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Supplemental file

Green synthesis and characterization of Ag nanoparticles in phytic acid/ascorbic acid/sodium hydroxide system and their application in electrochemical detection of H_2O_2

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1. Materials and methods

1.1. Orthogonal test

The factors and levels of the orthogonal test were shown in Table S1. Since the orthogonal experiment involved 6 factors and 5 levels, the orthogonal table ($L_{25}(5^6)$) as shown in Table S2 was designed. Then, the factors and levels were corresponding to the orthogonal table ($L_{25}(5^6)$) to obtain the experimental program, as shown in Table S3.

Table S1. Factors and levels of orthogonal test.

Factor / Level	A	B	C	D	E	F
	Concentration of $AgNO_3$ (mM)	PA dosage	AA dosage	The pH of solution A	The pH of solution C	Reaction temperature (°C)
1	7	0.5:1	0.5:1	7	7	25
2	8	1:1	1:1	8	8	40
3	9	2:1	2:1	9	9	50
4	10	3:1	3:1	10	10	60
5	11	4:1	4:1	11	11	70

Note: PA and AA dosage were expressed as n(PA):n($AgNO_3$) and n(AA):n($AgNO_3$), respectively.

Table S2. Orthogonal table ($L_{25}(5^6)$).

Test number	Column					
	1	2	3	4	5	6
1	1	1	1	1	1	1
2	1	2	2	2	2	2
3	1	3	3	3	3	3
4	1	4	4	4	4	4
5	1	5	5	5	5	5
6	2	1	2	3	4	5
7	2	2	3	4	5	1
8	2	3	4	5	1	2
9	2	4	5	1	2	3
10	2	5	1	2	3	4
11	3	1	3	5	2	4
12	3	2	4	1	3	5

13	3	3	5	2	4	1
14	3	4	1	3	5	2
15	3	5	2	4	1	3
16	4	1	4	2	5	3
17	4	2	5	3	1	4
18	4	3	1	4	2	5
19	4	4	2	5	3	1
20	4	5	3	1	4	2
21	5	1	5	4	3	2
22	5	2	1	5	4	3
23	5	3	2	1	5	4
24	5	4	3	2	1	5
25	5	5	4	3	2	1

Table S3. Testing program.

Test number	Combination of levels	Test condition					
		Concentration of $AgNO_3$ (mM)	PA dosage	AA dosage	The pH of solution A	The pH of solution C	Reaction temperature (°C)
1	$A_1B_1C_1D_1E_1F_1$	7	0.5:1	0.5:1	7	7	25
2	$A_1B_2C_2D_2E_2F_2$	7	1:1	1:1	8	8	40
3	$A_1B_3C_3D_3E_3F_3$	7	2:1	2:1	9	9	50
4	$A_1B_4C_4D_4E_4F_4$	7	3:1	3:1	10	10	60
5	$A_1B_5C_5D_5E_5F_5$	7	4:1	4:1	11	11	70
6	$A_2B_1C_2D_3E_4F_5$	8	0.5:1	1:1	9	10	70
7	$A_2B_2C_3D_4E_5F_1$	8	1:1	2:1	10	11	25
8	$A_2B_3C_4D_5E_1F_2$	8	2:1	3:1	11	7	40
9	$A_2B_4C_5D_1E_2F_3$	8	3:1	4:1	7	8	50
10	$A_2B_5C_1D_2E_3F_4$	8	4:1	0.5:1	8	9	60
11	$A_3B_1C_3D_5E_2F_4$	9	0.5:1	2:1	11	8	60
12	$A_3B_2C_4D_1E_3F_5$	9	1:1	3:1	7	9	70
13	$A_3B_3C_5D_2E_4F_1$	9	2:1	4:1	8	10	25
14	$A_3B_4C_1D_3E_5F_2$	9	3:1	0.5:1	9	11	40
15	$A_3B_5C_2D_4E_1F_3$	9	4:1	1:1	10	7	50
16	$A_4B_1C_4D_2E_5F_3$	10	0.5:1	3:1	8	11	50
17	$A_4B_2C_5D_3E_1F_4$	10	1:1	4:1	9	7	60

