Abstract: The hydrogenation of 4-nitrophenol (4-NP) has attracted much attention, since it is typically used as a model reaction for evaluating newly developed catalysts, but its mechanism is still debated. Herein, Co(OH)$_2$-modified CuO catalyst (Co(OH)$_2$/CuO) was used for the reduction of 4-NP to 4-aminophenol (4-AP) in an aqueous sodium borohydride (NaBH$_4$) solution. The reaction mechanism was investigated by UV-Vis spectroscopy (UV-Vis), high-performance liquid chromatography (HPLC), HPLC-Q-orbitrap high-resolution mass spectrometry (LC-MS/MS), and $^1$HNMR spectroscopy ($^1$HNMR) as an integrated technology at different concentrations of NaBH$_4$. Samples were taken at specified time intervals and monitored using UV-Vis, HPLC, LC-MS/MS, and $^1$HNMR. With the help of comprehensive analysis, eight intermediates, including azo and azoxy compounds, were effectively captured, and the variation tendency of each intermediate was determined, revealing that the hydrogenation of 4-NP proceeds via a coexistence of the direct and condensation routes. The integrated analysis methods were powerful technical supports for the study of the catalysis mechanism.

Keywords: 4-nitrophenol hydrogenation; intermediates; mechanism; Co(OH)$_2$/CuO bimetallic nanocatalyst; integrated analysis method

1. Introduction

The reduction process is an important chemical transformation in organic synthesis and industrial chemistry. The catalytic reduction of 4-nitrophenol (4-NP) to 4-aminophenol (4-AP) by sodium borohydride (NaBH$_4$) is an industrially important reaction which is commonly used as a model reaction for evaluating the activities of newly synthesized catalysts [1–4]. Toxic 4-NP is responsible for serious environmental pollutions; however, it can be converted into harmless 4-AP, which is a very significant intermediate for accessing pharmaceuticals and dyes via the reduction process. Regarding the greener aspect of the catalytic process, it is highly desirable to pay attention to the catalytic process and intermediates in aqueous media to avoid secondary hazards to human health and the ecological environment [5–7].

For a model reaction, it is critical to develop a comprehensive framework illustrating the precise 4-NP reaction mechanisms and behavior. Sevda Dehghani et al. [8] took advantage of the computational-chemistry-designed Pd@ligand-decorated halloysite. The resulting catalyst, which exhibited high catalytic activity for the hydrogenation of nitroarenes, confirmed that the catalyst could successfully promote the hydrogenation of a...
sterically demanding substrate. Zhang et al. [3] studied the reduction of 4-NP with NaBH₄ catalyzed by silver nanoclusters supported on TiO₂ and provided a mechanistic investigation which implied that it was modeled in terms of the Langmuir–Hinshelwood mechanism. Kundu et al. and Bhui et al. [1,9] provided the surface reaction mechanism of 4-NP to 4-AP by studying the reaction kinetics. The kinetics of the reaction were modeled in terms of a Langmuir–Hinshelwood mechanism, where both reactants needed to be adsorbed on the surface prior to the reaction, and the rate-determining step was governed by the reaction of the adsorbed species. However, Liu et al. [10] proposed a mechanism concerning the reduction of nitro compounds with NaBH₄ catalyzed by a Ag NPs/Psi chip. In this case, the Ag NPs serve as the mediators of the electron transfers. The electrons are transferred from the nitro groups to the hydrogen groups, and ultimately the hydrogen is activated, forming metal hydride. The reduction proceeds when nitroaromatics and activated H₂ are absorbed chemically on the surface of the Ag NPs/Psi chip. The catalytic hydrogenation of the absorbed nitro compound and the removal of the water molecule yield the nitroso compound. N-hydroxylaniline is obtained via successive catalytic hydrogenation, which is converted into aromatic amine finally through subsequent hydrogenation and dehydration. As is seen from the proposed mechanism, the activation of the produced H₂ by the Ag NPs/Psi chip plays a major role.

