

Preparation of nitric oxide in carbon dioxide reference gas for food industry

Delong Hu, Xuemei Yang, Tingting Ren*, Qian Zou

Chongqing Academy of Metrology and Quality Inspection, No. 1 Yangliu North Road, Yubei District, Chongqing, China

Abstract: This article presents a weighing preparation method for reference gases of nitric oxide (NO) in carbon dioxide (CO₂). High-purity standard gases - NO and CO₂ are used as raw materials. The relative expanded uncertainty of concentration analytic results of NO in CO₂ reference gases is 3% ($k=2$), which proves excellent uniformity and stability of the prepared reference gases that can be used for accuracy evaluation of analytic instruments applied to detection of NO in food additive CO₂. The preparation method can also be applied to prepare other reference gases with variety of concentrations and components.

1. Introduction

Carbon dioxide (CO₂), as a gas food additive, is widely used in carbonated beverages, beer, and food preservation. Impurities such as nitric oxide (NO) in food additive CO₂ are easily adsorbed by human bodies through food, causing irreversible harm to human health. NO is unstable, and when absorbed by the human body, it quickly oxidizes into NO₂, which is highly corrosive and toxic and can cause certain harm to human respiratory tract [1-2]. According to National food safety standard GB 1886.228-2016 Food additive - Carbon dioxide, the content of NO in food additive CO₂ should not be more than 2.5 µL/L. Therefore, the content of NO in food additive CO₂ must be strictly controlled.

At present, the commonly used method for measuring the content of NO in CO₂ is chemical absorption method, which used spectrophotometric instruments to measure the absorbance of solutions [3-5]. To ensure accurate measurement results of analytical instruments for determination of NO in CO₂, reliable reference materials should be used for calibration of such instruments [6-7]. The existing research on preparation of CO₂ reference materials in China all adopt only one component and have high uncertainties in standard concentration values. In order to accurately calibrate spectrophotometric instruments used for the determination of NO in food additive CO₂, this article uses high-purity CO₂ and NO as raw materials and prepared a series of NO in CO₂ standard gas using weighing method [8-10]. The prepared standard gas has good pressure release and long-term stability, as well as satisfying uncertainty, and can be used as brand new reference material which contains two components for the confirmation and evaluation of spectrophotometric instruments, which will provide stronger technical support for the standardization of food additive CO₂ than the current-in use reference materials only containing one component.

2. Preparation of NO in CO₂ standard gas

2.1. Instruments and raw materials

Type PQZ-II gas distribution device, and type LP1200S-M electronic balance were adopted as standard gas preparation instruments. High purity CO₂ certified reference material (CRM) whose authorization No. is GBW(E) 060045 with concentration of 99.999% and high purity NO CRM whose authorization No. is GBW(E) 060042 with concentration of 99.99% were used as raw materials. 8 L aluminum alloy gas cylinders were used as containers of the prepared gas.

2.2. Purity analysis of standard gases used as raw materials

High purity CO₂ standard gas was analyzed with center cutting method by type GOW-MAC gas chromatograph (GC), which was equipped with DID detector and 5A stainless steel chromatographic column (3 mm×4 m). High purity helium was adopted as carrier gas, and column flow was 30 ml/min. Column temperature was set at 70°C and temperature of the detector was set at 150°C. The sample was injected by automatic valve and the injection volume was controlled by 0.5 mL sample loop. The analytical results of the high purity CO₂ is: concentration of CO₂ is 99.999%, concentration of O₂ is no more than 0.01 µmol/mol, concentration of Ar is no more than 0.02 µmol/mol, concentration of H₂ is no more than 0.01 µmol/mol, concentration of CH₄ is no more than 0.01 µmol/mol, concentration of N₂ is no more than 0.01 µmol/mol, concentration of CO is no more than 0.01 µmol/mol, and concentration of H₂O is no more than 0.05 µmol/mol. The selected high purity CO₂ standard gas can

*Corresponding author's email: 755245093@qq.com

meet the requirement of preparation of NO in CO₂ standard gas as raw material.