18	$A_4B_3C_1D_4E_2F_5$	10	2:1	0.5:1	10	8	70
19	$A_4B_4C_2D_5E_3F_1$	10	3:1	1:1	11	9	25
20	$A_4B_5C_3D_1E_4F_2$	10	4:1	2:1	7	10	40
21	$A_5B_1C_5D_4E_3F_2$	11	0.5:1	4:1	10	9	40
22	$A_5B_2C_1D_5E_4F_3$	11	1:1	0.5:1	11	10	50
23	$A_5B_3C_2D_1E_5F_4$	11	2:1	1:1	7	11	60
24	$A_5B_4C_3D_2E_1F_5$	11	3:1	2:1	8	7	70
25	$A_5B_5C_4D_3E_2F_1$	11	4:1	3:1	9	8	25

1.2. Stability test of Ag NPs@PA

1.2.1. Stability of storage

In order to clarify the storage stability of Ag NPs@PA sol at 4 °C, in this experiment, the sols stored at 4 °C for different times (0, 1, 3, 5, and 6 weeks) were tested using UV spectrophotometer. The samples were diluted 20 times for each test, and three parallel experiments were performed and the average value was taken. The relative stability is the ratio of the absorption peak intensity of each test to the absorption peak intensity of the initial storage time.

1.2.2. pH stability

To verify the stability of Ag NPs@PA under different harsh conditions, some experiments were carried out on the sol under different pH environments [1]. Firstly, deionized water with different pH (6−14) was configured. The samples were then diluted 20-fold with deionized water of different pH, and the mixture was incubated for 2 hours at room temperature. Finally, the mixture was transferred to a quartz cuvette for absorbance measurement, and three parallel experiments were performed for each group and the average value was taken. The highest value was defined as 100% relative stability.

1.2.3 Stability of temperature

In addition to different pH environments, further experiments were carried out at different temperatures [1]. The samples were first diluted 20-fold, and then the dispersions were incubated at different temperatures (4, 25, 30, 60, 70 °C) for 2 h. Finally, absorbance measurements were performed in a quartz cuvette. Similarly, three parallel experiments were performed for each group and the average value was taken. The highest value was defined as 100% relative stability.

1.3. Selectivity of electrochemical H_2O_2 sensors

The time-current test was used to detect 6 substances including 0.1 mM H_2O_2, 1 mM NaCl, 1 mM citric acid (CA), 1 mM urea, 1 mM glucose (Glu) and 1 mM ascorbic acid (AA) using sensors. The test potential was -0.5 V vs SCE.

1.4. Anti-interference of electrochemical H_2O_2 sensor

Sensors were used to detect the changes of the electrochemical response signal of 1 mM H_2O_2 in the presence and absence of 1 mM NaCl, 1 mM citric acid, 1 mM urea, 1 mM glucose and 1 mM ascorbic acid.

1.5. Repeatability and reproducibility of electrochemical H_2O_2 sensors

The same sensor was used to detect 1 mM H_2O_2 for four times, and the electrochemical response signal was compared to verify the repeatability of the sensor. In addition, three electrodes were made by the same method to detect 1 mM H_2O_2, and the relative standard deviation (RSD) was calculated to study the reproducibility of the sensor.

1.6. Test of electrochemical H_2O_2 sensor in real samples

In order to evaluate the feasibility of the simple electrochemical H_2O_2 sensor in practical application, this sensor was used to detect the electrochemical response signal when 500 μM H_2O_2 was added to real samples such as purified water, tap water and Fenhe water, and the recovery rate of each water sample was studied.

2. Results and discussion

2.1. Optimization of preparation conditions for Ag NPs@PA

Table S4. Analysis of test results.

