

"AN INVESTIGATION INTO THE LOSS OF PRESERVATIVES
FROM OPHTHALMIC SOLUTIONS, DURING
FILTRATION STERILIZATION."

A Thesis presented for the Degree
Master of Science (Pharmacy)

at

Rhodes University

by

N. T. NAIDOO

B. Sc. (Pharm.) B. Sc. (Hons.)

1970

ACKNOWLEDGEMENTS

My foremost thanks go to my director, Professor C.H. Price, and my gratitude is also due to Professor T.J. McCarthy, of the University of the Western Cape, for his valuable help and encouragement during the time of his supervision.

I N D E X

	<u>Page</u>
List of Reagents used.	(v)
<u>Part I</u>	
Chapter One - Introduction	1
Chapter Two - Bacterial Filter Media	5
A) Depth Filters	5
Sintered Glass	6
Unglazed Porcelain Candles	9
Asbestos Pads	11
B) Screen Filters	13
Membrane Filters	13
Summary	16
Chapter Three - The Mechanisms of Bacterial Filtration	17
Summary	23
Chapter Four - Preservatives used in Ophthalmic Solutions	24
Chlorhexidine ('Hibitane')	27
Phenylmercuric Nitrate	29
Benzalkonium Chloride	31
Phenylethylalcohol	32
Summary	33
<u>Part II</u>	
Chapter Five - The Filtration Unit	34
Filters Used	36
Filter Units	37
a) Sintered Glass Filters	37
b) Unglazed Porcelain Candles	37
c) Asbestos Pads	38
d) Membrane Filters	39
Summary	40

	<u>Page</u>
Chapter Six - Adsorption of Chlorhexidine	41
Preparation of Beer's Law Graph	42
a) Adsorption onto Sintered Glass Filters	42
Results and Discussion	46
b) Adsorption onto Doulton-Pasteur Unglazed Porcelain Candles	62
Results and Discussion	64
c) Adsorption onto Asbestos Filter Pads	75
Results and Discussion	79
d) Adsorption onto Sartorius Membrane Filters	88
Results and Discussion	90
Summary	94
Chapter Seven - Adsorption of Phenyl-Mercuric Nitrate	98
Preparation of Beer's Law Curve	99
a) Adsorption onto Sintered Glass Filters	101
Results and Discussion	102
b) Adsorption onto Doulton-Pasteur Unglazed Porcelain Candles	109
Results and Discussion	111
c) Adsorption onto Asbestos Filter Pads	116
Results and Discussion	119
d) Adsorption onto Sartorius Membrane Filters	125
Results and Discussion	128
Summary	135
Chapter Eight - Adsorption of Benzalkonium Chloride	139
Preparation of Beer's Law Graph - Method (a)	142
Method (b) - Complexation of Benzalkonium Chloride with Bromophenol Blue	143

	<u>Page</u>
a) Adsorption onto Sintered Glass Filters	145
Results and Discussion	148
b) Adsorption onto Doulton- Pasteur Unglazed Porcelain Candles	156
Results and Discussion	159
c) Adsorption onto Asbestos Filter Pads	167
Results and Discussion	171
d) Adsorption onto Sartorius Membrane Filters	182
Results and Discussion	185
Summary	196
Chapter Nine - Adsorption of Phenyl- ethanol	203
Preparation of Beer's Law Graph	203
a) Adsorption onto Sintered Glass Filters	204
Results and Discussion	205
b) Adsorption onto Doulton- Pasteur Unglazed Porcelain Candles	205
Results and Discussion	206
c) Adsorption onto Asbestos Filter Pads	206
Results and Discussion	208
d) Adsorption onto Sartorius Membrane Filters	208
Results and Discussion	210
Summary	211
<u>Part III</u>	
Chapter Ten - Concluding Discussion	213
Bibliography	220

LIST OF REAGENTS

The Preservatives and Chemical reagents used in the experiments in this work are as follows:-

Chlorhexidine Acetate B.P.C. ('Hibitane' - Imp.Chem. Ind.)

Phenylmercuric Nitrate laboratory grade

Benzalkonium Chloride 50% w/v

2-Phenyl-ethanol (British Drug Houses Ltd.)

Dithizone (Diphenylthiocarbazone)

Bromophenol Blue (Merck)

Chloroform (Analytical grade)

1,2-Dichloroethane (Merck -Analar. extra pure grade)

Sodium Acetate Anhydrous (Merck, A.R.)

Sodium Dihydrogen Phosphate (Merck, A.R.)

Sodium Phosphate Anhydrous (Merck, A.R.)

Sodium Edetate (Diodium salt of Ethylene-diamine-tetra-acetic acid) (B.D.H., Analar.)

Sodium Chloride (B.D.H., Analar.)

Sodium Carbonate (A.R.)

Sodium Nitrate (B.D.H., Analar.)

Glacial Acetic Acid

Dilute Hydrochloric Acid (lab. grade)

Concentrated Sulphuric Acid (lab. grade)

Dilute Nitric Acid (lab. grade)

PART I

Most eye drops are dispensed in multi-dose containers, hence protection should be given against microbial contamination during use. To remain sterile during it's use, the solution should contain a preservative in such concentration so that it kills micro-organisms in a short time. It has been stated in the literature^{7,8,9} that the instillation of contaminated eye drops constitutes a greater danger than the intravenous injection of a contaminated solution, since the cornea is non-vascular and lacks the normal antimicrobial defences of the blood stream.¹⁰ Amongst the preservatives used, those included in the official formulations for eye drops are Chlorhexidine Acetate, Phenyl Mercuric Nitrate, Benzalkonium Chloride, and to some extent Phenylethanol, which may be used in combination with Benzalkonium Chloride.

The main purpose of this investigation has been to determine the adsorption of these preservatives by different filters during sterilization by filtration. The filters used for sterilization are known as bacteriological, bacterial, or bacteria-proof filters and several types^{6,11,12} are available. These may be of sintered glass, which is made from finely ground borosilicate glass, unglazed porcelain candles, compressed mats composed largely of asbestos fibres, and porous membranes of cellulose nitrate or other esters.

The essential feature of a sterilizing filter is that the pore size should be sufficiently small to prevent the passage of the smallest bacteria. However the pore size of the filter does not necessarily need to be smaller than that of the micro-organisms to be removed,¹³ and in some instances the average pore diameter may be about three times that of the micro-organisms. It is obvious therefore that the process does not depend on simple mechanical filtration and other factors that play a part include the thickness of the filter bed, capillarity, electrical charges on the filter and the bacteria, and the hydrogen-ion concentration of the solution being filtered.

The phenomenon of the possible adsorption of organic or inorganic compounds onto polymeric materials such as Nylon¹⁴ polyethylene, cellulose dialysis membranes and regular filter paper has been well recognized in the past. Filter pads made from cellulose esters or similar polymeric materials have been widely used in both analytical and biological work.¹⁵⁻²⁵ However, the possible adsorption of chemicals onto these filters has been generally underestimated or not noticed by research workers. A systematic investigation by Win and Smith²⁶ of the potential effect of these filters on both qualitative and quantitative chemical analysis, was begun after an accidental discovery of the surprisingly high adsorption of a water-soluble organic compound after filtration.

Van Ooteghem and Herbots²⁷ determined the adsorption of preservatives used in ophthalmic solutions onto membrane filters, during bacterial filtration, and observed that adsorption takes place principally at the beginning of the filtration, and the amount adsorbed differs for each preservative. On investigating the effect of particulate solids on various types of preservatives, McCarthy^{28, 29} observed a loss of Chlorhexidine Acetate, from solution onto filter paper. Consequently Chlorhexidine Acetate as used in official eye drops, was filtered through a Millipore membrane, a 5 on 3 sintered glass filter³⁰ and filter paper. The unexpected and significant losses which were observed, thus led to a comprehensive investigation of the adsorption of preservatives by filtering media.

CHAPTER TWO

BACTERIOLOGICAL FILTER MEDIA

Bacteriological filters are made in such a variety of shapes, sizes and media, that success in terms of satisfactory performance will often hinge upon the correct choice of filter medium with reference to subtle differences in characteristics. Sterilizing filtration is one example where even 99.995% efficiency is not good enough, and every viable organism must be removed, with the minimum amount of adsorption of medicaments or preservative from solution. Two general classes^{31, 32} of filters are currently available for bacterial filtration. These are:

- A) The DEPTH type filter
- B) The SCREEN type membrane filter.

A) DEPTH FILTERS.

They consist usually of fibres, particles, or fragmented material of some sort that has been pressed or bonded together to form a torturous maze of flow passages. Although the pores of the depth filter can be made coarse or fine, depending on the degree of compaction or the size of the fibres, flow channels are inherently random and variable in size. The principal mechanism of bacterial retention is random adsorption and entrapment. Retention is generally controlled by changing the density of the structure with pressure or heat, or sometimes impregnating fibres

with resinous compounds.

In general, depth filters are characterized by their increased filter thickness, random adsorption of dissolved or colloiddally dispersed active principles, coloring matter, and undesirable contaminants, and the absorption of a sizeable portion of the liquid being filtered. With depth filters there is usually a decrease in flow rate with time, caused by the build up in the amount of material retained within the layers and channels of the filter itself. Examples^{1,6,11,13} of bacterial retention on depth filters used in the investigation, were ultrafine porosity sintered glass, unglazed porcelain candles and asbestos pads.

1) Sintered Glass.