High purity NO standard gas was analyzed by type AO2020 UV absorption spectrum gas analyzer, and the injection flow was (300-500)ml/min. The analytical results of the high purity NO is: concentration of NO is 99.99%, and concentration of NO₂ is no more than 20 μmol/mol. The selected high purity NO standard gas can meet the requirement of preparation of NO in CO₂ standard gas as raw material.

2.3. Preparation process of NO in CO₂ standard gas

Weighing method is adopted to prepare the gas. Firstly, the empty cylinder is subjected to pre-treatment such as drying, flushing, and vacuuming (the cylinder pressure shall be below 5.0 Pa). Then, certain amount of high-purity CO₂ standard gas is filled into the cylinder, and the mass of CO₂ filled into the cylinder was determined by the weight difference between the cylinder before and after filling. Fill another cylinder with high-purity NO using the same method. Inflate the prepared NO into the cylinder filled with prepared CO₂ through the inflation device, weigh and record the mass of the cylinder, calculate the mass of each component gas filled into the cylinder, and make the concentration of NO in CO₂ 49.7 μmol/mol, which is the standard value of the prepared high-concentration NO in CO₂ standard gas.

Prepare pre-treated empty cylinders, vacuum the cylinders and rinse it with high-purity CO₂ for three times, then vacuum them until their inner pressure is below 5.0 Pa, and record the weight of the empty cylinders. Fill the cylinder with certain amount of CO₂ standard gas, and weigh its mass after its temperature cools to room temperature. Inflate the prepared NO in CO₂ with high concentration (49.7 μmol/mol) into the cylinder filled with prepared CO₂ through the inflation device, weigh and record the mass of the cylinder, calculate the mass of each component gas filled into the cylinder, and make the concentration of NO in CO₂ about 25 μmol/mol, which is the standard value of the prepared mid-concentration NO in CO₂ standard gas. Repeat the above steps, and make the

concentration of NO in CO₂ about 5 μmol/mol, which is the standard value of the prepared low-concentration NO in CO₂ standard gas.

Weighing data and uncertainty of NO in CO₂ prepared by weight method are demonstrated in Figure 1.

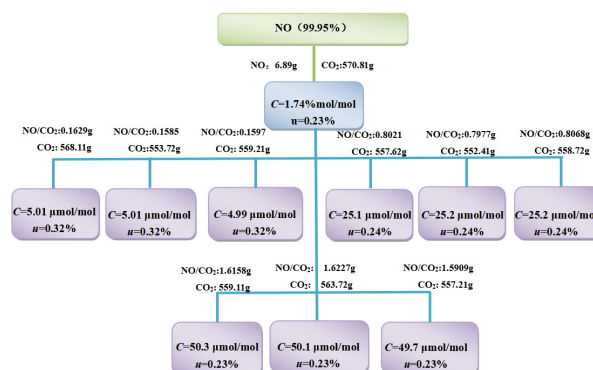


Fig. 1. Weighing data and uncertainty of NO in CO₂

During the whole preparation process, always ensure that the pressure inside the pipeline is higher than the pressure inside the receiving bottle. Throughout the entire preparation process, operators should ensure that the aluminum alloy cylinder is externally sealed. Keep the cylinder clean and handle it with care to avoid loss of quality in aluminum alloy gas cylinders.

3. Experimental validation and discussion

3.1. Mixing experiment

In order to mix the gas in the cylinder evenly, place the cylinder filled with prepared NO in CO₂ gas on the cylinder rolling device, continuously roll it for two hours, and stand it for 24 hours. Carry out concentration determination experiments 30 minutes, 60 minutes, 3 hours, 1 day, 3 days, 7 days, 12 days, and 20 days after mixing, using type AO2020 UV absorption spectrum gas analyzer, and the experimental results are shown in Table 1.

Table 1. Changes in concentration of prepared NO in CO₂ gas over time.

