Title
Comparison of Permeation of Cyclohexanol Through Single and Multiple Layers of a Disposable Nitrile Glove

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Comparison of Permeation of Cyclohexanol

through Single and Multiple Layers of a Disposable Nitrile Glove

A thesis submitted in partial satisfaction

of the requirements for the degree Master of Science

in Environmental Health Sciences

by

Xingmei Liu

2019

ABSTRACT OF THE THESIS

Comparison of Permeation of Cyclohexanol

Through Single and Multiple Layers of a Disposable Nitrile Glove

by

Xingmei Liu

Master of Science in Environmental Health Sciences

University of California, Los Angeles, 2019

Professor Shane S. Que Hee, Chair

The hypothesis was that multiple layers of disposable nitrile gloves would provide more protection against cyclohexanol than a single layer relative to Standardized Breakthrough Time (SBT) and Steady State Permeation Rate (SSPR). The aims of this research were (1) to determine if a disposable nitrile glove resisted cyclohexanol, and (2) to determine if multiple glove layers provided more protection. In this study, cyclohexanol was used because of its high boiling point and solubility in water to enable closed-loop permeation cell water collection. Lavender Nitrile Powder-Free Exam Gloves were used because they are the least expensive and thinnest disposable nitrile gloves from Kimberly-Clark. The American Society for Testing and Materials (ASTM) Method F739-12 for permeation resistance under continuous contact was used in this study through single, double and triple layers of Lavender disposable nitrile glove pieces. Samples were taken

from the collection side of the permeation cells and later analyzed by gas chromatography-mass spectrometry. The Standardized Breakthrough Times (SBTs) of single, double and triple layers samples were 0.25 ± 0.25 minutes, 45 ±15 minutes, and 180 ± 60 minutes, respectively, which were Not Recommended, Good and Excellent according to Kimberly Clark safety rating. The SSPR of single layer samples was 363.3 ± 4.0 $\mu g/cm^2/min$, which rating was Poor according to Kimberly-Clark safety rating and Fair according to Ansell safety rating. The diffusion coefficient was 257 ± 29 $*10^{-8}$ cm^2/min. Double and triple layers samples never reached SSPR during the 8-hour tests. The results proved that multiple layers did provide more protection against cyclohexanol, where double layers were 180 times more protective than single layer, and triple layers were 720 times more protective than a single layer relative to standardized breakthrough time. The limitations of this study were: (1) glove pieces swelled, so that the calculation for diffusion coefficients may not be valid; (2) the duration between each sample was not short enough to have accurate average permeation rate at the times less than 10 minutes and greater than 60 minutes; (3) the shaking water bath could not simulate exactly hand motions; (4) this study did not test other Kimberly Clark disposable nitrile gloves, such as Blue and Purple.

The thesis of Xingmei Liu is approved.

Yifang Zhu

Wendie Robbins

Shane S. Que Hee, Committee Chair

University of California, Los Angeles

2019

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1. INTRODUCTION

1.1 *Hypothesis and Aims*

The hypothesis was that multiple layers of disposable nitrile gloves would provide more protection against cyclohexanol than a single layer relative to Standardized Breakthrough Time (SBT) and (Steady State Permeation Rate) SSPR. The aims of this research were (1) to determine if a single disposable nitrile glove resisted cyclohexanol using the method of American Society for Testing and Materials (ASTM) F739-12, and (2) to determine if multiple glove layers provided more protection relative to SBTs and SSPR.

1.2 *Background*

1.2.1 *Glove Permeation*

Disposable nitrile gloves are inexpensive barriers used for skin protection. Although chemically resistant glove should be worn for optimum personal protection against chemicals, disposable gloves may be the only available choice because of cost and the situation. However, information is usually inadequate for their chemical resistance. Disposable nitrile gloves are usually used in food industry and medical purposes because it is resistant to oil and fats. Thinner gloves usually provide more comfort and are cheaper. However, the thickness can differ for every glove material tested, and thinner materials allow more permeation than thicker ones of the same material. (1, 2) Permeation is the process through which chemical substances move at the molecular level through the glove material. Research in this field is limited and often relies on manufacturers to provide data on how protective their products are against different types of chemicals. (1, 2) According to NIOSH, skin disease is the second most common type of occupational disease. It was estimated that more than 13 million U.S. workers may be exposed to chemicals absorbed through the skin in

2013. Dermal exposure to hazardous agents can lead to a variety of occupational diseases, including occupational skin diseases and systemic toxicity. (3) Skin adverse effects, such as dermatitis, could also be caused by contact with chemicals if the gloves worn are not resistant (4, 5, 6). Deaths are known to have occurred when gloves thought to be protective were not (30). Therefore, standard test methods are necessary to provide more accurate information about how effective disposable nitrile gloves are in protecting hands in order to effectively protect workers from dermal exposures (1).

Double gloving is commonly used for medical purposes especially for surgeons and dentists to reduce surgical cross-infection (38). Triple gloving is required when dealing with microorganisms that cause serious infectious diseases, such as Ebola virus (39).

There are some previous studies about double gloving permeation. It was found in the study of Waegemaekers et al. 1983 that double layers of latex surgeon's gloves were more protective against methyl methacrylate based on lag time and permeation rate (32). Connor 1984 found that double thicknesses of glove material (latex and polyvinyl chloride, especially of the thicker polyvinyl chloride) reduced the permeation of the chemotherapy drug carmustine (33). Another study from Connor 1995 showed that Perry® Style 42 glove material that was tested as a double thickness was impermeable to five cancer chemotherapy drugs (doxorubicin, cyclophosphamide, 5-fluorouracil, carmustine, and cisplatin) (34). Jordan et al. 1996's study found that the time to first breakthrough of double layers of latex gloves against glutaraldehyde increased to 3 to 4 hours from 45 minutes (35). In 2003, Mäkelä et al. found that the breakthrough time values for double layered gloves were about five times longer than for the single layered gloves of similar thicknesses against 70% isopropyl alcohol in the ASTM F739 test and about 16 times longer in the EN374 test (36). In 2012, Capron et al.'s study showed that none of the evaluated double-gloving combinations displayed any detected permeation of chemotherapeutic agents at 43 °C, confirming that the technique can be used safely during hyperthermic intraperitoneal chemotherapy (37).

There are very limited studies about triple gloving permeation. Song 2017 study found that double and triple layers of Kimberly-Clark Blue disposable nitrile gloves were more protective than single gloving against 2-butoxyethanol. SBTs were found between 5 to 10 minutes for single, between 10 to 20 minutes for double, and between 30 to 40 minutes for triple layers (6).

1.2.2 *Permeation Theory*

The process of permeation through a glove also involves degradation and penetration considerations. Degradation is physical deterioration of the material due to an agent contact. The material may become harder, more rigid, brittle, softer, weaker, swell or shrink. (4, 6) Penetration results when the chemical leaks into the material either already in the material through seams, pinholes and other imperfections or after degradation. (6, 8) Permeation takes place when the chemical molecules adsorb onto the contact surface of the material, diffuse through the material, and then desorb at the opposite surface of the material (7, 9). Fick's First Law can be applied to express the diffusion of chemicals through the glove material as in Equation 1-1. (10)

$$J=D*A*(dc/dl)\ldots\ldots\ldots\ldots(1\text{-}1)$$

Where J is the permeant mass transfer rate (μg/min);

D is the diffusion coefficient (cm^2/min);

A is the exposed area (cm^2);

c is the permeant concentration (μg/cm^3);

l is the glove thickness (cm).

The permeation rate can be determined by using a standard test method, such as ASTM Method F739, European Permeation test EN 374, and the International Standard Organization test ISO 6529. (4)

Equation 1-1 can also be expressed as in Equation 1-2:

$$J= D*A*(C1- C2)/l\text{…………}(1\text{-}2)$$

Where C1 and C2 are analyte concentration or masses in the challenge material cell at time t;

D is independent of the concentration gradient and thickness if the material is isotropic. (4)

The diffusion coefficient (D) can be calculated in a closed-loop system by extrapolating the mass or mass/area versus time permeation steady state region to zero mass at lag time (L_T, minute) and using Equation 1-3:

$$D= l^2/ 6*L_T \text{……………….}(1\text{-}3)$$

Equation 1-3 is not obeyed if significant swelling or shrinking of the material occurs.

1.2.3 *Test Compound*

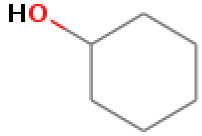
This study used cyclohexanol because of its high boiling point of 160 °C making it a suitable semi-volatile chemical for testing with little loss from volatilization and its solubility in water making it suitable for closed-loop water collection testing and GC-MS analysis. It also had available previous open loop data from glove manufacturers. (1)

1.2.3.1 *Physical Properties*

IUPAC Name: Cyclohexanol

CASRN: 108-93-0

2D Structure:



Molecular Formula: $C_6H_{11}OH$

Molecular Weight: 100.158 g/mol

Physical Description: a deliquescent colorless liquid or white crystals with a camphor-like odor

Density: 0.9624 at 20 °C /4 °C relative to water (11)

Melting point: 25.93 °C

Flash point: 62.8 °C (closed cup); 67.8 °C (open cup) (12)

Lower explosive limit: 2.7 % by volume

Upper explosive limit: 12 % by volume (18)

Boiling point: 161.84 °C (11)

Solubility: 3.6 % (wt/wt) in water at 20 °C; miscible with ethyl acetate, linseed oil, petroleum solvents. (13)

Vapor pressure: 0.657 mm Hg at 25 °C (14)

1.2.3.2 *Uses*

Cyclohexanol is used in soap making to incorporate solvents and phenolic insecticides (15). Pure cyclohexanol or admixed with cyclohexanone as KA-oil, is in the production of caprolactam, which is used in the manufacture of nylon-6 polymer. (16, 17) It is also a solvent for alkyd resins, alcohol-soluble phenolic resins, ethyl cellulose for manufacturing celluloid, finishing textiles and used in insecticides. (13) Cyclohexanol is a chemical intermediate as a stabilizer and homogenizer for soaps and synthetic detergent emulsions and solvent for lacquers, varnishes, paints, finish removers, leather degreasing, polishes, plasticizers, plastics, and germicides. (15)

1.2.3.3 *Standards/Recommendations:*

OSHA Permissible Exposure Limit (PEL) -8-hr Time Weighted Average (TWA): 50 ppm ($200 mg/m^3$) (19, 28)

NIOSH Recommended Exposure Limit (REL)-TWA: 50 ppm ($200 mg/m^3$), skin

NIOSH Immediately dangerous to life or health (IDLH): 400 ppm (20)

ACGIH Threshold Limit Value (TLV)-TWA: 50ppm (skin), eye irritation, central nervous system impair (21)

1.2.3.4 *Toxic Effects*

Through skin absorption, inhalation and ingestion, it can be absorbed into the body. A harmful air contamination will not or will only be achieved very slowly when this substance is evaporated at 20 °C, the headspace concentration at equilibrium being 864.47 ppm at 25 °C. The eyes, skin and respiratory tract are irritated by cyclohexanol. It can affect the central nervous system. Cyclohexanol liquid affects the skin. It may cause headache, nausea, vomiting, dizziness and unconsciousness at high concentrations. Cyclohexanol removes natural oils from the skin causing dryness, cracking, and dermatitis. Long or high exposures may cause damage to the liver, kidney, and lung (18, 22). Fiserova-Bergerova et al. 1990's study showed that the flux of cyclohexanol through skin was $0.3274 mg/cm^2/hr$, rated as potential for dermal absorption.

