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Research Article

The Influence of Phosphate Buffer on the Formation of N-Nitrosodimethylamine from Dimethylamine Nitrosation

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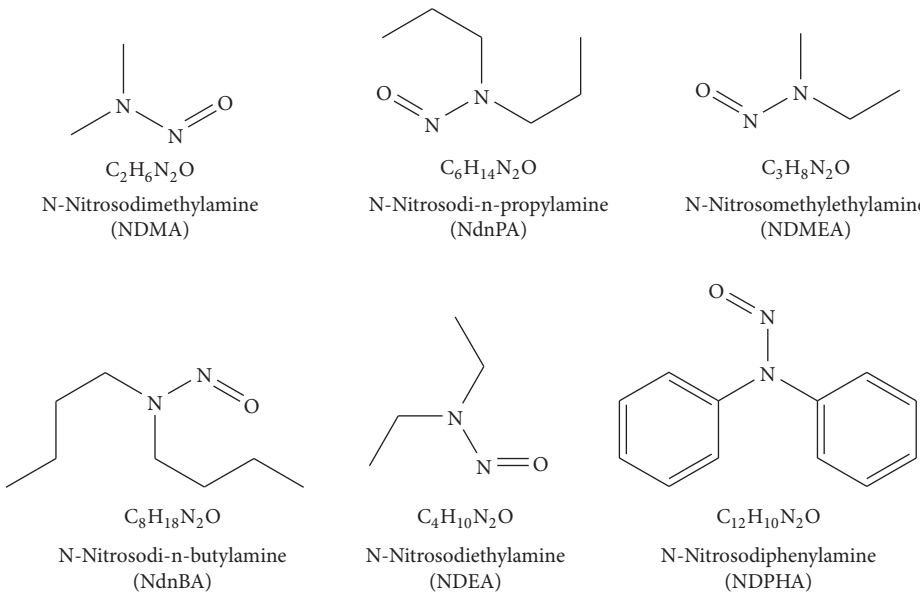
Buffer solutions were widely used for almost all the investigations concerning N-nitrosodimethylamine (NDMA), a member of powerful mutagenic and carcinogenic compounds which are ubiquitous in the environment. However, whether or how the buffer matrixes influence NDMA formation is still unknown. The effect of buffer solutions on NDMA formation from the nitrosation of dimethylamine (DMA) by nitrite ($NaNO_2$) was investigated at pH 6.4 in four kinds of buffer solutions, that is, $Na_2HPO_4/C_6H_8O_7$, $Na_3(C_6H_5O_7)/C_6H_8O_7$, NaH_2PO_4/NaOH, and NaH_2PO_4/Na_2HPO_4. Our observations demonstrate an unexpected inhibitory effect of the buffer solutions on NDMA formation and the phosphate buffer plays a more significant role in inhibiting NDMA formation compared to the citrate buffer. Moreover, the amount of the phosphate in the buffer was also found to greatly impact the formation of NDMA. A further investigation indicates that it is the interaction between NaH_2PO_4 and reactant $NaNO_2$ rather than DMA that leads to the inhibitory effect of phosphate buffer during the DMA nitrosation reaction. This study expands the understanding of the influence of buffer solution on nitrosamines formation through the nitrosation pathway and further gives a hint for water plants to reduce the formation of nitrosamines.

1. Introduction

N-Nitrosamines are undesired industrial and environmental pollutants with rising concerns due to their widespread observation in foods, soil, industrial workplace environments, and cosmetics [1–5] as well as due to their high carcinogenic risks [6–8]. US Environmental Protection Agency (U.S. EPA) has prescribed six kinds of nitrosamines (Scheme 1) as probable B2 carcinogenic compounds to humans. Nitrosodimethylamine (NDMA), as the simplest molecule in structure among all the nitrosamines, has been demonstrated to be one of the most carcinogenic, mutagenic, and teratogenic nitrosamines [9]. In the U.S. EPA Integrated Risk Information Service (IRIS) database, NDMA has been identified to have an estimated 10^{-6} lifetime cancer risk level at a concentration as low as 0.7 ng/L [10]. Although no national regulation controlling NDMA has been established yet, the Ontario Ministry of the Environment [11] and the California Department of Public Health [12] have a maximum allowable concentration (MAC) of 9 and 10 ng/L, respectively.

Such a low MAC of NDMA has attracted great interest in the investigation of the formation mechanisms of NDMA. Generally, there are two types of pathways contributing to the NDMA formation, that is, oxidation of UDMH or chlorinated unsymmetrical dimethylhydrazine (UDMH-Cl) derived from the dimethylamine (DMA) pathway and nitrosation of the DMA pathway. The former pathway was proposed recently by Choi and Valentine [13, 14] and Mitch and Sedlak [15] based on their findings that NDMA emerged during the chlorine and chloramine disinfection of drinking water and sewage, in which DMA was first chlorinated to form UDMH or UDMH-Cl, which then underwent oxidation to yield NDMA. The later nitrosation pathway involving a reaction between nitrite and common organic nitrogen precursors, such as a DMA, has been known for a while and has commanded much attention because it could occur almost everywhere especially *in vivo*. Until now, it has been



Scheme 1: Structures of six possibly human carcinogenic N-nitrosamines (U.S. EPA).