Now there are two probable routes (the direct route and the condensation route) for the reduction of the nitro groups to amino groups, based on the electrochemical model reaction presented by Haber [11,12]. In the direct route, the aromatic nitro compound is reduced to nitroso, and then to hydroxylamine, in two very fast and consecutive steps. Finally, the hydroxylamine is reduced to aniline in a rate-determining step. The second route involves the condensation of one molecule of the nitroso compound with a molecule of hydroxylamine to give the azoxy compound, and then reduced to azo, hydrazo, and aniline compounds in a series of consecutive steps. Gelder et al. [13] claimed that the Haber mechanism is not enough to completely explain the experimental data, and performed a detailed gas chromatography–mass spectrometer (GC-MS) analysis on the reaction mechanisms of nitrobenzene and nitrosobenzene, and showed that nitrosobenzene is not an intermediate in the hydrogenation of nitrobenzene. Nitrobenzene was reduced to phenylhydroxylamine directly, and thus a new mechanism was proposed. Corma et al. [14] studied the hydrogenation of nitrobenzene and reaction intermediates by in situ Fourier transform infrared spectroscopy (FT-IR) and demonstrated that the direct route was the preferred pathway for the hydrogenation of aromatic nitrocompounds on Au/TiO₂, i.e., R-NO₂→R-NHOH→R-NH₂. Biraj Jyoti Borah et al. [15] proposed that NaBH₄ nitrohydrogenation takes place via the direct route over Cu@Fe₂O₃ NPs, as observed by UV–visible and GC-MS analyses. Xiangkai Kong et al. [16] proposed six different intermediates with no condensed roots to depict the catalytic process by paper-assisted ultrasonic spray ionization mass spectrometry on N-doped graphene and Ag nanoparticles in the presence of NaBH₄. Keya Layek et al. [17] proposed a probable surface reduction mechanism based on the Langmuir–Hinshelwood model without going into the intricate kinetic details and stoichiometric equations, using NAP-Mg–Au(0) and sodium borohydride as the catalytic system, and concluded that the direct route involving nitrobenzene→nitrosobenzene→phenylhydroxylamine→aniline is the most likely path followed for the reduction of the aromatic nitro group involving the NAP-Mg–Au(0) catalyst. Kasibhatta J. Datta et al. [18] synthesized the magnetite microspheres and, by employing hydrazine hydrate as the reductant, efficiently transferred a variety of nitroarenes to respective anilines, and confirmed that the reduction of nitroarene proceeded via the direct route as R-NO₂→R-NO→R-NHOH→R-NH₂. García Sánchez [19] synthesized Pd nanoparticles glued with PDA on inorganic supports, and tested the hydrogenation of 4-nitrophenol using NaBH₄ as the hydrogenating agent, by applying the multivariate curve resolution factorial methodology to show the reduction of the functional groups NO₂ to NO and NO to NH₂, thus indicating that the reaction followed the direct route by a slower reduction of NO to NH₂, which constitutes the rate-limiting step.
Although the reduction of 4-NP by borohydride has emerged as one of the most widely used model reactions for accessing the catalytic activity of nanostructures [4,20], it is more about the catalytic surface reaction mechanisms, as the different perceptions of the intermediates in the reaction process lead to different views on the reaction mechanisms. Most studies evaluate the hydrogenation of 4-NP to 4-AP by monitoring the decrease in the absorbance peak at 400 nm by UV-Vis or analyzing the intermediates by high-performance liquid chromatography (HPLC) [21,22]. Although these methods are intuitive and simple, and rapidly obtain data, it is difficult to determine all the intermediates produced in the reaction. However, the degradation of pollutants is not always translated or mineralized completely and may produce more toxic degradation resistance or metastable intermediates in the transformation process. Therefore, a comprehensive analysis using integrated analytical methods and online tracking is important to solve these problems.

In our previous studies, Co(OH)$_2$/CuO were synthesized by a simple coprecipitation method and was successfully applied for the rapid removal of RhB in the Co(OH)$_2$/CuO/PS system under visible light and the water oxidation reaction [23,24]. The catalyst showed superior catalytic activity for the reduction of 4-NP to 4-AP, providing us a good opportunity to investigate the mechanism by online UV-Vis, high-performance liquid chromatography (HPLC), HPLC-Q-orbitrap high-resolution mass spectrometry (LC-MS), and $^1$HNMR spectroscopy ($^1$HNMR) as an integrated technology.

To capture the intermediates produced in the reaction, the critical time nodes of the reaction were first determined by the in situ detection of changes in the UV-Vis spectra, and then samples were taken at the determined time nodes for the online LC-MS/MS and $^1$HNMR testing. Q-orbitrap high-resolution mass spectrometry (HRMS) has extremely high resolution and sensitivity for the rapid monitoring of the reaction process and short-lived reaction intermediates, offers unprecedented capabilities to probe the molecular composition, and provides molecular formula assignments [25].

In this paper, the catalytic reduction of 4-NP and sodium borohydride with different concentrations of Co(OH)$_2$/CuO was designed. To overcome the uncertainty of a single monitoring process under the same experimental conditions, samples with different reaction time nodes were taken for online detection. Using four different analytical methods, the molecular and structural information of all intermediates involved in the reaction solution have been captured completely and described in detail for the first time. The mechanism of the model reaction in the aqueous solution was proposed by analyzing the order and intensity of the species in online detection. Finally, a new and reasonable reaction mechanism was proposed in which both the direct and the condensation routes coexisted. This has not been done in previous reports, and this study has been carried out using an ample combination of experimental techniques, which is a strong point in favor of this work.

2. Results and Discussion

2.1. Catalytic Performance for the Hydrogenation of 4-Nitrophenol

The as-synthesized Co(OH)$_2$/CuO was characterized by powder X-ray diffraction (PXRD) (Figure S1c, Supporting Information (S)), which suggests that the synthesized nanostructure is composite. However, XRD patterns did not provide a clear indication about the presence of Co(OH)$_2$. In the FESEM images (Figure S1a,b), the morphology of the Co(OH)$_2$/CuO catalyst is shuttle-like, and the approximate width and the length of the nanoparticle was 30 nm and 80 nm, respectively. As is shown in the XPS analysis in Figure S2, the results demonstrate the bonding configuration of both the copper and cobalt metals, and also provide information about the composition of the as-synthesized bimetallic catalyst, ensured by the existence of a small amount of Co(OH)$_2$ in the bimetallic catalyst.

