

Environmental Science Nano

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Environmental Significance Statement:

The environmental impact of nanomaterials can be strongly modulated by their chemical transformations. Despite the critical role that redox chemistry plays in biology, the influence of specific biologically relevant electron transporters such as nicotinamide adenine dinucleotide (NADH) and glutathione (GSH) on the transformation of engineered nanoparticles, and the reciprocal influence of nanoparticles on the oxidation state of these molecules, have not been widely considered previously. The present work shows that the exposure of LiCoO₂ nanoparticles to nicotinamide adenine dinucleotide (NADH) accelerates release of Co²⁺ by reducing cobalt from its highly insoluble Co³⁺ form to the soluble Co²⁺ form, and the LiCoO₂ concurrently oxidizes NADH to NAD⁺. Similar results are observed using glutathione. Transformations of nanoparticles driven by redox chemistry of molecules involved in biological electron transport pose two important mechanisms for toxicity in the environment. First, the reduction of Co(III) and other high-valence metals to lower-valence states increases their solubility and leads to faster release into the aqueous phase. Secondly, the corresponding reciprocal oxidation of NADH, GSH, and other molecules relevant to electron transport and cellular homeostasis can serve as a direct pathway to biological impact.

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ARTICLE

Reciprocal Redox Interactions of Lithium Cobalt Oxide Nanoparticles with Nicotinamide Adenine Dinucleotide (NADH) and Glutathione (GSH): Toward a Mechanistic Understanding of Nanoparticle-Biological Interactions

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Among high-valence metal oxides, LiCoO₂ and related materials are of environmental importance because of the rapidly increasing use of these materials as cathodes in lithium ion batteries. Understanding the impact of these materials on aqueous environments relies on understanding their redox chemistry because Co release is dependent on oxidation state. Despite the critical role that redox chemistry plays in cellular homeostasis, the influence of specific biologically relevant electron transporters such as nicotinamide adenine dinucleotide (NADH) and glutathione (GSH) on the transformation of engineered nanoparticles has not been widely considered previously. Here we report an investigation of the interaction of LiCoO₂ nanoparticles with NADH and GSH. Measurements of Co release using inductively coupled plasma-mass spectrometry (ICP-MS) show that exposing LiCoO₂ nanoparticles to either NADH or GSH increases solubilization of cobalt, while corresponding spectroscopic measurements show that NADH is concurrently oxidized to NAD⁺. To demonstrate that these effects are a consequence the high-valence Co(III) in LiCoO₂ nanoparticles, we performed control experiments using Co(III)-containing Co(OH)₂ and LiCoPO₄, and dissolved Co²⁺/Li⁺ ions. Additional experiments using molecules of similar structure to NADH and GSH, but that are not reducing agents, confirm that these transformations are driven by redox reactions and not by chelation effects. Our data show that interaction of LiCoO₂ with NADH and GSH induces release Co²⁺ ions and alters the redox state of these biologically important transporters. Observation of NADH binding to LiCoO₂ using x-ray photoelectron spectroscopy (XPS) suggests a surface catalyzed reaction. The reciprocal reduction of LiCoO₂ to enable release of Co²⁺ and corresponding oxidation of NADH and GSH as model redox-active biomolecules has implications for understanding the biological impacts of high-valence metal oxide nanomaterials.

1. Introduction

The interactions of nanomaterials with environmental and biological systems involve a complex suite of interaction mechanisms. While physical interactions can be important, many emerging nanoparticles of technological utility have chemical compositions that are reactive, leading to new mechanisms such as release of solubilized metals^{1, 2} or surface-driven formation of reactive oxygen species. For example, nano-Ag(0) can be oxidized to Ag(I), which then has significant antimicrobial activity. Metal oxides constitute the most widely

used class of engineered nanomaterials and are frequently used in high oxidation states, with the corresponding metal hydroxides having much lower solubility than those formed from the lower oxidation states. For example, at pH = 6, the solubility of Fe(OH)₃ is ~10⁻¹³ M while Fe(OH)₂ is highly soluble. Because of the low solubility of high-valence metals, redox-active small organic acids can enhance dissolution of metal oxides by reducing the metals to lower oxidation states,³⁻⁷ and biologically-induced reduction processes impact biogeochemical cycling of metals such as Fe and Mn.⁸⁻¹⁰ The interaction of biomolecular reducing agents with nanoparticles could induce biological impacts both by enhancing release of transition metals and by altering the cellular redox potential and associated homeostasis. Despite the critical role that redox chemistry plays in cellular homeostasis, the influence of specific biologically relevant electron transporters such as nicotinamide adenine dinucleotide (NADH) and glutathione (GSH) on the transformation of engineered nanoparticles has not been widely considered previously.

Among high-valence metal oxides, LiCoO₂ and a broader family of “NMC” compositions (LiNi_xMn_yCo_{1-x-y}O₂, x, y < 1) are of particular environmental importance because of the rapidly

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†Electronic Supplementary Information (ESI) available: (1) Nanoparticle synthesis procedures, (2) description of XPS calculations, (3) DFT computational details, (4) minimal medium composition and chemical information, (5) changes in solution pH before/after nanoparticle exposure, (6) fluorescence and ICP-MS calibration, (7) TEM images of LiCoO₂ nanoparticles, (8) quantified [Li] release determined by ICP-MS, (9) comparison of NADH and NAD⁺ fluorescence spectra, (10) representative x-ray photoelectron spectra for NADH binding to nanoparticles, (11) additional control experiments regarding NADH fluorescence, and (12) additional control experiments regarding NADH/NAD⁺ UV-visible spectra. See DOI: 10.1039/x0xx00000x

increasing use of these materials in micro- and nano-structured form as cathodes in lithium-ion batteries. Because the use of lithium ion batteries in consumer electronics is rapidly expanding and their recycling is limited,¹¹ understanding the chemical transformations of LiCoO₂ has significant potential for environmental risk assessment and provides fundamental insights of a broader class of high-valence transition metal nanoparticles. Released cobalt from LiCoO₂ is hazardous to cells in aqueous environments because it is carcinogenic, induces oxidative stress, competes with the intended ions (e.g. Fe²⁺) for binding sites in metalloproteins, induces a hypoxia-like state, and has increased mobility once in the Co(II) form.^{12, 13} Cobalt is released in a manner strongly dependent on the oxidation state. At pH = 6, the solubility of Co³⁺ is $\sim 1 \times 10^{-20}$ M compared with $> 10^{-5}$ M for Co²⁺, based on the experimental solubility products of Co(OH)₃ and Co(OH)₂.^{14, 15} Among redox-active biomolecules that could impact LiCoO₂ dissolution, the NADH/NAD⁺ and GSH/GSSG redox couples, depicted in Figure 1, are among the most important. NADH can be oxidized to NAD⁺, while GSH can be oxidized to glutathione disulfide (GSSG) by forming a disulfide dimer. Both NADH/NAD⁺ and GSH/GSSG act as coenzymes and substrates for various metabolic processes and maintain an appropriate redox state within the cell (e.g. antioxidants).^{16, 17}

Here we report an investigation of the interaction between LiCoO₂ nanoparticles with NADH and GSH. Between these molecules, we choose to primarily study NADH with some comparison to GSH. Prior studies have shown surface-mediated oxidation of GSH on nanocarbon surfaces.^{18, 19} NADH or GSH may interact in such a way with LiCoO₂, which to our knowledge has not been reported. Additionally, the redox-dependent fluorescence of NADH/NAD⁺ allows for easy detection, making the molecule an appealing choice. Quantitative measurements of Co release using inductively coupled plasma-mass spectrometry (ICP-MS) show that exposure of LiCoO₂ nanoparticles to either NADH or GSH increases solubilization of cobalt, while corresponding spectroscopic measurements show that NADH is concurrently oxidized to NAD⁺. To demonstrate that these effects are a consequence of the high valence of Co(III) in LiCoO₂, we performed control experiments using Co(II)-containing Co(OH)₂ and LiCoPO₄. Additionally, by testing interaction of LiCoO₂, etc. with molecules of similar structure to NADH and GSH, but that are not reducing agents, we find the NADH oxidation / Co reduction effect due to a redox reaction and not chelation of a specific functional group with Co or the dissolved ions. This coupled transformation of LiCoO₂ and redox-active biomolecules has implications in the biological impacts of high-valence metal oxide nanomaterials and the redox-driven weathering of metal oxides.