known that a series of reaction factors impact the NDMA formation including pH value, reaction time, temperature, and catalysts. Mirvish [16] demonstrated that NDMA formation via DMA nitrosation is a highly pH-dependent reaction and the reaction occurs most rapidly at pH 3.4. Choi and Valentine [14] and Mitch and Sedlak [15] found that the NDMA formation via oxidation of the UDMH(-Cl) pathway appears to increase with pH, sharply increasing to about pH 8. Several catalysts such as formaldehyde, chloral, carbon dioxide, hypochlorite, and fulvic acid [17–21] have been proved able to catalyze the nitrosation reaction at a neutral or even basic pH. Andrzejewski and Nawrocki [22] even verified the formation of NDMA from the reaction of potassium permanganate and DMA via the nitrosation pathway. Very recently, Padhye et al. [23] found that the most commonly used activated carbon during the preconcentration steps of NDMA analysis can catalyze transformation of DMA to yield trace levels of NDMA under ambient aerobic conditions. Their results show that some unexpected potent errors could definitely be induced by reaction conditions and analytical methods. Thus, it is significantly important to obtain more information about the influence of various reaction conditions and analytical methods on the NDMA formation.

It is well known that the NDMA-forming reaction is highly dependent on pH; thus, a specific buffer [24–33] solution has to be used to regulate the suitable pH value for almost all the investigations concerning NDMA. However, to the best of our knowledge, little attention has been paid to the influence of buffer matrices on the formation of NDMA, in spite of its widespread employment during these studies. Only Schreiber and Mitch [34] reported that the elevated phosphate buffer is capable of decreasing NDMA formation during chloramination. Due to its widespread application, phosphate buffer is one of the most favorable buffer matrixes; therefore it is of significant importance to understand the influence of phosphate buffer on the formation of NDMA. Otherwise, as Padhye et al. [23] reported, without such knowledge, nitrosamine analysis will continue to be susceptible to this potential error. However, up to now, this lack of attention results in an uncertainty whether phosphate buffer affects the NDMA formation and how it executes its influence.

Taking into account what was previously mentioned, batch experiments on NDMA formation from the reaction of DMA and nitrite were conducted in the four kinds of buffer solutions, that is, $Na_2HPO_4/C_6H_8O_7$, $Na_3(C_6H_5O_7)/C_6H_8O_7$, NaH_2PO_4/NaOH, and NaH_2PO_4/Na_2HPO_4. The results distinctly illuminated the influence of buffer matrix on the formation of NDMA from the nitrosation of DMA by nitrite. Because of the widespread applications of buffer matrices in the nitrosamines research, this finding will expand our understanding of the influence of buffer matrices on N-nitrosamines formation and will remind the researcher to pay more attention to minimize the potent errors in the later investigations.

As a preliminary work to identify the influence of phosphate buffer on NDMA formation, more systematical investigations are underway to further illustrate roles of more buffer matrices on the formation of N-nitrosamines.

2. Experimental Part

2.1. Materials. Standard-grade NDMA (solution in methanol, 100 ng/μL) was obtained from Dr. Ehrenstorfer GmbH and diluted using dichloromethane to desired concentrations. Dimethylamine hydrochloride (DMA·HCl, 99%+) and sodium nitrite ($NaNO_2$, 99%+) were purchased from Sinopharm Chemical Reagent Co. Ltd. and Beijing Chemical Works, respectively. Methanol,

hexane, and dichloromethane were of Chromatographic purity and were used during the extraction process. Deuterated N-nitrosodimethylamine (NDMA-d_6, 1000 μg/mL in methanol) was purchased from Cambridge Isotopes (Andover, MA) and diluted to the required concentration. Buffer solutions (pH = 6.4) containing $Na_2HPO_4/H_3(C_6H_5O_7)$, $Na_3(C_6H_5O_7)/C_6H_8O_7$), NaH_2PO_4/NaOH, and NaH_2PO_4/Na_2HPO_4, which were all of analytical laboratory grade, were prepared using ultrapure water (18.2 MΩ cm at 25°C) from a Millipore Mili-Q water purification system. All chemicals were used without further purification.

2.2. Preparing the Solid Phase Extraction (SPE) Cartridges. Materials used for the Solid Phase Extraction (SPE) cartridges were purchased from Agela Technologies, Tianjin. Methods for preparing the SPE column were the same as previously reported [35]. The SPE cartridge was kept airtight until it was used for the extraction of NDMA in aqueous solution.

2.3. NDMA Formation. All glassware used in these experiments were washed with an ultrasonic method and air-dried prior to use. NDMA formation experiments from DMA (10 mM) and $NaNO_2$(4 mM) were conducted in graduated tubes at 90°C placed in a temperature-controlled water bath. The tubes were sealed with a Teflon-lined screw cap and wrapped by black plastic to shield them from light, so as to avoid NDMA photolysis. Reactions were performed at pH 6.4 maintained by four buffer solutions: $Na_2HPO_4/C_6H_8O_7$, $Na_3(C_6H_5O_7)/C_6H_8O_7$, NaH_2PO_4/NaOH, and NaH_2PO_4/Na_2HPO_4. Reaction solutions were prepared by the addition of the desired amount of dimethylamine stock solution and $NaNO_2$ stock solution into the buffer solution with the total volume of 10 mL.

Dependence of NDMA formation on reaction conditions was assessed by varying reaction time, buffer species as well as the form and concentration of buffer components. All batch experiments were performed at least triplicated to ensure the repeatability of the results.