Analysis results	Cylinder NO.			
	152371	638976	435614	
Concentration (μmol/mol)	5	25	50	
Concentration of prepared NO in CO ₂ gas over time (μmol/mol)	30min	5.05	25.1	50.12
	60 min	5.08	25.1	50.1
	3 h	5.06	25.3	50.2
	1 d	5.11	25.4	50.3
	3 d	5.12	25.4	50.2
	7 d	5.12	25.4	50.2
	12 d	5.13	25.4	50.3
20 d	5.13	25.5	50.4	
Average (μmol/mol)	5.10	25.3	50.2	
RSD (%)	0.627	0.588	0.206	

The relative standard deviation of the measurement results determines mixing effect. According to Table 1, RSD of the prepared NO in CO₂ gas is within the range of

0.206%~0.627% over time from 30 min to 20 d, indicating good mechanical mixing of the prepared gases.

3.2. Pressure release stability test

The prepared NO in CO₂ gas is deflated at the pressure of (4/3/2/1.5/1/0.5) MPa through a pressure reducing valve. The concentration of the prepared gas is analyzed using

AO2020 gas analyzer. The concentrations of the prepare series of gases with the same mass are tested for equal numbers of times. The results are shown in Table 2 (cylinder 422345) and Table 3 (cylinder 438923).

Table 2. Analysis results 5 μmol/mol prepared gas under different pressures (μmol/mol).

NO. of times	Pressure					
	4MPa	3MPa	2MPa	1.5MPa	1MPa	0.5MPa
1	5.05	5.03	5.04	5.04	5.00	5.00
2	5.08	5.05	5.05	5.05	4.99	5.01
3	5.06	5.05	5.06	5.03	4.98	5.03
4	5.11	5.04	5.04	5.08	5.00	5.05
5	5.07	5.04	5.00	5.03	4.97	5.04
Average	5.07	5.04	5.04	5.05	4.99	5.03

According to Table 2, it can be concluded that $m=6$, $n=5$, $\nu_1=5$, $\nu_2=24$, $Q_1=0.0039873$, $Q_2=0.0086000$, $F=2.23$. $F_{(0.05, 5, 24)}=3.38$, $F < F_{(0.05, 5, 24)}$, which shows that pressure

variability has no significant impact on the stability of the prepared gas with concentration of 5 μmol/mol.

Table 3. Analysis results 50 μmol/mol prepared gas under different pressures (μmol/mol).

NO. of times	Pressure					
	4MPa	3MPa	2MPa	1.5MPa	1MPa	0.5MPa
1	50.2	50.2	50.5	50.4	50.6	50.3
2	50.4	50.1	50.2	50.2	50.2	50.5
3	50.3	50.5	50.3	50.3	50.8	50.4
4	50.2	50.2	50.0	50.2	50.3	50.2
5	50.3	50.6	50.2	50.3	50.4	50.3
Average	50.3	50.3	50.2	50.3	50.5	50.3

According to Table 3, it can be concluded that $m=6$, $n=5$, $\nu_1=5$, $\nu_2=24$, $Q_1=0.0296000$, $Q_2=0.6600000$, $F=0.22$. $F_{(0.05, 5, 24)}=3.38$, $F < F_{(0.05, 5, 24)}$, which shows that pressure variability has no significant impact on the stability of the prepared gas with concentration of 50 μmol/mol.

The long-term stability test select January, February, March, May, July, September, and December as the time points for stability test. The test results are shown in Table 4 and Table 5.

3.3. Long-term stability test

Table 4. Analysis results of long-term stability test

Analysis results		Cylinder NO.		
		154345	472923	621612
Concentration (μmol/mol)		5	25	50
Concentration of prepared NO in CO ₂ gas over time (μmol/mol)	January	5.13	25.3	50.1
	February	5.14	25.1	50.2
	March	5.12	25.4	50.1
	May	5.08	25.5	50.2
	July	5.11	25.4	50.3
	September	5.10	25.1	50.3
	December	5.09	25.3	50.4
Average (μmol/mol)		5.11	25.3	50.2
RSD (%)		0.423	0.604	0.222

Table 5. Calculation results of long-term stability test.