2. EXPERIMENTAL

2.1 *Apparatus*

Lavender Nitrile Powder-Free Exam Gloves (Kimberly-Clark Global Sales, LLC, Roswell, GA 30076 USA, LOT: BY82391314, Date of Manufacture: August 2018, Expiration Date: July 2023) were used to study the glove permeation of cyclohexanol. An analytical balance (Mettler AE 260 DeltaRange®) was used to measure the weights of each glove specimen and a micrometer gauge (Marathon, catalog No. CO 030025) was used to measure the thickness. A RadioShack® Illuminated Microscope 60-100x magnification (63-1133) was utilized to detect pinholes in the glove to avoid penetration.

Desiccators contained a saturated solution of sodium dichromate ($Na_2Cr_2O_7 \cdot 2H_2O$) to generate a relative humidity (RH) of 52% at 20 °C to condition the glove pieces before and after each permeation run (23). The permeation test cell (ASTM type I-PTC-600, Pesce Lab Sales, Immersible closed loop chamber) is a two-chambered cell for contacting the specimen with the chemical (10 mL) on the specimen's outside surface and with a collection medium (10 mL water) on the inside surface, which were used as shown in Figure 1. A torque wrench was used to tighten permeation cell bolts after material layering with a force of 10 ft-lb. The chambers were placed into a calibrated shaking-tray constant temperature water bath (Thermo Scientific, Model 2870) at 35.0±0.5°C operated at 70.33 ± 0.86 revolutions/min. 100 µL Eppendorf pipet with long tips (Fisherbrand™ Extended-Length Tips Catalog No.02-681-418) was used to transfer 0.1-mL samples from the collection side of the permeation cell into 1.5-mL vials to store at 0 °C. A 10-µL syringe (Hamilton Company) was used to inject samples into the gas chromatograph-mass spectrometer (GC-MS), an Agilent 6890N with a moderately polar HP-5MS capillary column

60.0m * 320 μm inner diameter * 1.00 μm film thickness connected to an Agilent 5973 Mass Spectrometry.

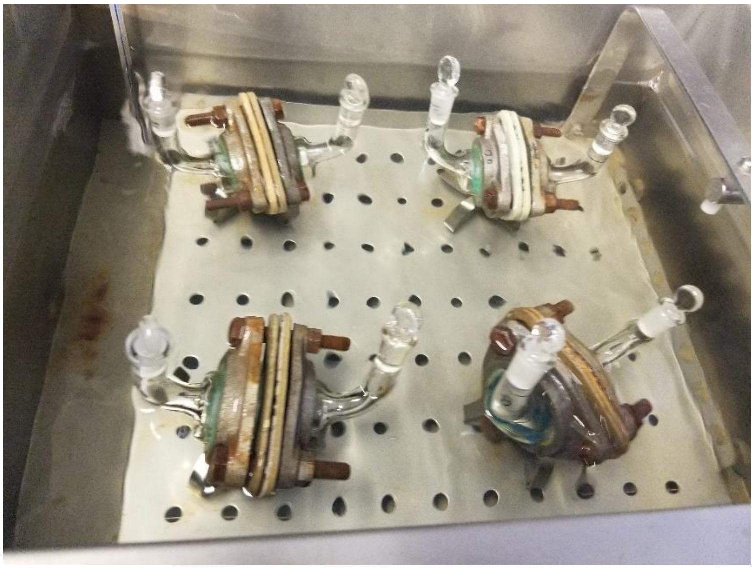


Figure 1. Test Cell Chambers in the Water Bath

2.2 *Chemicals*

Chemicals used are H_2O (Milli-Q deionized and organics-free water), Cyclohexanol (Sigma Aldrich, 99%), 4- bromophenol (4-BP) (Sigma Aldrich), Acetone (Fisher Scientific), and liquid neutral detergent (AJAX).

2.3 *Procedures*

The method used in this study was the American Society for Testing and Materials (ASTM) F739-12, (7) 'Standard Test Method for Permeation of Liquids and Gases through Protective Clothing Materials under Conditions of Continuous Contact.' There were two differences: the permeation cells were assembled with two challenge chambers; and the temperature of the water bath was 35 °C, not 27 °C. The closed-loop system was not a circulating type. By measuring the standardized breakthrough time (SBT), steady-state permeation rate (SSPR), and cumulative permeation over a period of time, the permeation of a chemical through a protective clothing material can be evaluated. The following steps were performed:

(1) Used a hand microscope to detect pinholes in the glove.

(2) Drew and cut out circular pieces of gloves (diameter = 49mm for single layer specimens, diameter = 43mm for double and triple layers specimens. Due to minor leaks during single layer experiment, shrinking the diameter helped to decrease absorption and desorption of cyclohexanol from and to the water bath through the glove pieces out of the gaskets).

(3) Preconditioned the gloves in the desiccator for at least 24 hours prior to the test.

(4) Weighed the specimen.

(5) Measured thickness by using the micrometer screw gauge at three locations within the palm cutout. The diameters exposed to the mixture and collection water solvent were both 2.54 cm (1 inch).

6) Placed the specimen between two test-cell chambers and tightened the cell bolts with a 10-ft-lb torque wrench.

7) Assembled four test cells (3 cyclohexanol, 1 blank-water collection alone) and deposited 10 mL deionized water in the collection side of each cell and placed the cells in the calibrated constant temperature shaking water bath for 30 minutes at 35.0±0.5 °C. Any back-permeation of water led to starting over again.

8) Pipetted into the challenge side a volume of 10 mL of cyclohexanol into 3 cells, air for the blank cell, started the shaker, and timer.

9) Removed a volume of 0.1 mL of sample from each collection side at 0, 5, 10, 20, 30, 60, 120, 240, 360, and 480 minutes.

10) Stored samples in capped 1.5-mL vials in a 0 °C freezer until measurement.

After each test run:

1) Poured collection side of each cell into a labeled graduated cylinder.

2) Discarded the liquid in the challenge side.

3) Disassembled the test-cell chamber.

4) Transferred the specimens into plastic petri dishes to dry and recondition in the desiccator for 24 hours.

5) Weighed the specimen and measured its thickness.

6) Submerged the test-cell chambers, Teflon gaskets, bolts and nuts into tap water with liquid neutral detergent overnight.

7) Rinsed at least three times with distilled water or until no more detergent foaming was evident, then cleaned and dried with acetone.

For GC-MS analysis:

1) Thawed the 0.1 mL samples to room temperature.

2) Added 4-BP internal standard to a concentration of 0.1 µg/µL in each sample by 10 µL syringe.

3) Injected a volume of 2.0 µL into the GC splitless injection port at 250 ^{o}C.

4) Generated the standard curve with cyclohexanol concentrations of 0, 0.3, 0.5, 1.0, 5.0, 10.0, 40.0 ng/µL in deionized water for the lower linear range, and 0, 40, 100, 300, 500 ng/µL for the upper linear range using linear regression model.

5) Diluted the sample to 10 % when it exceeded the upper range standard curve for GC-MS analysis.

2.4 *GC-MS Analysis*

The samples were analyzed by capillary gas chromatography-selective ion monitoring (SIM) mass spectrometry (GC-MS) by the method of internal standards (cyclohexanol peak area divided by 4-bromophenol, 4-BP) peak area at a constant 4-BP sample concentration of 0.1 µg/µL versus collection side concentration. The GC column temperature started at 90 °C for 6 minutes and increased to 280 °C at 120 °C/min with the injector at 280 °C. There was a 6.0-min solvent delay. The column MS-ionization inlet was at 250 °C. The inlet ion source and quadrupole ion filter were 230 °C and 150 °C, respectively. The SIM mode allowed quantitation and was used to enhance sensitivity and selectivity by the internal standard method with ions m/z 57 and 82 for cyclohexanol and m/z 172 for 4-BP internal standard. The helium flow rate was at 2.5 mL/min. (17)

2.5 *Data Analysis*

The collected data were computerized and statistically analyzed with Microsoft Excel (Microsoft® Office 365ProPlus) and RStudio (Version 1.1.456, RStudio, Inc.).

The Student t-test was used to analyze the statistical differences between the averages and standard deviations of the triplicate weights and thicknesses of glove specimens before and after the experiments at $\alpha = 0.05$. Linear regression was used to characterize the internal standard curves.

Cumulative mass permeations were plotted with amount in collection side versus time. The lag time (L_T) was measured by extrapolating the linear steady state section of the permeation curve to the horizontal time axis where the mass/area (y - axis) zero, enabling the diffusion coefficient (D) to be calculated from Equation 1-3. The SBT at which the permeation rate reached 0.1 μg/cm^2/min was determined by the time when the collection side concentration was 0.255 ng/μL.