2.4. Mechanical Studies. Experiments were conducted to elucidate how the buffer matrices inhibit NDMA formation from DMA and $NaNO_2$. The DMA/$NaNO_2$ ratio was definitely controlled as either 100 or 0.01 in the two sets of reactions. In one case, 1 mM NaH_2PO_4 was added into the citrate buffer solution containing 0.4 mM $NaNO_2$ and 40 mM DMA, whereas in the other case, 1 mM NaH_2PO_4 was added into a citrate buffer solution containing 40 mM $NaNO_2$ and 0.4 mM DMA·NaH_2PO_4 was added after all the other components were completely mixed. Then the mixtures were treated as the same procedure described in NDMA Formation section.

2.5. NDMA Analysis. The analysis of NDMA was performed via a method using a Thermo Fisher Scientific GC-MS equipped with EI source (J&W scientific DB1701 column 30 m by 0.25 mm I.D. by 0.25 μm film thickness) after solid-phase extraction (SPE). Prior to extraction, the reaction solution was cooled to room temperature and all samples were dosed with the deuterated NDMA-d_6. Extractions were performed with SPE cartridges. In brief, the SPE method for extracting NDMA in aqueous solution mainly includes methanol activation, sample addition, hexane leaching, and methylene dichloride elution [35]. In every step, the SPE cartridge was drained to dry thoroughly, and the flow rate was maintained at about 2-3 mL/min. After eluting, the extract was concentrated to 3 mL with dichloromethane and the determination of NDMA from the concentrated extract was achieved by using an isotopic dilution method based on the mass detection of molecular ion (m/z = 74) and the characteristic molecular ion of NDMA-d_6 (m/z = 80). NDMA was analyzed by both full scan and selected ion monitoring (SIM). The GC/MS operating conditions were set as follows: splitless injections of 1 μL at 200°C for inlet, 230°C for transfer line, and 250°C for ion source, respectively. Initial temperature of oven was set at 45°C (5 min) wand then ramped at 10°C/min to 200°C (held for 5 min); mass spectrometry was performed in electron ionization mode. Helium carrier gas was maintained at 1.0 mL/min and the retention time for NDMA was 6.9 min. The instrument and method detection limits for NDMA by using SPE-GC/MS method are 1 and 5 ng/L, respectively.

2.6. Computational Details. Quantum chemistry calculations were conducted to gain more insights into the effect of a phosphate buffer on the formation of NDMA in the "DMA + NO_2^-" reaction system. All structures and energy optimization calculations of reactants and products were performed with Gaussian03W using the B3LYP method in conjunction with 6-311+G(d,p) basis set [36]. Minima and transition states were optimized and characterized by harmonic vibrational frequency analysis. Intrinsic Reaction Coordinate (IRC) [37] calculations were performed to confirm that every transition state connected the corresponding reactant and product through the minimized-energy pathway.

3. Results and Discussion

The yield of NDMA formation from DMA nitrosation affected by many reaction conditions included reactant concentration, pH, and reaction time. Thus, before embarking on this work, it is necessary to optimize some reaction conditions.

3.1. Effect of Reaction Time on NDMA Formation via DMA Nitrosation. In order to choose an appropriate reaction time, experiments to evaluate the effect of a time scale on NDMA formation were performed. In this case, NDMA concentrations of the reactions of 10 mM DMA with 4 mM $NaNO_2$ at pH 6.4 phosphate buffer were monitored every 1 h up to 9 h.

Figure 1 shows the results of time-dependent NDMA formation and exhibits a nonlinear correlation between the NDMA concentration and time scale. The yield of NDMA appears to increase rather slowly within the first hour, whereas it increases dramatically from 1 to 4 h with the value

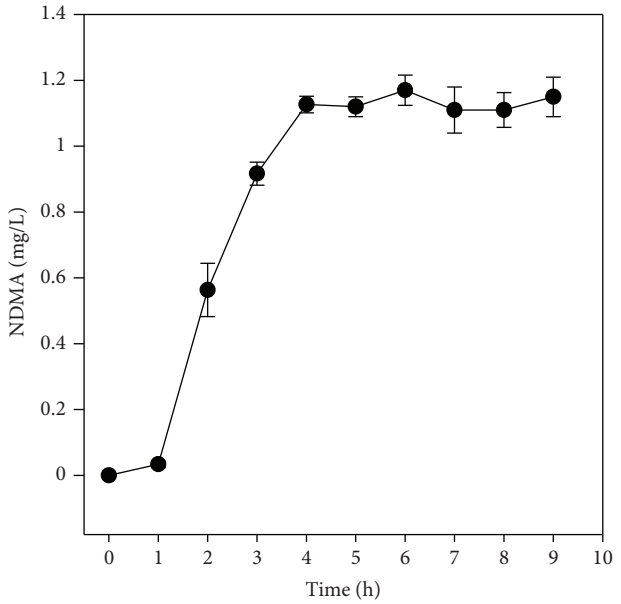


Figure 1: NDMA formation concentrations in the reaction of 10 mM DMA and 4 mM $NaNO_2$ at 90°C in the pH 6.4 phosphate buffer with the reaction time from 1 to 9 h. The vertical bars represent the standard deviation ($n = 3$).