Item	Nominal value (μmol/mol)		
	50	25	5
β_1 (slope of the fitted line)	-0.0025	-0.0084	0.0048
β_0 (intercept of the fitted line)	9.9982	10.556	10.0101
S (regression residue)	0.0275	0.0376	0.0602
$S(\beta_1)$ (Standard deviation of β_1)	0.0026	0.0034	0.0056
$t_{0.95, n-2}$	2.571	2.571	2.571
$t_{0.95, n-2} \times S(\beta_1)$	0.0066	0.0088	0.0143
Trend analysis	No obvious trend	No obvious trend	No obvious trend
Spanning time (month)	12	12	12

It can be seen from Table 4 and Table 5 that there is no significant trend, and the prepared gas is stable within its validity period.

4. Uncertainty evaluation

4.1. Uncertainty introduced by weighing method preparation

The secondary dilution method was used to prepare 49.7 $\mu\text{mol/mol}$ NO in CO_2 gas, and the relative uncertainty introduced during the preparation process Δm_i was analyzed using the following mathematical model as demonstrated in equation (1), equation (2), and equation (3).

$$y=f(m_1, m_2, u_1, u_2, u_3, u_4, u_{d1}, u_{d2}, u_{d3}, u_{d4}, M_i, M_{\text{NO}}) \quad (1)$$

$$m_i = P_i + b_i + F_i \quad (2)$$

$$\Delta m_i = \sqrt{2\Delta P_i + \Delta b_i + \Delta F_i} + W \quad (3)$$

where m_i is the mass of the filled gas, P_i is the gas mass weighed with an electronic balance, b_i is the correction value of the electronic balance, F_i is correction value of buoyancy during cylinder weighing, Δm_i is uncertainty of gas mass weighing, ΔP_i is random uncertainty of the electronic balance, Δb_i is uncertainty of the electronic balance calibration, ΔF_i is uncertainty of buoyancy correction value during cylinder weighing, and W is the maximum mass change between disassembly and assembly during gas cylinder filling. Therefore, $\Delta m_i=11.53\text{mg}$ is obtained.

The combined standard uncertainty introduced by weighing method preparation u_{pre} is close to equation (4).

$$u_{\text{pre}} = \frac{\Delta x_i}{x_i} < \frac{\Delta m_1}{m_1} + \frac{\Delta m_2}{m_2} + \frac{\Delta u_1}{u_1} + \frac{\Delta M_{\text{mix}}}{M_{\text{mix}}} + \frac{\Delta M_{\text{NO}}}{M_{\text{NO}}} \quad (4)$$

where ΔX_i is mole uncertainty of the mixed gas, x_i is molar mass of the mixed gas, m_1 is the mixed gas mass measured by electronic balance during the first dilution, Δm_1 is uncertainty of the mixed gas mass measured by electronic balance during the first dilution, m_2 is the mixed gas mass measured by electronic balance during the second dilution, Δm_2 is uncertainty of the mixed gas mass measured by electronic balance during the second dilution, M_{mix} is relative molecular mass (RMM) of the mixed gas, ΔM_{mix} is RMM uncertainty of the mixed gas, M_{NO} is NO RMM, and ΔM_{NO} is RMM uncertainty of NO. Therefore,

$$u_{\text{pre}} = \frac{\Delta x_i}{x_i} < 1.82\%. \text{ Due to the infinite proximity of } u_{\text{pre}} \text{ to}$$

1.82%, $u_{\text{pre}}=1.82\%$ is taken. Take the inclusion factor k as 2, the uncertainty introduced by weighing method preparation $U_{\text{pre}} = \frac{u_{\text{pre}}}{k} = \frac{1.82\%}{2} = 0.91\%$ is obtained.