This medium is popular for sterilizing small volumes.¹² The manufacture and uses of sintered (fritted) glassware has been described by Cooper⁶ and Sykes.¹ It is manufactured by powdering high-grade borosilicate glass in a ball mill, separating the finest particles by air-elutriation, and sintering appropriate sizes into discs in suitable moulds, by heating to a temperature just below fusion point, thus causing the particles to adhere together but leaving the disc porous. The discs are usually fused into Buchner-type glass holders or sometimes into pipe-line glass holders when used for clarification purposes. (Fig 2.1)

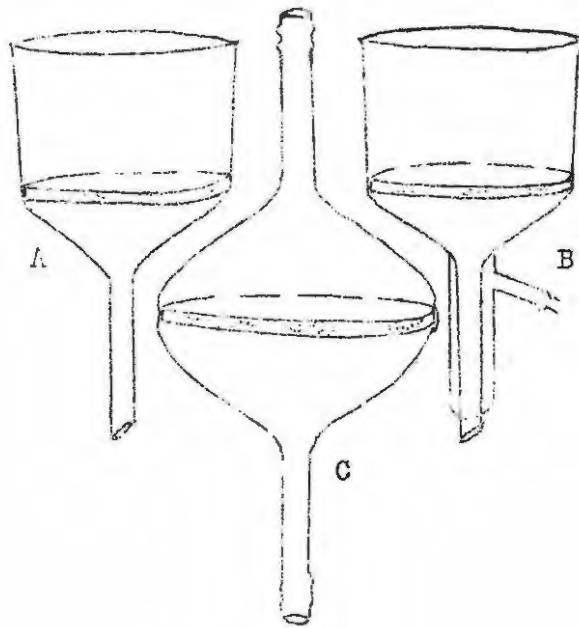


Fig. 2.1

- A. Buchner type. B. Buchner type with side arm.
 C. Line filter.

They are graded according to pore size and are available in a range of porosities, the coarsest grade 00 have pore sizes normally from 250 - 400 microns and the finest, grade 5, from 1 - 1.5 microns. A British Standards specification (B.S. 1752) gives specifications for quality, pore size and permeability. Their standard porosities are described in the following table.

TABLE 2.1

<u>Porosity</u>	<u>Pore Size Range</u> (microns)	<u>Pore Size (microns)</u> <u>Generally Supplied</u>
No. 00	Between 200-500	250-400
" 0	" 150-200	120-200
" 1	" 90-150	110-130
" 2	" 40-90	55-75
" 3	" 15-40	20-30
" 4	" 5-15	7-12
" 5 on 3	" 0.7-3	1-1.5

Filters of porosity No. 3 and 4 are widely used in pharmacy for the clarification of ophthalmic and parenteral solutions, whilst grade 5 (maximum pore size not more than 2 microns) is used for removing bacteria. Filtration is slow through filters in which the maximum pore size is less than 1.5 microns. This is also the case when the disc is thick, but because thin discs cannot withstand the pressures and vacua of bacterial filtration, it is customary to use a combination filter by supporting a thin layer of grade 5 on a thicker layer of grade 3 (a clarifying grade). Consequently, bacteria-proof sintered glass filters are usually known as 5-on-3 (pore size 0.7 - 3.0 microns). (Fig 2.2)



Fig. 2.2

5 on 3 sintered glass filter.

The efficiency of sintered glass filters is chiefly determined by their microstructure, which cannot be easily made visible by normal electron microscope methods. Also by reflected light microscopy the details of their microstructure

are not made evident in a satisfactory manner because of the low depth of the field. Micrographs³³ obtained from an inclined-beam illumination technique³³ using thin sections, indicated a sphere packing arrangement for sintered glass filters.

Sintered glass filters carry a negative charge which is mainly responsible for the adsorption phenomenon. They are made from borosilicate glass, and do not alter the pH of solutions passing through them, nor do they yield any soluble or particulate matter, such as fibres to solution filtrates. Their porosity is not affected by heat sterilization, and they are easily cleaned by passing through strong sulphuric acid, followed by thorough washing through with distilled water, and drying.

2) Unglazed Porcelain Candles.

Early filters of this type such as the Pasteur-Chamberland, were produced by sintering a mixture of kaolin (China Clay) and quartz sand, but modern forms are made from finely ground unglazed porcelain. Examples⁶ are the Doulton candles. Stock sizes of Doulton Candles range from 55mm long 10mm diameter to 205mm long and 25mm diameter. Their walls are about 3mm thick. Some have glazed porcelain mountings (Fig. 2.3 a and b) to facilitate incorporation into filtration units. These mounts are attached by glazed joints to avoid the use of cement, which is a potential source of weakness. Unmounted candles with a glazed top

(Fig. 2.3 c) are also available.

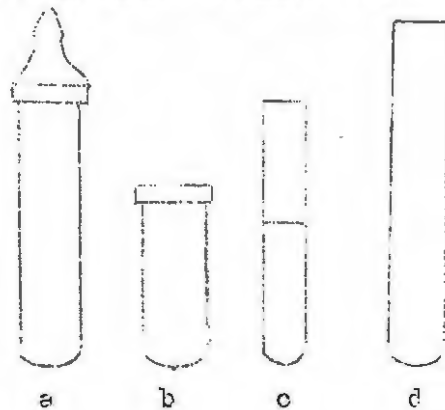


Fig. 2.3

Unglazed porcelain filters.

The filters are obtainable in a number of grades of porosity, only one or two of which are made as sterilizing filters, e.g. the Doulton No. 1 Pasteur Candle. The maximum pore size is usually between 2 and 2.5 microns. The flow rate through a filter of maximum pore size, 0.75 micron is only about 12% of the rate when the maximum pore size is 2 μ .

In general the filters will retain organisms about $\frac{1}{4}$ the size of the pore. They are robust and stand up well to handling, to filtration pressures and vacua, and to drastic cleaning methods. They are usually cleaned with hot dilute Hydrochloric Acid, to remove adsorbed substances, followed by a thorough washing through with distilled water, and subsequent drying. Enlargement of the pore size with use is slight and the life of the filter may be considerable.

3) Asbestos Pads.

The asbestos pad provides positive clarification and sterile filtration for most solutions.^{34, 35} These are soft pads about 3mm thick, usually round but occasionally square, consisting largely of compressed asbestos, but other fibrous or granular materials such as wood cellulose and alkaline earths such as magnesium, are admixed as binders and fillers. They are manufactured in sheets which are cut into discs of diameter 3.6, 6 and 14 cms or squares, which are available in several grades,³⁶ the finest being suitable for bacterial filtration. Several sterilizing grades (see table 2.2) e.g. grades EKS (S.E.) and S. 10 (G.S.) are manufactured by Carlson-Ford and are known as "Ford's Sterimats".

TABLE 2.2

Grades and Classifications of Asbestos Filter Pads.

<u>Grades</u>	<u>Classification</u>
PYR EKS2 EKS EK S10	Sterilizing
S9 8 7 7A	Fine Clarifying
6 6A 5 5A 4	Medium
3 2 2A 1 1A 0	Coarse

The asbestos pads are easily susceptible to distortion and cracking, because of their softness and pliability, especially if wet, and are used once and then discarded.

One particular drawback with this type of filter is that on account of its chemical composition it imparts alkali, and sometimes traces of iron to the filtrates. There have been many reports¹ of basic substances in solution near their isoelectric point, being precipitated on passing through an asbestos filter pad because of its alkalinity. This could be overcome by prewashing³⁷ the filter with a dilute acid solution. Another difficulty encountered with this type of filter is that it tends to shed fibres continuously into the filtrate, a matter of some importance when final clarity of the solution is concerned, (e.g. ophthalmic and parenteral solutions), and it is therefore often followed by a grade 3 or 4 sintered glass disc. Nevertheless these filters offer a distinct advantage in their ability to hold back a variety of particulate matter including pyrogenic material which would normally pass through the newer screen type filters, but asbestos being negatively charged¹ (see Mechanism of Bacterial Filtration Chapter 3) and fibrous in nature, is strongly adsorptive, causing significant adsorption⁶ of medicaments and preservatives from solution.

B) SCREEN FILTERS.

A screen filter is a geometrically regular porous matrix which retains micro-organisms and particles on its surface primarily by a sieving mechanism. The geometry of the filter can be controlled in production, so that it is possible to state with precision, the size of the holes in the structure and therefore, the maximum size particle or micro-organism that will be able to pass through with the filtrate. Anything larger must be retained and the filter is given an efficiency rating on the basis of the largest passage that exists. A screen or a wire mesh is a good example of this kind of filter. Examples of this type of bacteria retention screen filter are the membrane filters.

Membrane Filters.

These are thin sheets of microporous plastic which contain millions of microcapillary pores of fairly uniform size (especially in the upper 10% of the filter) per unit of filter surface. They may consist of pure cellulose acetate, (Oxoid membrane filters), a mixture of cellulose esters such as Cellulose acetate and nitrate (Millipore filters manufactured by the Millipore Filter Corporation, U.S.A.) or mixtures of cellulose esters and regenerated cellulose (Membranfiltern manufactured by Sartorius Werke A.G., Germany). Ultra filters composed of nitrocellulose are also available, in porosities from 5 - 10 microns.

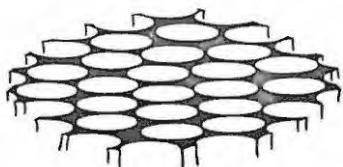
All membrane filters are used once and then discarded.