T-Values	Peak position (nm)						Half-peak width (nm)						Peak intensity (a.u.)					
	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
T_1	2056.5	2300	2089.5	2091.5	2056	2106.5	1159.73	1218.33	921.56	1041.59	988.65	1476.37	6.441	8.877	6.596	7.221	8.147	3.675
T_2	1981	2018	1992.5	2100	2026	2144.5	787.34	973.53	953.68	1258.97	1101.13	1108.69	8.259	8.214	7.445	6.305	7.956	6.471

T_3	2012	2003.5	1980.5	2008.5	2148.5	2067.5	971.64	921.55	741.95	879.96	986.76	1109.63	8.349	7.684	9.727	7.93	6.232	6.883
T_4	2076	1955.5	2059	2081.5	1984.5	1963	1013.22	885.63	1287.33	1265.59	1029.29	772.21	7.05	6.437	6.523	6.793	7.503	9.58
T_5	2152	2000.5	2156	1996	2062.5	1996	1106.8	1039.69	1134.21	592.62	932.29	571.83	7.908	6.795	7.716	9.758	8.169	11.398
t_1	411.3	460	417.9	418.3	411.2	421.3	231.946	243.666	184.312	208.318	197.73	295.274	1.2882	1.7754	1.3192	1.4442	1.6294	0.735
t_2	396.2	403.6	398.5	420	405.2	428.9	157.468	194.706	190.736	251.794	220.226	221.738	1.6518	1.6428	1.489	1.261	1.5912	1.2942
t_3	402.4	400.7	396.1	401.7	429.7	413.5	194.328	184.31	148.39	175.992	197.352	221.926	1.6698	1.5368	1.9454	1.586	1.2464	1.3766
t_4	415.2	391.1	411.8	416.3	396.9	392.6	202.644	177.126	257.466	253.118	205.858	154.442	1.41	1.2874	1.3046	1.3586	1.5006	1.916
t_5	430.4	400.1	431.2	399.2	412.5	399.2	221.36	207.938	226.842	118.524	186.458	114.366	1.5816	1.359	1.5432	1.9516	1.6338	2.2796
Relatively excellent level	A_2	B_4	C_3	D_5	E_4	F_4	A_2	B_4	C_3	D_5	E_5	F_5	A_3	B_1	C_3	D_5	E_5	F_5
Combination of levels	$A_2B_4C_3D_5E_4F_4$						$A_2B_4C_3D_5E_5F_5$						$A_3B_1C_3D_5E_5F_5$					

2.2. Morphology of Ag NPs@PA

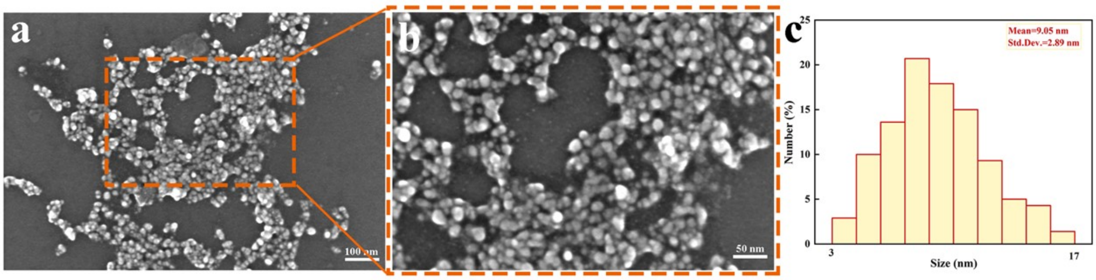

Figure S1. SEM images of Ag NPs@PA at (a) 100000、(b) 200000 magnification times and their (c) particle size distribution.

Figure S1a, b displayed SEM images of Ag NPs@PA at different magnifications. It can be seen from the figure that the morphology of Ag NPs@PA is almost spherical, and the particles are monodisperse, which was the same as the conclusion obtained in TEM. In addition, statistics were also carried out for the nanoparticles in Figure S1b, and the particle size distribution results were shown in Figure S1c. The particle size distribution ranged from 3-17 nm and the average particle size is 9.05 nm, which was consistent with the conclusion of TEM.

