Electronic Supplementary Material (ESI) for Nanoscale Advances.

Supplementary Information

Quantification of Amine Functional Groups on Silica Nanoparticles: A Multi-Method Approach

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Table S1 Characterization of silica NPs provided by the manufacturer or measured at NRC (BET only).

$SiO_2@C_3NH_2$	Diameter (nm) [TEM] [a]	Hydrodynamic Diameter (nm) [DLS]	Zeta Potential (mV) [b]	Estimated Surface Area (m^2/g) [c]	BET Specific Surface Area (m^2/g) [d]
B1 50 nm	47 ± 3	88	+ 34		
B1 80 nm	79.7 ± 5.9	108.7	+ 43.4		
B1 100 nm	97 ± 6	144	+ 64		
B2 20 nm	22.6 ± 2.7	64	+ 20.5	135	
B2 50 nm	48 ± 3	83	+ 33.9	63.8	80
B2 80 nm	79.7 ± 5.9	108.7	+ 43.4	38.4	33
B2 100 nm	97 ± 6	144	+ 64	31.6	
B2 120 nm	118 ± 8	145.1	+ 44.7	25.9	36
B3 50 nm	48 ± 3	83	+ 33.9		
B3 80 nm, dry	80 ± 6	-	-		
B3 100 nm, dry	97 ± 6	-	-		

[a] Average TEM diameter, with standard deviation as a measure of the breadth of the particle size distribution.

[b] Most values were measured in 2 mM acetate in ethanol; exceptions are B2, 120 nm and B1, 80 nm measured in ethanol and B2, 80 nm measured in water.

[c] Surface area was estimated from the average TEM diameter and a silica density of 1.96 g/cm^2, using the following approach, with r = radius.

Surface area (m^2/g) = (# particles/g) x (surface area/particle) where

surface area/particle = $4\pi r^2$

#particles/g = 1/(mass/particle) = 1/(volume/particle x density) = $1/(4/3(\pi r^3)$ x density)

[d] BET specific surface area measured by adsorption of nitrogen with a gas sorption analyzer Nova 2200e (Quantachrom). Prior to analysis samples were degassed under vacuum at 115 °C for 16 hours. 7-point nitrogen sorption isotherms were measured at -196 °C and the specific surface area was calculated by the multipoint BET method.

Table S2 Redispersion of NPs for colorimetric assays. A suspension of 100 nm NPs (nominal diameter) was measured by dynamic light scattering before and after solvent exchange and redispersion.

Sample	Z-average, nm	PdI
NPs, as received in ethanol	158	0.078
NPs, manufacturer data sheet	152.5	-
NPs, after solvent exchange to 60% ethanol + redispersion (NP-R)	140	0.019
NP-R, 15 min bath sonication	136	0.026
NP-R, 30 min bath sonication	137	0.016

Table S3 Determination of amine content for silica NPs using the ninhydrin colorimetric assay.

	Amine content, µmol/g		
Experiment[a]	120 nm, NH2	50 nm, NH2	100 nm, bare
1A	275	494	1
1B	305	525	1
1C	265	442	2
1D	267	480	1
1 Average	**278 ± 16**	**485 ± 30**	**1 ± 1**
2A	258	472	0
2B	271	446	
2C	338	461	
2D	262	444	
2 Average	**282 ± 34**	**456 ± 12**	**0**
3A	271	441	
3B	277	435	
3C	278	464	
3D	270	437	
3 Average	**274 ± 4**	**444 ± 12**	
Average	**278 ± 23**	**458 ± 27**	**1**

[a] Experiments 1A-1D are 4 replicates done in parallel on the same day. Experiments 2A-2D and 3A-3D are replicates from two different days.

Table S4 Determination of amine content for silica NPs measured using the ninhydrin colorimetric assay.