2. Experimental

2.1 Nanoparticle synthesis and characterization

We synthesized sheet-like nanoparticles of cobalt hydroxide, Co(OH)₂, and LiCoO₂ following procedures we described previously.^{20, 21} Co(OH)₂ nanosheets were prepared via a

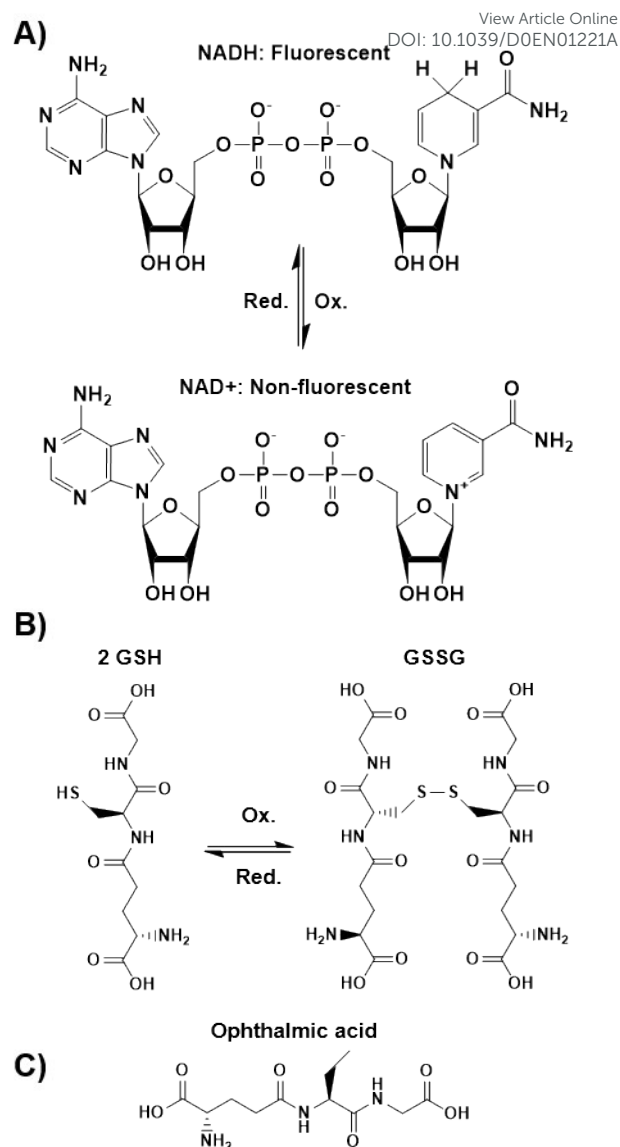


Figure 1. Oxidation-reduction equilibria (H⁺ not shown) of the biomolecules A) NADH and NAD⁺, B) glutathione (GSH) and glutathione disulfide (GSSG), and C) structure of ophthalmic acid (OA), a molecule similar to glutathione except lacking the redox-active thiol moiety.

precipitation method. The Co(OH)₂ precursor was converted to Li_xCoO₂ using a molten salt flux of 6:4 molar ratio of LiNO₃:LiOH at 200 °C. As a control sample, we synthesized rod-like Cmc₂ LiCoPO₄ nanoparticles using a microwave-assisted solvothermal method adapted from a method published previously.²² All solutions in this study were prepared from nanopure water (Barnstead Genpure System, $\rho \geq 18.2$ M Ω -cm). Detailed procedures for nanoparticle syntheses and chemical information (Table S1) can be found in the Electronic Supplementary Information (ESI[†]).

Powder X-ray diffraction (XRD) patterns were obtained for each sample using a Bruker D8 Advance diffractometer equipped with a copper K α source and 6 mm slit width. Samples for XRD analysis were prepared by affixing nanoparticle powder onto a B-doped silicon crystal zero-diffraction plate (MTI

Corporation) with vacuum grease. Scanning electron microscope (SEM) images were collected using a LEO Supra55 VP field-emission scanning electron microscope. Samples for SEM were prepared by drop-casting a dilute solution of nanoparticles suspended in ethanol onto a conductive B-doped silicon wafer. Specific surface area measurements of ~ 0.1 g vacuum-dried samples were determined by nitrogen physisorption (Micromeritics Gemini VII 2390 surface area analyzer) and Brunauer-Emmett-Teller (BET) analysis.²³ Lastly, we used dynamic light scattering (DLS) and laser Doppler microelectrophoresis with a Malvern Zetasizer Nano ZS to measure particle aggregate size and surface charge (respectively) in the solution of interest. Each particle type was suspended in minimal medium with dextrose (see section 2.2) at $100 \text{ mg}\cdot\text{mL}^{-1}$ in approximately 10 mL. These stock solutions were then diluted to $5 \text{ mg}\cdot\text{mL}^{-1}$. Particle suspensions were sonicated for 1 h in a cup ultrasonicator with cooling water (10 s on, 10 s off for 30 min total sonication time) immediately before analysis. The results reported are averages and standard errors of the mean of at least three measurements of size (number mean) and zeta potential for each sample.

2.2 Dissolution studies with nanoparticles and biomolecules

A model bacterial growth medium with minimal constituents ("minimal medium" with dextrose) was prepared in nanopure water with various salts, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer, and dextrose. See Table S1 for chemical information and Table S2 for the full composition of minimal medium with dextrose in the ESI[†]. We recognize that minimal medium used may not fully replicate the complex environment inside cells, perhaps warranting further investigations. However, this medium does provide a relatively simple matrix to investigate the specific interactions of NADH and other molecules with the nanomaterials of interest. Additionally, while NADH is typically unable to transverse membranes, there is evidence that lower concentrations of NADH can exist in extracellular matrices via transmembrane protein transport or other mechanisms.²⁴ It follows that our conclusions do not rely on nanomaterials entering cells.