2.3. Stability of Ag NPs@PA

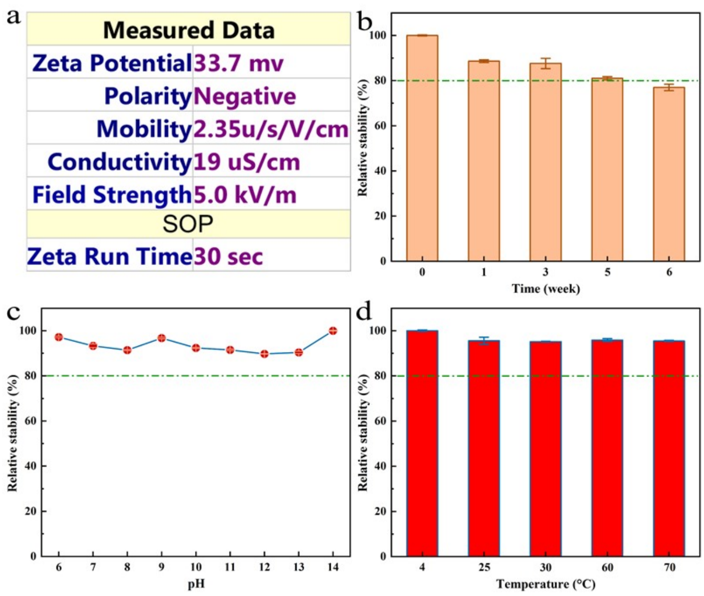

Figure S2. (a)ZETA potential of Ag NPs@PA and their stability under different conditions: (b)time, (c)pH, (d)temperature.

The stability of sol is closely related to the convenience of storage and further application. It has been reported that nanoparticle sol is stable when its ZETA potential range is beyond ±20 mV [2]. Figure S2a exhibited that the ZETA potential of the Ag NPs@PA sol is -33.7 mV, indicating that the Ag NPs@PA sol has sufficient stability, which was the result of PA acting as dispersant and stabilizer to play a steric hindrance and the same charge repulsion between particles to avoid agglomeration.

In addition, as shown in Figure S2b, the Ag NPs@PA sol was continuously monitored for 6 weeks, and it was found that the relative stability of the sol remained above 80% after 5 weeks, which also indicated that the sol was very stable. This storage stability also depended on the effective coating of Ag NPs by PA and was significantly affected by the antioxidant properties of PA molecules. Its antioxidant ability was derived from the fact that each PA molecule can provide 6 pairs of hydrogen atoms to form a stable structure for the electrons of free radicals, thus replacing Ag NPs as the oxidized molecules.

In order to investigate the stability of the sol in practical application, two external environmental factors such as pH and temperature were also arranged to test the Ag NPs@PA sol. As can be seen from Figure S2c, the relative stability of the sol was much higher than 80% in the wide range (pH=6−14) of weak acid, neutral and basic, indicating that the sol has a strong environmental adaptability in terms of pH. This is because PA had a high degree of ionization in the pH range of weak acid, neutral and basic. The strong electrostatic repulsion between the PA coated on the surface of different Ag NPs ensured the stability of Ag NPs@PA. Figure S2d showed the stability test of Ag NPs@PA sol at different temperatures. No matter in low temperature storage environment, room temperature or near high preparation temperature, its relative stability was much higher than 80%, indicating that its stability did not depend on external temperature. Under the low temperature storage environment of 4 °C, the free movement of particles was very inactive, and agglomeration was difficult to occur under the obstruction of PA. With the increase of external temperature, the Brownian motion intensified and the probability of collision between particles increased. However, since Ag NPs were coated by PA, the agglomeration phenomenon was not obvious.

2.4. Selectivity of electrochemical H_2O_2 sensors

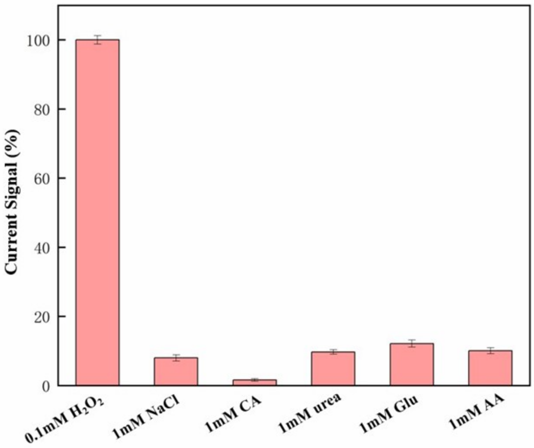

Figure S3. Selectivity of sensor.

To study the selectivity of the electrochemical H_2O_2 sensor, the sensor was used to detect H_2O_2, NaCl, citric acid, urea, glucose and ascorbic acid, as shown in Figure S3. It can be found from the response signal in the figure that even though the concentration of H_2O_2 was only 1/10 of the other interfering substances, its response signal was still much stronger than NaCl, citric acid, urea, glucose and ascorbic acid, which was enough to manifest that the sensor has excellent selectivity for the detection of H_2O_2 [3].

