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The transport of nitric oxide through various polymeric matrices

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Abstract

The transport of nitric oxide (NO) in various plasticized and unplasticized polymeric films (silicone rubber, plasticized and unplasticized polyurethane, plasticized poly(vinyl chloride) and plasticized cellulose triacetate) is examined, and apparent NO diffusion coefficients are reported for each of the polymer compositions studied. Diffusion coefficients are found to range from ca. 2.5×10^{-6} cm^2/s in low weight percent plasticizer films to 3.0×10^{-5} cm^2/s in silicone films. The variation in NO diffusion rates through different polymer systems is explained by considering the characteristics of the polymers and, when applicable, the influence of plasticizer content. The newly measured values reported here should promote the rational choice of appropriate polymeric matrices for specific NO mass transfer applications. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Over the last decade, the volume of scientific research involving nitric oxide (NO) has grown exponentially as many biological and environmental roles of NO continue to be discovered [1]. Several of these research efforts involve NO transport through polymeric materials. For example, inlet devices have been designed using Silastic® tubing to deliver NO to a mass spectrometer as a means of measuring NO concentrations [2,3]. In addition, various electrochemical [4–7] and optical [8] NO sensors use polymer membranes in their design as outer gas diffusion barriers. Nitric oxide inhalation therapy for hospital patients with respiratory distress has become routine and involves passing NO through a polymer tubing or membrane-based oxygenator [9,10]. Nitric oxide diffusion from polymer films has also been demonstrated to improve the biocompatibility of intra-arterial sensor membranes [11] and cardiac shunts [12]. In all cases, nitric oxide diffusion through a polymeric material is a critical parameter affecting the suitability of the polymer for its designed application.

Given the importance of NO diffusion through polymeric materials, there is a need for reference values for such a transport. However, only one diffusion coefficient value for NO can be found in the literature, and this is for NO through Silastic® tubing [2]. Studies reported here were, therefore, undertaken to measure NO transport through various polymer matrices (silicone rubber, plasticized and unplasticized polyurethane, plasticized poly(vinyl chloride) and plasticized cellulose triacetate), and to determine how polymer film composition affects the apparent diffusion coefficient of NO within these materials. Assessment of the data set presented reveals that the trends for NO transport through polymer matrices are similar to those reported for other gases [13]. Therefore, many of the mechanistic explanations previously invoked to explain differences in gas transport through polymer systems [14–16] are not reiterated here. Rather, it is hoped that reference values reported here for NO will enable the rational choice of specific polymer matrices for given applications where NO transport is important.

The apparent diffusion coefficient measured in these studies is a composite term, dependent upon several factors, including size, shape and polarity of the penetrant gas, the solubility of the gas in the polymer matrix, and characteristics of the polymer (e.g. mean free volume and density) [14]. As the penetrant gas (NO) is constant for all results described herein, the differences in the apparent diffusion coefficient can be attributed to variations in the polymer systems studied. The apparent diffusion coefficient is calculated based on Fick's First Law of diffusion, from an equation derived for the experimental diffusion cell shown in

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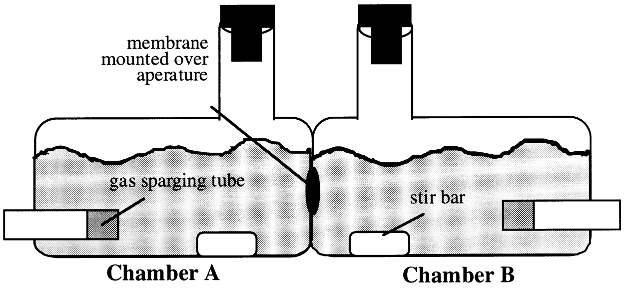

Fig. 1. Schematic of diffusion cell used for making NO diffusion coefficient measurements through polymeric films.

Fig. 1 [17]:

$$\ln\left(\frac{C_0}{(C_0 - C)}\right) = \frac{DA}{lV}t \qquad (1)$$

where C_0 is the NO concentration in chamber A (assumed constant—mM); C is the NO concentration in chamber B (mM); D is the diffusion coefficient (cm^2/s); A the membrane area (cm^2); 1 is the diffusion path length (membrane thickness—cm); V is the volume of chamber B (cm^3); and t is the time (s).

By experimentally measuring the concentration of NO in chamber B at specific time intervals and creating a plot of $\ln(C_0/(C_0 - C))$ versus time, the slope of the linear regression is used to calculate the diffusion coefficient. Notably, the above equation does not treat partitioning of NO into the membrane as a separate step; hence, the diffusion coefficient is only an apparent value, dependent upon both partitioning between the aqueous solution and organic film, and NO diffusion within the film.