NADH reduced disodium salt, NAD⁺ free acid, L-glutathione reduced, and ophthalmic acid were obtained from Sigma Aldrich and used as received. Ophthalmic acid (OA) is nearly identical to glutathione in structure, except it lacks the redox-active thiol group (Figure 1C). Solutions were prepared for each molecule of interest by dissolving 0.5 mM in minimal medium. This concentration was chosen because it is relevant to concentrations of these molecules found in certain cells²⁵⁻²⁷ and produces suitable fluorescence and absorbance signals for NADH/NAD⁺ solutions. Nanoparticles were introduced to each solution at $1 \text{ mg}\cdot\text{mL}^{-1}$ nanoparticle concentration, using vials of approximately 3 mL. The sealed vials were covered from light and shaken for 24 h. The samples were then centrifuged at $13,100 \times g$ for 20 min (Eppendorf MiniSpin plus). Finally, the supernatant was collected and filtered through $0.1 \mu\text{m}$ porosity

syringe filter cartridges (Millex VV) to ensure nanoparticle removal. Preliminary experiments in which particles were isolated only by centrifugation saw remaining particles cause light scattering in fluorescence measurements, particularly for LiCoO₂. Light scattering was eliminated with the introduction of the additional syringe filter isolation step. While the $0.1 \mu\text{m}$ porosity dimension is larger than certain particle dimensions, most particles are likely blocked as aggregates while navigating the filter. All experiments were performed in at least duplicate and error bars represent standard error of the mean.

We performed another form of dissolution experiment to study the reaction kinetics of NADH and LiCoO₂ in a single solution over time. LiCoO₂ was introduced at $1 \text{ mg}\cdot\text{mL}^{-1}$ to a single 30 mL solution of NADH in minimal medium in a larger reaction vessel. The reaction was stirred for 48 h total, and 1-mL aliquots were removed at 2 h, 6 h, 12 h, 24 h, and 48 h (five total). The $1 \text{ mg}\cdot\text{mL}^{-1}$ concentration for LiCoO₂ does change vary greatly from aliquot removal because the volume change (1 mL) is small relative to the total volume (30 mL) and the stirred solution is moderately homogeneous. The aliquots were centrifuged and filtered in the same way as the above samples.

2.3 Quantification of NADH concentrations

The concentration of NADH in samples was determined using fluorescence measurements using an ISS K2 photon-counting spectrofluorimeter, using measurement conditions similar to those reported previously.^{28, 29} Samples were placed in a fused silica cuvette at room temperature, excited at $\lambda_{\text{ex}} = 338 \text{ nm}$, and the emission spectrum was measured between $\lambda_{\text{em}} = 400$ and $\lambda_{\text{em}} = 550 \text{ nm}$ with a step size $\Delta\lambda = 1 \text{ nm}$ and integration time of 1 s per step. NADH exhibits an emission peak at $\lambda_{\text{ex}} \approx 455 \text{ nm}$, whereas NAD⁺ does not. The intensity was background-subtracted using a blank consisting of minimal medium with dextrose. Spectra were also normalized to the fluorescence of a solid tetraphenylbutadiene (TPB) standard to account for day-to-day variations in lamp intensity. NADH was quantified by converting the fluorescence intensity to concentration using a calibration curve (Figure S1, ESI[†]). As a secondary method for detecting NADH and NAD⁺, UV-visible absorbance spectra were obtained of selected NADH and NAD⁺ solutions using a Shimadzu UV2401 dual-beam spectrophotometer.

2.4 Determination of dissolved ion concentrations

We used inductively coupled plasma mass spectrometry (ICP-MS, Shimadzu 2030) to measure dissolved ion concentrations in samples after centrifugation/filtration. Samples were acidified in 2.5% HNO₃ before analysis. Additionally, a 500:1 dilution was required to reduce salt concentrations from the minimal medium. ⁷Li and ⁵⁹Co intensities were measured and referenced to internal standards of ⁹Be and ⁶⁹Ga, respectively. Each sample was measured in triplicate by ICP-MS and the average intensities were used for quantification. Concentrations of Li and Co were determined using calibration curves (Figure S2, ESI[†]). Standard solutions of Li and Co (combined) were

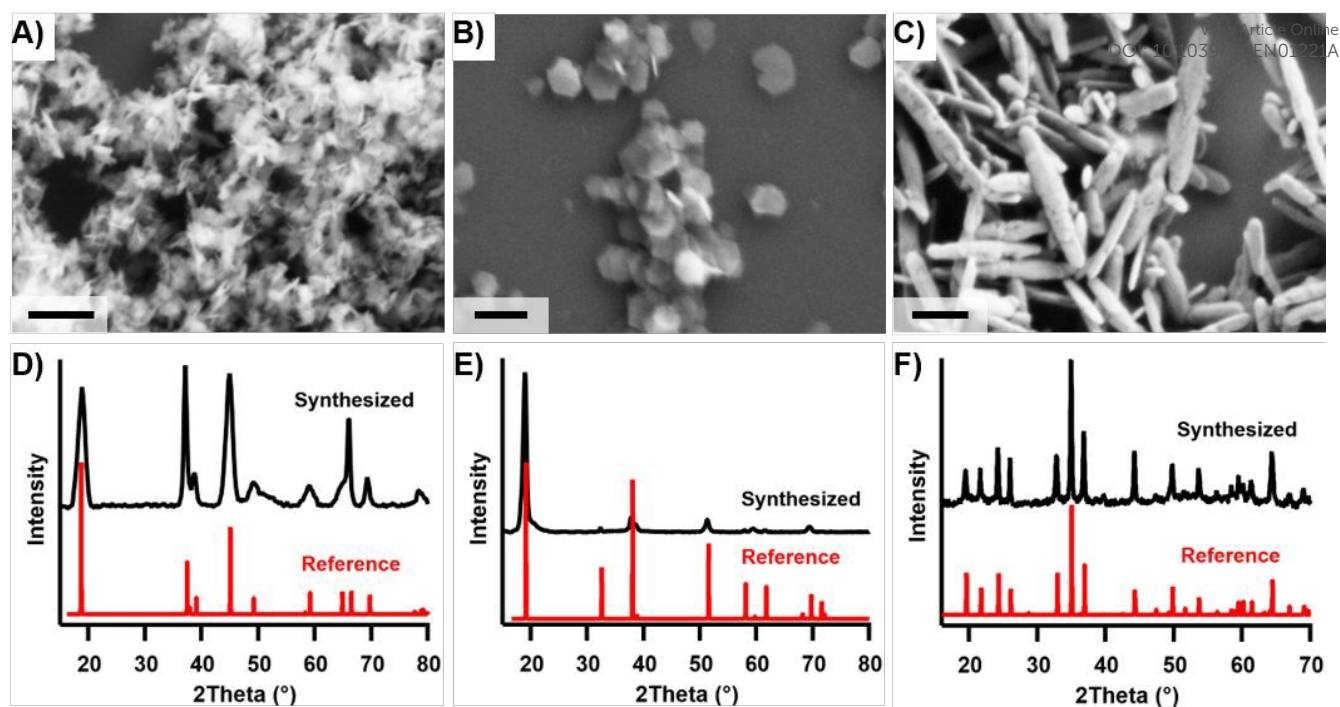


Figure 2. Scanning electron microscopy images and x-ray diffraction patterns for A/D) LiCoO₂, B/E) Co(OH)₂, and C/F) LiCoPO₄ nanoparticles, respectively. Scale Bars = 200 nm. Experimental XRD patterns (black) are compared to reference patterns (red). See text for references.

prepared by serial dilution from 1 g·L⁻¹ certified reference materials. Standards were diluted and acidified in the same manner as the unknowns.