Calculation was as follows:

c = PR * A * T/V

= 0.1 μg/cm^2/min * π* $(2.54\ cm)^2$ * 5 min / (4* 10 ml) = 0.255 μg/ml = 0.255 ng/μL

Where PR was the permeation rate at 0.1 μg/cm^2/min;

A was the exposure area which is 1-inch diameter circle;

T was the time interval which is 5 minutes at least;

V was the volume in the collection side which is 10 ml.

SSPR was determined as the maximum constant rate of permeation after the breakthrough during the steady-state permeation period.

The amount of cyclohexanol in each sample was calculated by the equation 2-1:

$$A_i=C_i*V_i+0.1*\sum_{1}^{i-1} C_{i-1} \ldots\ldots\ldots\ldots(2\text{-}1)$$

Where A_i is the amount of cyclohexanol in sample i (i is from 1 to 10);

C_i is the concentration of cyclohexanol in sample i;

V_i is the volume of collection side when sample i was taken.

3. RESULTS

3.1 *GC-MS*

The retention times for cyclohexanol and the internal standard (4-BP) were 8 and 11.5 mins, respectively, which agreed with previous research (1). The total run time for each injection was 12.58 minutes.

The internal standard regressions for cyclohexanol in water were $y=0.006761x+0.003786$ ($R^2=0.9919$, p-value=$1.289*10^{-6}$) in the lower range (0, 0.3, 0.5, 1.0, 5.0, 10.0, 40.0 ng/µL) and $y=0.01774x-0.43800$ ($R^2=0.9543$, p-value=0.00272) in the upper range (0, 40, 100, 300, 500 ng/µL), where x is the concentration of cyclohexanol in ng/µL and y was the peak area ratio of cyclohexanol to 4-BP.

3.2 *Weights and Thicknesses of the Gloves*

Tables 1, 2 and 3 show the glove weights and Tables 4, 5 and 6 show the glove thickness data. For each glove specimen before and after the experiment, weights and thicknesses were measured three times. Most of the weights of glove specimens had statistically significant differences at $\alpha = 0.05$. None of the thicknesses of glove specimens of blank cells had statistically significant differences. For double layers experiment, the order of assembling the glove specimens was collection chamber – 1 – 2 – challenge chamber. For triple layers experiment, the order of assembling the glove specimens was collection chamber – 1 – 2 – 3 – challenge chamber. The glove pieces were marked with 1, 2 and 3, and the analysis results are shown in Tables 2, 3, 5, and 6.

3.2.1 Weights

The weights of the glove specimens before and after the single layer, double layers, and triple layers experiments are shown in Tables 1, 2 and 3, respectively. All the glove pieces tested had significant differences in weights between before and after the experiment at α = 0.05. The most weight increase occurred was for cell 3 of single layer experiment with an increase at 12.34%. The least weight increase occurred was for cell 12 of triple layers experiment, which was a blank cell, with an increase at 7.54%. The average weight gains were not statistically greater than for the blank.

Table 1. Weights of the Glove Specimens before and after the Single Layer Experiment

	Before the experiment Mean (g) ± SD	After the experiment Mean (g) ± SD	Increase	Student t	p-value
Cell 1*	0.1349 ± 0.0001	0.1480 ± 0.0001	9.71%	227	0.000019
Cell 2*	0.1398 ± 0.0003	0.1537 ± 0.0001	9.94%	69.5	0.00021
Cell 3*	0.1402 ± 0.0001	0.1575 ± 0.0001	12.34%	173	0.000033
Cell 4 (Blank)*	0.1389 ± 0.0002	0.1551 ± 0.0001	11.66%	122	0.000067

*: There was a statistically significant difference of the weights before and after the experiment.

Table 2. Weights of the Glove Specimens before and after the Double Layers Experiment

	Before the experiment Mean (g) ± SD	After the experiment Mean (g) ± SD	Increase	Student t	p-value
Cell 5-1*	0.0953 ± 0.0001	0.1049 ± 0.0001	10.07%	289	0.000012
Cell 5-2*	0.0939 ± 0.0001	0.1039 ± 0.0001	10.65%	173	0.000033
Cell 5*	0.1892 ± 0.0001	0.2088 ± 0.0002	10.36%	295	0.000012
Cell 6-1*	0.1075 ± 0.0001	0.1184 ± 0.0001	10.14%	326	0.0000094
Cell 6-2*	0.1020 ± 0.0001	0.1113 ± 0.0001	9.12%	278	0.000013
Cell 6*	0.2095 ± 0.0002	0.2296 ± 0.0001	9.59%	302	0.000011
Cell 7-1*	0.0949 ± 0.0001	0.1048 ± 0.0001	10.43%	171	0.000034
Cell 7-2*	0.1112 ± 0.0001	0.1228 ± 0.0001	10.43%	175	0.000033
Cell 7*	0.2061 ± 0.0002	0.2276 ± 0.0001	10.43%	244	0.000017
Cell 8-1 (Blank)*	0.1074 ± 0.0001	0.1174 ± 0.0002	9.31%	114	0.000077
Cell 8-2 (Blank)*	0.1153 ± 0.0001	0.1271 ± 0.0001	10.23%	355	0.0000079
Cell 8 (Blank) *	0.2227 ± 0.0002	0.2446 ± 0.0003	9.83%	182	0.000030

*: There was a statistically significant difference of the weights before and after the experiment.

Table 3. Weights of the Glove Specimens before and after the Triple Layers Experiment

	Before the experiment Mean (g) ± SD	After the experiment Mean (g) ± SD	Increase	Student t	p-value
Cell 9-1*	0.0943 ± 0.0001	0.1030 ± 0.0001	9.23%	262	0.000015
Cell 9-2*	0.1014 ± 0.0001	0.1105 ± 0.0001	8.97%	272	0.000014
Cell 9-3*	0.0948 ± 0.0002	0.1022 ± 0.0001	7.81%	112	0.000080
Cell 9*	0.2904 ± 0.0002	0.3157 ± 0.0002	8.71%	379	0.0000070
Cell 10-1*	0.0981 ± 0.0001	0.1062 ± 0.0001	8.26%	121	0.000068
Cell 10-2*	0.0987 ± 0.0001	0.1082 ± 0.0001	9.63%	283	0.000012
Cell 10-3*	0.1050 ± 0.0001	0.1143 ± 0.0001	8.86%	139	0.000052
Cell 10*	0.3018 ± 0.0002	0.3286 ± 0.0002	8.88%	223	0.000020
Cell 11-1*	0.0905 ± 0.0001	0.0976 ± 0.0001	7.85%	215	0.000022
Cell 11-2*	0.1028 ± 0.0001	0.1113 ± 0.0001	8.27%	253	0.000016
Cell 11-3*	0.0858 ± 0.0001	0.0929 ± 0.0001	8.28%	215	0.000022
Cell 11*	0.2791 ± 0.0002	0.3018 ± 0.0002	8.13%	258	0.000015
Cell 12-1 (Blank)*	0.1042 ± 0.0001	0.1117 ±0.0002	7.20%	24.1	0.0017
Cell 12-2 (Blank)*	0.1091 ± 0.0001	0.1176 ± 0.0001	7.79%	254	0.000016
Cell 12-3 (Blank)*	0.1021 ± 0.0002	0.1100 ± 0.0001	7.74%	79.0	0.00016
Cell 12 (Blank) *	0.3155 ± 0.0003	0.3393 ± 0.0002	7.54%	412	0.0000059

*: There was a statistically significant difference of the weights before and after the experiment.

3.2.2 *Thicknesses*

The thicknesses of the glove specimens before and after the single layer, double layers, and triple layers experiments are shown in Tables 4, 5 and 6, respectively. The glove pieces from cells 5, 6, and 7 from double layers experiment and those from cells 9, 10, and 11 from triple layers experiment had significant differences in thickness between before and after the experiment at $\alpha = 0.05$. None of glove pieces from the blank cells had significant differences in thickness between before and after the experiment at $\alpha = 0.05$. The most swelling that occurred was for cell 3 of single layer experiment with a swelling at 23.21 %. The least swelling that occurred was for cell 12 of triple layers experiment, which was a blank cell, with a swelling at 2.65 %. The average swelling was statistically greater than for the blank.

Table 4. Thicknesses of the Glove Specimens before and after the Single Layer Experiment

	Before the experiment Mean (g) ± SD	After the experiment Mean (g) ± SD	Swelling	Student t	p-value
Cell 1	0.055 ± 0.005	0.067 ± 0.003	22%	2.53	0.127
Cell 2	0.060 ± 0.004	0.069 ± 0.001	15.0%	3.58	0.0701
Cell 3	0.056 ± 0.007	0.069 ± 0.001	23%	3.54	0.0712
Cell 4 (Blank)	0.060 ± 0.002	0.060 ± 0.002	0.0%	1.00	0.423

From Table 5 and 6, since the glove pieces marked with 2 (for double layers experiment) or 3 (for triple layers experiment) were placed closer to the challenge sides, while the differences of thicknesses between before and after the tests were larger (glove pieces were more swollen), which proved that the direction of permeation was from the challenge sides to the collection sides.

Table 5. Thicknesses of the Glove Specimens before and after the Double Layers Experiment

	Before the experiment Mean (g) ± SD	After the experiment Mean (g) ± SD	Swelling	Student t	p-value
Cell 5-1	0.059 ± 0.001	0.062 ± 0.003	5.1%	1.47	0.270
Cell 5-2*	0.060 ± 0.002	0.065 ± 0.002	8.3%	13.0	0.00587
Cell 5*	0.120 ± 0.002	0.127 ± 0.002	5.8%	4.58	0.0445
Cell 6-1	0.064 ± 0.001	0.065 ± 0.001	1.6%	2.50	0.130
Cell 6-2*	0.059 ± 0.001	0.064 ± 0.001	8.5%	14.0	0.00506
Cell 6*	0.123 ± 0.001	0.129 ± 0.001	4.9%	19.0	0.00276
Cell 7-1	0.063 ± 0.002	0.065 ± 0.001	3.2%	3.46	0.0742
Cell 7-2*	0.071 ± 0.001	0.074 ± 0.002	4.2%	5.50	0.0315
Cell 7*	0.134 ± 0.002	0.139 ± 0.003	3.7%	17.0	0.00344
Cell 8-1 (Blank)	0.062 ± 0.003	0.064 ± 0.002	3.2%	2.00	0.184
Cell 8-2 (Blank)	0.063 ± 0.002	0.066 ± 0.001	4.8%	1.75	0.222
Cell 8 (Blank)	0.125 ± 0.001	0.129 ± 0.002	2.8%	3.61	0.0691

*: There was a statistically significant difference of the weights before and after the experiment.

