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UTILITY OF GAS PERMEATION AS A MEANS OF PROTECTION FOR OXYGEN SENSITIVE SYSTEMS

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ABSTRACT

In this study, the utility of a gas permeator for decreasing the dissolved oxygen content of aqueous vehicles was determined at ambiant temperatures. Since decarbonation of water also takes place, the pH of the resulting decarbonated, deoxygenated water was very nearly neutral. Oxidative decomposition of two model compounds, phenylephrine and hydroquinone, was determined for several methods of protection such as nitrogen flushing and the addition of a chelating agent versus depletion of dissolved oxygen by permeation. It was found that permeated vehicles compared favorably to the other methods in relative protection of oxygen sensitive moieties.

INTRODUCTION

Because the complex reactions involved in oxidative decomposition of drug moieties interfere with a complete understanding of the degradation mechanism, extensive data and interpretation thereof are not readily available. The most common form of oxidative degradation in drug systems is autoxidation via free radical, chain mediated reactions, inasmuch as the unsaturated aromatic character of many drug molecules allows stabilization of free radicals by resonance. Molecular oxygen is involved in the propagation step of chain reactions, but since it is required in extremely low concentrations, its effect on the rate of autoxidative decomposition is not readily apparent. Schou, (1), has indicated that oxygen levels are rarely considered because, with presently available equipment, it is not possible to change the concentration enough to effect a change in reaction rates.

Deoxygenation via polymer membranes may provide a means for reducing oxygen concentration to levels such that it can be considered. An outcome of membrane separation techniques, gas permeators, consist of a large number of hollow polymer fibers encased in a noncontaminating metal sheath. The liquid to be deoxygenated is forced through the fibers; and permeation of selected molecules occurs through the walls of the fibers. A nitrogen flush or vacuum system around the fiber bundle permits removal of the permeant. Preliminary tests with such equipment indicate that this method is capable of reducing dissolved oxygen levels to about 0.1 ppm (2). The simplicity of design, coupled with these results suggest that this technique may be applicable to reduction of oxygen in liquid dosage forms, with a resulting increase in the shelf-life of the product.

EXPERIMENTAL

I. Preparation of Water Vehicles

Distilled water was passed through a mixed bed demineralizer[1] to remove cationic and anionic contaminants. The water was treated by one of five methods, (Table I), and initial dissolved oxygen levels (ppm) were determined with a Delta 106 Dissolved Oxygen Analyzer[2] prior to preparation of the buffered test systems.

TABLE I

A Summary of the Treatment Methods
for the Water Vehicles

Method	Treatment	D.O. ppm
Control	distilled deionized H_2O	8.32
Chelation	0.05% DiSodium EDTA[3]	8.31
N_2 flush[4]	15 min @ 250 ml/min	0.75
Gas Permeation-1	1 pass thru permeator	0.77
Gas Permeation-2	2 passes thru permeator	0.14