4.2. Uncertainty introduced by long-term stability

The uncertainty introduced by long-term stability U_{sta} is calculated according to equation (5).

$$U_{\text{sta}} = S(\beta_1) \times \frac{t}{x} \times 100\% \quad (5)$$

where $S(\beta_1)$ is the standard deviation of slope β_1 , t is the validity period of the prepared gas, and x is the prepared value of the gas prepared by weighing method. Therefore $U_{\text{sta}} = 0.64\%$ is obtained using the experimental data obtained from long-term stability testing.

4.3. Uncertainty introduced by pressure release stability

The uncertainty introduced by pressure release stability U_{rls} is calculated according to equation (6).

$$U_{\text{rls}} = \sqrt{\frac{(MS_s + MS_d)}{n}} \quad (6)$$

Where MS_s is the mean square of repeated measurement results under the same pressure, MS_d is the mean square of repeated measurement results under different pressures, and n is the number of repeated measurements. Therefore, $U_{\text{rls}}=0.93\%$ is obtained using the experimental data obtained from pressure release stability testing.

4.4. Uncertainty introduced by the undetermined prepared gas

The uncertainty of the undetermined prepared gas u_c is introduced by weighing method preparation, long-term stability and pressure release stability. Therefore, the combined uncertainty of the undetermined prepared gas is calculated according to equation (7).

$$u_c = \sqrt{u_{\text{pre}}^2 + u_{\text{sta}}^2 + u_{\text{rls}}^2} = \sqrt{0.0091^2 + 0.0064^2 + 0.0093^2} = 1.5\% \quad (7)$$

Take the inclusion factor k as 2, the uncertainty of the undetermined prepared gas $U = ku_c = 2 \times 1.5\% = 3.0\%$ is obtained.

5. Conclusion

According to the preparation and value determination method presented in this article, the content of the NO in CO_2 gas prepared in this article is $(5-50) \times 10^{-6} \mu\text{mol/mol}$ ($U=3.0\%$, $k=2$), with validity period of 1 year. During the preparation process, enough attention should be paid to cylinder vacuum, raw material gas purity, and weighing accuracy, which can greatly influence the accuracy of the prepared gas. The preparation can also be used to prepare other reference gases with variety of concentrations and components.

References

1. Zhang, X., et al, (2008). Development of reference gas for automotive working condition emissions. Advanced Measurement and Laboratory Management, 02: 17-18.
2. Wang, Y. J., (2023). Preparation of nitric oxide gas reference material in nitrogen. Low Temperature and Specialty Gases. 41(4): 27-31.

3. Mao, Y. W., et al, (2019). Preparation of gas reference material by gravimetric method. *Contemporary Chemical Industry*, 48(6): 1358-1361, 1368.
4. Zhang, D. L., et al, (2012). Development of standard gas sample of methane in nitrogen (air). *Low Temperature and Specialty Gases*, 30(5): 30-33.
5. Liu, Y., et al, (2010). Preparation of gas reference material for detecting automobile emission. *Low Temperature and Specialty Gases*, 28(1): 20-22, 43.
6. Zhang, W., et al, (2008). Research on the influencing factors and analysis methods of the calibration of trace carbon monoxide gas standard substances in nitrogen. *Measurement Technique*, 05: 35-38.
7. Wang, X., et al, (2023). The development of n-butane standard gas in air. *Low Temperature and Specialty Gases*, 41(3): 27-30, 37.
8. Wu, G. Z., et al, (2023). Preparation of ethylene gas reference material in nitrogen by gravimetric method. *Low Temperature and Specialty Gases*, 41(3): 31-37.
9. Dong, L. Y., et al, (2023). Preparation of trace water vapor in nitrogen gas reference material. *China Measurement & Test*, 49(5): 69-73.
10. Bao, G. Q., et al, (2023). Development of n-undecane reference material in air. *Shandong Chemical Industry*, 52: 12-18, 21.