Aminated silica	Amine, µmol/g
50 nm, Batch A, suspension	447 ± 15
50 nm Batch B, suspension	76 ± 4
50 nm Batch C, suspension	63 ± 2
80 nm, Batch A, dry	75 ± 7
80 nm, Batch B, dry	105 ± 2
100 nm, Batch B, dry	40 ± 2
100 nm, Batch B, dry	128 ± 5

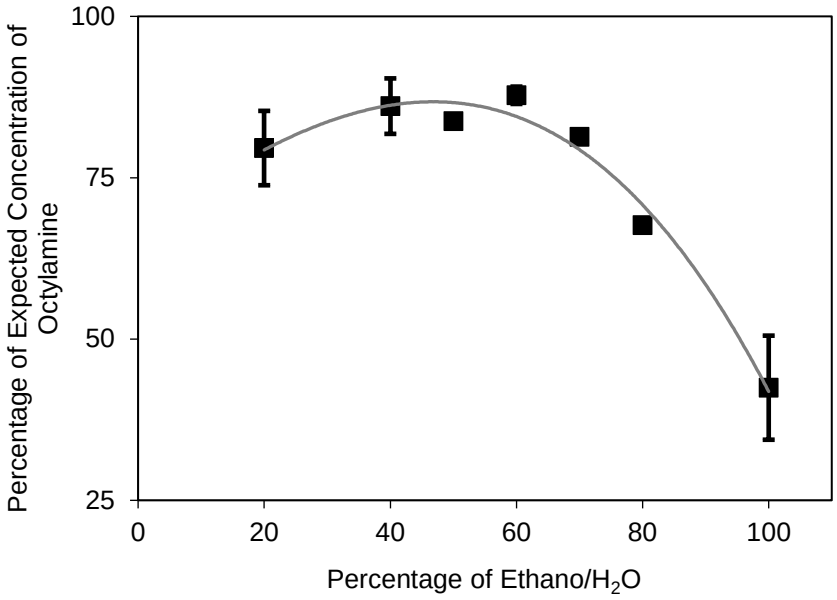

Fig. S1 Effect of solvent on the ninhydrin reaction for octylamine in aqueous ethanol mixtures; 2, 2, 1, 6, 1, 2 and 6 for 20, 40, 50, 60, 70, 80 and 100% respectively.

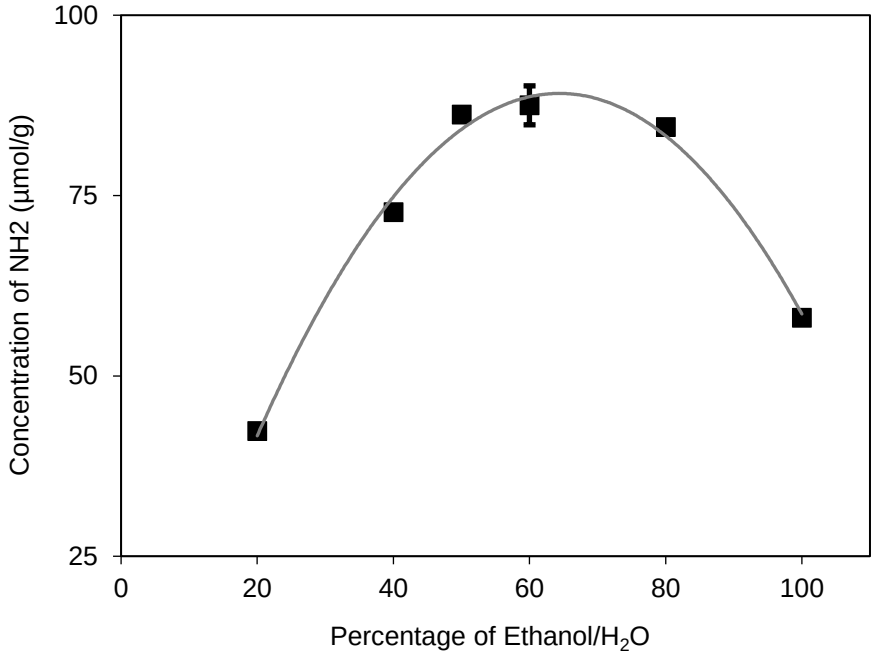

Fig. S2 Effect of solvent on amine determinations for 50 nm aminated silica NPs measured using the ninhydrin assay in aqueous ethanol mixtures. All points are single determinations except for 60% aq ethanol, n = 3.