For a deeper insight into the reaction mechanism, systematic UV-Vis analysis is firstly carried out for Solution 1. Normally, 4-NP exhibits a typical absorption peak at 317 nm (Figure 1c). After adding NaBH$_4$ to the solution, a red shift immediately occurs from 317 nm to 400 nm, and the color of the solution changes from light yellow to yellow, indicating the
formation of 4-nitrophenolate ions (NP ion). Thus, the progress of the reaction is monitored by tracking the changes in the absorption spectra at 400 nm. However, in the presence of Co(OH)$_2$/CuO, the yellow color turns colorless within 7 min, and the intensity decreases rapidly (Figure 1a). A concomitant appearance of a new peak at 300 nm matches well with that of the standard 4-AP, whereas Solution 2 turns colorless in 40 s (Figure 1d). The results indicate that Co(OH)$_2$/CuO can successfully catalyze the reduction of 4-NP (yellow color) to 4-AP (colorless), especially in an excess amount of NaBH$_4$.

Figure 1. Cont.
Figure 1. UV-Vis absorption spectra of 4-NP reduction of (a) Solution 1, (b) at a wavelength 230 nm of Solution 1, (c) at the same molarity of 4-NP, 4-NP ion, and 4-AP, (d) in Solution 2 and (e) the hydrogenation scheme of the reaction.

In previous studies, the decrease in the absorbance intensity at 400 nm was the main focus which indicated the fading of 4-NP, while ignoring the change in the absorption peak of aminophenol at 300 nm. No one was concerned about the changes in absorption at 230 nm.
nm, revealing the variation in the intermediates. By comparing the UV absorbance changes at 230 nm (Figure 1b,d), the results indicate that the catalytic reduction process may not follow the previously reported routes. Here, Solution 1 is selected as the research target. When Co(OH)$_2$/CuO is added to the solution, the absorbance at $\lambda_{\text{max}} = 400$ nm decreases from 3.8 to 0, while a new peak emerges at $\lambda_{\text{max}} = 300$ nm (Figure 1a). An increase in the absorption peak at 230 nm suggests that the intermediates are benzene ring compounds. Careful study of the spectral changes (Figure 1b) can preliminarily find that the absorbance changes at 230 nm are evident, while at 300 nm, they are not obvious, suggesting that the intermediates are produced after some time and that 4-AP is not immediately formed, as indicated in Figure 1e. Therefore, the key time nodes of Solution 1 are determined at 0, 1, 2, 3, 5, 7, 10, 30, and 60 min by online UV-Vis monitoring. Similarly, the online HPLC, LC-MS/MS, and $^1$H NMR monitoring are performed on the key time nodes to capture the reduced products.

2.2. UHPLC-Q-Orbitrap-HRMS Analysis

It is convenient to capture the reaction intermediates using LC-MS/MS due to the following advantages. Firstly, no preprocessing is required for all samples analyzed during the reduction process, thus reducing the loss of the trace components. Secondly, this technique has low requirements for chromatographic separation. Even if the HPLC separation of the compounds overlaps each other, it does not affect the analysis results. Thirdly, it also detects compounds with no UV absorption characteristics, eliminating the deficiencies of UV-Vis, HPLC, and other detection methods.

HRMS is used to analyze the standard compounds of 4-NP, 4-AP, and 4-nitrosophenol (4-NO) (intermediate), both in positive and negative ion modes, respectively. Figure S3a–f shows the MS/MS spectra of 4-NP, 4-NO, and 4-AP, respectively. The extracted mass of 4-NP is at 138.01933 [M$^+$H$^-$], indicating C$_6$H$_4$O$_3$N as the main MS/MS fragments (Figure S3b). The molecular mass of 4-AP is at 110.06012 [M+H]$^+$, as shown in Figure S3c, suggesting an elemental composition of C$_6$H$_8$ON, and Figure S3d shows its main MS/MS fragments. In Figure S3e, an m/z of 124.03921 is the [M+H]$^+$ of 4-NO in positive ion mode, indicating an elemental composition of C$_6$H$_6$O$_2$N, and its MS/MS is shown in Figure S3f. As no standards are available for other intermediates, we analyzed the fragments one by one using fragmentation information and the ion cleavage law by secondary mass spectrometry information. The fragment ion information at each key time node is given in Table 1.