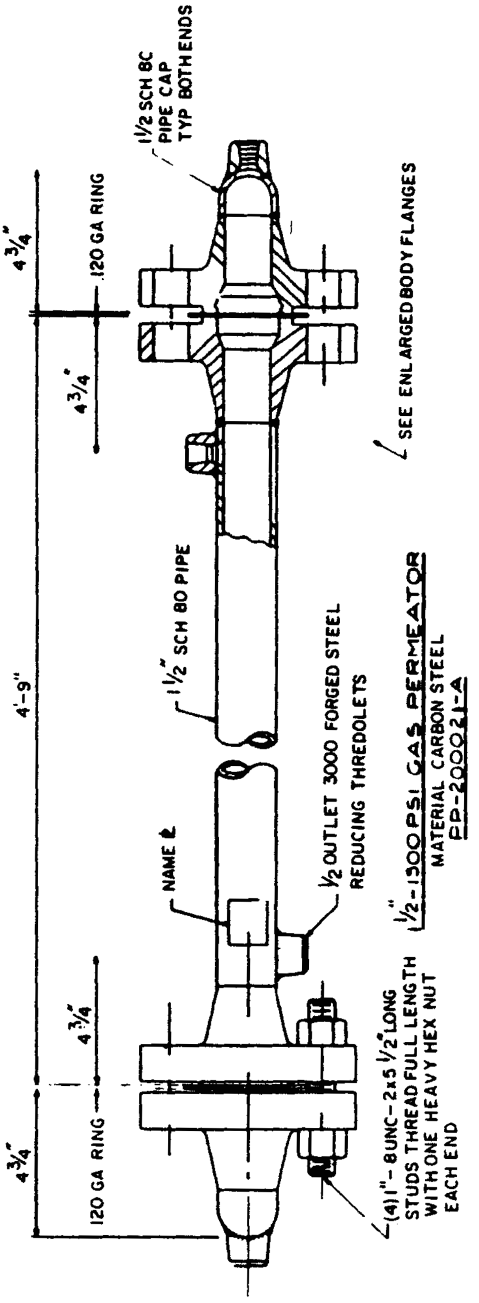

Figure 1. Schematic Diagram of Gas Permeator.

II. Preparation of Water Using Gas Permeation

Figure 1 depicts the gas permeator[5] used in this study. For more detailed description, refer to previous communication (2). Distilled, deionized water in a pressure tank[6] was forced through the permeator under one hundred pounds of nitrogen pressure. A continuous nitrogen flush was employed around the fiber bundle to remove permeants. Deoxygenated water was collected at a rate of thirty milliliters per minute in a type I glass collection flask[7] under a nitrogen hood to prevent air ingress. A second pass through the permeator was accomplished by placing the deoxygenated water in the pressure tank with a positive nitrogen siphon, and repeating the permeation procedure.

III. Determination of Dissolved Oxygen Levels

Dissolved oxygen levels in the waters prepared by each treatment method were determined with the dissolved oxygen analyzer rated at 0.01 ppm dissolved oxygen sensitivity. Botles of solvent water were sealed[8] to prevent air ingress and stored with the test systems to monitor dissolved oxygen levels, and to test the effectiveness of the sealing technique. Dissolved oxygen levels determined in water samples before and after the study varied only slightly, within the range of experimental error. There were no discernible patterns of oxygen consumption in the test systems.

IV. Determination of Protection Efficiency

The efficiency of each method of water protection against oxidative decomposition was evaluated using phenylephrine and hydroquinone test systems. All chemicals used in the study were of reagent grade quality. Melting points were used as a check for purity[9]. Equimolar concentrations of phenylephrine hydrochloride[10] and sodium hydroxide[11] reacted to form the phenylephrine base. The resulting product was washed with cold, deionized water to remove reactants and by-products. The melting point range of 169-171° C agreed closely with literature values. The phenylephrine base was dissolved in the appropriately treated water, and the solution was buffered to pH 8.5 as a phosphate buffer system. The resulting 0.01M solution was sealed and stored at 55°C to insure a reasonable rate of decomposition.

Hydroquinone[12] was recrystallized from hot water, and washed with cold, deionized water to remove impurities. A melting point range of 170-172°C agreed well with literature values. Appropriately treated waters were used to prepare 0.01M hydroquinone solutions at pH 6.9. The storage temperature selected for this molecule was 30°C.

Duplicate sets of solutions were prepared for each test molecule and each water preparation method studied. Samples of the buffered solutions prepared by nitrogen flushing or gas permeation were prepared in a glove box[13] under a nitrogen atmosphere to prevent air ingress into the deoxygenated solutions. All solutions were capped and sealed with wax mixture prior to storage in a constant temperature room[14] at the appropriate temperature. Analyses were performed periodically to determine the rate of decomposition. The procedures of Troup, Koshy, and Mitchner (3, 4) were used to determine phenylephrine content; that of Belyslov (5) was employed for hydroquinone content.

Values in Table II are the averages of twenty-four individual samples at ambient temperature. The dissolved oxygen levels were determined over the course of the study to monitor air ingress. No discernable differences were noted in the stored samples for the duration of the experiment.