Table 6. Thicknesses of the Glove Specimens before and after the Triple Layers Experiment

	Before the experiment Mean (g) ± SD	After the experiment Mean (g) ± SD	Swelling	Student t	p-value
Cell 9-1	0.062 ± 0.001	0.069 ± 0.003	11.3%	3.05	0.0928
Cell 9-2*	0.063 ± 0.001	0.071 ± 0.001	12.7%	13.9	0.00517
Cell 9-3*	0.061 ± 0.002	0.069 ± 0.002	13.1%	25.0	0.00160
Cell 9*	0.187 ± 0.004	0.210 ± 0.003	12.3%	7.67	0.0166
Cell 10-1	0.062 ± 0.002	0.070 ± 0.003	12.9%	2.52	0.128
Cell 10-2	0.062 ± 0.001	0.072 ± 0.003	16.1%	4.00	0.0572
Cell 10-3*	0.063 ± 0.003	0.071 ± 0.003	12.7%	6.38	0.0237
Cell 10*	0.188 ± 0.004	0.213 ± 0.003	13.3%	4.40	0.0480
Cell 11-1	0.060 ± 0.001	0.065 ± 0.001	8.3%	3.50	0.0728
Cell 11-2*	0.062 ± 0.002	0.069 ± 0.001	11.3%	4.35	0.0491
Cell 11-3*	0.059 ± 0.002	0.064 ± 0.001	8.5%	4.91	0.0390
Cell 11*	0.181 ± 0.004	0.198 ± 0.002	9.4%	5.03	0.0373
Cell 12-1 (Blank)	0.063 ± 0.002	0.064± 0.001	1.6%	1.89	0.199
Cell 12-2 (Blank)	0.063 ± 0.002	0.064 ± 0.002	1.6%	0.87	0.478
Cell 12-3 (Blank)	0.063 ± 0.002	0.065 ± 0.000	3.2%	2.65	0.118
Cell 12 (Blank)	0.189 ± 0.003	0.194 ± 0.001	2.7%	3.27	0.0820

*: There was a statistically significant difference of the weights before and after the experiment.

3.3 *Permeation*

3.3.1 *Permeation Curves*

The cumulative permeation versus time data for each single, double, and triple gloving data are shown in Tables 7 through 9. The cumulative permeation curves of each test cell are shown in Figures 2 through 7.

Table 7. Cumulative Permeation of Cyclohexanol through Single Layer over Time

Permeation Time (mins)	Mass in Collection Side (µg)			
	Cell 1	Cell 2	Cell 3	Mean ± SD
0	6.97	5.80	6.23	6.34 ± 0.59
5	6.98	5.98	6.30	6.42 ± 0.51
10	7.12	6.75	6.37	6.74 ± 0.37
20	7.22	6.86	6.44	6.84 ± 0.39
30	50.7	152	44.1	82 ± 60
60	$2.98*10^3$	$4.50*10^3$	$6.74*10^2$	$(2.7 \pm 1.9)*10^3$
120	$8.12*10^3$	$1.68*10^4$	$7.12*10^3$	$(10.7 \pm 5.3)*10^3$
240	$4.91*10^4$	$6.98*10^4$	$4.08*10^4$	$(53.2 \pm 1.5)*10^3$
360	$2.97*10^5$	$3.03*10^5$	$2.18*10^5$	$(27.2 \pm 4.8)*10^4$
480	$5.40*10^5$	$5.56*10^5$	$3.90*10^5$	$(49.5 \pm 9.1)*10^4$

Table 8. Cumulative Permeation of Cyclohexanol through Double Layers over Time

Permeation Time (mins)	Mass in Collection Side (µg)			
	Cell 5	Cell 6	Cell 7	Mean ± SD
0	0.886	1.31	0.889	1.03 ± 0.24
5	1.02	1.80	1.18	1.33 ± 0.41
10	1.25	1.91	1.21	1.46 ± 0.39
20	1.86	2.00	1.58	1.81 ± 0.21
30	2.32	2.23	1.73	2.09 ± 0.32
60	6.61	12.0	4.79	7.8 ± 3.7
120	$2.51*10^2$	$5.09*10^2$	$2.90*10^2$	$(3.5 \pm 1.4)*10^2$
240	$9.55*10^3$	$1.68*10^4$	$1.18*10^4$	$(12.7 \pm 3.7)*10^3$
360	$2.49*10^4$	$4.18*10^4$	$2.71*10^4$	$(31.3 \pm 9.2)*10^3$
480	$8.16*10^4$	$1.00*10^5$	$1.21*10^5$	$(10.1 \pm 2.0)*10^4$

Table 9. Cumulative Permeation of Cyclohexanol through Triple Layers over Time

Permeation Time	Mass in Collection Side (µg)			
(mins)	Cell 9	Cell 10	Cell 11	Mean ± SD
0	0.377	0.0703	1.15	0.53 ± 0.56
5	1.09	0.800	1.18	1.02 ± 0.20
10	1.20	1.17	1.65	1.34 ± 0.27
20	1.21	1.37	2.43	1.67 ± 0.66
30	1.27	1.40	1.40	1.36 ± 0.075
60	2.04	1.44	1.44	1.64 ± 0.35
120	2.16	2.65	2.39	2.40 ± 0.25
240	$5.27*10^2$	$7.09*10^2$	$8.10*10^2$	$(6.8 \pm 1.4)*10^2$
360	$2.78*10^3$	$3.86*10^3$	$2.56*10^3$	$(30.6 \pm 6.9)*10^2$
480	$9.99*10^3$	$1.32*10^4$	$1.03*10^4$	$(11.2 \pm 1.8)*10^3$