<table>
<thead>
<tr>
<th>NO</th>
<th>$t_R$ (min)</th>
<th>[M+H]$^+$</th>
<th>[M−H]$^-$</th>
<th>Formula</th>
<th>Error (ppm)</th>
<th>MS/MS Data (m/z, Relative Abundance, %)</th>
<th>Label</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23.72</td>
<td>-</td>
<td>138.01968</td>
<td>C$_6$H$_4$O$_3$N</td>
<td>8.07</td>
<td>138(28), 108(100), 95(3)</td>
<td>4-NP</td>
</tr>
<tr>
<td>2</td>
<td>5.84</td>
<td>140.03409</td>
<td>-</td>
<td>C$_6$H$_4$O$_3$N</td>
<td>−0.93</td>
<td>-</td>
<td>4-NOOH</td>
</tr>
<tr>
<td>3</td>
<td>5.95</td>
<td>124.03942</td>
<td>-</td>
<td>C$_6$H$_4$O$_3$N</td>
<td>0.93</td>
<td>124(10), 107(8), 96(100), 94(10), 79(8), 66(5)</td>
<td>4-NO</td>
</tr>
<tr>
<td>4</td>
<td>5.95</td>
<td>125.04717</td>
<td>-</td>
<td>C$_6$H$_4$O$_3$N</td>
<td>0.34</td>
<td>125(10), 124(20), 96(100), 83(8), 89(5)</td>
<td>4-NOH</td>
</tr>
<tr>
<td>5</td>
<td>5.95</td>
<td>126.05502</td>
<td>-</td>
<td>C$_6$H$_4$O$_3$N</td>
<td>0.48</td>
<td>−126(6), 125(12), 108(96), 96(72), 80(100)</td>
<td>4-NOH</td>
</tr>
<tr>
<td>6</td>
<td>6.04</td>
<td>109.05281</td>
<td>-</td>
<td>C$_6$H$_4$ON</td>
<td>5.45</td>
<td>109(8), 108(22), 93(5), 80(100), 65(8)</td>
<td>4-NH</td>
</tr>
<tr>
<td>7</td>
<td>6.04</td>
<td>110.06023</td>
<td>-</td>
<td>C$_6$H$_4$ON</td>
<td>1.75</td>
<td>110(50), 109(20), 93(33), 82(10), 65(100)</td>
<td>4-AP</td>
</tr>
<tr>
<td>8</td>
<td>6.14</td>
<td>231.07610</td>
<td>-</td>
<td>C$_6$H$_11$O$_2$N$_2$</td>
<td>−1.39</td>
<td>231(12), 230(20), 214(44), 198(8), 186(100)</td>
<td>AOB</td>
</tr>
<tr>
<td>9</td>
<td>6.14</td>
<td>215.08133</td>
<td>-</td>
<td>C$_6$H$_11$O$_2$N$_2$</td>
<td>−0.81</td>
<td>215(12), 214(60), 198(100), 187(70), 95(58)</td>
<td>AB</td>
</tr>
<tr>
<td>10</td>
<td>6.14</td>
<td>216.08916</td>
<td>-</td>
<td>C$_6$H$_12$O$_2$N$_2$</td>
<td>−0.79</td>
<td>-</td>
<td>HAB</td>
</tr>
</tbody>
</table>

To obtain the structures of the intermediates involved in the reaction process, all four Solutions are analyzed by LC-MS/MS at their appropriating key time nodes. Solutions 1 and 2 are used for the detailed analysis, and the data are collected at full scan MS/MS mode. The compounds are determined according to the calculated mass and molecular composition calculated by HRMS. Here, the LC-MS/MS data of Solution 1 is taken to analyze the structures of the intermediates. As shown in Figure 2, when $t = 2$ min, there are 10 compounds with different m/z ratios. Compound 1 in the total ion current diagram (TIC) ($t_R = 23.72$ min), with
a molecular ion m/z of 138.01968 [M−H]−, indicates an elemental composition of C$_6$H$_4$O$_3$N$^-$, and its fragment ions in MS/MS are listed in Table 1, which are the same as in Figure S3b, suggesting that 4-NP (C$_6$H$_4$O$_3$N) is not completely transformed. The molecular ion peak at t$_R$ = 5.02–6.30 min in the TIC corresponds to compounds 2–10 in positive mode. The molecular ion peak for compound 2 (t$_R$ = 5.84 min) is present at m/z 140.03409 [M+H]$^+$, and it is speculated that the initially formed intermediate is C$_6$H$_5$O$_3$N (4-NOOH). The molecular ion peak of compound 3 is present at m/z 124.03942 [M+H]$^+$ (t$_R$ = 5.95 min), which indicates an elemental composition of C$_6$H$_3$O$_2$N, and its fragments are consistent with Figure S3f, confirming it as C$_6$H$_5$O$_2$N (4-NO). The MS/MS fragment ion of compound 4 (t$_R$ = 5.95 min), with an m/z of 125.04717 [M+H]$^+$, is at m/z 125, m/z 124, m/z 96, m/z 83, and m/z 79, which differs from the intermediates mentioned above, and it is speculated to be C$_6$H$_4$O$_2$N (4-NOH). Compound 5 (t$_R$ = 5.95 min) corresponds to an m/z of 126.05502 [M+H]$^+$, and its MS/MS fragments are shown in Table 1, speculated to be another important intermediate N-phenylhydroxylamine C$_6$H$_5$O$_2$N (4-NHOH). Compound 6 (t$_R$ = 6.04 min) shows a molecular ion peak at m/z of 109.05281 [M+H]$^+$, with a [M-1] peak at m/z of 108.04478. According to the fragment ions information in Table 1, it is the intermediate iminophenol C$_6$H$_5$ON (4-NH) produced by the reduction of N-phenylhydroxylamine in the previous step. The molecular ion peak of 110.06023 [M+H]$^+$ of compound 7 (t$_R$ = 6.04 min), with its MS/MS fragment ions shown in Table 1, are the same as the fragment of aminophenol (Figure S3d) and is suggested as C$_6$H$_7$ON (4-AP). When these intermediates are captured, it is also found that the condensation routes coexist. The molecular ion peak of compound 8 (t$_R$ = 6.14 min) at an m/z of 231.07610 [M+H]$^+$ is assumed to be azoxy phenol C$_{12}$H$_{10}$O$_3$N$_2$ (AOB) (fragments information are shown in Table 1). Compound 9 (t$_R$ = 6.14 min), with an m/z at of 215.08333 [M+H]$^+$, is deduced to be azo phenol C$_{12}$H$_{10}$O$_2$N$_2$ (AB) [26]. Compound 10 shows an m/z at 216.08916 [M+H]$^+$, and it is speculated to be hydrazine azo phenol C$_{12}$H$_{12}$O$_2$N$_2$ (HAB). From this data, it can be inferred that the reaction follows two routes, namely the direct route and the condensed route, involving eight intermediates.