Oxoid Membranes (Oxoid manual 1961) are made in diameters from 1.7 to 14cms. The 1.7cm and 2cm sizes are issued in centrifuge and syringe type holders. The discs are about 120 microns thick and the pores range from 0.5 - 1 micron on the upper surface, and gradually widen to from 3 - 5 microns on the lower surface.

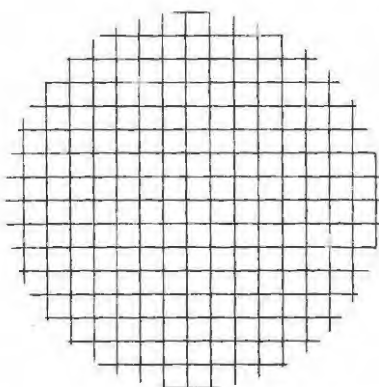
Millipore filters are available in 12 grades,³⁸ covering pore sizes from 8 - 10 microns. The HA grade which has a pore size of 0.45 (\pm 0.02) microns is generally used for bacterial filtration; but an alternative grade G.S. of pore size 0.22 (\pm 0.02) microns is recommended for elimination of very small bacterial contaminants, as some micro-organisms such as Pseudomonas aeruginosa when growing in a somewhat less optimal environment do not attain their normal size and remain underdeveloped. These micro-organisms are thus able to pass through the membrane and such instances have been reported³² to occur with the use of the H.A. grade of pore size 0.45 microns.

The German Sartorius membranes which were used in this investigation are produced³⁹ in several grades ranging from 1.0 - 0.1 microns in pore size. The membranes are available with imprinted 3.08mm grid - marked surfaces, facilitating statistical counts of particles of microbial colonies on the filter surface.

The pore volume of most membrane filters represents approximately 70 -85% of the total volume of the membrane (Fig. 2.4). This results in the relatively high flow rates of membrane filters.



Schematic illustration approximates large open volume (80%) of membrane filters in relation to solid filter material (20%).



This membrane filter contains close to a billion pores

Fig. 2.4.

Membrane filters are not attacked by water, dilute acids or alkalis and most hydrocarbons, but they are dissolved by ketones, esters, ethers, alcohols and nitroparaffins. The retention efficiency of these filters is unaffected by flow rate, pressure differential or by physico-chemical characteristics and amount of compatible suspended

matter.

Electron microscopic studies⁴⁰ of eight different membrane filters showed that some types possess a different surface topography on each side, probably originating in the manufacture of the filter. Remarkable differences in the distribution of the surface pore sizes in the tested filters were also evident. According to Nash³² the flow rates in selected filters from different batches can vary by as much as 50% from batch to batch.

In essence each pore acts as a direct channel through the filter. Material in the affluent, greater than the pore diameter will be collected on the upper surface of the membrane and will not be absorbed within the filter itself.

Unlike depth filters, membranes are normally inert and nonreactive, and will not alter the pH value of filtered aqueous solutions. They do not lose their integrity nor do they produce fibres or debris in the effluent or filtrate. There is relatively little adsorption of materials from solution and little absorption or liquid retention within the membrane.

SUMMARY

Two classes of bacteriological filter media have been outlined. These are Depth filters comprising, sintered glass discs, unglazed porcelain candles and asbestos pads, and Screen filters which are the membrane type.

CHAPTER THREE

THE MECHANISMS OF BACTERIAL FILTRATION

The exact mechanism by which a bacterial filter is able to function with such a high degree of efficiency is not clearly understood.

Fine porosity membrane filters retain microorganisms because their pores are smaller than bacteria. The cellulosic fibres in fibrous pads imbibe water from aqueous solutions. This causes the pad matrix to swell, with consequent reduction in the size of the interstices, and more efficient retention of bacteria. If this sieving process was the only mechanism, sintered media and fibrous pads in which the majority of the pores are larger than bacteria would not be bacteria-proof.

This immediately leads to the question as to how filter media which have mean pore sizes ranging from 5 - 20 microns are able to eliminate bacteria, some of which will be well below the 5 micron size. This could be due to the fact that other factors than that of the pure sieving effect come into play. These factors may be defined as a Depth effect,³⁶ and an Adsorption effect.

The depth effect is self-explanatory, i.e. the greater the depth, of the filter medium, then, for constant pore size, the greater the possibility of a particle becoming entrained in the capillary system rather than passing right through. An organism passing along a pore in a sintered or

fibrous filter travels a tortuous path, that has a very uneven surface. It has been estimated that there are about 2000 irregularities / cm of pore, in an unglazed porcelain candle. Bacteria may be stopped by or trapped in these hazards. This theory suggests an explanation for the penetration of sintered and fibrous media after prolonged filtration. Bacteria, originally arrested in projections in the upper part of the filter become dislodged as filtration proceeds and, after a series of further temporary retentions, eventually appear in the filtrate.

The adsorptional effect can probably be best described as either an electrostatic, or more accurately an electrokinetic effect, or possibly both. Attention was first drawn to the possible significance of electrostasis by Mudd (1922) who demonstrated by means of the selective adsorption of basic against acidic dyes, that most bacterial filters carry a negative charge. From this it was freely assumed that bacteria are adsorbed on the filter and held there by electrical attraction or "adsorption", a phenomenon which could also be applied to the adsorption of certain compounds from solution. But bacteria themselves are negatively charged, therefore any electrostatic effect must be one of repulsion rather than attraction. On the other hand, proteins play a large part in the life of the bacterial cell and they are amphoteric in nature. It is possible

therefore, that they may be adsorbed on the filter through their basic components and so cause the whole organism to be held. Acidic groups, also predominate in all bacterial species. However the overall charge of the micro organism may not be the important factor and retention may occur through the basic groups of cell components, namely the proteins mentioned.

In extenuation of this argument is the fact that the velocity of flow of water through a candle filter is only of the order of 0.1 cm/sec.

(Ronca 1950) and so, there is not a great flow pressure thrusting the micro organism through. Under these circumstances quite small changes on the filter material and on the bacteria, whether of mutual attraction or repulsion, might exert a significant influence on the passage of a particle through the filter, and so would effect its efficiency considerably.

The pH of the filtering solution would be expected to influence the mechanism involving electrostatic adsorption, by altering the basicity or acidity of the proteins. It has been shown⁶ that asbestos sterimats grade S.B. (BKS) can be made permeable to bacteria by soaking in oil before filtering an aqueous suspension of micro-organisms. It has been suggested that the oil insulates the charges on the filter. A similar effect was also shown by candle filters. Some surface active agents e.g. certain soaps and bile

salts increase the permeability of filters possibly by affecting the surface charge.

According to Cooper⁶ and Sykes¹, asbestos carries a negative charge, and is thus strongly adsorptive. However the manufacturers of the asbestos pads, Carlson-Ford³⁶ claim that asbestos fibres are positively charged, and they have demonstrated that when water, streams past asbestos fibres, an electro positive potential is created. Because micro organisms carry a negative charge, they have assumed that with the asbestos pads, the interplay of these opposite electric charges is a major factor. They have demonstrated this by filtering a suspension of yeast that had been grown on a medium containing, as phosphate, a radioisotope of phosphorus (P 32), through a filter sheet containing asbestos fibres. After the filtration the pad was sectioned and placed on a suitable photographic slide, which on development showed that the yeast cells were adjacent to the asbestos fibres in the filter sheet. A similar experiment performed with Chromobacterium prodigiosum gave the same result i.e. the bacteria that had been filtered out were all in close proximity to the asbestos fibres in the filter sheet.

A further postulate¹ to account for the "adsorption" phenomenon is based on purely mechanical considerations, which could also be considered as a sort of depth effect. The

structure of a filter can be likened to a large number of thin porous diaphragms layered in intimate proximity, each layer carrying a multitude of holes or pores. These pores are not all of the same dimensions (their actual sizes and range of sizes depend on the nature of the filter material), and so the porous nature of the filter body is determined partly by their coincidence in the proximal layers. The chances of a series of large holes co-inciding in more than a few successive layers, and so of forming a continuous uninterrupted pore over any appreciable length, must be very remote, and the chances of such a pore extending through a filter of normal thickness are virtually non-existent. Therefore, the overall picture of the structure of a filter is one of an indeterminate number of pores or short passages all of varying size and tortuousness. Furthermore, the pores themselves, by reason of the nature of the filter material, must be entirely irregular in shape; they are neither circular in cross-section, nor have they any other constant dimensions. Thus, when an organism enters a filter it comes up against numerous hazards and restrictions all of which it must negotiate successfully if it is to pass through, and in any one of which it may become lodged and trapped. It is therefore, these factors which determine the efficiency of a filter on mechanical considerations.

By viewing the various theoretical principles involved in bacterial filtration, the nature of various filter media becomes more apparent. It could possibly be supposed that the mechanisms which apply to filter out bacteria could, probably also hold true for the removal of active principles such as medicaments and preservatives from the solution being filtered.

Some compounds of very high molecular weight could be retained by the filter through absorption into the medium itself, or merely by a sieving effect. This however does not appear to be too realistic for compounds in solution, and depends on whether a depth or screen type of filter is being used, but it does seem that the factor most likely to play a major role in the withdrawal of active principles from solution, is that of the electrostatic charge of the filter. Certain compounds like bacteria may have an overall positive or negative charge, whilst other compounds may be cationic in nature, and these are likely to be held by the filter surface through electrostatic adsorption.