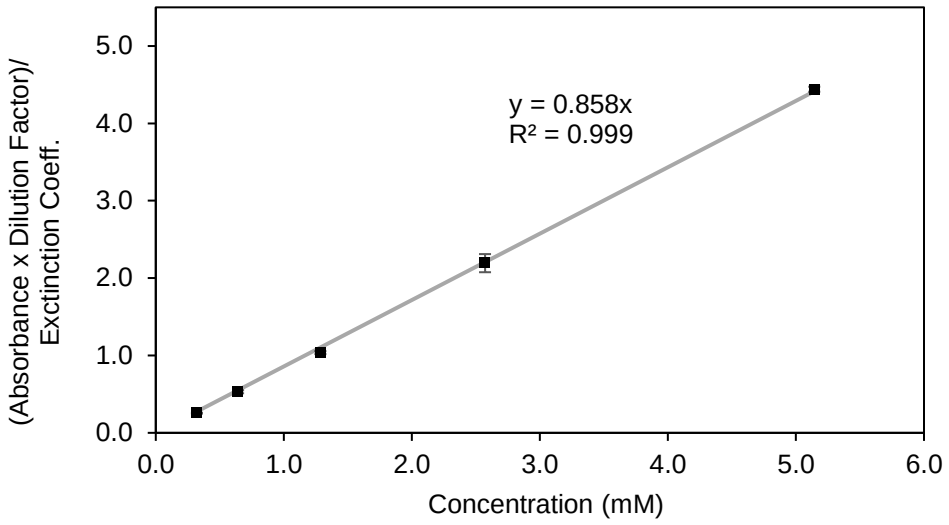

Fig. S3 Ninhydrin calibration curve performed with octylamine in 60 % ethanol/H_2O. Each data point represents an average of 4 measurements.

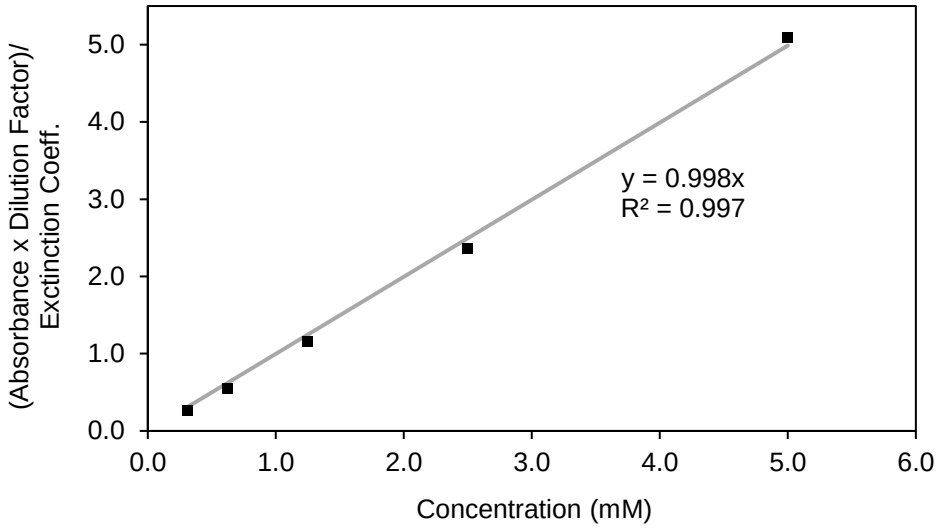

Fig. S4 Ninhydrin calibration curve performed with amino acid leucine in in 60 % ethanol/H_2O.

Estimation of detection limits for colorimetric assays. Detection limits were estimated by assuming that an absorbance value of 0.1 (1 cm path length) was required to quantify amine using either the ninhydrin or 4-NBA assay. For the 5 mg (dry mass) silica samples used here, this corresponds to quantification limits of 0.8 and 1.2 μmol amine/g silica.