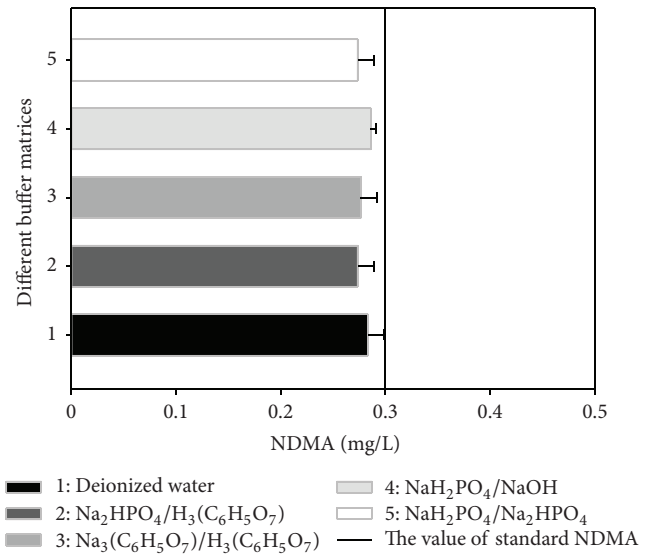


Figure 2: Concentrations of 300 μg/L standard NDMA after storing at 90°C in ultrawater (blank), $Na_2HPO_4/C_6H_8O_7$, $Na_3(C_6H_5O_7)/C_6H_8O_7$, $NaH_2PO_4/NaOH$, and NaH_2PO_4/Na_2HPO_4 buffer solutions (pH = 6.4) for 4 h. The horizontal bars represent the standard deviation ($n = 3$).

reaching approximately 0.026 mM. Thus, the NDMA yield at 4 h reaches around 0.26% of the initial amount of DMA concentration. During 4 to 9 h, the NDMA concentration is almost the same. Although a small enhancement of NDMA formation occurs at 6 h, there is little change when the experimental errors were taken into account. Thus, the yield of NDMA can be considered to reach the maximum at 4 h and be stable after this time. Therefore, 4 h was selected as the reaction time throughout all the experiments involved in this study. Similarly, when discussing the formation of NDMA from TMA and DMA, Scanlan et al. [31] also chose 4 h as the reaction time.

3.2. Effect of Buffer Matrix on NDMA Formation. To the best of our knowledge, although the influence of several parameters on NDMA formation has already been made during these years, little research work has been systematically performed on the influence of buffer matrices on NDMA formation. Thus, four commonly used buffer matrices, that is, $Na_2HPO_4/C_6H_8O_7$, $Na_3(C_6H_5O_7)/C_6H_8O_7$, NaH_2PO_4/Na_2HPO_4, and $NaH_2PO_4/NaOH$, have been investigated in this study.

First of all, it is necessary to rule out the influence of buffer matrix on the already formed NDMA. To achieve this goal, a standard 300 μg/L NDMA was put into deionized water as control and into four buffer matrices studied here; then the NDMA concentrations were monitored after 4 h. The concentrations of the standard NDMA in five different solutions were shown in Figure 2. As shown in Figure 2, the average NDMA concentrations were 278, 273, 276, 287, and 273 μg/L for deionized water, $Na_2HPO_4/C_6H_8O_7$, $Na_3(C_6H_5O_7)/C_6H_8O_7$, $NaH_2PO_4/NaOH$, and NaH_2PO_4/Na_2HPO_4 buffer, respectively. It is clear that all the values of the NDMA concentrations in the solutions are slightly lower than the 300 μg/L original standard NDMA. This decrease results from the SPE extraction recovery. However, the results show that the NDMA concentrations in the four buffer solutions are almost the same as that of deionized water. Thus, it can be concluded that the four types of buffer solutions employed in this study wholly have little effect on the standard NDMA. Therefore, it is reasonable to preclude the influence of buffer matrix on the already formed NDMA.

Now it is time to see whether the buffer matrix has an influence on NDMA formation during the reaction of $NaNO_2$ and DMA. The NDMA concentrations formed from the reactions of 10 mM DMA with 4 mM $NaNO_2$ in $Na_2HPO_4/C_6H_8O_7$, $Na_3(C_6H_5O_7)/C_6H_8O_7$, NaH_2PO_4/Na_2HPO_4, and $NaH_2PO_4/NaOH$ buffer solutions were shown in Figure 3. The results of Figure 3 demonstrate an obvious difference among the NDMA formation concentrations in the four buffer solutions, whose values are 1.18, 1.66, 1.16, and 2.42 mg/L for $Na_2HPO_4/C_6H_8O_7$, $Na_3(C_6H_5O_7)/C_6H_8O_7$, NaH_2PO_4/Na_2HPO_4, and $NaH_2PO_4/NaOH$ buffer solutions, respectively. The NDMA yield for the four buffers ranged from 0.26% to 0.54% of the initial amount of DMA concentration and the largest different yield induced from the different buffer reached 0.28% which is almost in the same level of the lowest yield. By comparing the yields of NDMA in the first column with that of the second, that is, in the $Na_2HPO_4/C_6H_8O_7$ and $Na_3(C_6H_5O_7)/C_6H_8O_7$ buffer solutions, the NDMA yield in the buffer solution involved an amount of phosphate was