The fact that the pH of the solution being filtered, alters the basicity or acidity of bacterial proteins leads one to believe that the number of micro-organisms held by the filter through electrostatic adsorption, could in some way be influenced. Thus if a solution to be filtered has a reasonable level of contaminants,

these contaminants are likely to affect the number of adsorption sites available for the adsorption of active principles from the solution being filtered. Thus the fewer bacteria held by the filter surface the greater the possibility of the active constituents being adsorbed, due to the availability of the adsorption sites.

On the other hand a great deal depends on the charges held by bacteria, active constituents and the filter surface, at the time of filtration. The possibility of these active constituents in the solution being filtered, being adsorbed by bacteria or other particles in solution, which are in turn adsorbed by the filter surface, should not be overlooked.

SUMMARY

The theoretical mechanisms by means of which bacteria are retained by bacteriological filter media have been discussed. Electrostatic adsorption appears to play a major role in bacterial filtration. The possible bearing of these mechanisms on the withdrawal of active constituents such as medicaments and preservatives, from solution, during bacteriological filtration have also been considered.

CHAPTER FOUR

PRESERVATIVES USED IN OPHTHALMIC SOLUTIONS

A commonly found, and hazardous contaminant in ophthalmic solutions is Pseudomonas aeruginosa. The latter causes a serious type of corneal ulcer in ophthalmology often resulting in the loss of an infected eye. Thus among the factors to be considered in the formulation of eye drops, the most important is that of sterility.^{41, 42, 43}

Contamination⁹ of eye drops can occur during preparation, (from the water, ingredients, container, apparatus, atmosphere, or operator), or during storage and use (from atmosphere, patient or lack of care during use).

A review article by Foster⁴⁴ on the selection of a satisfactory preservative for ophthalmic solutions, indicates that this can be a very complex problem. The primary requirement is that the chosen agent shall be shown to be effective against Pseudomonas aeruginosa, since this micro-organism is both one of the most resistant to chemical destruction and at the same time one of the most serious ocular pathogens. Eye solutions can also become contaminated⁴⁵ by other bacteria (Escherichia coli, Bacillus subtilis, Staphylococcus aureus and Proteus vulgaris), and micro-organisms such as fungi, moulds and viruses, which will often result in severe ocular infections⁴⁶ which may culminate in blindness.

Thus eye drops are sterilized immediately on preparation in a manner similar to that used for the preparation of parenteral injections, (sterilization filtration or heating) and must contain an efficient bacteriostat, that will prevent microbial proliferation, or preferably a bactericide, that will deal with any contamination introduced during use.

From the data available in the literature^{45, 47, 48} it would seem that most of the available preservatives have some points to recommend them, but frequently points in their favour, are outweighed by considerable disadvantages.

Esters of para-hydroxybenzoic acid^{46, 49} are effective against Pseudomonas aeruginosa only at high irritating concentrations. Chlorocresol is an effective bactericide, but is also too irritating, and should never be used where there is corneal damage. Chlorobutol⁵⁰ has long been used as an ophthalmic preservative, however it is heat labile, slow acting, and is effective at concentrations near its saturation point. Phenylethanol (Phenylethylalcohol) although not widely used, may have value particularly in combination with organic mercurials, and certain quaternary ammonium compounds. Phenylmercuric Nitrate and Thiomersal are effective preservatives but reports³⁰ of some side effects such as mercurialentis, when used over long periods, have deterred some users. Benzalkonium Chloride appears to be a safe and

reliable preservative^{46, 51, 52} but its solubilizing effect on the corneal epithelium could be an obvious drawback. Chlorhexidine^{52, 53, 54} is an effective antibacterial but is incompatible with several inorganic salts.

Jechman⁵⁵ in a review of the modern manufacturing of ophthalmic solutions, discusses the use of Phenylmercuric Borate, Phenylmercuric Nitrate, Benzalkonium Chloride, Polymixin B. Sulphate, Thiomersal and Chlorbutol. He quotes them as being the most popular germicides, whilst Go and Vrij⁵⁶ recommend the use of Phenylethylalcohol, Phenylmercuric Nitrate, Phenylmercuric Acetate and Benzalkonium Chloride, for preserving and sterilizing eye drops. Anderson⁵⁷ however found that aqueous solutions of Cetrimide (1:20,000), Benzalkonium Chloride (1:20,000), Chlorhexidine Acetate (1:10,000), Phenylmercuric Nitrate (1:10,000), Phenylethanol 0.6% and Chlorbutol 0.5%, were not entirely satisfactory in destroying Staphylococcus aureus and Pseudomonas aeruginosa. Chaikovs'ka⁵⁸ found that the mercurials and Cetylpyridinium Chloride were effective in preserving solutions containing various alkaloidal salts, against Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtilis.

There is, it seems, no single compound that will satisfactorily cope with all the formulations and conditions of use. The selection of a suitable preservative will be governed by a number of factors including compatibility with the medicaments and

the method of sterilization, which may lead to inadequate preservation of the final product.

The preservatives used in this investigation were those mentioned in the British Pharmaceutical Codex 1968, which states that aqueous eye drops are to be prepared in a vehicle which is bactericidal and fungicidal, for which purpose Phenylmercuric Nitrate (or Phenyl mercuric Acetate) (.002% w/v), Benzalkonium Chloride (0.01% w/v) and Chlorhexidine Acetate (0.01% w/v) are generally suitable. Phenyl-ethylalcohol was also included in the investigation. CHLORHEXIDINE ('HIBITANE') - (Bis-p-chlorophenyl-biguanohexane).

This compound is described as being highly effective⁵⁹ against Gram-negative micro-organisms as well as against Gram-positive ones. It has a relatively low toxicity and is claimed to cause no discomfort to the conjunctiva at concentrations up to 0.1% (diacetate salt); the digluconate is reported safe up to 0.2%. Anderson and Crompton⁵³ state that it is the best preservative for ophthalmic solutions. It has also been examined and reported on favourably by Hugo⁵⁰, Brown⁵² and Lawrence.⁶⁰

This compound has however several disadvantages.⁴⁴ At a really effective concentration of 0.05% w/v, it will precipitate most bicarbonates, borates, phosphates and sulphates,^{61,62} as well as fluorescein⁵³ and physostigmine over prolonged storage. The other serious disadvantage is that its activity is

seriously diminished, if not, completely inhibited in the presence of serum and organic matter, especially those of a lipid nature. In addition there is evidence that it is not so active in the presence of ophthalmic medicaments as it is in plain saline; however, Jeffs⁶³ describes the successful use of twenty-eight ophthalmic formulations which incorporate Chlorhexidine as a bacteriostatic agent. Furthermore it has been possible to increase the resistance of Pseudomonas aeruginosa to Chlorhexidine, by subculture on media containing the compound.

However, despite these disadvantages, it seems that they may not completely rule out the use of this compound, provided that they are recognized and preparations containing it are formulated accordingly. It is now included in the British Pharmaceutical Codex 1968 at 0.01% w/v as a vehicle for eye drops.

Difficulties may arise when these preparations are autoclaved in order to sterilize them, as under these conditions, Chlorhexidine may decompose to yield breakdown products such as chloroaniline. The influence of heat sterilization on the stability of Chlorhexidine has been examined by Polack⁶⁴ and that of different sterilization temperatures and pH, by Jaminet,⁶⁵ who observed that the thermal decomposition increases with the pH value of the solution, which indicates that the method of bacterial filtration would be more suitable,

provided that the adsorption effects are not critical. Van Ooteghem²⁷ and Herbots on investigating the adsorption of preservatives onto membrane filters observed varying losses of Chlorhexidine Gluconate, in some cases up to 35% from 10 ml. of solution. The effect of using sintered glass was determined by McCarthy²⁸ who observed losses up to 28% of Chlorhexidine diacetate from 10 ml. of solution. The relatively high loss of Chlorhexidine thus presents the problem of how much loss occurs when eye-drop solutions are sterilized by filtration.

PHENYLMERCURIC NITRATE

Organic mercurial compounds such as Phenylmercuric nitrate are of value as preservatives in ophthalmic solutions where no incompatibility occurs.⁵⁰ and, their action is bacteriostatic rather than bactericidal. Phenylmercuric nitrate in a concentration of 0.002% w/v and having a pH value of 4.44 to 7.40, has been described by Adamski⁶⁶ as being an effective preservative for ophthalmic solutions containing Pilocarpine Hydrochloride. The effectiveness of Phenylmercuric nitrate 0.01% w/v in eye drops, in experimental infections induced by Pseudomonas aeruginosa, Streptococcus pyogenes, Staphylococcus aureus, and Candida albicans, was studied by Smazynski,⁶⁷ by the determination of self-sterilization times in the presence of zinc sulphate and Boric Acid; it was found to be favourable.

Concentrations of Phenylmercuric nitrate up to 0.004% have been suggested for use as a preservative in ophthalmic solutions by Hind and Szekely.⁶⁸ Baker⁶⁸ recommends this concentration provided antibiotics and halides are absent in an ophthalmic formulation. According to Foster⁵⁰ and Soehring,⁶⁹ at this concentration the preservative could prove irritant. Anderson⁵⁷ found aqueous solutions of Phenylmercuric nitrate (1:10000) to be unsatisfactory in destroying Staphylococcus aureus, and Pseudomonas aeruginosa.

Like all the organic mercurials it is effective in high dilutions. Martindale⁶⁸ and the British Pharmaceutical Codex 1968 recommend concentrations of 0.002% w/v for the preservation of ophthalmic solutions.

In spite of its apparent excellence there have been reports³⁰ of the deposition of metallic mercury in the lens capsule of the eye (mercurialentis), especially when use is over a prolonged period. Since most eye drops are used for only a short time, this is unlikely to present a serious problem.