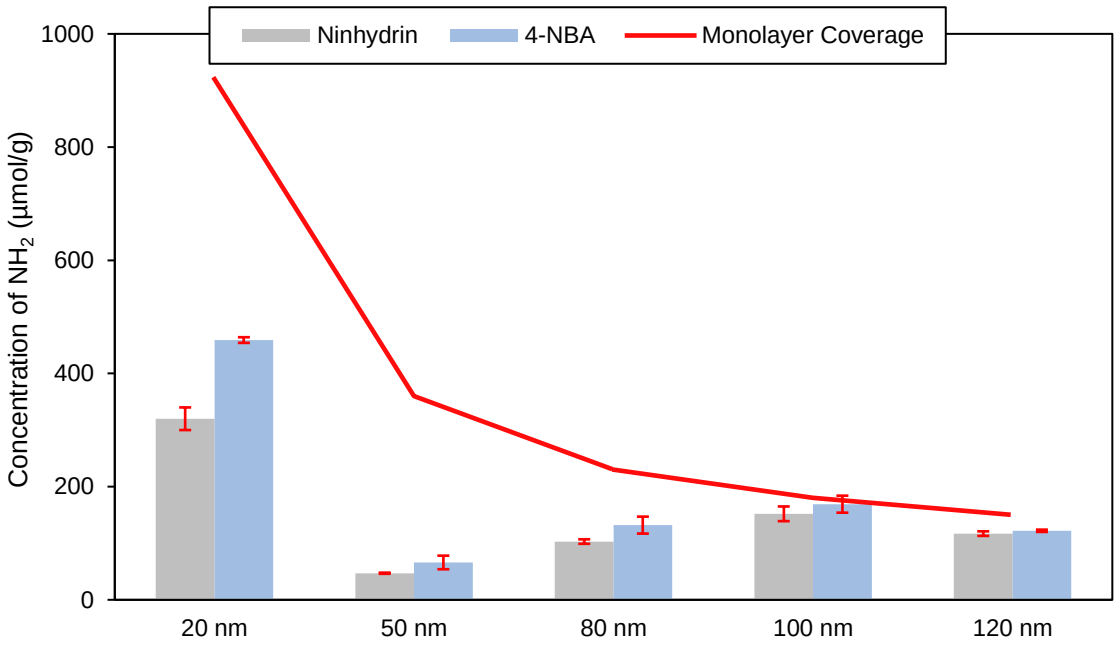

Fig. S5 Comparison of ninhydrin and 4-nitrobenzaldehyde colorimetric assays for a single batch (B2) of commercial silica nanoparticles. Monolayer coverage is shown assuming 4 aminopropyl siloxane/nm^2. Data taken from Table 1.

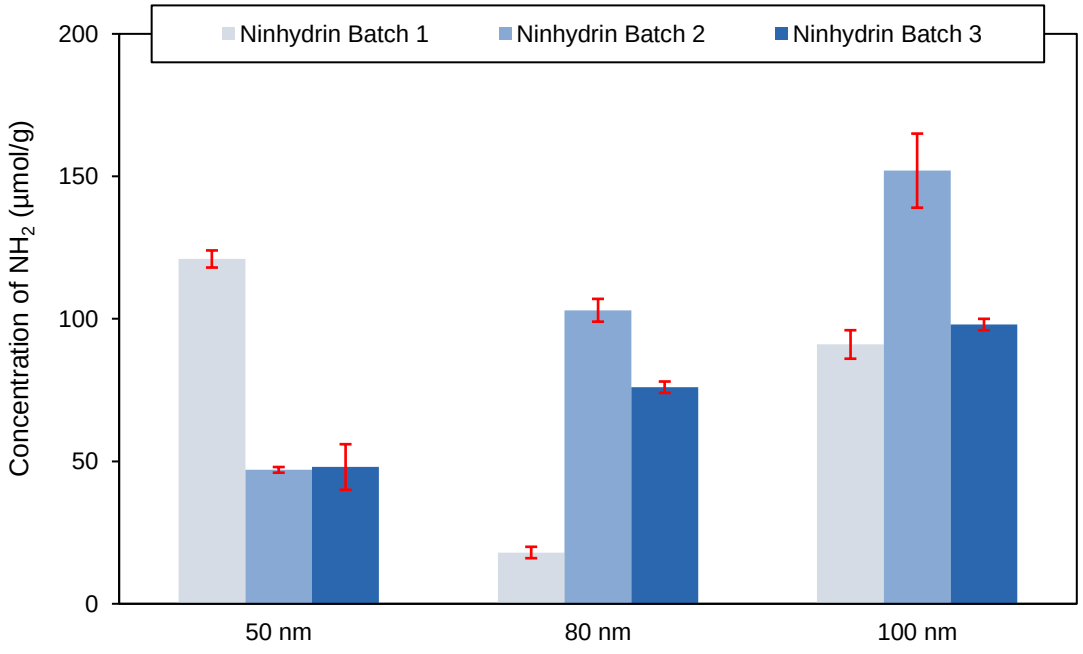

Fig. S6 Variability of surface amine content between commercial batches of functionalized silica nanoparticles, as determined by the ninhydrin assay. Data taken from Table 1.