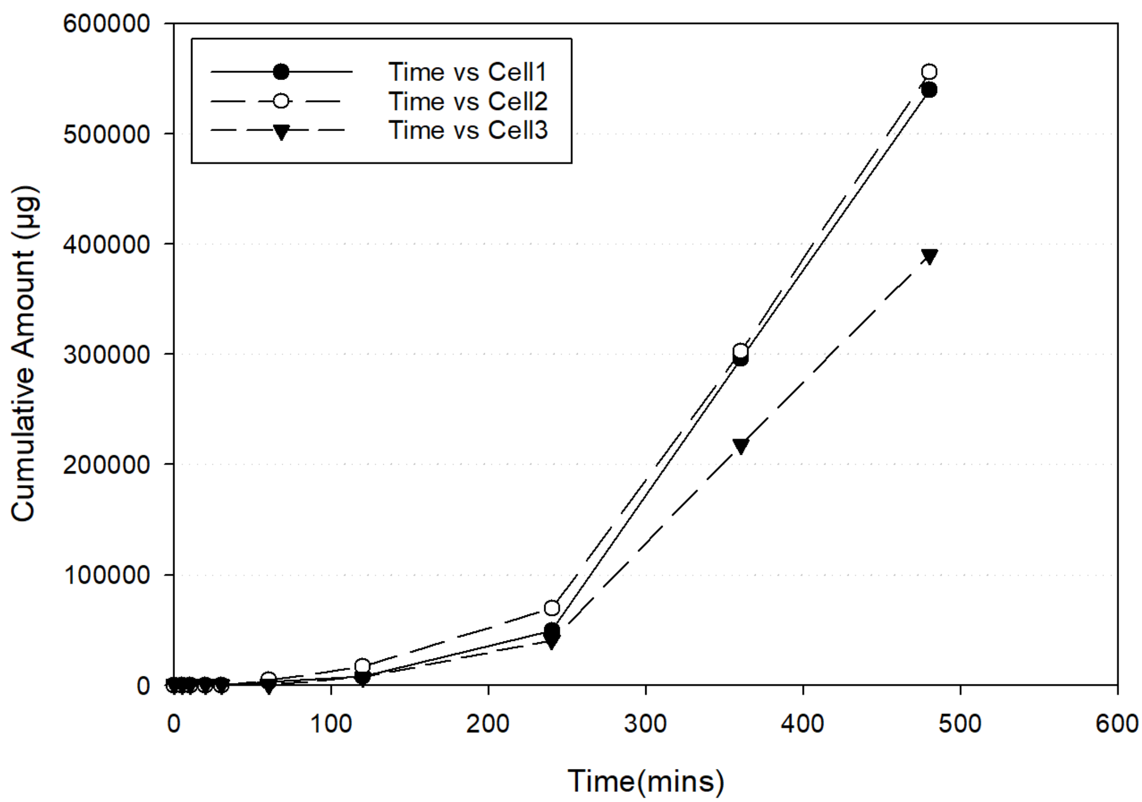


Figure 2. Cumulative Permeation of a Single Layer

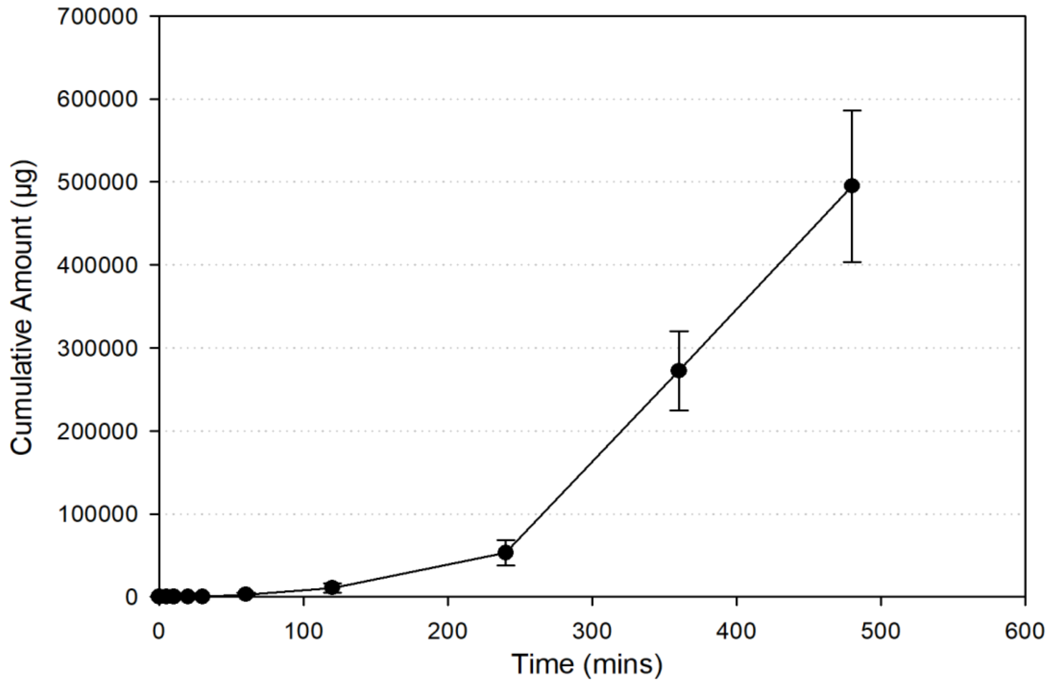


Figure 3. Average Cumulative Permeation of a Single Layer

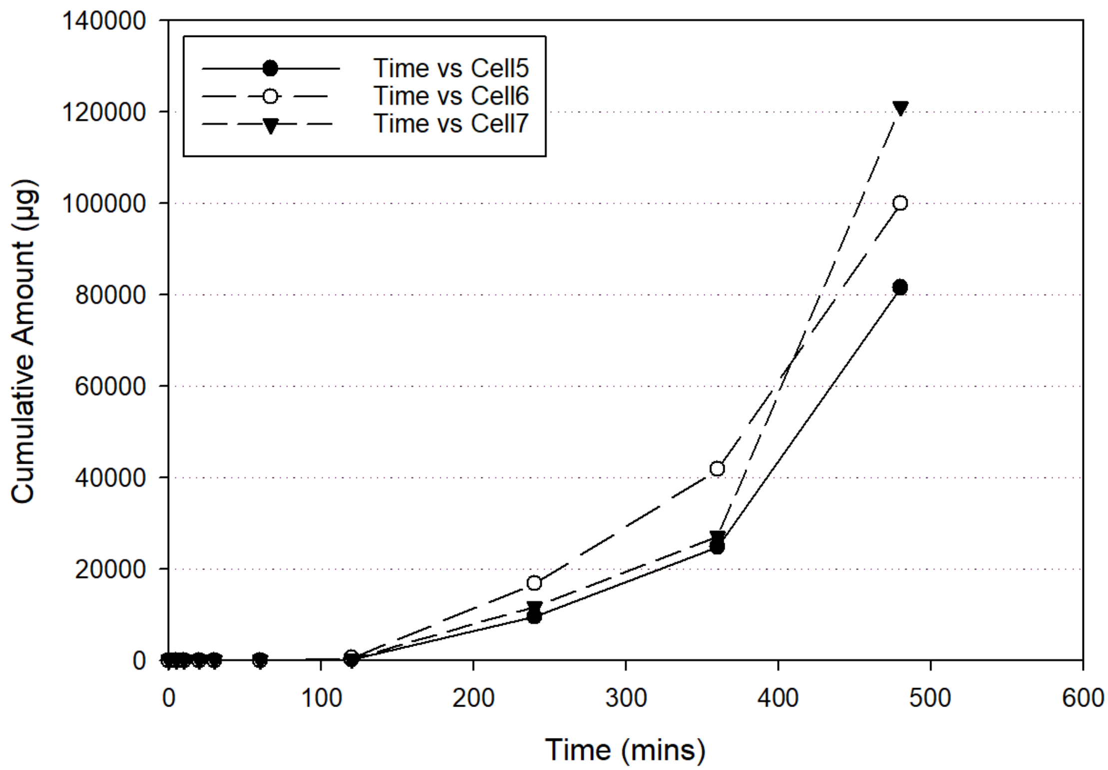


Figure 4. Cumulative Permeation of Double Layers

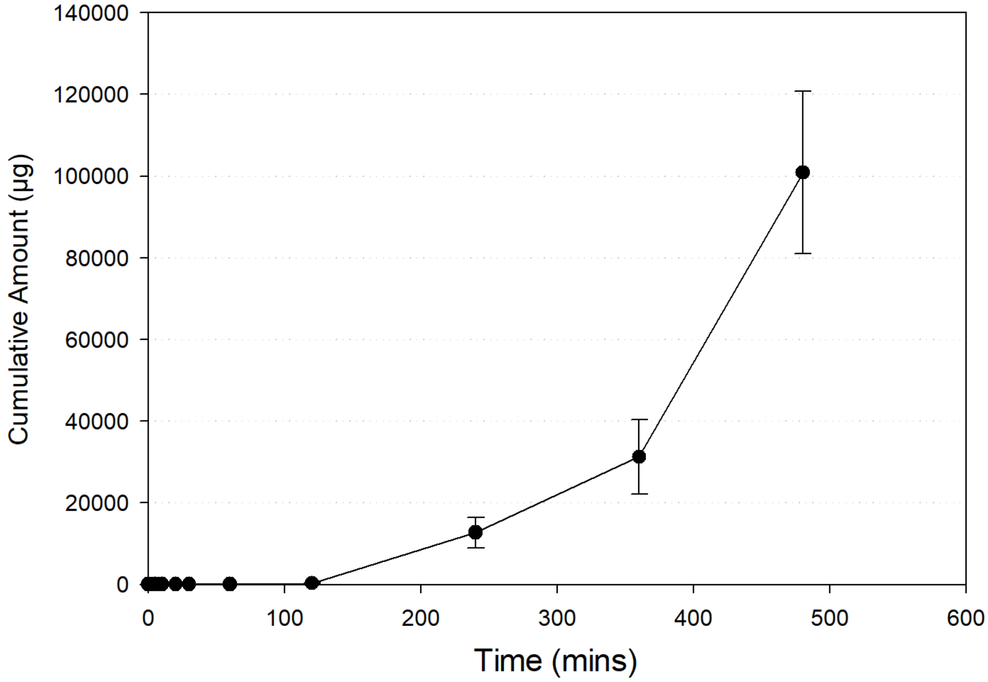


Figure 5. Average Cumulative Permeation of Double Layers

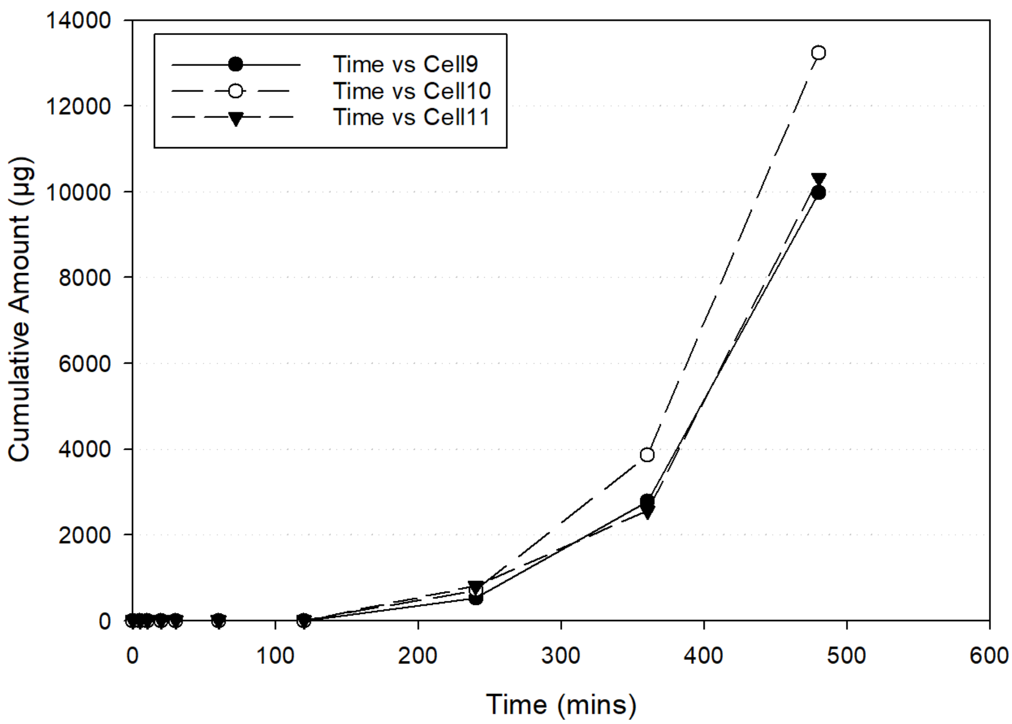


Figure 6. Cumulative Permeation of Triple Layers

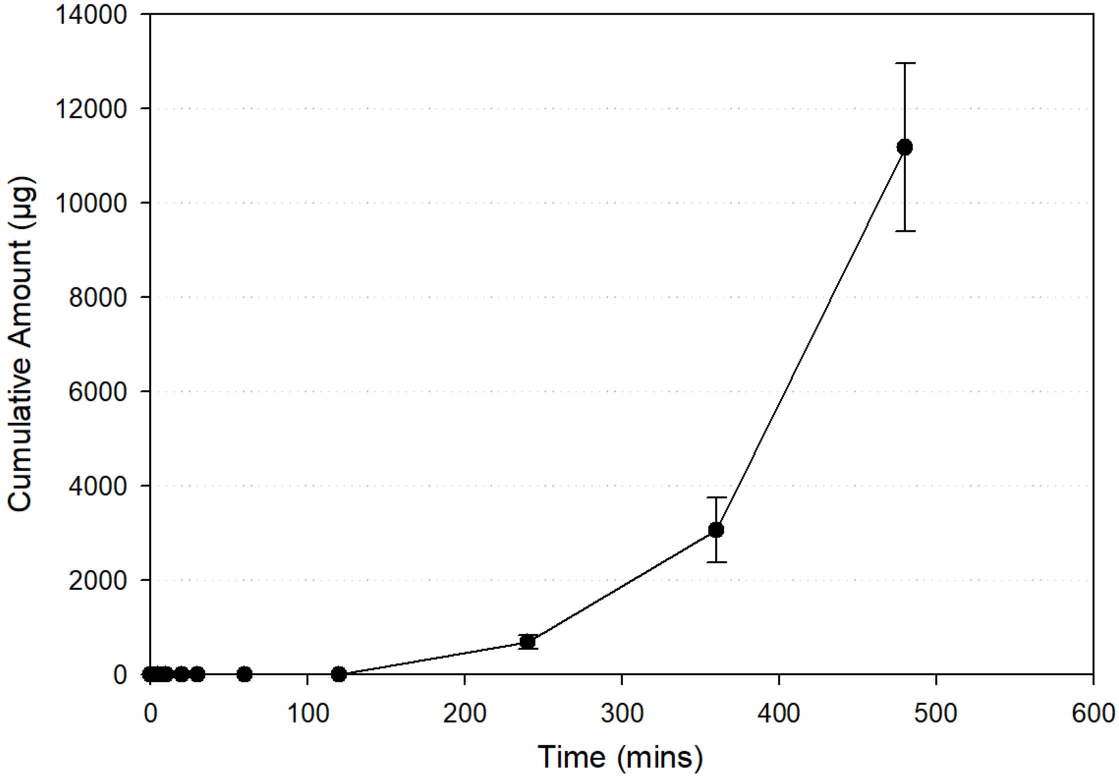


Figure 7. Average Cumulative Permeation of Triple Layers

The permeation rates versus time data for each single, double, and triple gloving data are shown in Tables 10 through 12. The permeation rates curves of each test cell are shown in Figures 8 through 13.