Table II is a summary of the deoxygenation efficiency for the various methods used in this study. The control method showed a dissolved oxygen content of approximately 8 ppm, as did the chelation method. The latter result was to be expected since the chelation method provides protection against oxidative decomposition via inactivation of catalytic trace metals rather

RESULTS AND DISCUSSION

TABLE II

A Summary of Deoxygenation Efficiency for the Methods Used to Decrease Dissolved Oxygen

Method	*D.O. ppm	Std. Dev.	Rel. % Reduction
Control	8.32	0.612	0.00
Chelation	8.31	0.207	0.00
N_2 flush	0.75	0.064	91.00
Permeation x 1	0.77	0.067	90.70
Permeation x 2	0.14	0.036	98.30

*Each value = average of 24 samples @ 25°C.

than by decrease of dissolved oxygen. The other methods all showed substantial reduction of dissolved oxygent content as demonstrated by the relative percent reduction in Table II. It should be noted that both a single and double pass permeation procedure reduced oxygen levels to a value less than 1 ppm.

The potential of gas permeation as a protection method for oxygen sensitive systems was evaluated by comparing decomposition rates of sensitive molecules in vehicles prepared by the various methods, including gas permeation. Phenylephrine was selected as a model system since it undergoes significant oxidative decomposition at elevated temperatures and alkaline pH conditions (6). Although phenylephrine is subject to complex oxidative and non-oxidative decomposition, our interest in this molecule was purely as a model to measure the relative protection afforded by various deoxygenation methods.

In figures 2 and 3 a plot of concentration vs. time is shown for the control and test systems; the curves follow apparent first order kinetics in terms of drug concentration. Figure 2 shows rates for the decomposition of phenylephrine as a function of time, predicated on the analytical determination of the loss of the intact phenolic functional group on the molecule. In figure 3, the rates of decomposition are based on the loss of the secondary amino group due to cyclization. Troup and Mitchner, (3), reported that this molecule undergoes simultaneous decomposition at these two sites at approximately the same rate. For this reason we felt it necessary to analyze both these sites.

In reference to Table III, it was found that the specific rate constants in the control were of approximately the same magnitude at both susceptible sites. The overall effect in a protective vehicle was to reduce the rate of decomposition at either one or the other site on the molecule. It was felt that the relative magnitude of the specific rate constants is indicative of the complexity of the system. It can be readily discerned from figures 2 and 3, and from Table III, that there was a reduction in the rate of decomposition whenever phenylephrine was placed in a protective

