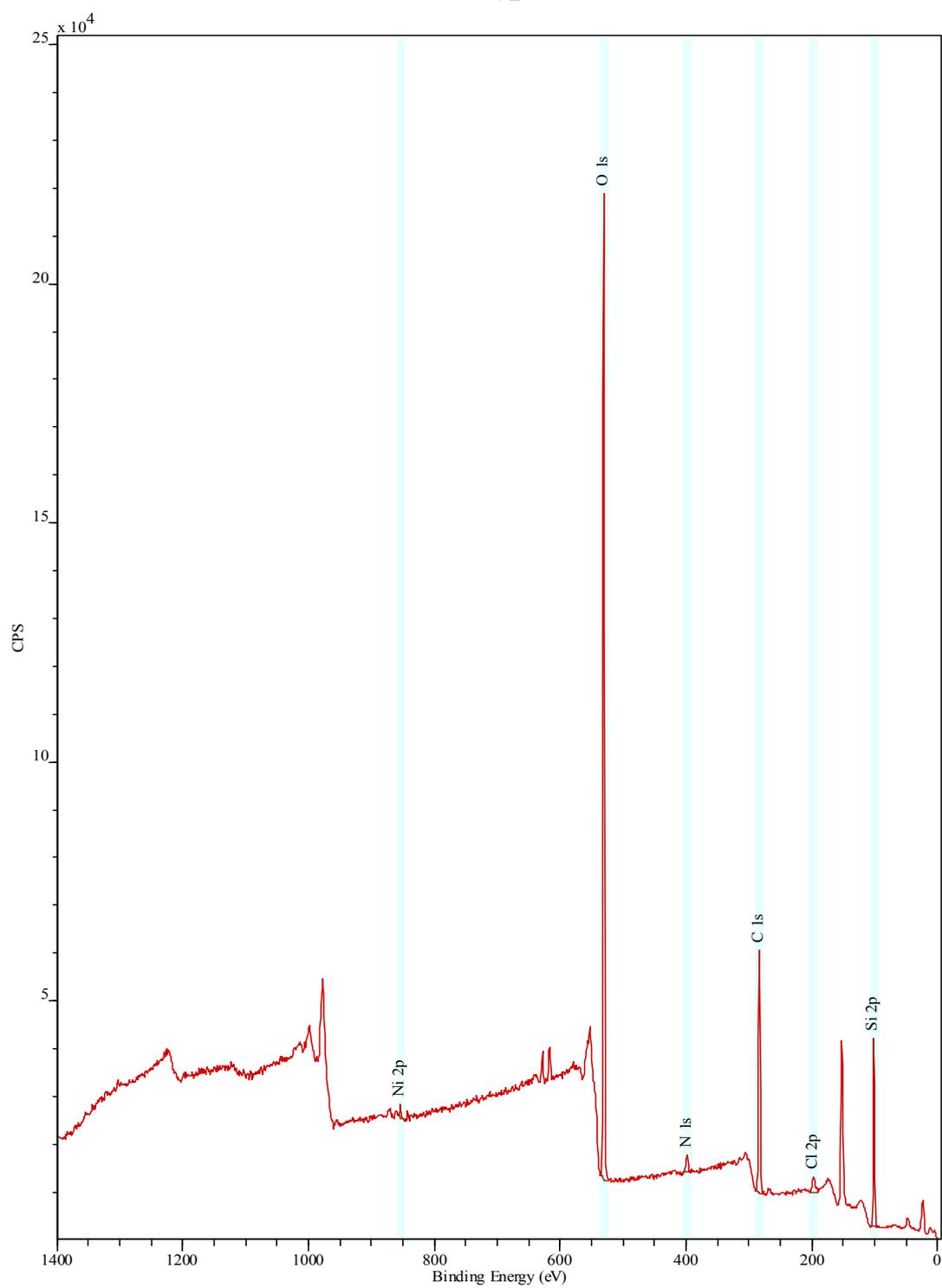
XPS

Survey_1/6



XPS spectrum for SBA-15-cyclam (Survey 1/6)

Survey_2/5



XPS spectrum for SBA-15-Ni-cyclam (Survey 2/5)

Quantification of high resolution XPS spectra

SBA-15-cyc

Name	Position (eV)	FWHM (eV)	R.S.F.	Area	% Conc.	
I 3d	618.5	1.4	19.9	13535	2.5	
O 1s	532.5	1.4	2.93	36548	45.4	
N 1s	399.4	1.5	1.8	1331	2.7	
C 1s	284.9	2.6	1	6698	24.4	
Si 2p	103.2	1.5	0.817	5606	25.0	
I 3d_1	618.5	1.4	19.9	7485	1.4	alkali iodide, HI
I 3d_2	620.5	1.4	19.9	5757	1.1	Alkyl iodide, I2
N 1s_1	399.4	1.4	1.8	715	1.4	C-N
N 1s_2	401.6	1.7	1.8	617	1.2	NH3+
C 1s_1	284.7	1.4	1	3438	12.7	C-C, C-H
C 1s_2	286.2	1.6	1	3192	11.7	C-N, C-O
Si 2p	103.2	1.6	0.817	5588	25.0	Silica,

SBA-15-Ni-cyc

Name	Position (eV)	FWHM (eV)	R.S.F.	Area	% Conc.	
I 3d	618.5	1.4	19.9	2467	0.3	
				4793		
O 1s	532.7	1.4	2.93	8	43.6	
N 1s	400.0	2.2	1.8	1012	1.5	
				1183		
C 1s	284.9	1.5	1	1	31.5	
Si 2p	103.3	1.6	0.817	6991	22.8	
Ni 2p	855.4	1.3	14.6	1303	0.2	
I 3d_1	618.6	1.4	33.6	2046	0.28	HI,
I 3d_2	620.3	1.7	33.6	355	0.05	Alkyl iodide, I2
N 1s_1	399.8	1.8	1.8	655	0.9	C-N
N 1s_2	401.6	2.3	1.8	378	0.5	NH3+
C 1s_1	284.8	1.2	1	7255	19.5	C-C, C-H
C 1s_2	286.2	1.8	1	4449	12.0	C-N, C-O
Si 2p	103.4	1.6	0.817	6978	22.8	Silica, Si(OMe) ₃
Ni						Ni in N-organic
2p5/2	855.6	2.1	14.6	833	0.2	complex

References

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Supplementary Material (ESI) for Chemical Communications

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