2.3. $^1$H NMR Spectroscopic Analysis for Further Confirmation of Intermediates

To further confirm the structure of the intermediates involved in the reaction process, Solution 1 was used for the online $^1$H NMR analysis. The molecular structure of the intermediate products was further verified by the changes of the online $^1$H NMR spectra sampled at key time nodes. The 4-NP were dissolved in deuterium water for the online $^1$H NMR analysis as a reference. As shown in Figure S6, the aromatic proton signals of 4-NP appear as a doublet at δ 6.82 ppm and 8.02 ppm, respectively. Then, the samples were taken at each key time node for online $^1$H NMR monitoring. Figure S7a–c shows the spectra at key time nodes of 2 min, 5 min, and 10 min, respectively. Significant changes are observed in the $^1$H NMR spectra at different key time nodes. Firstly, the doublet of 4-NP at δ 6.82 ppm and 8.02 ppm gradually disappears. At the same time, the single peak at δ 6.59 ppm gradually splits to multiple peaks between δ 6.75 and 6.81 ppm as the reaction progresses, indicating the aromatic proton signals of the intermediates and 4-AP [12]. Amino NH$_2$ and the other -NH proton signals of the intermediates were observed in the range of δ 4.50–4.75 ppm. However, the aromatic proton signals of the azo products appeared at δ 8.5 ppm. These results further confirm the rationality of the intermediates captured by LC-MS/MS and the changes in the UV-Vis spectrum. The correlation between these changes provides powerful data guidance to estimate the reaction mechanism.

2.4. Mechanistic Insight into the Reaction of 4-Nitrophenol Hydrogenation

Considering that the catalysts show different catalytic effects on 4-NP, most of the previous reports explained the reduction mechanism intangibly or theoretically. Recent studies are summarized in Table 2, indicating no consensus, but several different proposals for the reduction mechanism of 4-NP to 4-AP.
follows two routes, namely the direct route and the condensed route, involving eight intermediates.

Figure 2. Cont.
Figure 2. LC–MS spectrum of Solution 1 (a) Total ion current (TIC) and (b–f) LC–MS spectrum of each species monitored online at time node of 2 min.
Table 2. Different analytical methods used in the reduction of 4-nitrophenol and the proposed reaction mechanism.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Mass</th>
<th>Time (s)</th>
<th>4-NP (mM)</th>
<th>NaBH₄/4-NP</th>
<th>Methods</th>
<th>Pathway</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCB-Ni-Au</td>
<td>5 mg</td>
<td>160</td>
<td>0.20</td>
<td>50 times</td>
<td>LC-MS</td>
<td>direct</td>
<td>[27]</td>
</tr>
<tr>
<td>Cu/Co@NC-15</td>
<td>0.05 mg</td>
<td>90</td>
<td>0.10</td>
<td>200 times</td>
<td>UV-Vis</td>
<td>direct</td>
<td>[28]</td>
</tr>
<tr>
<td>Au NPs/CTS/AC</td>
<td>5 mg</td>
<td>800</td>
<td>0.20</td>
<td>200 times</td>
<td>UV-Vis</td>
<td>direct</td>
<td>[22]</td>
</tr>
<tr>
<td>Co@CuO NPs</td>
<td>2 mg/L</td>
<td>180</td>
<td>0.12</td>
<td>66 times</td>
<td>HPLC, UV-Vis</td>
<td>no mentioned</td>
<td>[12]</td>
</tr>
<tr>
<td>CuO NPs</td>
<td>5 mg</td>
<td>540</td>
<td>6.0</td>
<td>16 times</td>
<td>LC-MS</td>
<td>direct</td>
<td>[29]</td>
</tr>
<tr>
<td>Ni-CNTs</td>
<td>5 mg</td>
<td>600</td>
<td>4.0</td>
<td>100 times</td>
<td>UV-Vis</td>
<td>direct</td>
<td>[30]</td>
</tr>
<tr>
<td>Ni₀.₂₂/CB</td>
<td>1.0 mg</td>
<td>900</td>
<td>0.5</td>
<td>105 times</td>
<td>UV-Vis</td>
<td>direct</td>
<td>[31]</td>
</tr>
<tr>
<td>NAP-Mg:Au(0)</td>
<td>15 mg</td>
<td>420</td>
<td>0.21</td>
<td>476 times</td>
<td>UV-Vis</td>
<td>direct</td>
<td>[17]</td>
</tr>
<tr>
<td>Cu@Fe₂O₃ NPs</td>
<td>5 wt%</td>
<td>2700</td>
<td>1.0</td>
<td>20 times</td>
<td>GC-MS, UV-Vis</td>
<td>direct</td>
<td>[15]</td>
</tr>
<tr>
<td>Au/MTA(1 mol%Au)</td>
<td>20 mg</td>
<td>10,800</td>
<td>0.10</td>
<td>4–6 times</td>
<td>¹HNMR</td>
<td>direct</td>
<td>[32]</td>
</tr>
<tr>
<td>AuNPs</td>
<td>0.5 mM</td>
<td>50</td>
<td>0.20</td>
<td>50 times</td>
<td>UV-Vis</td>
<td>co-existing</td>
<td>[33]</td>
</tr>
<tr>
<td>Co(OH)₂/CuO</td>
<td>1.5 mg</td>
<td>420</td>
<td>3.72</td>
<td>7 times</td>
<td>UV-Vis, LC-MS, HPLC, ¹HNMR</td>
<td>co-existing</td>
<td>This work</td>
</tr>
<tr>
<td>Co(OH)₂/CuO</td>
<td>1.0 mg</td>
<td>40</td>
<td>0.76</td>
<td>70 times</td>
<td>Same as above</td>
<td>co-existing</td>
<td>This work</td>
</tr>
</tbody>
</table>