Van Ooteghem and Herbots included Phenylmercuric Borate in their investigation²⁷ on the adsorption of preservatives onto membrane filters, and in some cases observed losses of up to 45% from 10 ml. of solution using a cellulose nitrate membrane on cardboard, the losses being of a lower order for Sartorius and Millipore membranes.

BENZALKONIUM CHLORIDE (Alkyldimethylbenzyl ammonium chloride.)

The quaternary ammonium compounds have long been recognized as effective bacteriostats and were found to be one of the most effective in the presence of organic matter. There is ample evidence^{43,51,52,55,70} that it is an effective antibacterial agent, although it is not so effective against gram-negative micro-organisms,⁴⁴ the Pseudomonas species being one of the most resistant gram-negative types. For this reason formulations containing salts of ethylene-diamino-tetra-acetic acid (E.D.T.A.) are used in conjunction with Benzalkonium Chloride.

Benzalkonium Chloride is non-toxic, non-irritant, non-volatile and stable in solution. Its surface-active properties may be an aid to the absorption of the drug with which it is used.

An important disadvantage is incompatibility with nitrate ions and salicylates. There have also been reports⁴⁴ of harmful effects at high concentrations, caused by solubilization of the cement of the corneal epithelium. However it has been shown that Benzalkonium Chloride solution at 0.01% w/v has no deleterious effect on any tissues of the eye.

Heat sterilization of preparations containing Benzalkonium Chloride does not guarantee permanent sterility.⁴⁹ For this, the method of sterilization by filtration would appear to be more satisfactory

provided adsorption from solution is at a minimum. Van Ooteghem²⁷ and Herbots have reported that the adsorption of Benzalkonium Chloride onto membrane filters is quite pronounced. They observed losses of 7 - 25% from 10 ml. of solution, using different membrane filters, the Sartorius and Millipore types adsorbing lower amounts respectively.

All evidence however, seems to point to the fact that Benzalkonium Chloride is probably one of the most suitable preservatives for use in ophthalmic solutions. As such it is recommended in the British Pharmaceutical Codex 1968 at a concentration of 0.01% w/v for the preservation of eye drops, and in Martindale's Extra Pharmacopoeia at concentrations of 0.02% w/v to 0.005% w/v.

PHENYLETHYLALCOHOL (2-phenylethanol).

Although not classed as an official² preservative for eye drops, phenylethanol has some potential as a satisfactory antibacterial agent for ophthalmic solutions.⁵⁰ It is non-irritant and does not destroy the natural lysozyme present in the lachrymal secretion. It is particularly effective against gram-negative micro-organisms and inhibits the growth of Pseudomonas aeruginosa at a concentration of 0.3% to 0.5%. Although not widely used, it is sometimes used in conjunction with other bactericides and synergism⁴⁴ has been shown to occur, between

it and the organic mercurials. It is usually used at a concentration of 0.25% in conjunction with Benzalkonium Chloride.^{6,69} It is also compatible with most ophthalmic solutions which require preservatives.

SUMMARY

The suitability of various preservatives which are available for ophthalmic solutions, has been outlined. The preservatives used in this investigation were those that are mentioned for the preparation of eye-drops in the British Pharmaceutical Codex 1968; e.g. Chlorhexidine Acetate, Phenylmercuric Nitrate, and Benzalkonium Chloride. Phenylethylalcohol has also been included in the investigation. Each of these preservatives has been briefly discussed.

PART II

CHAPTER FIVE

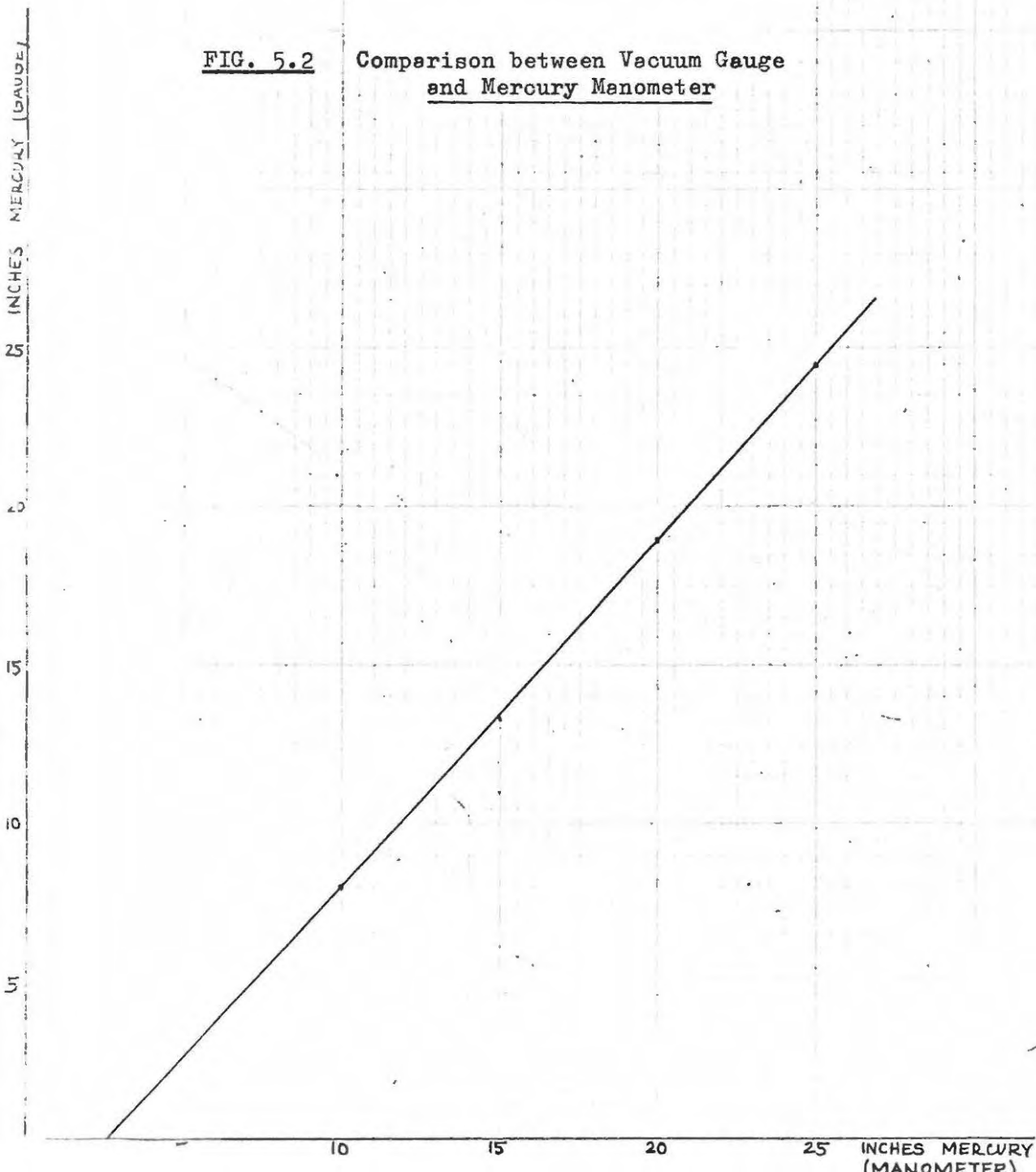
THE FILTRATION UNIT

Bacteria-proof filters are used in units designed to facilitate aseptic collection of the filtrate and, usually and desirably, aseptic distribution into the final sterile containers. Their basic design resembles an aseptic distribution unit but the tube through which liquid is admitted in the latter is either connected to or replaced by the filter.

Filtration through bacteria-proof media is very slow or negligible under atmospheric pressure, and must be accelerated by applying pressure to the liquid, or vacuum to the clean side of the filter. Pressure filtration has several advantages⁶ but nevertheless there are points in favour of vacuum filtration.⁶

All filtrations carried out in the investigation, were done under vacuum filtration. To effect this a controlled-vacuum filtration unit was set up. This consisted of an electrically operated motor driven vacuum pump, with a buffer flask or reservoir interposed between the filter unit (filter and receiver), and the pump, in order to protect the filtration medium and the joints of the unit from sudden, sharp, changes of pressure. A pressure-vacuum gauge was attached to the system, the vacuum being controlled by an adjustable tap. (Fig. 5.1). The filtration rates were determined

FIG. 5.2 Comparison between Vacuum Gauge
and Mercury Manometer



by means of a stop-clock.

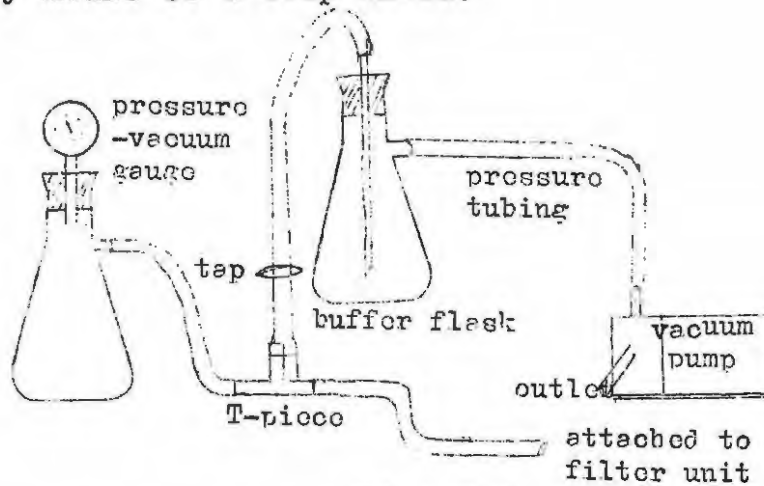


Fig. 5.1

Diagrammatic representation of filtration unit.