Table 10. Permeation Rate of Cyclohexanol through Single Layer over Time

Permeation Time (mins)	Permeation Rate (μg /cm^2/min) Cell 1	Cell 2	Cell 3	Mean ± SD
0	2.71	2.29	2.46	2.49 ± 0.21
5	0.000130	0.00722	0.00271	0.0034 ± 0.0036
10	0.00544	0.0303	0.00258	0.013 ± 0.015
20	0.00211	0.00217	0.00136	0.00188 ± 0.00045
30	0.857	2.86	0.743	1.5 ± 1.2
60	19.2	28.6	4.14	17 ± 12
120	16.9	40.6	21.2	26 ± 13
240	67.3	87.0	55.4	70 ± 16
360	407	383	291	360 ± 61
480	400	416	283	366 ± 72

Table 11. Permeation Rate of Cyclohexanol through Double Layers over Time

Permeation Time (mins)	Permeation Rate (μg /cm^2/min) Cell 5	Cell 6	Cell 7	Mean ± SD
0	0.350	0.517	0.351	0.406 ± 0.096
5	0.00542	0.0193	0.0115	0.0121 ± 0.0070
10	0.00877	0.00429	0.00136	0.0048 ± 0.0037
20	0.0122	0.00174	0.00704	0.0070 ± 0.0052
30	0.00894	0.00465	0.00302	0.0055 ± 0.0031
60	0.0283	0.0641	0.0201	0.038 ± 0.023
120	0.803	1.63	0.937	1.12 ± 0.45
240	15.3	26.8	18.8	20.3 ± 5.9
360	25.2	41.1	25.3	30.5 ± 9.1
480	93.2	95.8	155	115 ± 35

Table 12. Permeation Rate of Cyclohexanol through Triple Layers over Time

Permeation Time (mins)	Permeation Rate (μg /cm^2/min)			
	Cell 9	Cell 10	Cell 11	Mean ± SD
0	0.149	0.0278	0.455	0.21 ± 0.22
5	0.0282	0.0285	0.00114	0.019 ± 0.016
10	0.0193	0.0175	0.0641	0.033 ± 0.026
20	0.0143	0.0183	0.0159	0.016 ± 0.0020
30	0.0108	0.00940	0.0117	0.011 ± 0.0012
60	0.00979	0.00631	0.00558	0.0072 ± 0.0022
120	0.00221	0.00557	0.00507	0.0043 ± 0.0018
240	0.866	1.16	1.33	1.12 ± 0.23
360	3.70	5.18	2.88	3.9 ± 1.2
480	12.7	16.6	14.1	14.5 ± 2.0