To verify the hydrogenation pathway with NaBH₄ in the presence of Co(OH)₂/CuO, we accurately captured the intermediates of the other solutions at key time nodes via online LC-MS/MS analysis and obtained the same eight intermediates in all four solutions. To explain the results more clearly, Solution 2 is also discussed in detail (LC-MS spectra of Solution 2 are in Figures S4 and S5). In the following figures, only the time nodes with obvious changes are selected as the discussion objects.

The UV-Vis spectrum of Solution 2 (Figure 1d) reveals that the solution turned colorless within 40 s, and the absorbance at λₘₐₓ = 400 nm decreased from 4.8 to 0 with the progress of the reaction, while the absorption intensity at 300 nm and 230 nm remained same, as in the case of the 4-NP ion and 4-AP (Figure 3a). This suggests that the reaction is completely transformed to 4-AP. Changes in intensity for 4-NP and 4-AP with time are displayed in Figure 3b, indicating that 4-NP disappears altogether within 1 min in Solution 2. The variation with time for the eight intermediate species (4-NOOH, 4-NO, 4-NOH, 4-NHOH, 4-NH, AOB, AB, and HAB) during hydrogenation is shown in Figure 3c–e.

The intensity of 4-NOOH intermediate changes from 80,000 to 0 within 1 min with the disappearance of the 4-NP signal and, interestingly, the intensity of 4-NO increased after 1 min. Therefore, it can be inferred that 4-NOOH is obtained from the hydrogenation of 4-NP in the first step, which is quickly converted to 4-NO (Figure 3d). Figure 3c shows that 4-NHOH reaches the highest response when 4-NOH drops to the lowest point within 1 min, and then decreases with NOH content. Except for five intermediates at one time, the other three dimer intermediates, AOB, AB, and HAB, are formed and converted to products within 1 min (Figure 3e). These results demonstrate that the dimers are produced by intermediates, and some condensations in the reduction are also observed, suggesting two pathways, the direct route and the condensation route, in the reduction of 4-NP to 4-AP. Unlike the direct route, the condensation route is completed in 1 min. Since AOB and AB reach a peak value at 40 s, they quickly disappear within 1 min (Figure 3e), while the response of NOH reaches the highest value at 40 s and drops dramatically within 1 min. Obtained data suggest that 4-NOH is a participant of two routes, first acting as a precursor for the dimer AOB’s complete self-condensation, and then participates in the direct route to transform to 4-NHOH quickly between 40 s and 3 min. Meanwhile, the response of NO reaches the highest value at 1 min and drops, indicating that NO is also the participant of the two routes (Figure 3c,d). Thus, 4-NO and 4-NOH are considered as the common intermediates in the direct route and the condensation route. Based on the results, we conclude that the reduction involves the following crucial steps: (1) In the case of the direct route, the reduction of 4-NP to 4-AP follows six steps, and six different intermediates are formed. The capture of intermediate 4-NO indicates that the mechanism of the direct route
differs from that reported by Gelder et al., who suggested that it is possible to directly reduce 4-NP to 4-NHOH. (2) The condensation pathway shows the formation of AOB from 4-NOH, sequentially reducing in successive steps, such as $\text{AOB} \rightarrow \text{AB} \rightarrow \text{HAB} \rightarrow \text{NH} \rightarrow \text{4-AP}$, further verifying the coexistence of the condensation route. This is not the same as the Haber-reported mechanism that AOB is formed by the condensation of 4-NO and 4-NHOH. (3) The 4-NO is a necessary intermediate state for the hydrogenation of 4-NP, which differs from Gelder, who suggested that nitrosobenzene cannot be an intermediate in the hydrogenation of nitrobenzene. (4) The NH is not only obtained by NHOH hydrogenation, but also by the hydrogenation of HAB, further reducing to 4-AP.