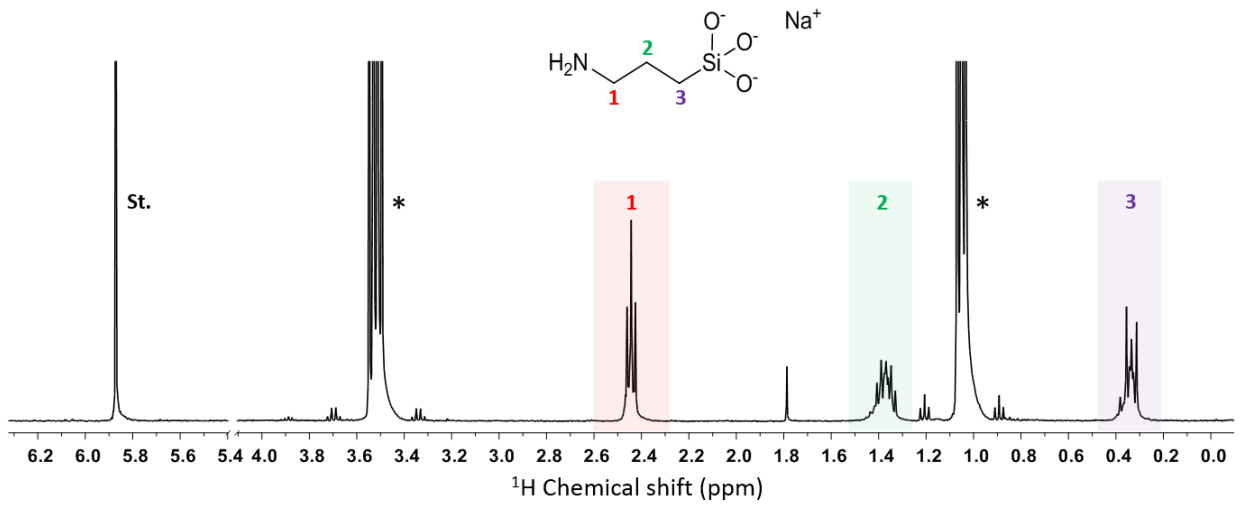

Fig. S7 ^{1}H NMR spectrum of 100 nm aminated silica NPs (B3) hydrolyzed in 0.4 M NaOD at 45 °C for 3 hours. The 3-aminopropyl siloxane group was quantified by comparison to maleic acid added as an internal standard (denoted as 'St.'). The water region (5.4 ppm - 4.1 ppm) was removed for clarity. The residual ethanol signals are marked with an asterisk.

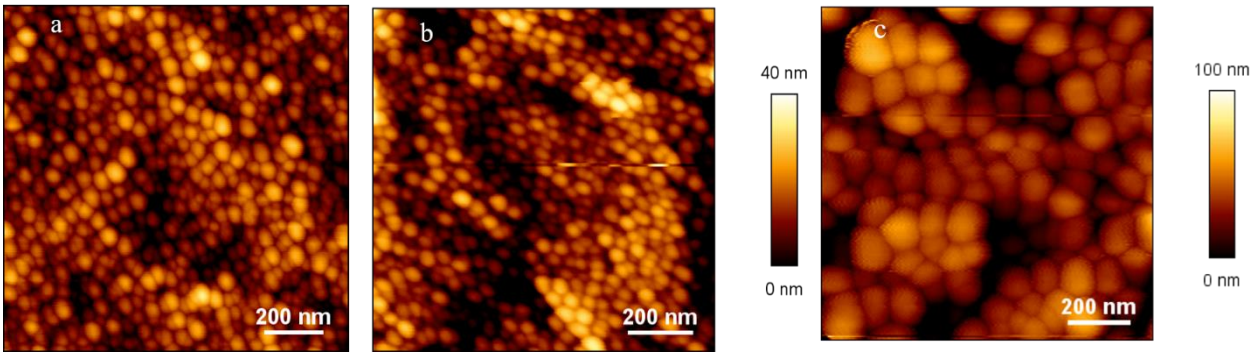

Fig. S8 AFM images of samples deposited on Au substrates for XPS measurements: 50 nm aminated silica NPs before (a) and after functionalization with BTFBA and 100 nm aminated silica after functionalization with BTFBA (c). Imaging was done on a JPK NanoWizard 2 in tapping mode with HQ:XSC11/Al BS cantilevers (42N/m, Mikromasch).