The gauge used was calibrated in inches of mercury, with a maximum reading of 30 inches. It was checked and recalibrated against an open-end U-tube, mercury manometer, which was connected to the system in place of the filter unit. Manometric readings of 10, 15, and 20 inches of mercury obtained by measuring the difference in heights between the mercury columns, were compared to gauge readings. (Table 5.1)

TABLE 5.1

Manometer Reading	Vacuum Gauge Reading
10 ins. mercury	8 ins. mercury
15 ins. mercury	13.25 ins. mercury
20 ins. mercury	19.00 ins. mercury
25 ins. mercury	*24.5 ins. mercury

(* obtained by extrapolation of graph - See Fig. 5.2)

TABLE 5.2

FILTER TYPE	GRADE	PORE SIZE obtained from literature ^{6,13,36}	DIMENSIONS
(a) Sintered Glass AG 3 x 5	5 on 3	1 - 1.5 microns	diameter - 25mm thickness - 3mm
Sintered Glass Pyrex G4*	4	7 - 12 microns	diameter - 30mm thickness - 1.5mm
(b) Doulton Pasteur unglazed porcelain candle	1	2 - 2.5 microns	80mm x 10mm x 3mm (30ml capacity)
(c) Asbestos Pads - Carlson-Ford filter sheets. (Ford's sterimats)	GS (S.10) SB (EKS)	5 - 20 microns	diameter - 3.6cms thickness - 3mm
(d) Sartorius membrane filter.	GmbH	0.45 microns	diameter - 5cms

*clarifying grade used for comparative purposes.

FILTER UNITS.

(a) Sintered Glass Filters:

The sintered glass discs are fused into a buchner type funnel, which is mounted by means of a rubber bung to a receiver flask, with a suitable means of attachment to the vacuum supply. Filtrates were collected in large Pyrex test tubes. (Fig. 5.3)

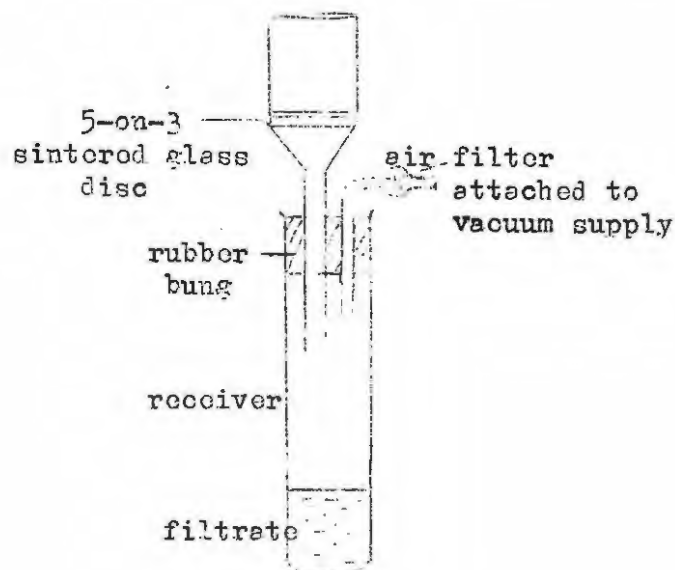


Fig. 5.3

(b) Unglazed Porcelain Candles;

These have a glazed porcelain mounting to facilitate incorporation into the receiver unit by means of a rubber bung. The receiver has a side-arm which is connected to the vacuum supply, as well as a tap for removing the filtrate. (Fig. 5.4)

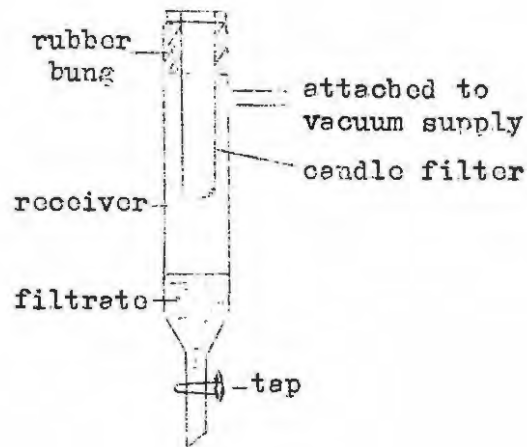


Fig. 5.4

(c) Asbestos Pads:

The filter pad is rough on the one surface and smooth and branded on the opposite side. The pad is usually supported on a perforated metal disc or grid, the rough unbranded side facing the incoming or unfiltered liquid. It is clamped into a buchner-funnel shaped metal holder, which is made from phosphor bronze or nickel-silver alloy, and silver plated. The unit may be separated into two parts by unscrewing the lower portion, containing the perforated grid. The holder has a capacity of 30ml and fits into a receiver with a suitable attachment to a vacuum supply. Large Pyrex test tubes were used as receivers. (Fig. 5.5)

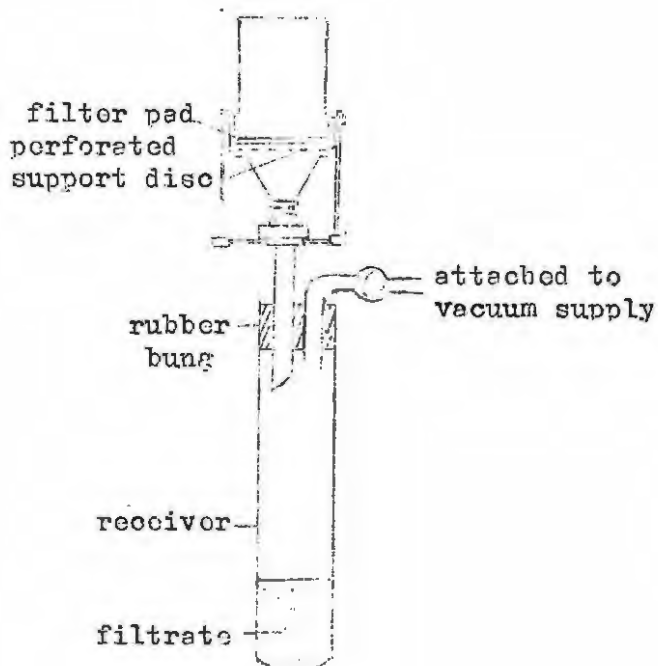


Fig. 5.5

(d) Membrane Filters:

Two types of Sartorius filter units were used:

- (i) The reservoir and receiver constructed from autoclavable polycarbonate, with a porous polycarbonate grid to support the membrane. The filtration reservoir and receiver, each have a capacity of 250ml and may be separated by unscrewing. The unit is fitted with a side arm, which is attached to the vacuum supply. (See Fig. 5.6d)
- (ii) An all glass filter holder consisting of a 250ml reservoir, and a sintered glass disc, fused into a funnel, to support the membrane; the two parts being held together by means of an external clamp.

The entire unit fits into a receiver by means of a rubber bung. The receivers used were 50ml Buchner tubes, with a fitted side arm, to which the vacuum was attached. (See Fig. 5.6c).

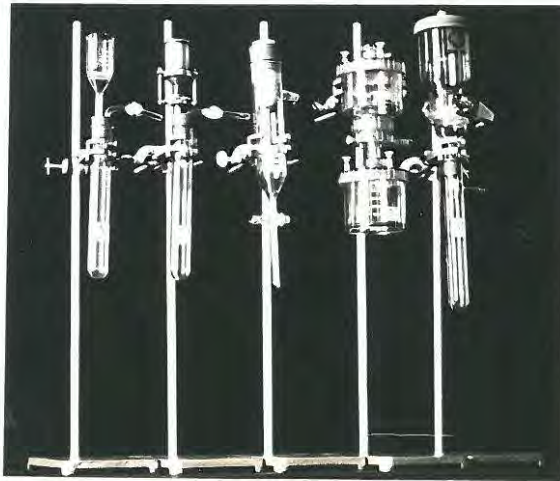


Fig.5.6a - Filter units - from left to right: Sintered Glass, Asbestos Pads, Pasteur Candle, Sartorius Membranes (Plastic and Glass units).

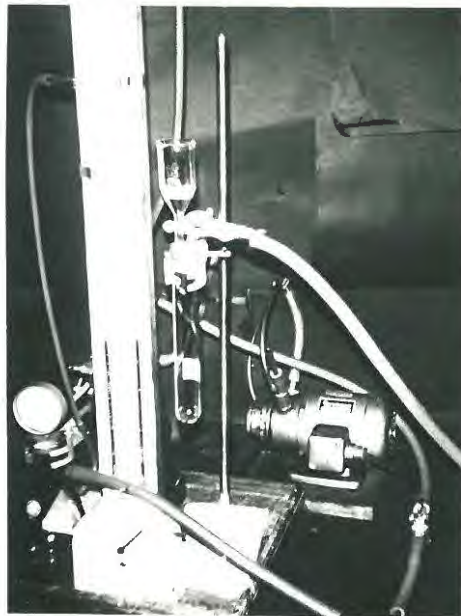


Fig.5.6b - Filtration unit incorporating the sintered glass filter.