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)

Figure 3. Cont.
According to the online UV-Vis, HPLC-MS/MS, and $^1$HNMR results, we propose that the reaction mechanism for the reduction of 4-NP by NaBH$_4$ in the presence of Co(OH)$_2$/CuO is different from Figure 4a,b. The reaction involves six steps, 4-NP→4-NOOH→4-NO→4-NOH→4-NHOH→4-NH and finally to 4-AP in direct way. The common intermediates 4-NOH and 4-NO exist in both routes, and 4-NOH reacts with itself to eliminate water and form azoxybenzene, whereas 4-NO condenses with the product 4-AP to form AB, and the dominant reactions of the condensation route, as well as the possible routes of the hydrogenation of 4-NP to 4-AP, are described as Figure 5. Therefore, the reduction mechanism of 4-NP by NaBH$_4$ in the presence of Co(OH)$_2$/CuO bimetallic nanocatalyst is proposed as Scheme 1.
Figure 4. The mechanism diagram proposed by Haber and Gelder [1,13,15] (a) The Haber mechanism and (b) reaction mechanism for nitrobenzene hydrogenation proposed by Gelder.

Direct Route:

\[
\begin{align*}
\text{PhNO}_2 & \rightarrow \text{PhNO} \rightarrow \text{PhNHOOH} \rightarrow \text{PhNH}_2 \\
\text{PhNH}_2 & \rightarrow \text{PhNH} \rightarrow \text{Ph-NH-NH-Ph}
\end{align*}
\]

Conensation Route:

\[
\begin{align*}
\text{PhNO}_2 & \rightarrow \text{PhNO} \\
\text{PhNH}_2 & \rightarrow \text{Ph-NH-NH-Ph}
\end{align*}
\]

Figure 5. The reaction mechanism for Co(OH)$_2$/CuO catalytic reduction of 4-nitrophenol in presence of NaBH$_4$ under nitrogen atmosphere.
3. Experimental Section

3.1. Material

The 4-NP and NaBH₄ were purchased from Aladdin. NaOH, cobalt (II) acetate tetrahydrate (Co(CH₃COO)₂·4H₂O), and Cu(CH₃COO)₂·H₂O were supplied by Sigma (Sigma-Aldrich, USA). MS grade methanol and ACN were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Deionized water was purchased from Watson’s (Hong Kong, Ltd., Hong Kong, China). All the purchased chemicals were used without any further purification.

3.2. Catalyst Preparation

Shuttle-like Co(OH)₂/CuO nanoparticles were synthesized by a simple coprecipitation method according to our previous work.

3.3. Characterization

Powder X-ray diffraction (PXRD) data on the catalyst was collected on a Rigaku D/max-ga X-ray diffractometer (Tokyo, Japan) with the scan rate of 6°·min⁻¹ in 2θ ranging from 5° to 80° with Cu Kα radiation (1.54178 Å). Field emission scanning electron microscopy (FESEM) images of the catalyst were investigated by employing a Hitachi SU8010 microscope (Tokyo, Japan) operated at 5 kV. X-ray photoelectron spectroscopy (XPS) experiments were performed using a Thermo Fisher Scientific XPS ESCALAB 250Xi (ThermoFisher Scientific, Waltham, MA, USA) instrument with an Al Kα (1486.8 eV) X-ray source.

3.4. Determination of Reaction Time Node

To comprehensively capture the intermediates, the reduction of 4-NP to 4-AP was first monitored by UV-Vis and then the reduced intermediates taken at each time node were determined by LC-MS/MS and ¹H NMR. Different concentrations of 4-NP and NaBH₄
were used under nitrogen at room temperature. In a typical experiment, 24 mL 4-NP aqueous solution (3.72 mM) was freshly prepared, and 30 mM NaBH₄ was added to the solution with continuous stirring. Then, 3.1 mg Co(OH)₂/CuO was uniformly dispersed in 1 mL deionized water under ultrasonication for several min and 500 µL of the dispersion was added to the mixture (labeled as Solution 1 below). Later, 100 mL 4-NP aqueous solution (0.7 mM) was freshly prepared, then 50 mL, 20 mL, and 10 mL were transferred to three different containers, respectively. Different concentrations of NaBH₄ (70, 35, and 16 times of 4-NP concentration, respectively) were added to the above solutions, followed by the addition of 1 mL (1.0 mg mL⁻¹) Co(OH)₂/CuO suspension. Firstly, online UV-Vis (UV-2450) was used to track the reaction process by taking a small amount of solution after a certain time interval and 0.22 µm membrane filtration. Results indicated good catalytic performance under different parameters, and at the same time, key time points for sampling were determined. In the latter three sets of experimental parameters, the solution with a NaBH₄ concentration 70 times of 4-NP concentration was marked as Solution 2.

3.5. Separation by HPLC

Samples taken at each key time node were analyzed by HPLC at a full wavelength scanning on the Dionex Ultimate 3000 instrument, and was performed on a Sunfire® C18 (5 µm, 4.6 mm × 250 mm) column (Waters, Ireland) at a flow rate of 0.6 mL/min at 25 °C. The mobile phase system consisted of water (solvent A) and acetonitrile (solvent B). The following gradient was used for separation: 0–5 min (5% B), 5–15 min (5–40% B), 15–25 min (40–65% B), 25–30 min (65% B), 30–35 min (65–100% B), 35–40 min (100% B). The injection volume was 10 µL and the balance time was 10 min.