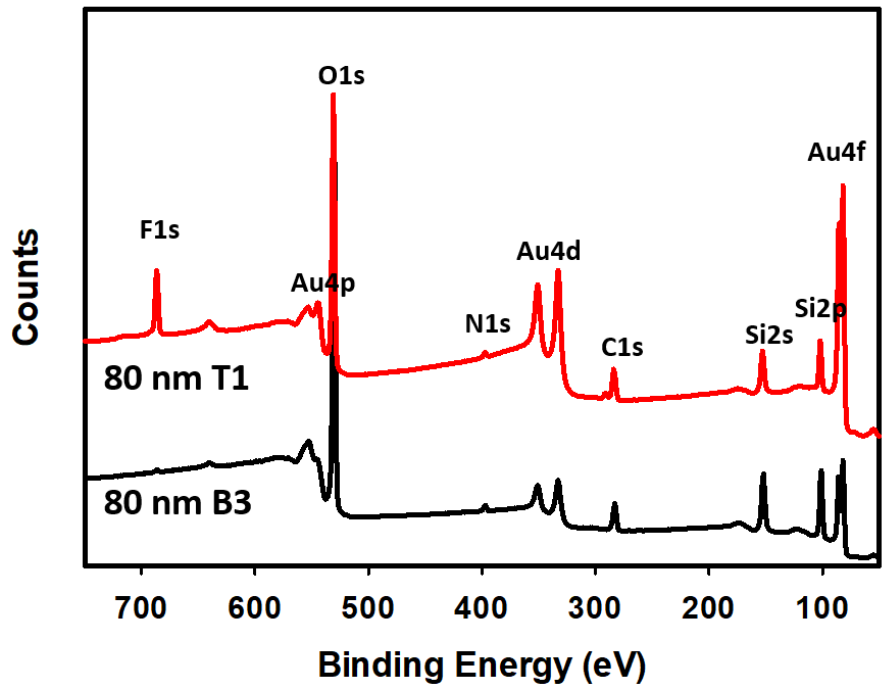

Fig. S9 XPS survey spectra of aminated silica NPs before (80 nm B3) and after (80 nm T1) labelling with BTFBA.

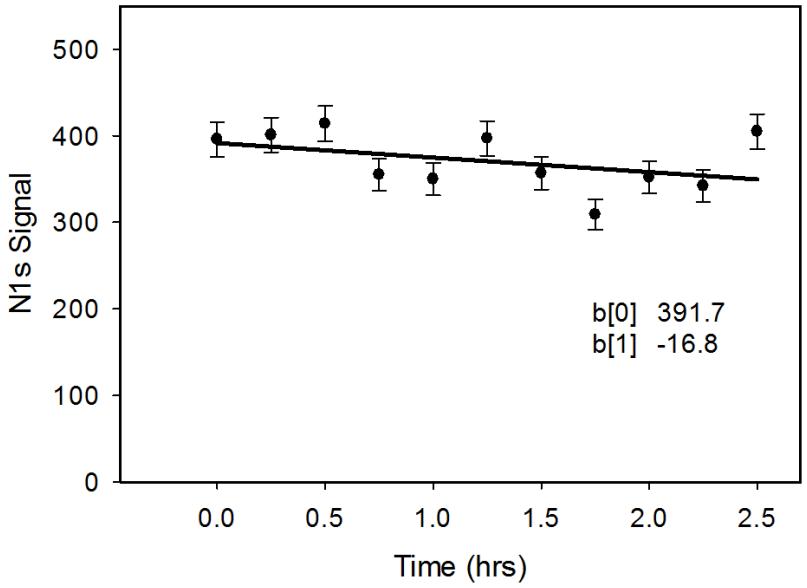

Fig. S10 Time dependence of N1s signal (integrated peak area after background subtraction as described above) for 50nm aminated silica NPs (B3, T2). The linear fit to the data indicates the signal loss is <10% during a 2.5 hr. scan, the time used for acquiring the data. This implies that the amount of nitrogen on the surface may be underestimated by up to 5%.