2.5 Nanoparticle-NADH binding experiments

To examine if NADH/NAD⁺ bind to nanoparticles surfaces, we used X-ray photoelectron spectroscopy to characterize the surface composition of nanoparticles that were exposed to NADH in nanopure water. Nanopure water was used instead of minimal medium to avoid the influence of dissolved salts on the XPS analysis. Nanoparticles at 1 mg·mL⁻¹ concentration were exposed to NADH solutions for 24 h in ~3 mL vials; the samples were then centrifuged (same as above), the supernatant removed, and the remaining nanoparticles particles were rinsed and centrifuged 3× with nanopure water to remove any weakly adsorbed species. The particles were then dried overnight in a vacuum oven. To determine elemental composition on the nanoparticle surfaces, the dried samples were pressed into indium foil and analyzed using a Thermo X-ray photoelectron spectrometer with a monochromatized Al K α X-ray source and hemispherical analyzer at an angle 45° relative to the surface normal. Survey spectra were obtained summing three scans with a binding energy step size of 1 eV and pass energy of 200 eV, and high-resolution spectra were obtained average 10 (Co(2p)), 20 (O(1s) and C(1s)), or 50 (N(1s) and P(2p)) scans with step size of 0.2 eV and pass energy of 50 eV. Casa XPS software was used for data analysis. For area quantification, peaks were fit to 70:30 Gaussian-Lorentzian functions with Shirley³⁰ background correction.

3. Results and discussion

3.1 Nanoparticle characterization

SEM images in Figure 2A-C show relatively monodisperse size and shape within each particle type, with the range in size from LiCoPO₄ > Co(OH)₂ > LiCoO₂. LiCoO₂ and Co(OH)₂ form hexagonal sheets and LiCoPO₄ forms cylindrical rods. Our specific surface area measurements (S) found $S_{\text{LiCoO}_2} = 126.5 \pm 0.4 \text{ m}^2\cdot\text{g}^{-1}$, $S_{\text{Co(OH)}_2} = 33.29 \pm 0.05 \text{ m}^2\cdot\text{g}^{-1}$, and $S_{\text{LiCoPO}_4} = 28.1 \pm 0.2 \text{ m}^2\cdot\text{g}^{-1}$. Differences in these specific surface areas are consistent with sizes observed in SEM. Since most of the experiments reported herein are conducted according to particle mass concentration (mg·mL⁻¹), this difference in surface area will be addressed in section 3.3.

Table 1 shows the results of characterizing particles in minimal medium via DLS size and zeta potential. DLS sizes for LiCoPO₄ and Co(OH)₂ are only slightly larger than compared to what is observed in SEM, indicating minor aggregation. On the other hand, the DLS mean diameter for LiCoO₂, is much larger than the primary particles observed in SEM, indicating significant aggregation. All three particle types possess a similarly negative surface charge, which is consistent with all of them being partially deprotonated and/or delithiated in the salt/buffer solution at circumneutral pH.

XRD diffraction patterns of all three particle types are consistent with previously published reference patterns (Figure 2D-F),³¹⁻³³ indexed to the R $\bar{3}m$ (LiCoO₂), P $\bar{3}m1$ (Co(OH)₂) and Cmc (LiCoPO₄) space groups. This agreement indicates that the particles are crystalline and have the correct structure. Slight broadening of XRD peaks is expected given their

nanoscale size.³⁴ Transmission electron microscopy (TEM) images of LiCoO₂ nanosheets before and after exposure to minimal medium reveal a layered structure ~8 nm thick that does not change significantly with solution exposure (Figure S3, ESI[†]).

Table 1 Size and surface charge of particles in minimal medium determined from DLS and laser Doppler microelectrophoresis.

| Particle | Mean d (nm) | Zeta potential (mV) |
|---------------------|-------------|---------------------|
| LiCoO ₂ | 364 ± 57 | -18.4 ± 0.7 |
| LiCoPO ₄ | 118 ± 13 | -16.5 ± 3.8 |
| Co(OH) ₂ | 130 ± 5 | -16.0 ± 2.0 |

3.2 Influence of NADH and GSH on Co release

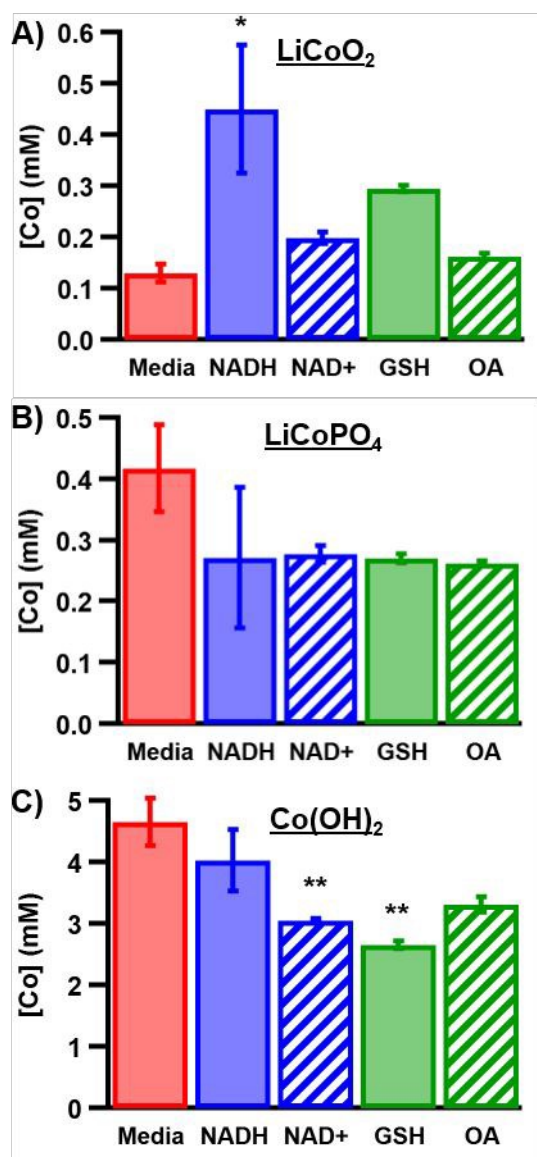


Figure 3. Dissolved Co concentrations in minimal medium solutions for A) LiCoO₂, B) LiCoPO₄, and C) Co(OH)₂ after 24-h exposure to each molecule under study. Molecule exposures are compared to media exposure with one-way ANOVA with Dunnett's multiple comparisons test: n = 3 or n = 4, * for p < 0.05 and ** for p < 0.01.

Figure 3 shows the concentrations of Co measured after LiCoO₂, LiCoPO₄, and Co(OH)₂ nanoparticles were introduced into minimal medium for 24 h. Similar data for Li are included in the ESI[†] (Figure S4). Figure 3 shows key differences across particles and across solutions tested. Figure 3A shows that exposing LiCoO₂ to NADH or GSH increases the release of Co: ~3× increase for NADH and ~2× increase for GSH compared that in minimal medium, with NADH being statistically higher than the media alone at 95% confidence. Since NADH and GSH contain functional groups (carboxylic acid and phosphate groups, respectively) that could complex with Co ions, we conducted further studies using NAD⁺ and ophthalmic acid (OA). These molecules are nearly identical in structure to NADH and GSA but lack the redox-active functional groups with the ability to reduce Co(III) in LiCoO₂. Furthermore, this experiment is necessary because in our experiments (see section 3.5) and in prior studies, phosphate is shown to have affinity for the LiCoO₂ surface. Figure 3A shows that NAD⁺ and OA exposure yields Co concentrations not statistically different from the media alone, and much lower than Co concentrations from NADH or GSH exposure. Based on these results, we conclude that the enhancement produced by NADH and by GSH arises from their oxidation-reduction properties and cannot be attributed to complexation of Co ions with the carboxylate or phosphate groups. Interestingly, NADH shows a greater effect on Co release compared to GSH. By comparing their reduction potentials at pH 7.0 vs. SHE ($E^0_{\text{NADH}} = -0.320 \text{ V}^{35}$ and $E^0_{\text{GSH}} = -0.252 \text{ V}^{36}$), NADH is the stronger reducing agent. This is consistent with 1) redox reactivity being the involved in increased Co release and 2) greater enhancement in Co release from NADH exposure compared to GSH exposure.