3.6. LC-MS/MS Analysis

Online HRMS analysis was combined with HPLC to obtain compounds retention time and to detect compounds without characteristic UV absorption. Thus, the accurate molecular weight and the multilevel fragment information of the compounds were obtained. The whole reaction process of the 4 solutions was monitored online by LC-MS/MS technology (Q Exactive Plus, Thermo, Bremen, Germany). Phenolic compounds were detected in negative ion mode, while amine compounds were ionized in positive ion mode. Thus, the qualitative analysis was performed both on negative ion mode and positive ion mode. Samples were taken at each key time node, filtered with a 0.22 µm membrane, and injected into the instrument. All 4 samples were monitored online according to their corresponding time nodes. The instrumental parameters were as follows: spray voltage was 3.85 kV; atomizing gas (N₂) flow rate was 35 arb; auxiliary gas flow rate was 10 arb; ion source temperature was set at 350 °C; the capillary temperature was 320 °C; step normalized collision energy (NCE) setting was 70 V; mass spectrum m/z scanning range was 50–750; fragment ion scanning range was 50–750; scanning resolution was 70,000. Data were analyzed using Thermo Xcalibur Qual Browser software (Version 4.0).

3.7. ¹HNMR Analysis

The samples were taken at key time nodes under the same experimental conditions. Deuterium water was used as the reaction solvent and the samples were filtered before online monitoring on ¹HNMR VARIAN 400-MR (Palo Alto, CA, USA). The obtained data were analyzed using MestReNova14.1(2019).

4. Conclusions

In this study, Co(OH)₂/CuO bimetallic nanocatalysts were used for the highly efficient hydrogenation of 4-NP in the presence of NaBH₄ within 40 s, and the reduction mechanism was studied by online UV-Vis, HPLC, LC-MS/MS, and ¹HNMR. Through LC-MS/MS qualitative analysis, the molecular weight and the possible structures of the intermediates were effectively and accurately identified in reaction solution. Furthermore, online ¹HNMR analysis provided good evidence for LC-MS/MS reaction reasoning and eliminated the
uncertainty of a single method. This compositional information was incredibly beneficial and essential to investigate the reaction mechanism, especially for the capture of the transition states in the reaction process.

By establishing an integrated online analysis method, eight intermediates, including azo and azoxy compounds, were effectively captured. At the same time, a new mechanism was proposed, suggesting the coexistence of the direct route and the concentration route, which is a more accurate and concise mechanism than the previously reported routes. This integrated technology involving multimethods could become a powerful tool to study the catalytic mechanism of reduction reactions. Furthermore, it will provide a promising idea for experimental design and methodological research for catalytic transformation technology in water and environmental remediation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/catal12050499/s1, Figure S1. (a,b) FESEM images of Co(OH)$_2$/CuO, (c) XRD patterns of Co(OH)$_2$/CuO; Figure S2. XPS of Co(OH)$_2$/CuO. Figure S3. HRMS data of references (a) 4-NP (c) 4-AP (e) 4-NO and their MS/MS spectra (b,d,f); Figure S4. Total ion current (TIC) and LC-MS spectra at key time node (t = 40 s) in solution 2; Figure S5. Total ion current (TIC) and LC-MS spectra at key time node (t = 2 min) in solution 2; Figure S6. $^1$HNMR of the 4-NP in deuterium water as reference; Figure S7. $^1$HNMR spectra of aliquot from the Solution 1 in different time nodes (a) t = 2 min, (b) t = 5 min and (c) t = 7 min.

Author Contributions: Conceptualization, M.W.; Data curation, M.W.; Formal analysis, M.W.; Funding acquisition, M.W., R.A. and J.W.; Investigation, M.W.; Methodology, M.W., R.A., Y.K. and N.A.; Project administration, H.A.A. and J.W.; Resources, H.A.A. and J.W.; Software, Z.L.; Supervision, H.A.A. and J.W.; Visualization, M.W.; Writing—original draft, M.W.; Writing—review and editing, R.A., H.A.A. and J.W. All authors have read and agreed to the published version of the manuscript.

Funding: Financial support was from the Special Project of Natural Science Foundation of Autonomous Region (no. 2020D01C041). The funding was from the National Natural Science Foundation of China (no. 21861035 and no. 32061133005) and the Technological Innovation and Functional Development Projects of the Instrument and Equipment of the Chinese Academy of Sciences (no. 2020gz014).

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors gratefully acknowledge the funding support provided by the Special Project of Natural Science Foundation of Autonomous Region and the National Natural Science Foundation of China, as well as the Technological Innovation and Functional Development Projects of the Instrument and Equipment of the Chinese Academy of Sciences.

Conflicts of Interest: The authors declare no conflict of interest.

References


15. Borah, B.J.; Bharali, P. Direct Hydrogenation of Nitroaromatics at Room Temperature Catalyzed by Magnetically Recoverable Cu@Fe2O3 Nanoparticles. *Appl. Organomet. Chem.* **2020**, *34*, e5753. [CrossRef]