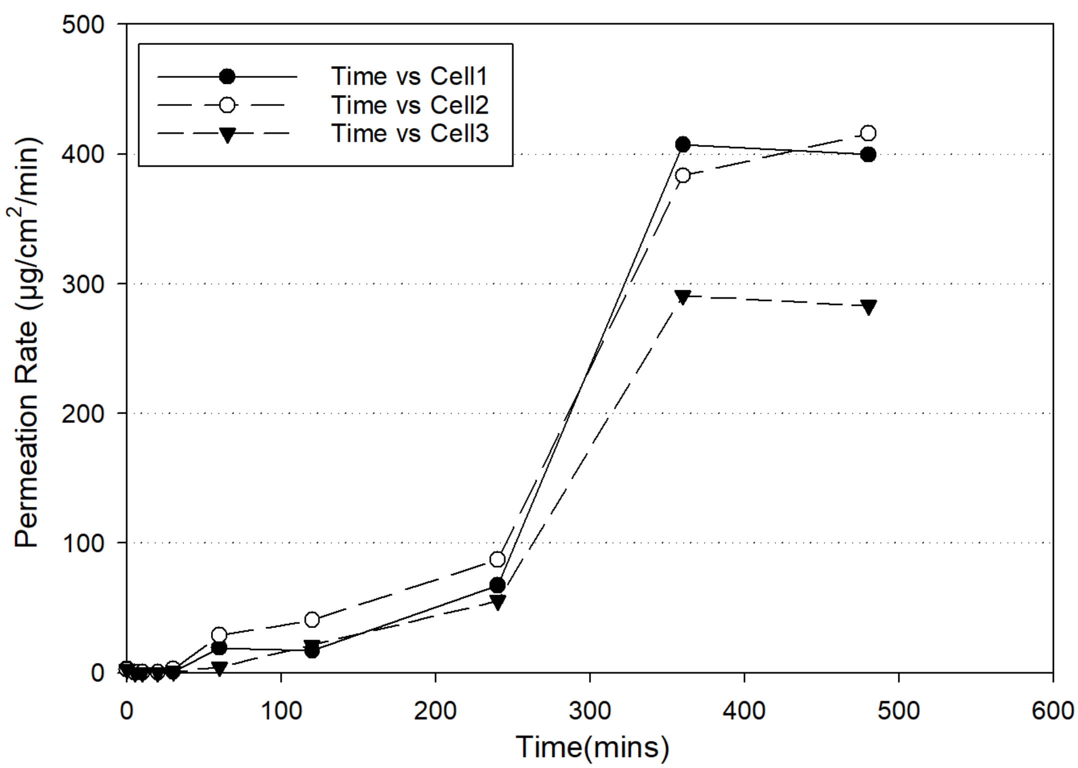


Figure 8. Permeation Rates of a Single Layer

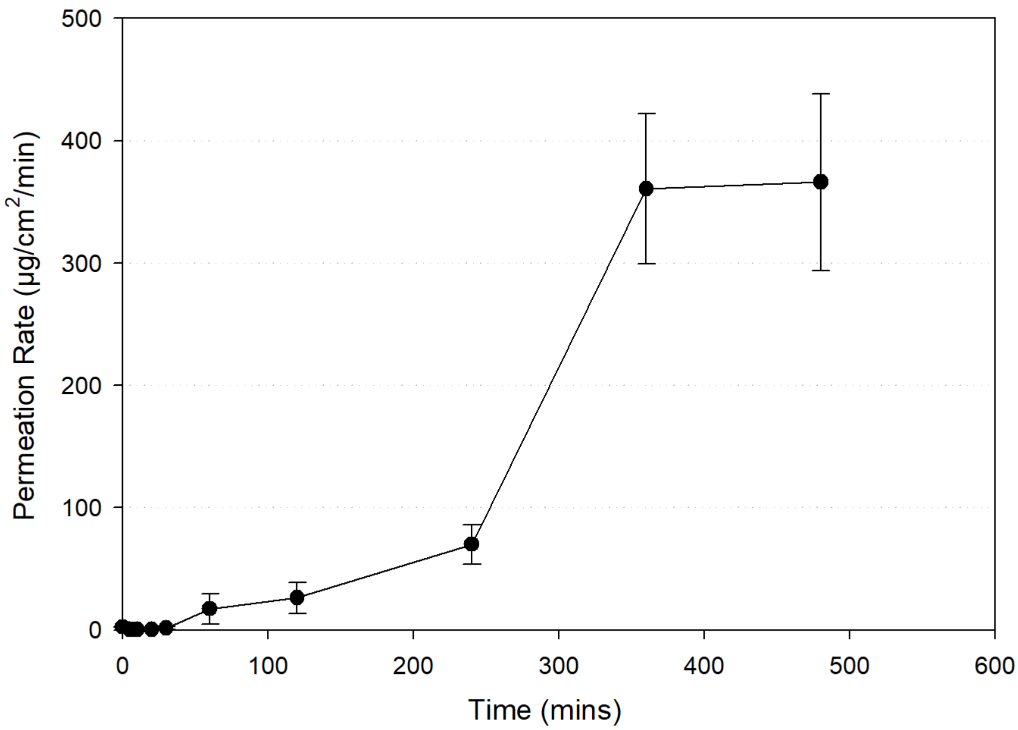


Figure 9. Average Permeation Rate of a Single Layer

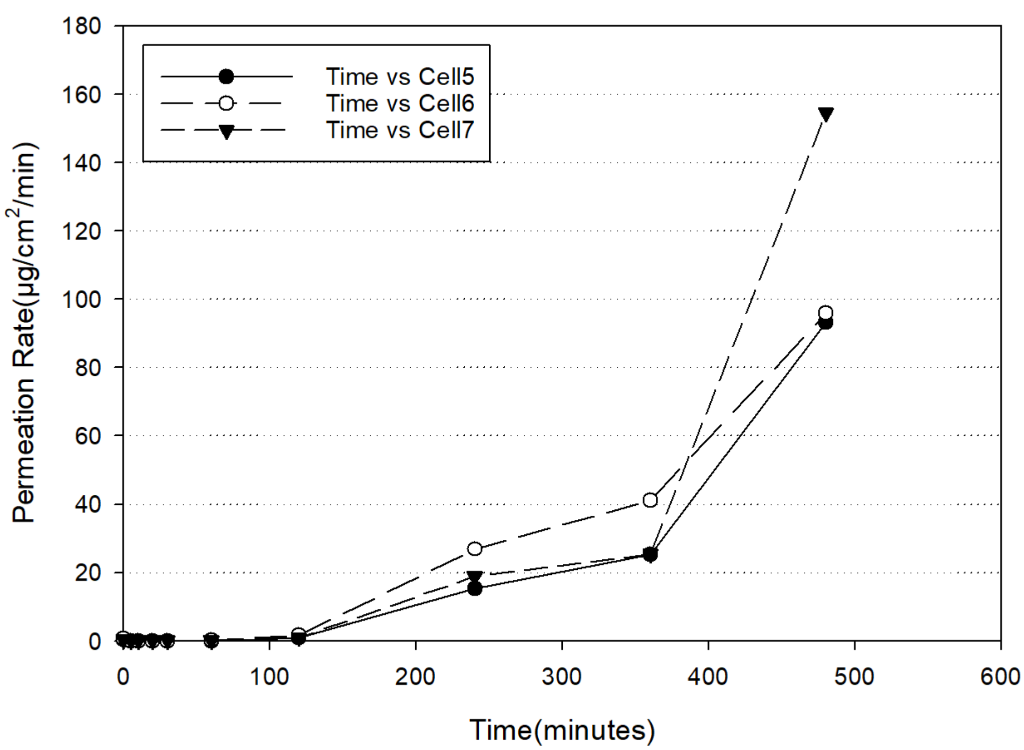


Figure 10. Permeation Rates of Double Layers

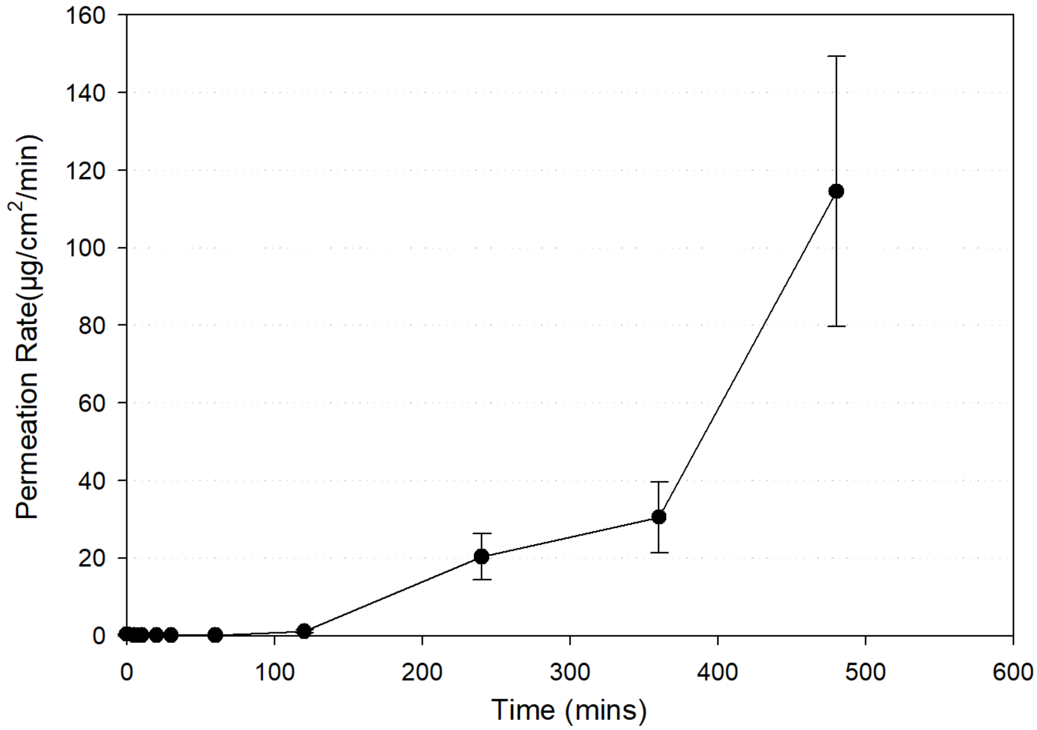


Figure 11. Average Permeation Rate of Double Layers

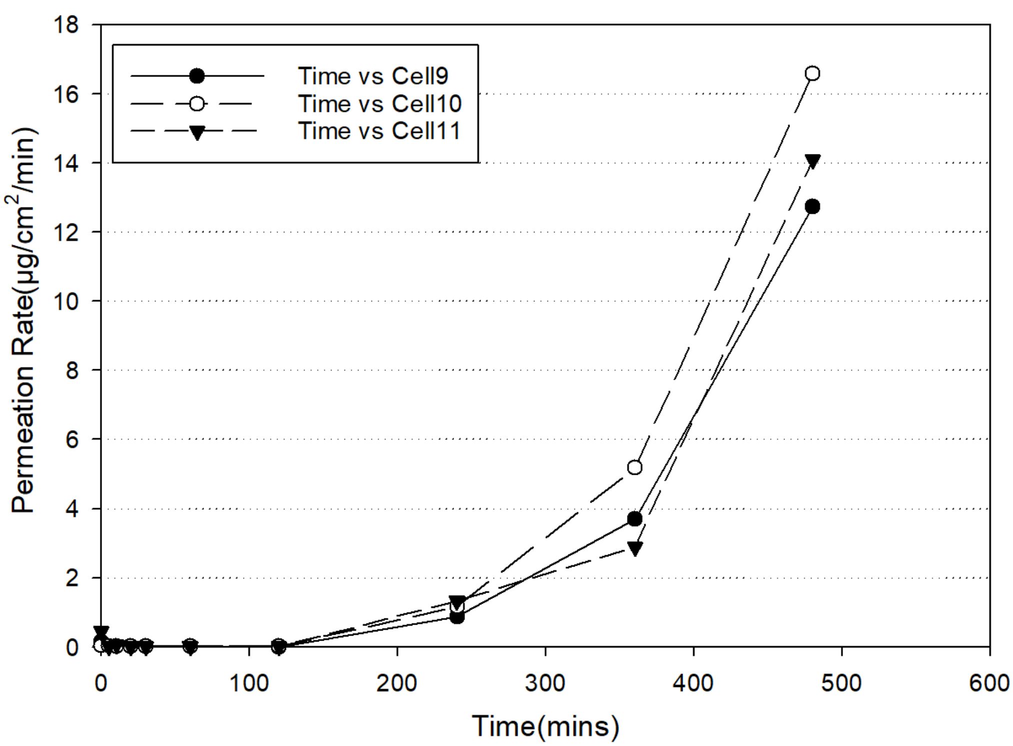


Figure 12. Permeation Rates of Triple Layers

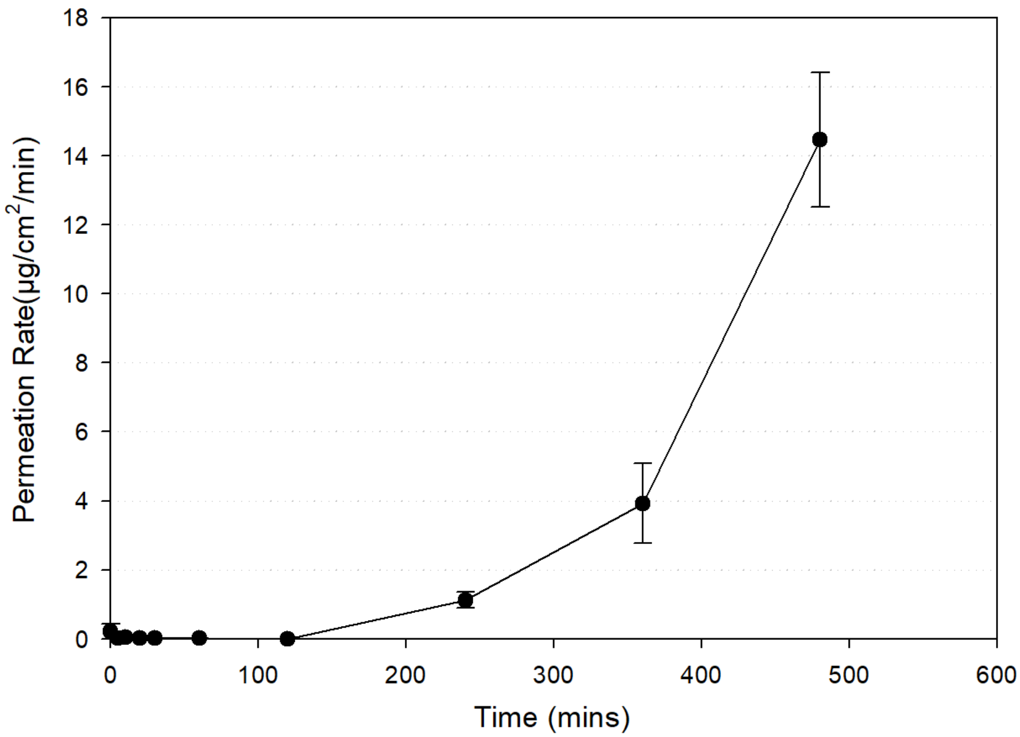


Figure 13. Average Permeation Rate of Triple Layers

3.3.2 *Permeation Parameters*

The permeation parameters of each single, double and triple layers cell are shown in Tables 13 and 14.