To further verify that the enhancement in Co release from LiCoO₂ induced by NADH and GSH is due to the ability of these molecules to reduce Co(III) (in LiCoO₂) to the more highly soluble Co(II), we conducted control experiments using two nanoparticle compositions in which Co exists in a Co(II) state: LiCoPO₄ (Figure 3B) and Co(OH)₂ (Figure 3C). Figures 3B-C show that for these compositions, the redox-active molecules NADH and GSH as well as the non-redox-active counterparts (NAD⁺ and OA) do not increase the amount of Co release compared with minimal medium. In the case of LiCoPO₄, all molecule exposures lead to slightly lower Co concentrations and none are statistically distinct from Co concentration of media alone at 95% confidence. Exposure to each molecule for Co(OH)₂ also shows slight decrease in Co concentration, with NAD⁺ and GSH statistically different from media alone at 95% confidence. Based on these data, we conclude that the influence of NADH and GSH on LiCoO₂ to enhance Co release can be ascribed to their ability to reduce Co(III) to Co(II). The slightly decreased Co release from Co(OH)₂ and LiCoPO₄ exposed to each molecule compared to with minimal medium alone suggests that these molecules may adsorb to the nanoparticles surfaces and partially restrict ion release (discussed in section 3.5).

Since LiCoO_2 and LiCoPO_4 are Li intercalation compounds, we also quantified the Li release to examine if it is impacted by the presence of each molecule (ESI[†], Figure S4). Dissolved Li concentrations for a given particle in Figure S4 are largely unaffected by changing the composition of the dissolution media. Consistent with prior results, the amount of Li released from these nanoparticles exceed that of Co, due to the fact that H^+ ions in solution can exchange with Li^+ ions in the solid without involving any redox processes.³⁷ The Li^+/H^+ exchange increases the pH of the solution, as shown by our pH measurements in the ESI[†], Table S3. Increase in pH occurs despite the medium being buffered with HEPES due to the high particle concentration ($1 \text{ mg}\cdot\text{mL}^{-1}$) used in our experiments. Notably, the pH values measured after 24-h exposure of LiCoO_2 nanoparticles to minimal medium and minimal medium with NADH, NAD⁺, GSH, and OA are identical within 0.2 pH units. Therefore, we conclude that while Li^+/H^+ exchange occurs and increases pH, this process is independent from the mechanism causing enhanced Co release in LiCoO_2 in the presence of NADH or GSH and not in the presence of NAD⁺ or OA.

3.3 NADH oxidation during LiCoO_2 dissolution

Our results indicate that the enhancement in Co release induced by the presence of NADH or GSH involves reduction of Co(III) to Co(II). In this case, the release of Co should be directly accompanied by an equivalent oxidation of NADH to NAD⁺ or possibly to other oxidation products. Figure 4 shows the NADH concentration remaining in solution after nanoparticles of the indicated compositions were introduced into the NADH solution for 24 h. These data show that [NADH] greatly decreases with exposure to LiCoO_2 , slightly decreases with exposure to Co(OH)_2 , and is statistically unchanged at 95% confidence when exposed to LiCoPO_4 . Also shown in Figure 4 are results from control samples prepared in an identical manner except lacking NADH; these samples show no significant fluorescence. We attribute the large decrease in [NADH] with LiCoO_2 to the oxidation of NADH coupled with Co release from LiCoO_2 . The slight decrease in observed [NADH] with Co(OH)_2 exposure is consistent with our results in Figure 7B showing NADH adsorbing onto the surface of Co(OH)_2 , as any adsorbed molecules would be removed from the solution with the particles before fluorescence analysis (see section 3.5).

Considering that the specific surface area of LiCoO_2 particles ($126.5 \pm 0.4 \text{ m}^2\cdot\text{g}^{-1}$) is approximately 4 - 4.5 times the specific surface areas of Co(OH)_2 ($33.29 \pm 0.05 \text{ m}^2\cdot\text{g}^{-1}$) and LiCoPO_4 ($28.1 \pm 0.2 \text{ m}^2\cdot\text{g}^{-1}$), we performed LiCoPO_4 dissolution experiments normalized by surface area instead of mass (i.e. $4.5\times \text{LiCoPO}_4$ particle concentration) to ensure that selective loss of NADH is not simply a surface area effect. The resulting [NADH] measured by fluorescence is shown in Figure 4 as “* LiCoPO_4 ”. The [NADH] for surface area normalized * LiCoPO_4 samples is nearly identical to the [NADH] for mass-normalized LiCoPO_4 and Co(OH)_2 samples and much higher than that for LiCoO_2 samples. This shows that the differences in dissolution behavior and

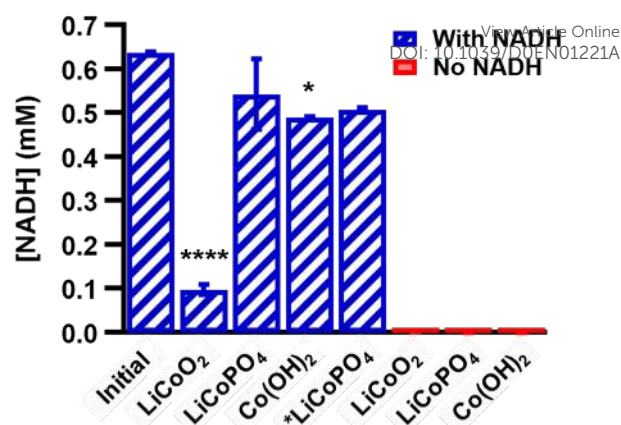


Figure 4. Concentration of NADH before (“initial”) and after exposure to the indicated nanoparticles for 24 h. “* LiCoPO_4 ” refers to NADH solutions after 24-h exposure to $4.5 \times [\text{LiCoPO}_4]$, a matching specific surface area exposure to LiCoO_2 samples. Particle solutions are compared to initial with one-way ANOVA with Dunnett’s multiple comparisons test: $n = 2$ or $n = 3$, * for $p < 0.05$ and *** for $p < 0.0001$. Also shown are fluorescence intensities of control samples prepared in an identical manner but without NADH (“No NADH”).

biomolecule interactions between LiCoO_2 and $\text{LiCoPO}_4/\text{Co(OH)}_2$ are due to differences in their chemical properties, not differences in surface areas.

While we anticipate that loss of NADH fluorescence is caused by oxidation to NAD⁺, we also considered whether loss of NADH might be caused by several other phenomena, including 1) a reaction of NADH with dissolved Co^{2+} or Li^+ ions, 2) adsorption of NADH onto the filter used to remove particles, and 3) interference in fluorescence spectra from residual particles scattering light. Details of these control experiments are presented in the ESI[†] (Figure S5). Notably, exposing NADH solution to dissolved $\text{Co}^{2+}/\text{Li}^+$ ions similar in concentration to experimental solutions or passing NADH solution through the syringe filter show no appreciable decrease in their fluorescence intensities. These control experiments uniformly confirm that NADH is oxidized to NAD⁺ by the nanoparticles.