2.5. Anti-interference of electrochemical H_2O_2 sensor

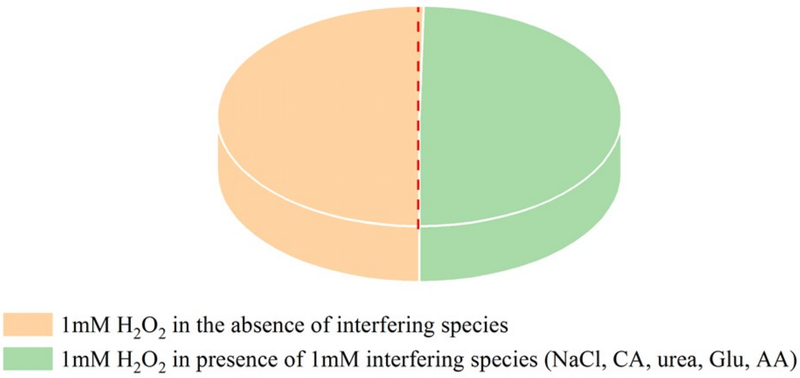

Figure S4. Anti-interference of electrochemical H_2O_2 sensor

Besides selectivity, the anti-interference ability of the sensor was also investigated. Figure S4 exhibited the comparison of the response signals of the sensor to detect H_2O_2 with or without interference. It can be seen intuitively that the response signal detected by the electrochemical H_2O_2 sensor had little change in the presence or absence of interfering substances, which confirmed that the sensor demonstrated excellent anti-interference ability for the detection of H_2O_2, which was conducive to its further practical application.

2.6. Repeatability and reproducibility of electrochemical H_2O_2 sensors

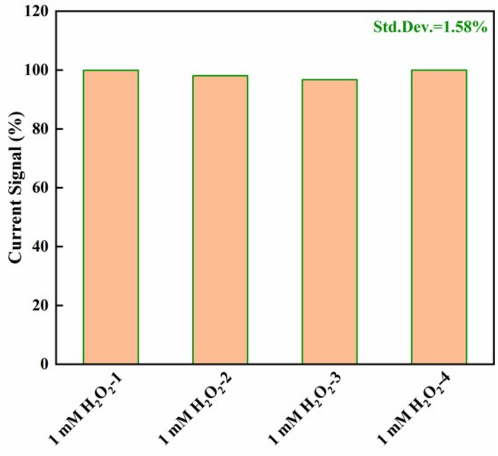

Figure S5. Repeatability of the sensor

Figure S5 revealed that the electrochemical H_2O_2 sensor possesses good repeatability. The response signal corresponding to the same sensor detecting the same concentration of H_2O_2 kept basically consistent, and the RSD of four tests was only 1.58%, indicating that the sensor has good repeatability and can carry out continuous detection, which was of great significance for field detection. Reproducibility is another important sensor performance parameter. Reproducibility is another important sensor performance parameter. The RSD of three electrodes made by the same method to detect the same concentration of H_2O_2 was 3.83%. Therefore, the reproducibility of the electrochemical H_2O_2 sensor was also acceptable.

2.7. Application of electrochemical H_2O_2 sensor in real samples

Table S5. Recovery rate (%) of H_2O_2 detected by electrochemical H_2O_2 sensor in actual samples.

Sample	H_2O_2 recovered (μL)	Recovery rate (%)	RSD (%)
PBS	500.0	100%	0
Purified water	504.5	100.09%	0.63
Tap water	505.1	101.02%	0.72
Fenhe water	463.8	92.76%	5.31

To evaluate the feasibility of the sensor in practical application, the electrochemical H_2O_2 sensor was used to detect the actual samples of purified water, tap water and Fenhe water [3]. A standard concentration of 500 μM H_2O_2 was added to a given actual water sample, and the recovery of each water sample was researched, as shown in Table S5. It is observed from the table that the recovery rate of the sensor in the three practical samples was relatively ideal, ranging from 92.77% to 101.02%, which can be applied in practical detection.

References

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