Table 13. Permeation Parameters of Cyclohexanol through Single Layer

	Cell 1	Cell 2	Cell 3
SBT (min)	0.25 ± 0.25	0.25 ± 0.25	0.25 ± 0.25
Average (min)		0.25 ± 0.25	
SSPR (µg/cm^2/min)	403.3 ± 5.3	399.5 ± 22.7	287.0 ± 5.6
Average(µg/cm^2/min)		363.3 ± 4.0	
L_T (min)	215	207	211
Average (min)		211 ± 4	
D*10^8 (cm^2/min) [a]	234	290	247
Average (cm^2/min)		257 ± 29	

*SSPR (Steady-state permeation rate)

*L_T (Lag time)

*SBT (Standardized breakthrough time)

*D (Diffusion coefficient): calculated from equation 1-3.

a: Not valid because of swelling

Table 14. Permeation Parameters of Cyclohexanol through Double and Triple Layers

Double Layers	Cell 5	Cell 6	Cell 7
SBT (min)	45 ± 15	45 ± 15	45 ± 15
Average (min)		45 ± 15	

Triple Layers	Cell 9	Cell 10	Cell 11
SBT (min)	180 ± 60	180 ± 60	180 ± 60
Average (min)		180 ± 60	

4. DISCUSSION

According to the Kimberly-Clark Nitrile Gloves-Chemical Resistance Guide, if the permeation breakthrough time is less than 1 minute, it is Not Recommended; 1-9 minutes, the rating is Poor; 10-59 minutes, Good and 60-480 minutes as Excellent. (24) The Kimberly Clark steady state permeation rate classification for CPC nitrile in μg/cm^2/min is <1, excellent;1-100, good; 100-10,000, poor; >10,000, not recommended. (25) The analogous Ansell steady state rate classification in μg/cm^2/min is (24): <0.9, excellent; 0.9-9, very good; 9-90, good; 90-900, fair; 900-9,000, poor; >9,000, not recommended. (26)

The single layer showed a SBT less than 0.5 minute, the rating of which is Not Recommended according to the Kimberly-Clark Nitrile Gloves-Chemical Resistance Guide, while double layers of Lavender disposable nitrile gloves had SBT at 45 ± 15 minutes the rating being Good, and triple layers of Lavender disposable nitrile gloves had SBT more than120 minutes, the rating being Excellent. The results in Figures 8 to 13 showed that only the single layer of Lavender glove reached SSPR. The SSPR in three cells were 403.3 ± 5.3 μg/cm^2/min, 399.5 ± 22.7 μg/cm^2/min, and 287.0 ± 5.6 μg/cm^2/min, respectively, and the average SSPR was 363.3 ± 4.0 μg/cm^2/min. However, the double and triple layers never reached SSPR in 8-hour experiments. Even though the single layer of Lavender nitrile gloves is not recommended for the usage of cyclohexanol, double and triple layers can protect well from cyclohexanol.

The summary of permeation parameters of single, double and triple layers against cyclohexanol is shown in Table 15.

Table 15. Summary of Permeation Parameters and Glove Safety Ratings for Cyclohexanol Challenging a Lavender Nitrile Glove

Tests	SBT [a] (min)	SSPR [b] ($\mu g/cm^2/min$)	D [c] $*10^8$ (cm^2/min)	More Protection (times) [d]
Single Layer	0.25 ± 0.25, Not Recommended	363.3 ± 4.0, Poor (KC), Fair (Ansell)	257 ± 29	1
Double Layers	45 ± 15, Good	Not applicable	Not applicable	180
Triple Layers	180 ± 60, Excellent	Not applicable	Not applicable	720

a: Kimberly-Clark (KC) safety rating follows the arithmetic mean and standard deviation

b: Ansell/Kimberly Clark safety ratings follow the arithmetic mean and standard deviation

c: Not valid because of swelling

d: Calculated based on SBTs relative to single layer data. Double layers were 45/0.25=180; Triple layers were 180/0.25=720.

There are five types of permeation behavior shown in figure 14 (7). In this study, it showed that the permeation behavior of cyclohexanol is type A, which agreed with Mathews' study (1).

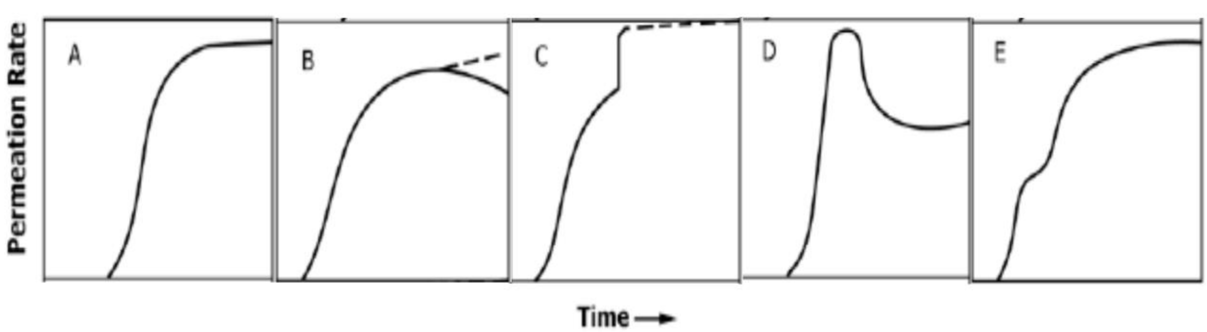


Figure 14. Five Types of Permeation Behavior

The diameters for the glove specimens used in single layer experiment were 49 mm, while in double and triple layers were 43 mm. During the experiments, there was minimal leaking for each cell. Even though the blank cells helped to minimize the impacts of the leaking, the amount in the blank cells were subtracted from the other cells. Changing the diameter for the glove specimens was one effort to decrease the leakages, because the glove pieces outside of the teflon gasket could absorb the water from the water bath or desorb the cyclohexanol into the water bath.

In this study, the torque wrench used was not 16 ft-lb as in the previous glove permeation studies (1, 6, 9), it was 10 ft-lb instead. According to Pesce Lab Sales (27), it should be finally tightened to 16 in-lb. During this study, 16 ft-lb was used to tighten the bolts first and the flanges kept breaking. After several tests, 10 ft-lb was applied to this study to make sure the force applied would not break the flanges and at the same time the leakage was acceptable.

Each permeation test was 8 hours, not 2 hours in this study. Usually, the disposable gloves would be only worn for 2 hours because there is usually a break between 2-hour work shift. However, there will always be reasons for not disposing the disposable gloves, such as cost, ignorance and accessibility. In this study, double and triple layers of Lavender nitrile gloves could provide more protection relative to single layer against cyclohexanol throughout 8-hour work shift. In terms of average SBT, double layers were 45/0.25=180 times and triple layers were 180/0.25=720 times more protective than a single layer. In terms of SSPR, the double and triple layers never reached steady state.

Lavender disposable nitrile gloves are the thinnest disposable nitrile gloves from Kimberly Clark. According to Mathews' study (1), Safeskin, Blue and Purple disposable nitrile gloves were already adequately protective against cyclohexanol throughout an 8-hour work shift, while rating of Sterling disposable nitrile gloves was poor, and it reached its SSPR. Lavender gloves are thinner than Sterling and the results agreed with the thicker the gloves were, more protective they would be. Double layers of Lavender disposable nitrile gloves were about the same as Safeskin, Blue and

Purple, but the SBT was longer than that of Safeskin, Blue and Purple according to Mathews' study (1). Triple layers of Lavender disposable nitrile gloves were much thicker and the SBT was much longer.

The results agreed with previous studies that triple and double gloving are more protective than single gloving (6, 32, 33, 34, 35, 36, 37). The method used in most of the previous studies was ASTM D6978-05 'Standard Practice for Assessment of Resistance of Medical Gloves to Permeation by Chemotherapy Drugs' (40) in accordance of F739-12 Test Method. There are several differences from ASTM F739-12 as follows. Firstly, a minimum of three glove samples should be tested. Secondly, at least nine currently used chemotherapy drugs should be tested. Thirdly, a minimum of 27 test samples should be tested. Another difference is that the experiment should be conducted at 35 ± 2℃. Most importantly, the test cells are the original ASTM F739 2-inch cells (35). 1-inch test cells were used in this study, not 2-inch test cells, since they provide equivalent permeation data and because less exposure area can reduce the possibility of penetration and produce less chemical waste. 50 ± 5% Relative Humidity was adopted from ASTM E171-94 'Standard Specification for Standard Atmospheres for Conditioning and Testing Flexible Barrier Materials' because it was more specific and in the range of 30% to 80% according to ASTM F739-12 (41). Most researchers and glove companies in the United States use the ASTM F739 to generate permeation data (1). The open loop mode of the test method with gas collection is most commonly used by glove manufacturers, and the downside is that low vapor weight may not volatilize enough (42). On the other hand, the ASTM closed loop method uses a set volume of liquid as a collection solvent and this allows for a more accurate and sensitive assessment of the permeation of semi/non-volatile chemicals, such as cyclohexanol, because the key factor is not analyte volatilization.

5. CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

There were limitations of this study. First was that glove pieces appeared to swell and the cyclohexanol exposed gloves swell significantly more than the blank, where some exceeded 10%, throughout the 8-hour permeation tests, so that the diffusion coefficients calculated based on the thicknesses before the tests were not valid. The second limitation was that the duration between taking each sample were not short enough (recommended for further research at times less than 30 min) to have accurate average SBTs. More data points are suggested in the last 6 hours. Thirdly, the shaking water bath could not simulate hand motions as well as robot hand and cannot test stretching of donned gloves. Another limitation was that this study did not test other Kimberly Clark disposable nitrile gloves, such as Kimtech Blue and Purple, and it did not test disposable nitrile gloves from other brands. The fifth limitation was that this study did not test other chemicals. The rating of cyclohexanol in Kimberly-Clark Kimtech Nitrile Gloves Chemical Resistance Guide (24) is excellent based on the permeation time at 112 minutes, which disagrees with the results in this study and Mathews' study (1). This is probably because the temperature used for Kimberly Clark resistance guide is room temperature, which is about 20-25 °C while cyclohexanol would be solid or semi-solid and would permeate much more slowly (the melting point of cyclohexanol is about 26 °C). The temperature used for each chemical should be at least over the melting point and 35 °C was recommended and used in this study because it was about the temperature of the skin when wearing disposable nitrile gloves (22, 32).

Another recommendation would be for Ansell Chemical Resistance Guide. The Ansell steady state rate classification is not in the 8th edition (29), but it exists in the 7th edition (26). It is understandable that without the classification would make the guide easier to understand, but this kind of technical data is important for scientific scrutiny.

This research investigation will help advance the science of Industrial/Occupational Hygiene through knowing the duration that workers can use disposable nitrile gloves working with cyclohexanol. In addition, this will help the workers to know that they can wear multiple layers of gloves to provide more protection. More work with other alcohols needs to be done to demonstrate how general the protective effect of multiple layers is.

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