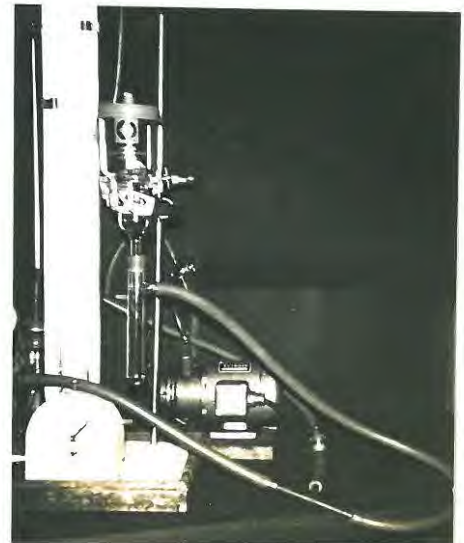


Fig.5.6c - Filtration unit incorporating the Sartorius Membrane (Glass unit).

SUMMARY

The filtration unit, incorporating the vacuum pump, vacuum gauge and, various filters and their respective units or holders, has been described.

CHAPTER SIX

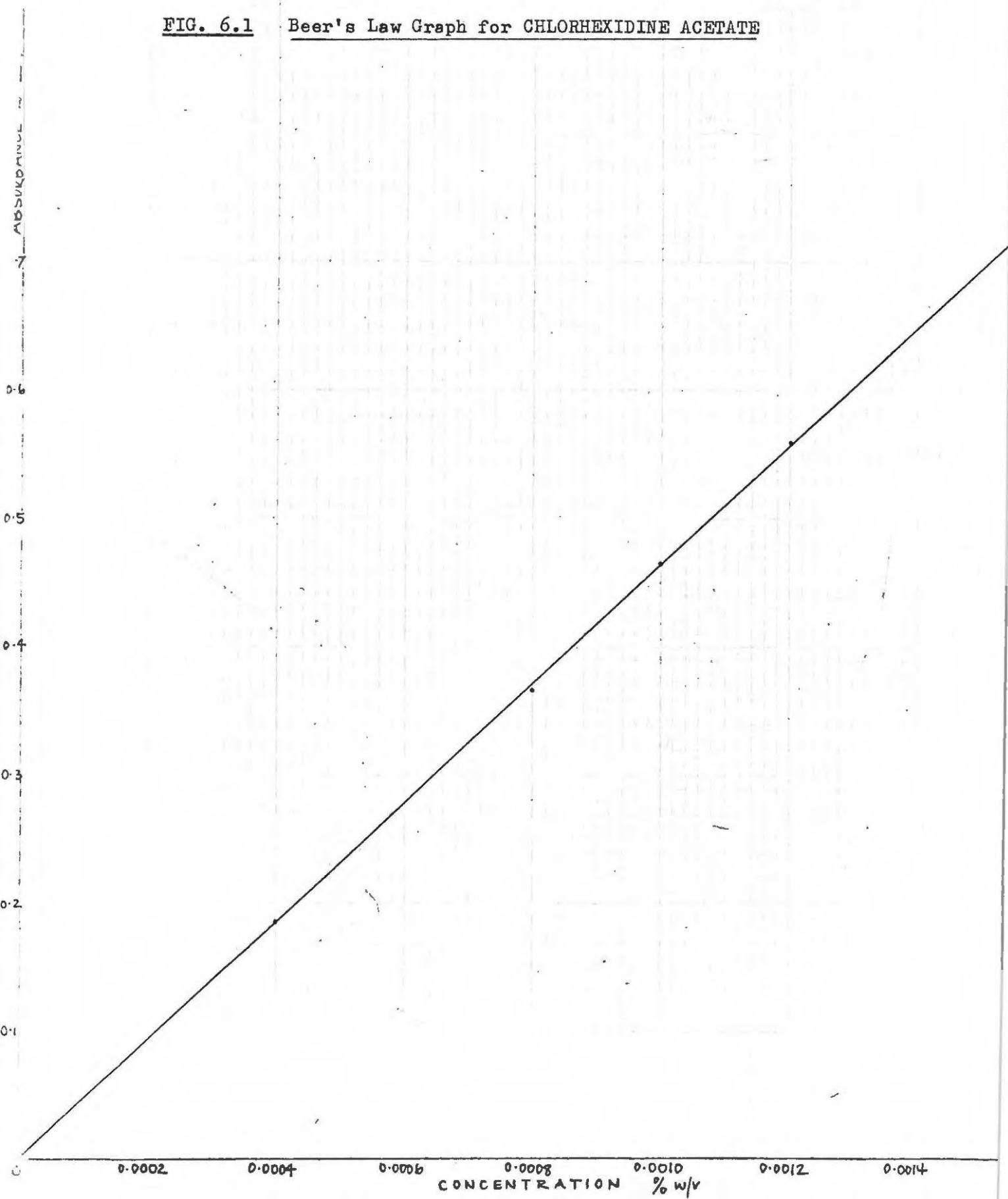
ADSORPTION OF CHLORHEXIDINE

Chlorhexidine Acetate ('HIBITANB' - manufactured by Imperial Chemical Industries) was used in all determinations concerning the adsorption of Chlorhexidine onto filter media. Chlorhexidine Acetate (1,6-Di(N-p-chlorophenyl diguanido) hexane acetate) of molecular formula $C_{22}H_{30}Cl_2N_{10}^2C_2H_4O_2$ and molecular weight 625.6, is a white to pale cream, odourless, micro-crystalline powder with a bitter taste. It is soluble 1 in 55 of water and 1 in 15 in alcohol, and is incompatible with soaps anionic emulgents, and at a concentration of 1 in 2000 with, borates, carbonates, chlorides, citrates, phosphates, sulphates and tartrates.

A stock solution of 0.2% w/v was prepared, from which the necessary dilutions were made to yield solutions of concentration 0.01% etc., when required; all solutions being prepared in distilled water.

Solutions were filtered through the various media and, all filtrates assayed spectrophotometrically by using the Beckman D.B. spectrophotometer, and measuring the spectral absorbance at a peak absorption wavelength of 254 nanometers (n.m.); Chlorhexidine Acetate, obeying Beer's law within the range 0.0004% w/v to 0.0016% w/v.

FIG. 6.1 Beer's Law Graph for CHLORHEXIDINE ACETATE



Preparation of Beer's Law Graph.

Solutions containing 0.0016% w/v, 0.0012% w/v, 0.0008% w/v and 0.0004% w/v of Chlorhexidine Acetate were prepared by appropriately diluting a stock solution containing 0.2% w/v of Chlorhexidine Acetate. The spectral absorbances for each of these dilutions were measured at a wavelength of 254 n.m. against a blank of distilled water, and the results plotted graphically after taking an average of readings between two determinations. (Fig. 6.1)

A 1 - 10 dilution of the original unfiltered solution containing Chlorhexidine Acetate 0.01% w/v, was found to have an absorbance reading of 0.465 at wavelength 254 n.m. The quantity of Chlorhexidine Acetate present in the filtrate was thus determined by preparing a 1 - 10 dilution of the filtrate, the spectral absorbance of which was measured, and from this, the quantity of Chlorhexidine lost through adsorption onto the filter, was calculated relative to a concentration of .001%, which gave an absorbance reading of 0.465 at 254 n.m.

(a) Adsorption onto Sintered Glass Filters.

Two similar sintered glass filters, A and B, of porosity No. 5 (3 x 5) were used for the determinations. Before every filtration, the filters were cleaned⁶ with 30ml of hot (80°C) concentrated Sulphuric Acid containing about 2% Sodium Nitrate to oxidise deposits, and then

washed, by flushing distilled water through, until the filtrates were acid-free and, showed no absorbance when measured spectrophotometrically at 254 n.m. The filters were then oven-dried at 150°C for 1 hour, and allowed to cool to room temperature before use. When ready for use they were attached to their receivers which were incorporated into the filtration unit.

The solutions to be filtered, were pre-assayed before every filtration. The filtrates were collected individually in large Pyrex test tubes and, the filtration rates for every filtration, were determined by means of a stop-clock.

The effect of different vacuums during filtration, on the adsorption of Chlorhexidine Acetate, was determined by filtering three 10ml aliquots of Chlorhexidine Acetate 0.01% w/v successively through each of the sintered glass filters, A and B, at vacua of 15 ins, 20 ins, and 25 ins of mercury.

The influence of the concentration of Chlorhexidine Acetate, on its extent of adsorption onto the sinters, was determined by filtering solutions containing 0.005% w/v, and 0.02% w/v through filters A and B, using a vacuum of 25 ins of mercury.

Multiple filtrations at a vacuum of 20 ins of mercury, using ten 10ml aliquots were carried out to determine the volume of Chlorhexidine Acetate 0.01% w/v, required to saturate the filters, so that on further filtration no further adsorption of Chlorhexidine would occur from

solution.

Desorption experiments were run to determine the quantity of distilled water required to desorb the Chlorhexidine from the filters, which were previously saturated with Chlorhexidine Acetate. In these desorption experiments, the filter funnel (reservoir and stem) was cleaned as best as possible, leaving Chlorhexidine Acetate only on the sintered glass disc. Thirty 10ml aliquots of distilled water were filtered successively at a vacuum of 20 ins of mercury, the filtrates being collected individually. The absorbances of the filtrates were measured directly i.e. without dilution, at 254 n.m.