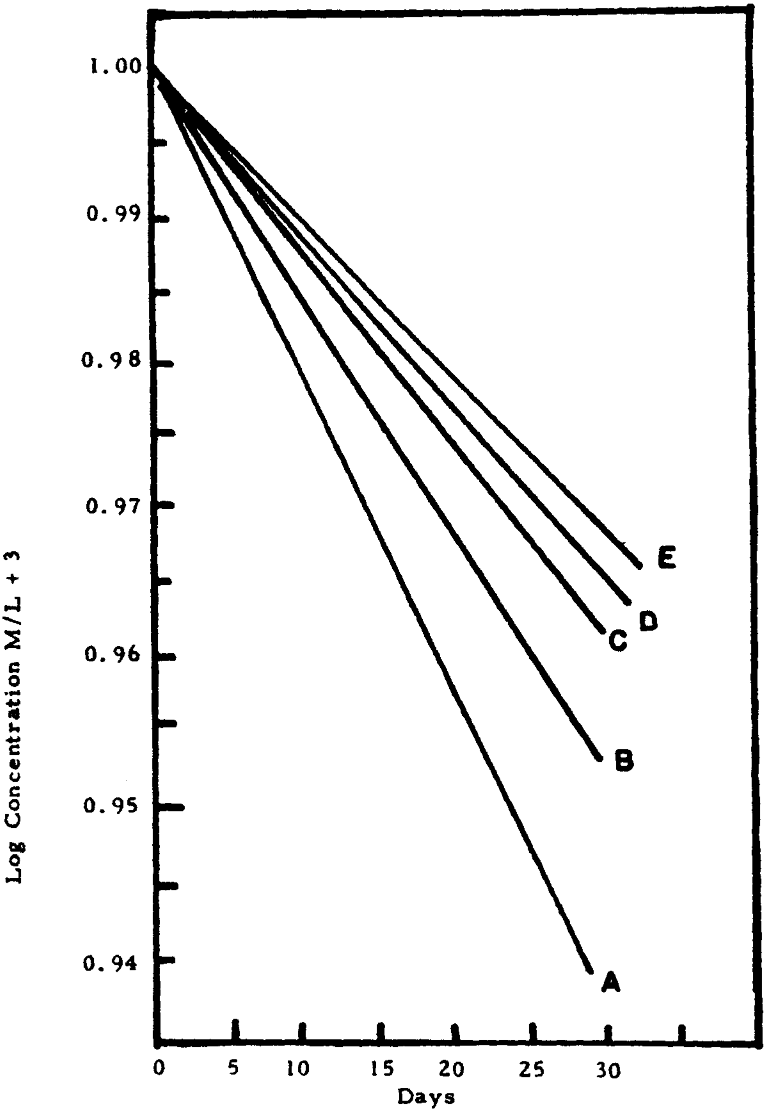

Figure 2. Decomposition of phenylephrine at 55 °C. and pH 8.5 based on analysis of hydroxyl group.

A - Control; B - Chelation; C - Nitrogen flash; D - 1 pass permeation; E - 2 pass permeation

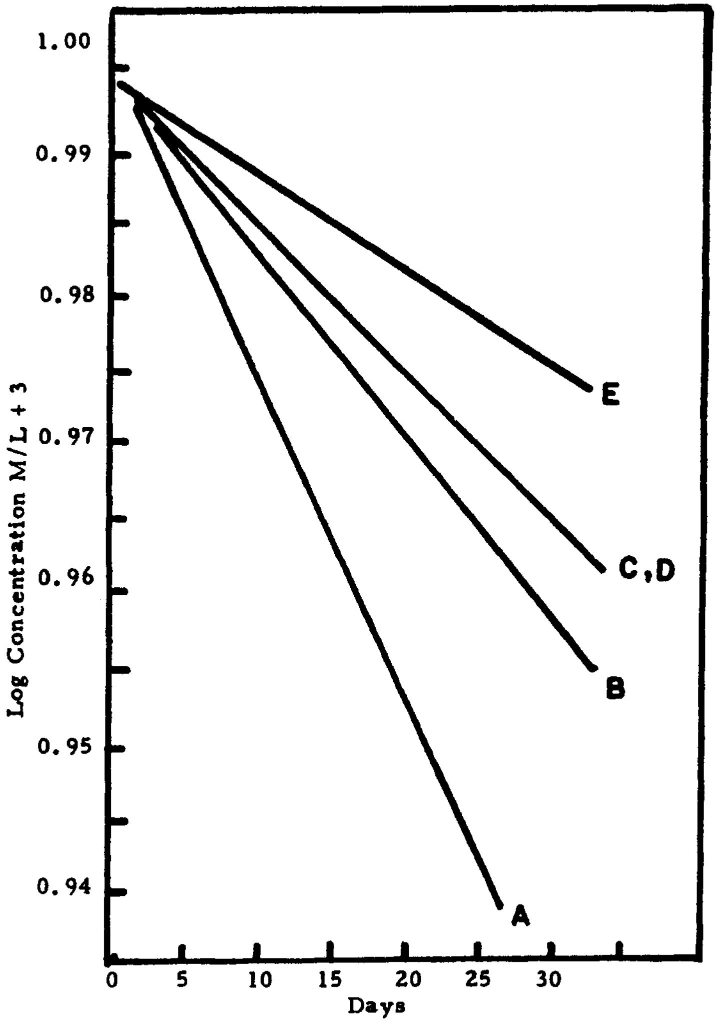

Figure 3. Decomposition of phenylephrine at 55°C. and pH 8.5 based on analysis of amino group. A - Control; B - Nitrogen flush; C - Chelation; D - 1 pass permeation; E - 2 pass permeation

TABLE III

A Summary of the Rates of Decomposition for Phenylephrine at pH 8.5 and 55 °C for the Various Protection Systems