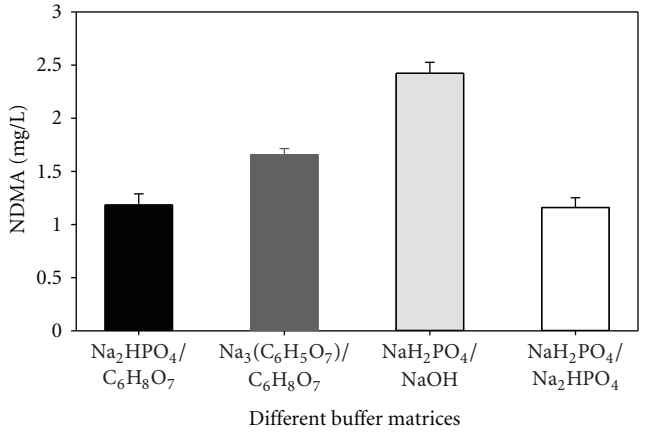


Figure 3: NDMA formation concentrations in the reaction of 10 mM DMA and 4 mM $NaNO_2$ in the $Na_2HPO_4/C_6H_8O_7$, $Na_3(C_6H_5O_7)/C_6H_8O_7$, NaH_2PO_4/NaOH, and NaH_2PO_4/Na_2HPO_4 buffer solutions (pH = 6.4). Reaction time = 4 h. Temperature = 90°C. The vertical bars represent the standard deviation (n = 3).

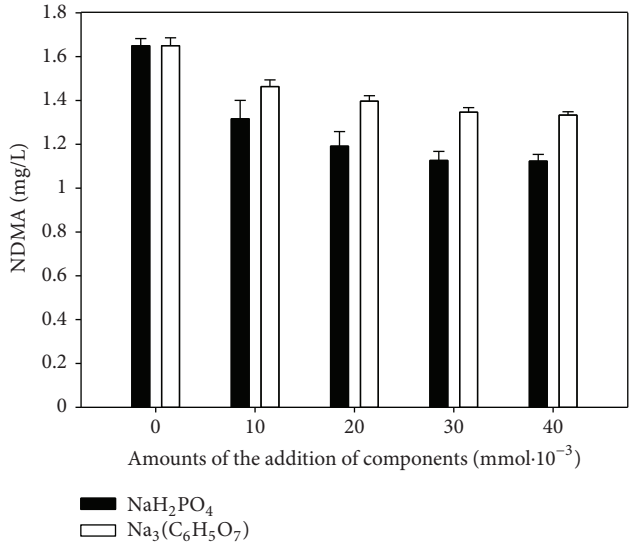


Figure 4: NDMA formation in the presence of variable amounts of NaH_2PO_4 and $Na_3(C_6H_5O_7)$ addition; 4 h reaction; 90°C. The pH was maintained at 6.4 with $Na_3(C_6H_5O_7)/C_6H_8O_7$ buffer. The vertical bars represent the standard deviation (n = 3).

relatively lower than that in the citrate buffer by around 28.9%. Similar results can also be found when a comparison between the yields of NDMA in $Na_3(C_6H_5O_7)/C_6H_8O_7$ and NaH_2PO_4/Na_2HPO_4 buffers was made. It indicates that the phosphate component of the buffer plays a significant role in inhibiting NDMA formation compared to the citrate buffer. Unfortunately, the concentration of NDMA in the NaH_2PO_4/NaOH buffer (the third column) is rather high, with the value of 2.42 mg/L, which seems completely contrary to the above conclusion. However, further analysis of the concentration of the phosphate listed in Table 1 demonstrates that the final concentrations of phosphate in $Na_2HPO_4/C_6H_8O_7$, NaH_2PO_4/Na_2HPO_4, and NaH_2PO_4/NaOH buffer solutions are approximately 0.14, 0.2, and 0.05 M, respectively. The final concentration of phosphate in the NaH_2PO_4/NaOH buffer is 2.8 times lower than that of the other groups. Therefore, the higher NDMA formation is possibly attributed to the less amount of phosphate in the NaH_2PO_4/NaOH buffer. It suggests that the amount of phosphate in the buffer may be a critical factor for NDMA formation during the nitrosation of DMA by nitrite. This conclusion is in a good agreement with the result reported by Schreiber and Mitch [34] that the elevated phosphate concentration can significantly decrease NDMA formation during DMA chloramination with the decrease being ~50% when the concentration increased from 5 to 50 mM.

Based on the results obtained it can be concluded that the phosphate buffer has an inhibitory effect for NDMA formation. However, which ingredient of the phosphate buffer is an inhibitory and how it inhibits NDMA formation will be discussed in the next section.

3.3. Role of Buffer Components in the NDMA Formation. In order to examine which ingredient of buffer components leads to the decrease of NDMA formation, experiments were conducted in which various concentrations of NaH_2PO_4 or $Na_3(C_6H_5O_7)$ were added into a pH 6.4 citrate buffer containing 10 mM DMA and 4 mM $NaNO_2$. Citrate buffer was selected as the buffer matrix because it appears to have little influence on NDMA formation. It should be noted that $H_2PO_4^-$ is the main existing form of the added NaH_2PO_4 in pH 6.4 buffer solution, which was accounted for the dissociation constants of phosphoric acid (pKa_1 = 2.16, pKa_2 = 7.21, pKa_3 = 12.32).

