**Background:** Mylar balloons are used to collect exhaled air for analysis of fractional nitric oxide concentration (FENO).

**Aim:** We studied the effect of storage conditions on the stability of nitric oxide (NO) in mylar balloons.

**Methods:** Exhaled air samples and calibration gases were stored in mylar balloons at 4, 21 and 37 °C, with or without silica gel. NO was measured after 0, 6, 9, 24 and 48 h. Scheffe F-tests were used to compare NO values.

**Results:** NO remained stable in balloons for 9 h at all temperatures, without silica gel. NO increased between 9 and 48 h, but only with low initial FENO. Silica gel increased variability.

**Conclusions:** FENO in mylar balloons is stable for at least 9 h. The storage temperature is not critical, but silica gel increases variability.

**Key words:** Exhaled nitric oxide, Stability, Mylar balloons, Silica gel, Temperature

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**Introduction**

Exhaled nitric oxide (eNO) has been proposed as a non-invasive marker to evaluate airway inflammation in atopic asthma.\(^1,2\) The fractional concentration of exhaled nitric oxide (FENO) can be measured on-line by direct exhalation into a chemiluminescence analyser. Recently, the American Thoracic Society and European Respiratory Society published guidelines for standardized FENO measurements in adults and children.\(^3,4\)

Off-line sampling of eNO and storage in a reservoir offers the possibility of remote and delayed analysis of FENO, independent of the immediate presence of a nitric oxide (NO) analyser, which offers advantages for epidemiological field studies and home monitoring of asthma.\(^5,10\) American Thoracic Society guidelines suggest that mylar balloons are suitable for the collection of eNO.\(^5\) However, little is known about the effect of storage conditions on the stability of NO in such balloons. The aim of this study was to evaluate the influence of storage time, temperature and air humidity on FENO, collected off-line in mylar balloons.

**Materials and methods**

**Exhaled NO measurements and study design**

Exhaled air was collected from three adult volunteers, two healthy and one atopic asthmatic (FENO 12.4, 21.0 and 34.0 ppb, respectively), with an off-line method according to American Thoracic Society guidelines.\(^3\) Subjects inhaled NO-free air and exhaled through a one-way valve into a device in which a dynamic flow restrictor limits flow at 50 ml/s.\(^6\) Mouth pressure was checked by a manometer and accepted between 5 and 20 cmH\(_2\)O. Dead space air (250 ml) was discarded by a manually operated switch and exhaled air was collected in 150 ml mylar balloons. Total duration of the exhalations was 8–12 s. Between exhalations there was a minimum of 30 s rest. Ambient NO levels were recorded during collection. Each volunteer filled 15 balloons with and 15 balloons without silica gel. To assess whether NO levels were affected by possible factors in exhaled air, 20 balloons were filled with dry calibration gas containing <1 ppb or 115 ppb NO.

Balloons containing exhaled air with and without silica gel were sealed and stored at temperatures of 4, 21 or 37 °C. Balloons containing dry calibration gases were stored at 21 °C (<1 ppb) and at 4, 21 and 37 °C (115 ppb). Balloons were sampled immediately after the exhalation and 6, 9, 24 and 48 h later. The NO concentration in balloons was measured with a chemiluminescence NO analyser (Sievers 280, Boulder, CO, USA), with a sensitivity of <0.1 ppb and a detection range of 0.1–500,000 ppb. The analyser was checked once daily, before the measurements, using certified NO-free gas and 115 ppb calibration gas, and calibrated when needed. Samples
FIG. 1. Changes of different NO levels (ppb) in mylar balloons without silica gel, stored at (a) 4°C, (b) 21°C and (c) 37°C, and in balloons with silica gel, stored at (d) 4°C, (e) 21°C and (f) 37°C during 48 h. Open symbols, exhaled air samples (○, low; △, medium healthy; □, high (asthmatic)); closed symbols, calibration gas. Note the non-normal x axis.
were obtained by passing the sample tube into the balloons, using a 175 ml/min sample flow for 10 s.

Statistical analysis

FENO levels immediately after collection and after 6, 9, 24, and 48 h were calculated as geometric means of five balloons with and five balloons without silica gel for each subject at each different ambient temperature. Data were compared by analysis of variance for repeated measurements and the levels of significance were verified by Scheffe F-test. A two-tailed \( p < 0.05 \) was considered significant.

Results

FENO remained stable in balloons for 9 h at all temperatures when no silica gel was added and irrespective of initial values (Fig. 1a–c); no significant differences were found between values at 6 and 9 h compared with 0 h. In balloons with low initial FENO, NO concentrations increased significantly between 9 and 48 h, compared with 0 h \(( p < 0.05 \) ). Under all storage conditions, silica gel increased variability (Fig. 1d–f) \(( p < 0.05 \) ). Stability was worst at 37°C, in the presence of silica gel (Fig. 1f).

Discussion

We explored the stability of NO in exhaled air during storage in a mylar balloon, and studied the influence of temperature and humidity. Our results indicate that FENO levels remain stable in mylar balloons for the first 9 h after collection, but may rise over 24 h. These findings are in agreement with previous work by Barreto \textit{et al.} and Silkoff \textit{et al.}, who showed that ambient temperature did not influence FENO levels in balloons present a progressive rise at 24 and 48 h after collection.\textsuperscript{8,10} These studies, however, did not evaluate the influence of environmental temperature and the possible effects of humidity, using silica gel to absorb water vapour, or comparing NO in exhaled air with NO in dry gas. We showed that ambient temperature did not influence the stability of NO in balloons for the first 9 h after collection.

Paredi and coworkers demonstrated that eNO levels of 15 and 60 ppb were stable for 24 h in mylar reservoirs containing silica gel.\textsuperscript{8} However, control experiments without silicagel were lacking.

The source of the increase in NO during long-term storage is unclear. Selective leakage of non-NO gases seems unlikely, as this would require considerable volume changes that were not observed. We speculate that NO is released from the balloon during storage, depending on intraluminal NO levels.

We conclude that off-line assessment of FENO in samples of exhaled air collected in mylar balloons is reliable, provided that samples are analysed within 9 h. The storage temperature is not critical, but silica gel should not be added as it reduces FENO stability.

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