System	D. O. ppm	*$K \times 10^3$/ day OH	$K \times 10^3$/ day NH	OH $\frac{K\text{ control}}{K\text{ system}}$	NH $\frac{K\text{ control}}{K\text{ system}}$
Control	8.37	4.25	4.59	1.0	1.0
N_2 flush	0.76	2.76	3.14	1.54	1.46
Chelation	8.76	3.38	2.21	1.26	2.08
Permeation x 1	0.70	2.54	2.24	1.61	2.05
Permeation x 2	0.14	2.45	1.87	1.73	2.45

*Determined from least squares generated slope of line from experimental data.

vehicle. The relative protection can be expressed as a ratio of the rates to the control rate. While the dissolved oxygen content was substantially reduced in several of these systems, decomposition still occurred at a somewhat diminished rate. Protection of the molecule was enhanced, but it was seemingly disproportionate to oxygen reduction. This was anticipated, since even very small concentrations of oxygen can form radicals to propagate the reaction via the production of oxygen free radicals or phenylephrine radicals.

There was some indication that reducing the dissolved oxygen content to very low values may allow for a larger protection index as seen in figures 2 and 3 for the single and double-pass permeation methods. The results which we are reporting are

TABLE IV

A Summary of Half-lives and t_{90} for Phenylephrine at pH 8.5 and 55°C for the Various Protection Systems

System	t (days) 90 (OH)	t (days) 90 (NH)	t (days) 50 (OH)	t (days) 50 (NH)
Control	24.5	22.7	163	151
N_2 flush	37.7	33.1	251	221
Chelation	30.8	47.1	205	314
Permeation x 1	40.9	46.4	273	309
Permeation x 2	42.4	55.6	283	371

considered to be only initial values of decomposition. Were decomposition allowed to proceed to a value greater than ten percent, the complexity of the decomposition pathways would make it difficult to interpret these values.

In Table IV, a summary of the calculated t_{90} and t_{50} for each of the test systems is shown for both of the analytical methods used for phenylephrine. In general, both the t_{90} and the t_{50} are seen to increase in value as a function of decreased dissolved oxygen level. Chelation is the exception, since it provides protection by inactivation of trace metals. It is included in this study for comparative purposes because it is a common method of protecting oxygen sensitive systems.

The complexity of the decomposition of phenylephrine led to the selection of hydroquinone as another oxygen sensitive molecule with well-documented oxidative pathways. Figure 4