The NDMA concentrations at various dosages of NaH_2PO_4 or $Na_3(C_6H_5O_7)$ were listed in Figure 4. Based on the results of Figure 4, either addition of NaH_2PO_4 or $Na_3(C_6H_5O_7)$ causes a decrease of NDMA formation. Moreover the decrease of NDMA caused by NaH_2PO_4 was more significant than that caused by $Na_3(C_6H_5O_7)$ with the same concentration. For instance, the NDMA concentration decreased from 1.66 mg/L at 0 mmol NaH_2PO_4 to approximately 1.31 mg/L at 0.01 mmol NaH_2PO_4, while 0.01 mmol $Na_3(C_6H_5O_7)$ decreased the NDMA concentration from 1.66 mg/L to approximately 1.49 mg/L. Therefore, it can be concluded that both of NaH_2PO_4 and $Na_3(C_6H_5O_7)$ are able to inhibit NDMA formation, but the inhibitory ability of NaH_2PO_4 is somewhat stronger than that of $Na_3(C_6H_5O_7)$. This finding is well consistent with the conclusion obtained from the above section. Furthermore, it is worth noting that the NDMA-inhibiting ability of NaH_2PO_4 and $Na_3(C_6H_5O_7)$ was also shown in a dose-dependent manner. As shown in Figure 4, with the amount of NaH_2PO_4 addition increased from 0 to 0.03 mmol, the concentration of NDMA formation decreased from 1.66 to 1.17 mg/L while it is changed from

Table 1: Buffer solution (pH = 6.4) preparation.

Buffer type	$Na_2HPO_4/C_6H_8O_7$, 0.1 M	$Na_3(C_6H_5O_7)/C_6H_8O_7$, 0.1 M	NaH_2PO_4/NaOH, 0.05 M	NaH_2PO_4/Na_2HPO, 0.2 M
Volume (mL)	13.85/6.15 (20, total)	2.0/18.0 (20, total)	5.0/1.26 (dilute to 20, total)	5.3/14.7 (20, total)

1.66 to 1.39 mg/L over the same range for $Na_3(C_6H_5O_7)$. In comparison to the addition of 0.03 mmol NaH_2PO_4 or $Na_3(C_6H_5O_7)$, the NDMA concentration shows little variation with the addition of 0.04 mmol. It indicates that the addition of around 0.03 mmol reaches an inhibitory saturation for the reagent concentrations applied in this study.

The results of these experiments clearly verify the inhibitory effects of NaH_2PO_4 buffer on NDMA formation. They also indicate that the choice of phosphate buffer can definitely impact on the analytical results in nitrosamine measurements.

3.4. Proposed Mechanism of NaH_2PO_4 Affecting NDMA Formation during DMA Nitrosation. To illustrate the inhibition mechanism of NaH_2PO_4 on NDMA formation, two kinds of experiments were designed to conduct in a citrate buffer to determine which reactant ($NaNO_2$ or DMA) can interact with NaH_2PO_4 in the DMA nitrosation reaction. In the first experiment, the concentration of $NaNO_2$ was chosen to be 40 mM which is far greater than that of DMA (0.4 mM), while in the second one, in contrast, the concentration of DMA was 40 mM which is far greater compared to that of $NaNO_2$ (0.4 mM). The concentration ratio of DMA/$NaNO_2$ for the two experiments was controlled to be 0.01 and 100, respectively. Since the concentration of NaH_2PO_4 was chosen to be 1 mM, it means that NaH_2PO_4 is in excess compared to DMA and $NaNO_2$ for the two experiments, respectively. The results for the two reactions are presented in Figures 5(a) and 5(b), respectively.

As shown in Figure 5(a), the yield of NDMA with the presence of NaH_2PO_4 was 577 μg/L while the control experiment (marked as blank) without the NaH_2PO_4 addition was 580 μg/L. The NDMA concentrations are approximately the same which reveals that there is no significant difference in NDMA formation caused by the NaH_2PO_4 addition for the first experiment. It also indicates that NaH_2PO_4 does not react or react slowly with DMA otherwise DMA would be completely exhausted because the concentration ratio of DMA/$NaNO_2$ is only 0.01, which would lead to the decline of NDMA concentration.

In the second experiment (DMA/$NaNO_2$ ratio controlled at 100), NaH_2PO_4 was found to reduce NDMA formation (Figure 5(b)). The NDMA concentration in the experimental group was around 53 μg/L, whereas that in the control experiment was 70 μg/L. This result shows that NDMA formation was almost lower by 24% after adding NaH_2PO_4. Since the concentration of DMA was in large excess over $NaNO_2$ in the second experiment, it can be inferred that it is the mutual interaction between $NaNO_2$ or its derivative and NaH_2PO_4 that resulted in the decrease of NDMA formation.

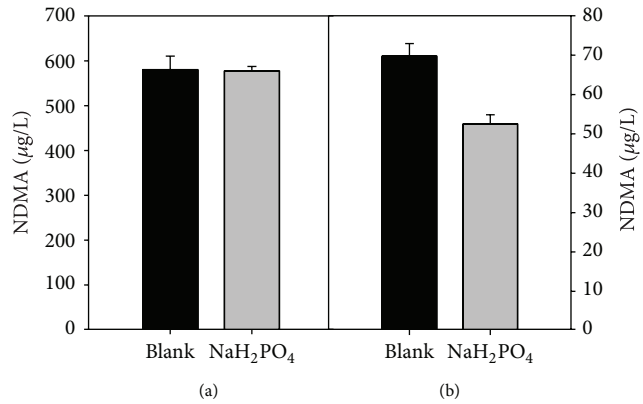


Figure 5: (a) Amounts of NDMA formed after 2 h from the reaction of a large excess of $NaNO_2$ (40 mM) with DMA (0.4 mM). (b) Amounts of NDMA formed from the reaction between a large excess of DMA (40 mM) and $NaNO_2$ (0.4 mM). The reaction solutions were buffered at pH 6.4 citrate buffer at 90°C with the concentration of NaH_2PO_4 being 1 mM. The vertical bars represent the standard deviation (n = 3).