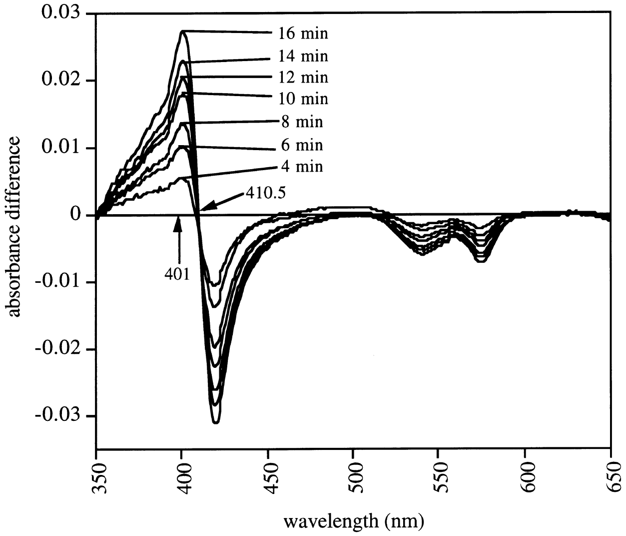

Fig. 2. Difference spectra of 1.0 ml of 1 μM oxyHb solutions after the addition of 50 μl aliquots of solution containing NO. The aliquots were removed from chamber B as a function of time during a diffusion experiment with a 66% SG-80A PU/34% DOS polymer film.

2. Experimental

2.1. Reagents

Tecoflex SG-80A and SG-60D polyurethane (SG-60D PU and SG-80A PU, respectively) were gifts from Thermedics, Inc. (Woburn, MA). High molecular weight poly (vinyl chloride) (PVC), cellulose triacetate (CTA), dioctyl sebacate (DOS) and 2-nitrophenyl octyl ether (NPOE) were purchased from Fluka (Ronkonkoma, NY). RTV3140 silicone rubber (SR) was from Dow Corning. Tetrahydrofuran (THF), dichloromethane and free-flowing granular potassium iodide were from Fisher (Fair Lawn, NJ). Sulfuric acid was purchased from Mallinckrodt (Paris, KY). Nitrite ion (1000 ppm) chromatography standard was obtained from EM Science (Gibbstown, NJ). Human ferrous oxyhemoglobin-A$_0$ (>98% Fe^{2+}) and 10 mM phosphate buffered saline (PBS), pH 7.4, containing 138 mM NaCl and 2.7 mM KCl were purchased from Sigma (St. Louis, MO).

2.2. Procedure

Polymer films were prepared by dissolving all the components (totaling 300 mg for silicone rubber films, 200 mg for all others) in 3 ml of THF (3 ml of dichloromethane for CTA). The cocktail solution was then cast into a glass ring on a glass plate and allowed to cure overnight. Silicone rubber films were cast in a Teflon ring on a Teflon plate and cured for at least two days prior to use. A 2 cm i.d. disk was cut from the center of each film and its thickness was measured using an optical microscope. The film was then mounted over the 1.15 cm i.d. aperture between the two chambers of the gas-tight diffusion cell shown in Fig. 1. The experiment was conducted by first filling both the chambers with 15.2 ml H_2O to condition the membrane for 3.5 h. After conditioning, 425 μl of fresh 4 M KI and 850 μl of 4 M H_2SO_4 were added to chamber A, and an additional 1.8 ml of H_2O was added to chamber B. Both chambers were purged with nitrogen gas while stirring with magnetic stir bars for 25 min. This purging step is critical for the success of the experiment as any residual oxygen present in the cell would oxidize NO, leading to erroneous diffusion coefficient values. The chambers were then sealed with rubber sleeve stoppers, the N_2 gas purging was discontinued and 500 μl of deoxygenated 1000 ppm nitrite ion chromatography standard was added to chamber A via a gas-tight syringe. In the presence of a large excess of KI and acid catalyst, the NO_2^- in chamber A was immediately converted to NO in a stoichiometric manner ($2I^- + 2NO_2^- + 4H^+ \rightarrow I_2 + 2NO + 2H_2O$) [18–20]. The temperature for all experiments was 22–24°C.