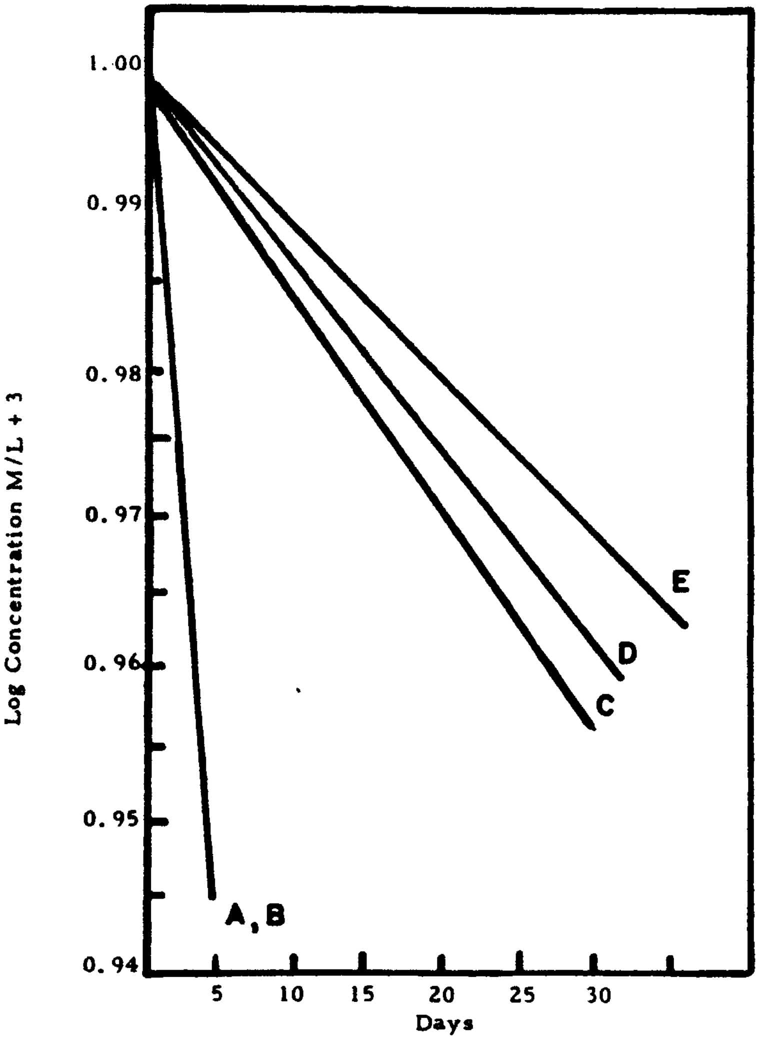

Figure 4. Decomposition of hydroquinone at 30°C. and pH 6.9

A - Control; B - Oxygen flush; C - Nitrogen flush; D - 1 pass permeation; E - 2 pass permeation

TABLE V

A Summary of Decomposition Rates, Half-lives and t_{90} for Hydroquinone at pH 6.9 and 30°C for the Various Systems Studied

System	*D.O. ppm	$K \times 10^3$ day	t day 90	t day 50	k control / k
Oxygen flush	15.7 ± 0.40	34.60	3.0	20	0.97
Control	8.3 ± 0.30	33.70	3.1	21	1.00
N_2 flush	0.73 ± 0.03	2.98	34.9	233	11.31
Permeation x 1	0.68 ± 0.20	2.66	39.1	265	12.67
Permeation x 2	0.16 ± 0.02	2.28	45.6	304	14.78

* - At least 24 individual determinations.

and Table V summarize the efficiency of the protective vehicles in retarding oxidative decomposition of this molecule. An additional test system, prepared by flushing the water with oxygen gas, was used to study the effect of increasing dissolved oxygen levels to double the normal level.

In the case of the hydroquinone systems, a much more dramatic decrease in the rates of decomposition with decreasing oxygen level resulted. It should be noted that degradation of the molecule did not cease at the lower dissolved oxygen levels, but still occurred at greatly diminished rates.

In the case of the oxygen flushing system, an approximate doubling of the oxygen content resulted in decomposition rates similar to the control system. This implies that, at the very least, normal oxygen concentration of about 8 ppm are more than sufficient to sustain oxidation at the control state. It should be noted that we are dealing only with initial rates of decomposition. At the end of thirty days, residual hydroquinone levels for the protected systems was still greater than ninety percent.

As seen with phenylephrine, further reductions in dissolved oxygen levels as seen with the double permeation afforded greater protection than the single permeation system. This would seem to imply that the complete absence of oxygen in a system might provide absolute protection; however, although complete deoxygenation would be desirable as a protective method, it may practically be unattainable. Deoxygenation via gas permeation in conjunction with less than usual amounts of antioxidant and chelating agents could perhaps provide this complete protection.

The efficiency of gas permeation and optimal operating conditions have only been introduced in this study. Some of these considerations will be investigated further, and our results will be made known in later communications.

FOOTNOTES

[1] Barnstead Demineralizer Model BD-1, Barnstead Still and Sterilizer Company

[2] Delta Scientific, Lindenhurst, NY.

[3] Baker Analyzed Reagent, J. T. Baker, Philadelphia.

[4] N_2 Gas high purity 0.001% O_2, Cranston Welding, Cranston, Rhode Island.

[5] Permasep, DuPont de Nemours & Co., Wilmington, Delaware.

[6] Millipore Corporation, 3 gal. capacity, Bedford, Mass.

[7] Pyrex, 2 gal. capacity.

[8] Wax mixture composed of carnauba, paraffin, and beeswax to result in M.P. = 65°C.

[9] Unimelt Melting Point Apparatus, Arthur H. Thomas, Phila.

[10] Ruger Chem., Irvington, NJ.

[11] Certified Reagent, Fisher Scientific, Fairlawn, NJ.

[12] Baker Allied Chemical, Morristown, NJ.

[13] Kewaunee Scientific, Adrian, Mich.

[14] Hotpack Corp., Phila., PA.

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