Based on the above two experiments, it can be concluded that the inhibition mechanism of NaH_2PO_4 on NDMA formation during the DMA nitrosation reaction results from the interaction between $NaNO_2$ or its derivative and NaH_2PO_4.

3.5. Theoretical Work on the Inhibition Mechanism of NaH_2PO_4. The experiments have shown that phosphate exhibits inhibitory effect on the formation of NDMA. Basically, the possibility of reaction between amine and phosphate accounting for the inhibition can be excluded, because the nucleophilic amine (DMA) is expected to resist the phosphate anion. Therefore, we focused on the interaction between phosphate and nitrosating agent N_2O_3 which is viewed as the effective nitrosating agent in the solvent nitrosation reactions [18]. The nitrosating agent N_2O_3 is produced via the protonation of the nitrite ion and further dehydration of two HNO_2 molecules (1), and this has been well established in previous studies [38, 39]

$$\begin{aligned} H^+ + NO_2^- &\longrightarrow HNO_2 \\ 2HNO_2 &\longrightarrow N_2O_3 + H_2O \end{aligned} \quad (1)$$

It should be noted that $H_2PO_4^-$ is the main existing form of the added NaH_2PO_4 in pH 6.4 buffer solution; therefore, only $H_2PO_4^-$ anion was considered in the calculations. The results show that $H_2PO_4^-$ reacts easily with N_2O_3. Therefore, $H_2PO_4^-$ is proposed to be responsible for the inhibition of NDMA formation through scavenging the nitrosating agent

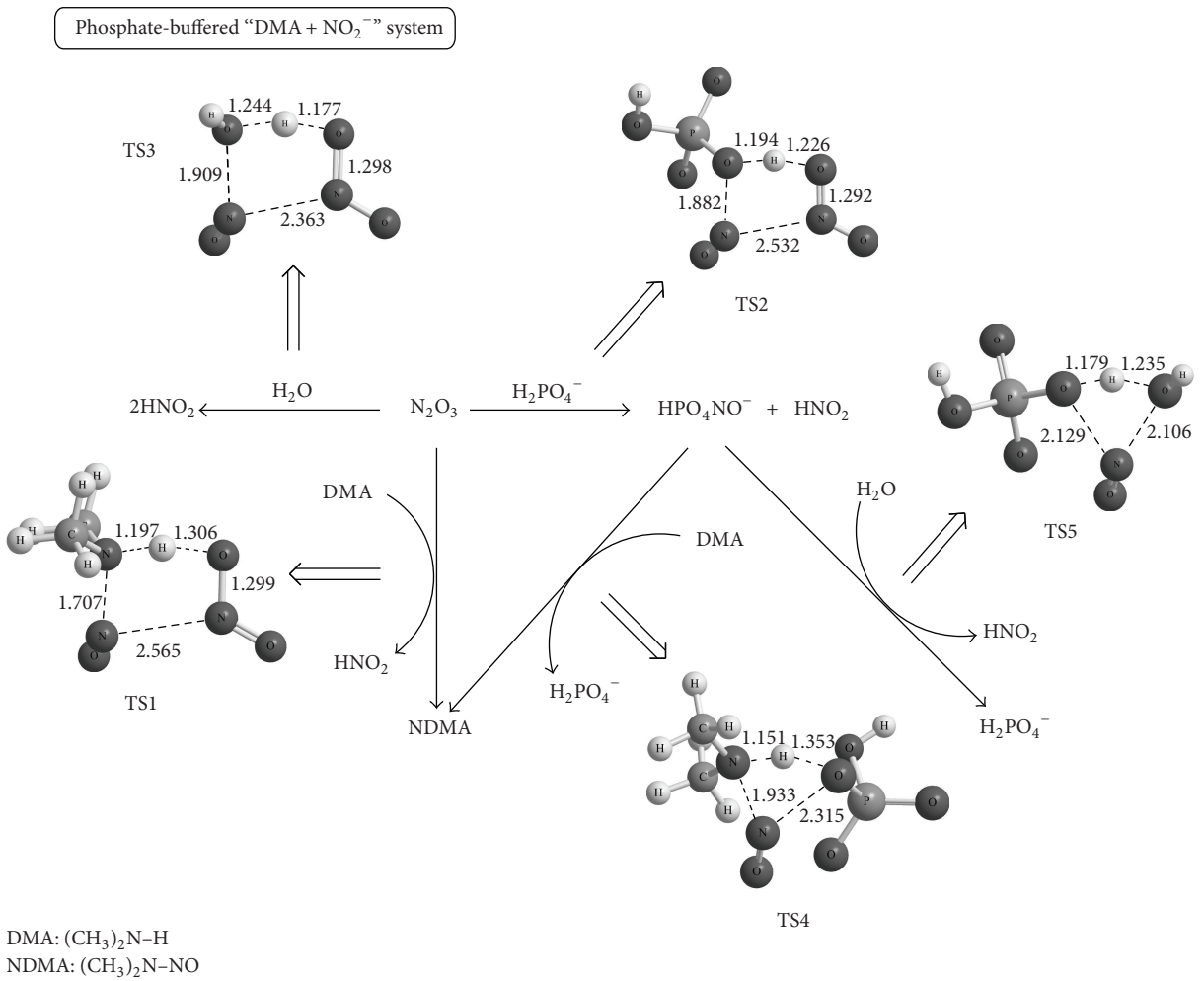


Figure 6: Possible reactions and transition structures (distances in angstroms) involved in the phosphate-buffered "DMA + NO_2^-" reaction system.