As a complement to measuring the loss of NADH by fluorescence, we also used UV-visible absorption spectra of NADH solutions with/without exposure to LiCoO_2 , LiCoPO_4 and Co(OH)_2 to assess the concentrations of NADH and NAD⁺ before/after nanoparticle exposure. NADH and NAD⁺ both exhibit peaks around 203 nm and 259 nm with similar molar absorptivities, whereas NADH alone exhibits a peak at 339 nm.³⁸ The ESI[†] contains a detailed analysis of NADH and NAD⁺ UV-visible absorbance spectra (Figure S6). Interestingly, we found concentration-dependent peak shifts at higher concentrations, perhaps due to molecule aggregation/stacking. Based on their spectral properties, we can identify the redox state of a NADH/NAD⁺ solution using absorbances at 259 nm and 339 nm. For a NADH solution exposed to particles, the three possible cases are: 1) to retain absorbance at 339 nm, NADH has not been transformed to NAD⁺, removed, or degraded, 2) to lose absorbance at 339 nm but retain absorbance at 259 nm, NADH

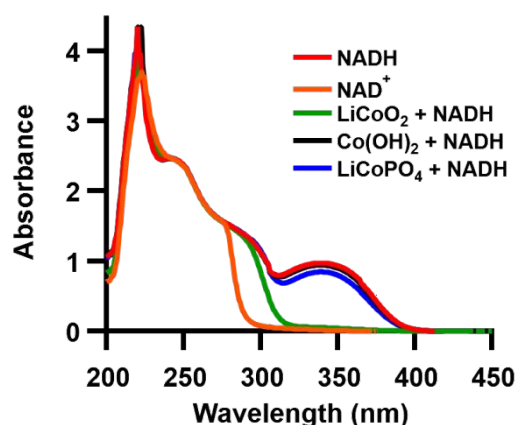


Figure 5. UV-visible absorption spectra of 0.5 mM solutions of NADH and NAD⁺, and 0.5 mM NADH solutions after 24-h exposure to LiCoO₂, LiCoPO₄, and Co(OH)₂. All solutions were prepared in minimal medium, which also served as the background. All 0.5 mM NAD⁺ samples exposed to nanoparticles yielded spectra nearly identical to the orange “NAD⁺” trace without nanoparticle exposure and are omitted for clarity.

has been oxidized to NAD⁺, or 3) to lose absorbance at both 339 nm and 259 nm, NADH has been removed from the system or severely degraded. Figure 5 shows a clear difference in which case is followed between solutions exposed to LiCoO₂ compared to solutions exposed to LiCoPO₄ or Co(OH)₂. With exposure to LiCoPO₄ or Co(OH)₂, NADH solutions maintain absorbance at 339 nm and 259 nm, indicating that NADH is unchanged (case #1). With exposure to LiCoO₂, absorbance at 339 nm is almost entirely lost, while absorbance at 259 nm is constant (case #2). Therefore, our UV-visible absorption results support the conclusion that NADH is oxidized to NAD⁺ with LiCoO₂ exposure, but not with LiCoPO₄ or Co(OH)₂ exposure. Since these data suggest NAD⁺ was present in our prior fluorescence studies, we confirmed that solutions of NAD⁺ with/without nanoparticle exposure yield negligible fluorescence compared to NADH solutions (Figure S7, ES1[†]).

Since NADH/NAD⁺ absorbance properties change with concentration, particularly at the 500 μM used for most dissolution experiments, additional dissolution experiments identical to those described in the experimental were performed exposing LiCoO₂ to 100 μM NADH in minimal medium. The results combining LiCoO₂ and 100 μM NADH show a decrease in [NADH] measured by fluorescence from 91.6 ± 6.6 μM to 2.21 ± 0.19 μM, and an increase in released Co measured by ICP-MS from 0.120 ± 0.002 mM to 0.139 ± 0.006 mM. These changes reaffirm that NADH enhances Co release and LiCoO₂ oxidizes NADH. Since results are consistent across the NADH concentrations tested, we conclude that the increase in released Co and oxidation of NADH at higher concentrations is not due to some specific spectral or aggregation effect of NADH, but through interaction with LiCoO₂.

The relative magnitudes of changes in NADH removal and Co release for 100 μM NADH are different compared to those of the 500 μM NADH experiments (i.e. Figures 3 and 4) because

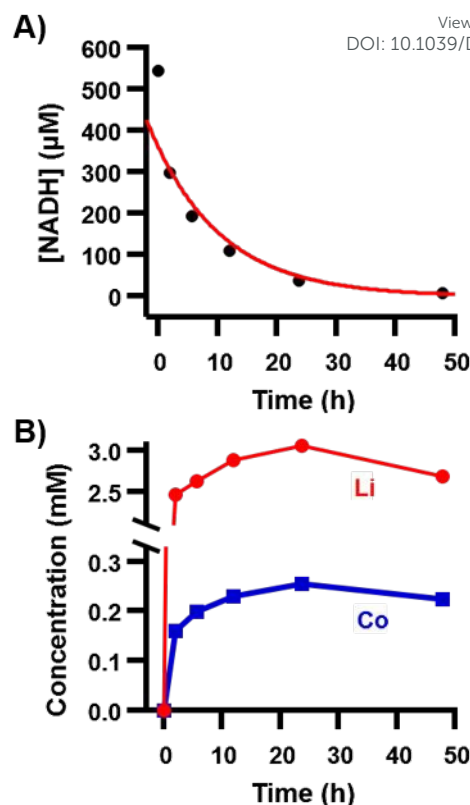


Figure 6. Concentrations measured over time for species in dissolution of 1 mg·mL⁻¹ LiCoO₂ with 0.5 mM NADH A) NADH measured by fluorescence with exponential decay fit of $Y = 350 \cdot e^{-0.084 \cdot X}$, and B) concentrations of Li (red circles) and Co (blue squares) measured by ICP-MS.

in the case of 100 μM, [LiCoO₂] ≈ 100×[NADH], whereas in the case of 500 μM, [LiCoO₂] ≈ 20×[NADH] (1 mg·mL⁻¹ LiCoO₂ for both). That is, in the extreme where [LiCoO₂] >> [NADH], a given amount of reaction yields a relatively large change in [NADH] and a relatively small change in released Co concentration.