A spectrophotometric oxyhemoglobin, $Hb(Fe^{2+})O_2$, (oxyHb) assay [21] was used to quantify the concentration of NO in chamber B at given time intervals. This assay is based on the stoichiometric reaction of NO with oxyHb to

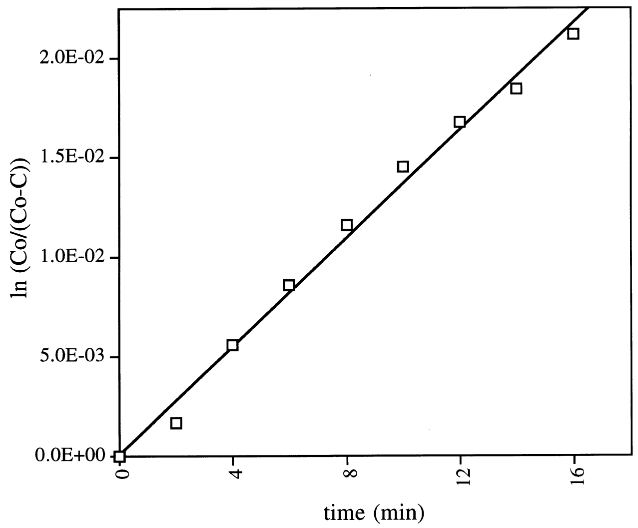

Fig. 3. Plot of $\ln(C_0 + (C_0 - C))$ vs. time (min) for a diffusion experiment with a 66% SG-80A PU/34% DOS film. The slope of the linear regression ($y = 1.356 \times 10^{-3}\, x + 3.601 \times 10^{-5}\ r = 0.996$) is used to calculate the apparent diffusion coefficient using Eq. (1).

produce nitrate and methemoglobin, Hb(Fe^{3+}), (metHb) ($NO + Hb(Fe^{2+})O_2 \rightarrow Hb(Fe^{3+}) + NO_3^-$). Solution aliquots were removed from chamber B at $t = 2$, 4, 6, 8, 10, 12, 14 and 16 min and added to a glass septum vial containing 1.0 ml of 1.0 μM of oxyHb (prepared in PBS). After 2 min reaction time, an absorbance spectrum of the oxyHb solution was recorded between 350 and 750 nm. The concentration of methemoglobin formed (equivalent to NO concentration) was determined by difference spectroscopy using Eq. (2):

$$\Delta\mathrm{metHb} = \frac{\Delta A_{401} + (1 - x)\cdot A_{0(401)}}{\Delta\varepsilon_{401(\mathrm{metHb-oxyHb})}} \tag{2}$$

where x equals $A_{1(410.5)}/A_{0(410.5)}$. Typical changes in the absorbance of 1.0 μM hemoglobin after the addition of aliquots from chamber B at different times in the experiment are illustrated in Fig. 2. The molar extinction coefficient for the difference in absorbance was determined experimentally to be 46.8 mM^{-1}/cm^{-1}.

Table 1
Apparent diffusion coefficients of NO through polymer matrices as a function of film formulation

Membrane type	Membrane thickness (μm)	Apparent diffusion coeff. × 10^{-5} (cm^2/s)
100% SR	329	2.97 ± 0.75
92% SR/8% DOS	378	3.00 ± 0.60
66% PVC/34% DOS	227	0.233 ± 0.07
34% PVC/66% DOS	315	1.80 ± 0.40
34% PVC/66% NPOE	315	1.63 ± 0.11
66% CTA/34% DOS	245	0.392 ± 0.14
100% SG-80A PU	298	0.274 ± 0.02
66% SG-80A PU/34% DOS	311	1.00 ± 0.19
34% SG-80A PU/66% DOS	287	2.01 ± 0.56
66% SG-60D PU/34% DOS	338	0.462 ± 0.06

Finally, to determine apparent diffusion coefficients, the concentration of NO in chamber B as a function of time was measured, and the diffusion coefficient was calculated from the linear regression of a plot of $\ln(C_0/(C_0-C))$ vs. time according to Eq. (1).

3. Results and discussion

A representative plot of $\ln(C_0/(C_0-C))$ vs. time for NO transport through a polymer film composed of 66% SG-80A PU/34% DOS is shown in Fig. 3 (typical correlation coefficient, r, for the linear regression was greater than or equal to 0.990). As indicated by the linear increase in NO concentration of chamber B over the short time frame of the experiment, steady-state flux has not yet been attained and, therefore, the experimental design is appropriate for measuring the NO diffusivity [22]. The measured apparent diffusion coefficients for different polymer films are summarized in Table 1. As expected, the apparent diffusion coefficient is largest for silicone rubber films. The addition of a small amount of plasticizer (92% SR/8% DOS), as is typical for chemical sensor applications, does not affect the diffusion coefficient through the film. These diffusion coefficients also compare favorably to the reported diffusion coefficient (2.3×10^{-5} $cm^2\ s^{-1}$) for NO through Silastic® tubing (Dow Corning) [2].