N_2O_3. Detailed discussion is given below to support this hypothesis. Reactions involved in the phosphate-buffered "DMA + NO_2^-" reaction system are illustrated in Figure 6. Corresponding energy data are collected in Table 2.

As shown in Figure 6, the fate of N_2O_3 is related to three different pathways for its further reactions: (1) reaction with DMA to form NDMA; (2) hydrolysis to regenerate HNO_2; (3) elimination by $H_2PO_4^-$ to give HPO_4NO^-.

Nitrosation of DMA by N_2O_3 occurs in a concerted step with a five-membered cyclic transition state (TS1 in Figure 6). NDMA forms via releasing nitrous acid (HNO_2). Activation enthalpy (Table 2) was calculated to be 6.87 kcal/mol, and the reaction is exothermic by 15.90 kcal/mol. NDMA forms easily via the N_2O_3-induced nitrosation of DMA, and this is consistent with previous studies [40, 41]. In addition, N_2O_3 undergoes hydrolysis (TS3-involved pathway in Figure 6) to reproduce HNO_2, and the activation energy (12.80 kcal/mol) is approximately twice as high as that (6.87 kcal/mol) of its reaction with DMA to form NDMA. Moreover, the hydrolysis is slightly endothermic by 1.50 kcal/mol. Therefore, N_2O_3 is more favored to be consumed by DMA to produce NDMA than to be hydrolyzed into HNO_2.

A very interesting finding is the reaction between N_2O_3 and the phosphate buffer (HPO_4^{2-}). Figure 6 shows that the reaction of $H_2PO_4^-$ with N_2O_3 occurs with a five-membered cyclic transition state TS2, and a new species (HPO_4NO^-) is generated via releasing HNO_2. Corresponding activation enthalpy (Table 2) was calculated to be 8.40 kcal/mol, which is very close to the activation enthalpy (6.87 kcal/mol) of the N_2O_3-induced nitrosation of DMA. This indicates that the reaction of N_2O_3 and DMA to give NDMA competes with its reaction with $H_2PO_4^-$ to produce HPO_4NO^-. Note that HPO_4NO^- is an ON^+ carrier which may also have nitrosating ability. Therefore, the nitrosation of DMA by HPO_4NO^- (TS4-involved pathway in Figure 6) was studied, however, the activation energy (22.05 kcal/mol) is much higher than that (6.87 kcal/mol) of the N_2O_3-induced nitrosation. Consequently, HPO_4NO^- is a weak nitrosating agent

Table 2: Calculated activation energies and reaction energies (in kcal/mol) for possible reactions involved in the phosphate-buffered "DMA + NO_2^-" reaction system at the B3LYP/6-311+G(d,p) level.

Reactions	$\Delta H^{\neq}$	$\Delta G^{\neq}$	ΔH	ΔG
DMA + N_2O_3 → NDMA + HNO_2	6.87	8.24	−15.90	−18.05
N_2O_3 + $H_2PO_4^-$ → HPO_4NO^- + HNO_2	8.40	10.83	−2.59	−2.37
DMA + HPO_4NO^- → NDMA + $H_2PO_4^-$	22.05	24.97	−28.39	−28.52
N_2O_3 + H_2O → $2HNO_2$	12.80	15.85	1.50	−0.06
HPO_4NO^- + H_2O → $H_2PO_4^-$ + HNO_2	25.41	28.63	−8.77	−8.15

when compared with N_2O_3. In addition, Lewis et al. [42] proposed that the hydrolysis of HPO_4NO^- is rapid which can account for the inhibitory effect of phosphate for the N_2O_3-induced nitrosation of DMA. However, the calculated result does not support this conclusion because the activation energy predicted for the hydrolysis of HPO_4NO^- (TS5-related pathway in Figure 6) is the highest (25.41 kcal/mol, Table 2) among the reactions of interest.

To conclude, the inhibited formation of NDMA in our experiment resulted from the fast elimination of the nitrosating agent N_2O_3 by $H_2PO_4^-$ to give HPO_4NO^-, and HPO_4NO^- is a "stable" species whose further reaction with DMA and hydrolysis are relatively slow in the studied "DMA + NO_2^-" system.

4. Conclusions

In this paper, batch experiments on NDMA formation from the reaction of DMA and nitrite were systematically conducted in the four kinds of buffer solutions, that is, $Na_2HPO_4/C_6H_8O_7$, $Na_3(C_6H_5O_7)/C_6H_8O_7$, NaH_2PO_4/NaOH, and NaH_2PO_4/Na_2HPO_4. Experimental data indicated that the phosphate component of the buffer plays a significant role in inhibiting NDMA formation compared to the citrate buffer and the inhibitory effect was proved to be in a dose-dependent manner. Moreover, it is the interaction between NaH_2PO_4 and reactant $NaNO_2$ rather than DMA that leads to the inhibitory effect of phosphate buffer during the DMA nitrosation reaction. Theoretical results found that a five-membered ring intermediate compound formed during the reaction of $H_2PO_4^-$ with N_2O_3, and the nitrosation ability of this intermediate was weaker than that of N_2O_3, which leads to the reduced amount of NDMA formation. This theoretical result supports the experimental conclusion and further points out why phosphate buffer inhibits the formation of NDMA during the nitrosation of DMA. This study discovered the inhibition of phosphate buffer solutions on NDMA formation and gave a hint to reduce the carcinogenic nitrosamines.

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