3.4 Reaction kinetics of NADH and LiCoO₂

To characterize the kinetics of the interaction between NADH and LiCoO₂, we measured the concentrations of NADH (reactant) and released Co (product) over time for a single reaction solution. Time-points were taken from 2-hr to 48-hr, spanning across the 24-hr time point used in other experiments. The data in Figure 6A reveal an exponential decrease in [NADH] over the course of reaction. The general rate equation for this reaction is described by Eq. 1:

$$\frac{d[\text{NADH}]}{dt} = k[\text{NADH}]^n[\text{LiCoO}_2]^m \quad (1)$$

where k is the rate constant and n and m are reaction orders for NADH and LiCoO₂, respectively. As noted above, under the conditions of our experiments we assume [LiCoO₂] >> [NADH] and $\Delta[\text{LiCoO}_2] \approx 0$. Because of this, even if $m \neq 0$ the rate expression in Eq. 1 can be simplified by combining [LiCoO₂] ^{m} within the rate constant, k :

$$\frac{d[\text{NADH}]}{dt} \approx k'[\text{NADH}]^n \quad (2)$$

From curve fitting graphs of $[\text{NADH}]$, $\ln[\text{NADH}]$, and $[\text{NADH}]^{-1}$ vs. time, it is clear that the reaction is first order with respect to NADH (i.e. $n = 1$). For example, in the case of $\ln[\text{NADH}]$ vs. time a linear regression yields $R^2 = 0.972$, whereas for $[\text{NADH}]$ vs. time linear $R^2 = 0.581$. We determined the pseudo-first-order rate constant of $k' = 0.084 \pm 0.007 \text{ hr}^{-1}$. Interestingly, the $t = 0$ point in Figure 6A deviates more significantly from the fit than most points. This suggests that a separate process, possibly adsorption of NADH onto the particles (see section 3.5) occurs in the initial stages of dissolution to remove more NADH than expected over the longer-term reaction.

Concentrations of dissolved Co and Li for the same dissolution experiment are shown in Figure 6B. As with single time point experiments, $[\text{Li}] \gg [\text{Co}]$ due to Li^+ -proton exchange. Both species are released at a greater rate in the initial stages of reaction, and plateau in concentration over time. The plot of Co concentration with time mirrors that of NADH with time, which we ascribe to solubilized Co being a product of the reaction of NADH with LiCoO_2 . The decrease in the rate of Co-production with time is consistent with our conclusion that the NADH- LiCoO_2 reaction depends directly on $[\text{NADH}]$.

3.5 Binding of NADH to nanoparticles

The data in Figures 3 and 4 suggest that some of the apparent loss of NADH is due to adsorption onto the nanoparticle surfaces. Additionally, the sharp decrease in $[\text{NADH}]$ at the first time point of our kinetics study suggests NADH adsorbs relatively quickly compared to the longer-term redox reaction. To determine the extent of NADH interaction with nanoparticle surfaces, we obtained XPS spectra of nanoparticles before and after exposure to NADH, using N(1s) emission to quantify the presence of NADH and using Co(2p) as an internal standard. Figure 7A shows representative N(1s) spectra from LiCoO_2 before and after exposure to NADH (0.5 mM, 24 h). Additional spectra are shown in the ESI[†]. Figure 7A clearly shows a pronounced increase in N(1s) emission intensity for the NADH-exposed sample compared to the unexposed sample. Figure 7B summarizes similar data for LiCoO_2 , LiCoPO_4 , and Co(OH)_2 . We note that while NADH is the species introduced to the nanoparticles, the XPS spectra cannot distinguish between adsorbed NADH or NAD^+ . We determined the surface coverage of N atoms bound to each surface (LiCoO_2 , LiCoPO_4 , and Co(OH)_2) from the N(1s) and Co(2p) photoelectron emission (Figure 7B), as described in the ESI[†]. Figure 7B shows the resulting surface coverages. The data show a statistically significant increase in N surface coverage with NADH exposure for Co(OH)_2 at 95% confidence, a moderate increase for LiCoO_2 , and no change for LiCoPO_4 . Using the known stoichiometry of 7 N atom per NADH/ NAD^+ molecule, the N coverages correspond to $0.37 \pm 0.07 \text{ NADH/NAD}^+ \text{ molecule}\cdot\text{nm}^{-2}$ for LiCoO_2 , $0.036 \pm 0.007 \text{ molecule}\cdot\text{nm}^{-2}$ for LiCoPO_4 , and $0.62 \pm 0.05 \text{ molecule}\cdot\text{nm}^{-2}$ for Co(OH)_2 . For comparison, assuming a density of closely packed NADH of $\sim 1.7 \text{ g}\cdot\text{cm}^{-3}$ (from solid NADH) and monolayer thickness of $\sim 0.5 \text{ nm}$ yields a maximum coverage of ~ 0.8

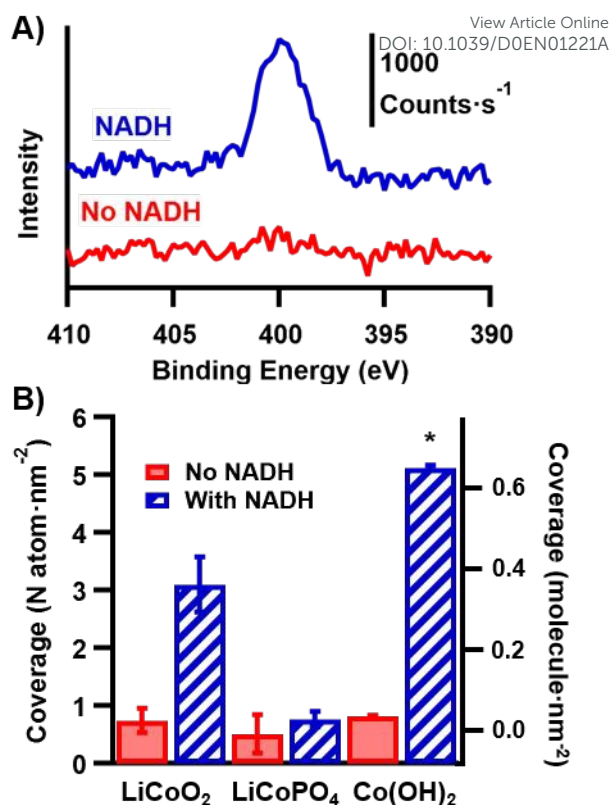


Figure 7. A) N(1s) XPS spectra of LiCoO_2 particles after 24-h soak in solutions with or without 0.5 mM NADH, and B) N atom surface coverages on nanoparticles after 24-h soak with or without 0.5 mM NADH, determined from N(1s) and Co(2p) peak areas. With/without NADH samples are compared for a given particle type with unpaired t-test with Welch's correction: $n = 2$ or $n = 3$, * for $p < 0.05$.

$\text{molecule}\cdot\text{nm}^{-2}$. A 0.5 nm monolayer thickness is estimated using literature³⁹ bond lengths and assuming a monolayer of NADH/ NAD^+ molecules bind via a phosphate moiety (see below) in a planar conformation roughly perpendicular to the surface. Thus, on LiCoO_2 and Co(OH)_2 the molecular coverages estimated from XPS are comparable to that expected from a monolayer of NADH/ NAD^+ .

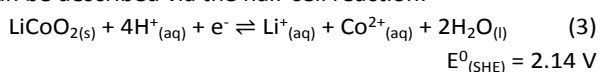
The presence of significant N coverage after three cycles of rinsing, mixing, and centrifuging indicates that NADH/ NAD^+ has a high affinity with LiCoO_2 and Co(OH)_2 surfaces. The strong binding we observe for NADH/ NAD^+ with LiCoO_2 and Co(OH)_2 suggests that the phosphate moieties in NADH/ NAD^+ bind to surface oxygen atoms on each particle surface, and that the phosphate-containing molecules do not bind to phosphate terminated particles (i.e. LiCoPO_4). This result is an agreement with prior work showing that phosphate binds strongly to LiCoO_2 ²¹ and to Fe(III) oxides⁴⁰ via an inner-sphere configuration on an oxygen-terminated surface. Notably, prior work reported that despite its attachment to the LiCoO_2 surface, phosphate does not increase LiCoO_2 dissolution.²¹ In contrast, we find that NADH significantly increases LiCoO_2 dissolution. Using our measured LiCoO_2 specific surface area, XPS surface density, and known solution volumes, we determined that adsorption onto

the LiCoO₂ nanoparticle surfaces accounts for decrease in total NADH concentration of (0.077 ± 0.014 mM). This value is much smaller than the actual decrease in [NADH] after 24-h LiCoO₂ exposure (0.539 ± 0.012 mM) that we observed and allows us to conclude that while NADH binds to LiCoO₂ nanoparticle surfaces, surface adsorption is not the primary origin of the decrease in [NADH] observed in Figures 4 and 5. On the other hand, the fact that NADH binds even more strongly with Co(OH)₂ can explain the much smaller decrease in [NADH] seen in Figure 4. Any NADH molecules adhering to the Co(OH)₂ particles are removed from the solution and would not contribute to the measured fluorescence.