In contrast to silicone rubber films, the apparent diffusion coefficient is comparatively small for high wt.% PVC and CTA films. As illustrated by the results presented in Table 1, one method of increasing the diffusivity of NO through PVC is to increase the amount of plasticizer in the film. In general, increased plasticizer content of a polymer leads to increased segmental mobility, decreased polymer viscosity and increased diffusion coefficients [14]. It is interesting to note, however, that no significant difference was observed in the apparent diffusion coefficient between highly plasticized PVC films prepared with DOS or NPOE, despite the large difference in polarity of the two plasticizers (dielectric constants of 3.9 and 23.9, respectively) [22]. These results suggest that although the plasticizer identity may affect the individual partitioning or solubility characteristics of NO for a given polymer, these differences are small and, thus, are not evident in the overall measured apparent diffusion coefficient.

The last group of polymer systems examined includes several polyurethane formulations. Polyurethanes are a large class of polymers containing three distinct regions repeating randomly throughout each polymer chain: an isocyanate, a macroglycol and a chain extender [23]. The macroglycol segments are commonly referred to as the soft segments while the other two regions comprise the hard domains. Both of the commercially available polymers studied are synthesized from the same reactants (methylene bis(cyclohexyl) diisocyanate (HMDI), poly(tetramethylene ether glycol) (PTMEG) and 1,4 butane diol chain extender), and differ only in their hard:soft segment ratio [24,25]. However, large differences in NO diffusion coefficients are obtained for SG-60D PU vs. SG-80A PU films having the same polymer/plasticizer ratio (see Table 1). Indeed, 66% SG-60D PU/34% DOS films have a dramatically smaller apparent diffusion coefficient for NO compared to 66% SG-80A PU/34% DOS films. This result can be attributed to the fact that the weight percent of hard segment for SG-60D PU (59%) is more than double that for SG-80A PU (27%) [25]. Notably, even for 66% SG-60D PU/34% DOS membranes, the apparent diffusion coefficient of NO is twice that obtained for PVC films of the same polymer/plasticizer ratio. Finally, in the case of SG-80A PU films, as the plasticizer content of the film is increased, there is a corresponding increase in the NO transport through the film.

For all studies described here, the experimental conditions were chosen such that the medium on each side of the sample membrane was an aqueous solution rather than gas. The apparent diffusion coefficient is dependent upon this experimental parameter. Not only is gas partitioning into the film dependent upon the phase of the medium surrounding the film, but the water adjacent to the film actually permeates into the polymer [26,27], yielding a significant water concentration within the film (e.g. 51–82 mM, for plasticized PVC, depending on plasticizer identity [26]). Indeed, oxygen diffuses up to several orders of magnitude more rapidly when a polymer film is surrounded by water rather than gas, due to water uptake by the polymer and oxygen's increased diffusivity through the water enriched film [28]. The surrounding water medium in these studies is likely to have a similar effect on NO diffusion as the diffusion coefficient for NO through the aqueous solution (4.8×10^{-5} cm^2 s^{-1}) [29] is larger than any of the values measured through the polymer systems studied. In fact, aqueous bathing solutions were chosen for these studies because such conditions hold for so many of the applications envisioned for NO diffusion through polymers (i.e. sensors for measurement of NO in aqueous media [5–8], polymeric NO delivery systems to cell culture media for studying DNA damage [3] or to whole blood for improving biocompatibility [11,12]).

4. Conclusions

The results described herein should provide guidance with regard to the selection of proper polymer materials for a given application where NO mass transfer through polymer films is important. The practical implications of the difference in the apparent diffusion coefficients for medical devices are evident by considering that the range of diffusion coefficients reported yield over an order of magnitude difference in the time required for NO gas to diffuse through a polymer film of a given thickness (using $x^2=2Dt$, where x equals distance in cm). By extension, electrochemical sensors requiring a polymer film as a diffusion barrier should be prepared with silicone rubber rather than with PVC for optimal sensor response times and sensitivity. In contrast, PVC films may be preferred in applications where a slow, sustained NO transfer across a polymer film is desired. Further, as the diffusion apparatus used in these studies (Fig. 1) is sufficiently simple, the measurement of the apparent NO diffusion coefficient for a specific polymer system other than those included here is quite feasible.

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