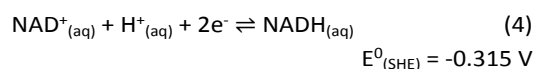
3.6 Coupled transformation of NADH and LiCoO₂

The data in Figure 3 show that in minimal medium alone (i.e., in the absence of NADH or other intentionally added reducing agents), the amount of Co released from LiCoO₂ is much less than the amount released from LiCoPO₄. This observation is consistent with the hypothesis that while LiCoPO₄ can directly release cobalt in the highly soluble Co(II) form, release of cobalt from LiCoO₂ is less favorable due to the highly insoluble nature of Co(III). The lower rate of release from LiCoO₂ in the absence of added reducing agents is further supported by density functional theory (DFT) and thermodynamics calculations of the initial steps (loss of first partial-monolayer) in release of Co from the lowest-energy surface faces of LiCoO₂ and LiCoPO₄ in water. These calculations (see ESI[†]) indicate that the initial release of Co from the LiCoO₂(001) surfaces in pure water at pH = 7 (with other water-soluble species set at 10⁻⁶ M) is endergonic with an energy change of ΔG = +1.15 eV/Co while initial release from the LiCoPO₄(010) surface is exergonic, ΔG = -0.87 eV/Co, in qualitative agreement the experimental results.

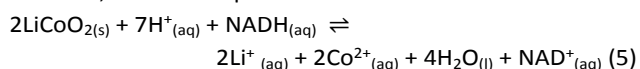
Our experiments demonstrate that NADH greatly increased the rate of Co release from LiCoO₂, while no corresponding increase release from LiCoPO₄. This observation strongly points to an electrochemically driven interaction between NADH and LiCoO₂. The electrochemical reduction of LiCoO₂ with release of Co²⁺ can be described via the half-cell reaction:



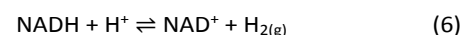
Here, the standard electrochemical reduction potential of E⁰ = +2.14 V at 25°C can be readily determined using the Gibbs free energy of formation of LiCoO₂ from the pure elements at standard state (-615 kJ·mol⁻¹)^{41, 42} and standard electrochemical potentials. The NADH/NAD⁺ couple is described by the electrochemical reduction:³⁵



Coupling these two together to form the electrochemically mediated reaction (Eq. 5) yields a net ΔG = -4.9 eV (-474 kJ·mol⁻¹) as written, or -2.45 eV per Co²⁺ ion released.



Additionally, our measurements of [NADH] vs. time and [Co] vs. time demonstrate the reaction is first order with respect to NADH and that the reaction rate is greatest when nanoparticles and NADH solution are first exposed to one another. While the stoichiometry of Eq. 5 suggests there should be two Co ions released for each molecule of NADH that is reduced, experimentally we observe that the Co release (0.32 ± 0.13 mM) is smaller than the loss of NADH (loss of 0.539 ± 0.012 mM). This greater than anticipated loss of NADH suggests that NADH may be simultaneously involved in other reduction reactions, such as:



with ΔG⁰ = +0.037 eV. A prior study reported evidence for direct surface oxidation of NADH at Co₃O₄ surfaces,⁴³ while other studies have shown that oxides made from metal oxides exhibit enhanced electrochemical reduction of NADH and have attributed this enhancement to the ability of metal oxide surfaces to adsorb protons.⁴³ As a result, we conclude that while release of Co(II) is directly attributed to NADH, the transformation of NADH and NAD⁺ may be further increased by additional surface-catalyzed reactions.

4. Conclusions

Our results show that biological molecules that are important in cellular respiration and electron transport can play an active role in the transformation of high-valence transition metal oxides through redox chemistry at nanoparticle surfaces. NADH and GSH interact with LiCoO₂ nanoparticles by redox chemistry at the solid-liquid interface: electrochemical reduction of Co(III) to Co(II) allows Co to be easily solubilized and released into solution while also oxidizing NADH to NAD⁺. We attribute this coupled redox transformation to redox reaction between Co(III) within LiCoO₂ and NADH/GSH reducing agents, and not a complexation/chelation/pH effect, based on five pieces of evidence. First, NADH and GSH produce similar effects when exposed to LiCoO₂ despite differences in their structures. Second, non-reducing analogues of NADH and GSH do not reproduce the same effects. Third, NADH is oxidized when exposed to LiCoO₂ but not when exposed to LiCoPO₄ or Co(OH)₂ (whether samples are normalized by mass or surface area) and is not oxidized when exposed to Co²⁺/Li⁺ ions. Fourth, Li release is relatively unaffected by the presence of the molecules, as this occurs primarily by H⁺ exchange, and pH change is found to not be the driver of changes in dissolution in our experiments. Lastly, measuring concentrations of NADH (reactant) and released Co (product) over time reveal a direct relationship between reaction rate and [NADH].

Transformations of nanoparticles driven by redox chemistry of molecules involved in biological electron transport pose two important mechanisms for toxicity in the environment. First, the reduction of Co(III) and other high valence metals to lower-valence states increases their solubility and leads to faster release into the aqueous phase. High concentrations of metals such as Co and Ni released from metal oxides have been shown

to induce toxicity towards various aquatic organisms.^{44, 45} Select microorganisms (e.g. *S. oneidensis* MR-1) are known to reduce high valent metals (Fe, Co, Mn) in minerals extracellularly,⁴⁶ increasing metal ion release.⁴⁷⁻⁴⁹ We find similar behavior from introduction of reducing biomolecules, suggesting that this metal-reduction phenomenon could occur to a lesser extent from non-metal-reducing organisms interacting with high surface area nano-scale metal oxides. Secondly, the corresponding oxidation of NADH, GSH, and other molecules relevant to electron transport and cellular homeostasis can serve as an additional pathway to biological impact. When redox-active molecules facilitate metal oxide nanoparticle dissolution, they can be transformed or degraded. In the case of NADH and GSH, severe unnatural oxidation surrounding a non-metal-reducing organism from interaction with a nanoparticle will alter the NADH:NAD⁺ and GSH:GSSG ratios in/around cells, entirely disrupting cellular redox homeostasis. Imbalances in GSH or NADH concentrations can have many toxic effects,⁵⁰ but in particular, removal of the reduced forms of these well-known antioxidants may lower cellular defenses against ROS. Several studies have observed ROS in the presence of nanomaterials,^{51, 52} including LiCoO₂.⁵³ In such cases, whether nanoparticle transformation or cellular machinery generates ROS, the ROS will more negatively impact cells with reduced anti-oxidant capability.

Conflicts of interest

There are no conflicts to declare.

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