The effect of temperature on the adsorption and desorption of Chlorhexidine Acetate, was determined by filtering six 10ml aliquots of Chlorhexidine Acetate 0.01% w/v through filter A at temperatures of 60°C and 90°C. Desorption studies at an elevated temperature were performed by desorbing the previously saturated filter A (saturation being effected by filtering 100ml of 0.01% w/v Chlorhexidine Acetate, followed by testing the filtrate for the original Chlorhexidine Acetate concentration) with 10ml aliquots of distilled water at 60°C and 90°C.

The inclusion of various salts in solutions could influence the extent of adsorption of Chlorhexidine, by affecting the surface tension⁷² of the solution being filtered, or possibly, by

affecting the adsorption sites that are available. A number of ophthalmic formulations containing Chlorhexidine Acetate 0.01% w/v and, Sodium Chloride 0.9% w/v, have been quoted by Jeffs.⁶³ Martindale⁶⁸ states that at a concentration of 0.05% w/v, Chlorhexidine Acetate is incompatible with Chlorides and may form salts of low solubility; more soluble salts however, being formed at dilutions of 1:10000 (0.01%) of Chlorhexidine Acetate. However, the effects of Sodium Chloride on the adsorption of Chlorhexidine was determined by including various concentrations of Sodium Chloride (0.9%, 0.05%, 0.01% and 0.001% w/v) in solutions of Chlorhexidine Acetate 0.01% w/v.

The effect of a buffer⁷³ such as Sodium Acetate having a pH value of 5.89 (measured on a Metrohm pH meter) (Chlorhexidine Acetate unstable at alkaline pHs) was also determined by incorporating Chlorhexidine Acetate 0.01% w/v in the buffer solution, which was made by, mixing 95 parts of 0.2 molar Sodium Acetate with, 5 parts of 0.2 molar Acetic acid.

In the above cases of Sodium Chloride and Sodium Acetate, 10ml aliquots were filtered successively through filter A at a vacuum of 20 ins of mercury with 10ml of distilled water. On assaying all the filtrates spectrophotometrically, corresponding blank solutions of Sodium Chloride and Sodium Acetate buffer were used respectively, in the reference cuvette.

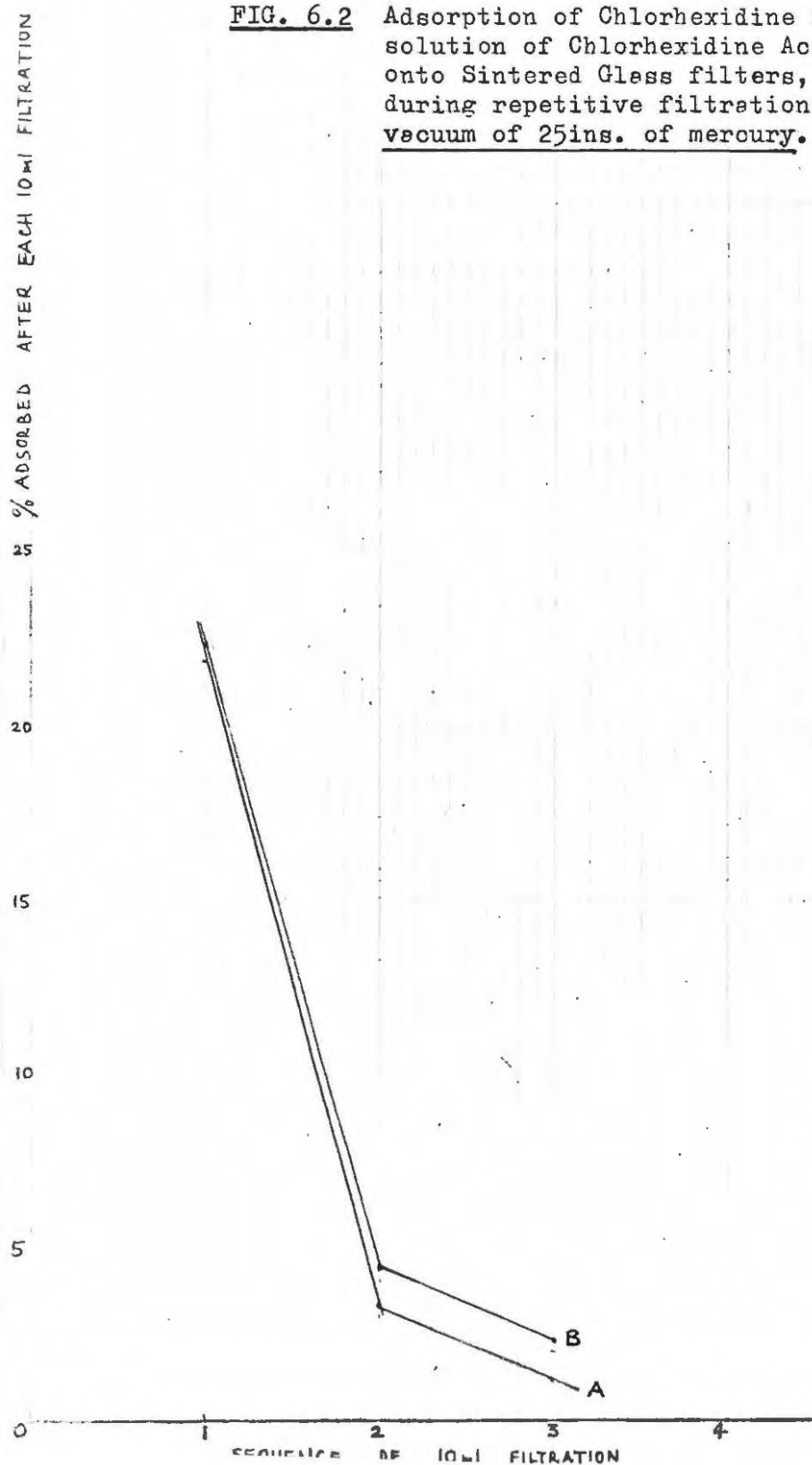
A proposed study on the effects of the Disodium salt of Ethylene-diamine-tetra-acetic acid (Sodium Edetate), on the adsorption of Chlorhexidine Acetate was considered. A solution containing Chlorhexidine Acetate 0.01% w/v and Sodium Edetate (Sodium Edetate Eye Drops⁶⁸) was prepared, but after three days a heavy precipitate was observed to have developed.

According to Chiou and Smith²⁶ the extent of adsorption seems to relate to the pore size of filters; the smaller the pore size, the greater the adsorption. This might be due to the higher specific surface area available for adsorption in the smaller pore size filters. The effect of this was demonstrated by successively filtering five 10ml aliquots of Chlorhexidine Acetate 0.01% w/v, through each of two (A and B) Pyrex G.4 (porosity 4, of maximum pore size 7-12 microns) sintered glass filters, which were previously cleaned and dried.

RESULTS and DISCUSSION

In all instances, adsorption was observed to take place principally at the beginning of filtration, the amount of adsorption decreasing as filtration proceeded. The effect of the vacuum used, on the adsorption of Chlorhexidine Acetate appeared to be insignificant, and even though the amount adsorbed did vary slightly, this variation could not be associated directly with the variation of the vacua of filtration

FIG. 6.2 Adsorption of Chlorhexidine from a solution of Chlorhexidine Acetate 0.01% w/v, onto Sintered Glass filters, A and B (porosity No.5), during repetitive filtration of 10ml aliquots at a vacuum of 25ins. of mercury.



and the rates of filtration. From Table 6.1 it may be seen that, the losses which occurred, seemed to show no correlation with the vacuums used for filtration, even though the filtration rates differed markedly. Filters A and B differed slightly in their extents of adsorption, the latter filter adsorbing more (Fig. 6.2).

The filtration rates between A and B were markedly different, filter B filtering at a rate which varied more proportionately with the vacuum used, than filter A. Generally, the filtration rates were observed to decrease as filtration proceeded.

Influence of the concentration of the solution filtered.

The amount of Chlorhexidine Acetate lost from solution was found to vary with the concentration of the solution filtered. Both filters A and B showed an increase in the amount adsorbed from solutions of a lower concentration; filter B again adsorbing a slightly greater amount than filter A (Table 6.2). The percent of adsorption occurring at different concentrations using filters A and B is shown in Fig. 6.3.

TABLE 6.1

MULTIPLE FILTRATIONS OF CHLORHEXIDINE ACETATE 0.01% w/v AT VARIOUS VACUA OF FILTRATION

Sintered Glass Filter A (porosity 5)	Vacuum (ins. mercury)	10ml aliquots of Chlorhexidine Acetate 0.01%	Time taken for filtration	Absorbance of 1-10 dilution at 254 n.m.	Percentage loss
	25	1	78 secs	0.365	21.7
		2	140 secs	0.450	3.23
		3	4m. 45 secs	0.460	1.07
	20	1	2m. 45 secs	0.370	20.43
		2	4m. 30 secs	0.443	3.65
		3	5m. 40 secs	0.463	0.43
	15	1	2m	0.363	21.93
		2	2m	0.452	2.8
		3	2m. 20 secs	0.458	1.5
Sintered Glass Filter B (porosity 5)	25	1	45 secs	0.362	22.15
		2	45 secs	0.445	4.3
		3	48 secs	0.455	2.15
	20	1	55 secs	0.360	22.58
		2	60 secs	0.443	4.73
		3	60 secs	0.450	3.22
	15	1	85 secs	0.375	19.35
		2	88 secs	0.448	3.65
		3	88 secs	0.452	2.79

FIG. 6.3 Adsorption of Chlorhexidine Acetate from solutions of different concentrations, onto Sintered Glass filters A and B of porosity No.5.

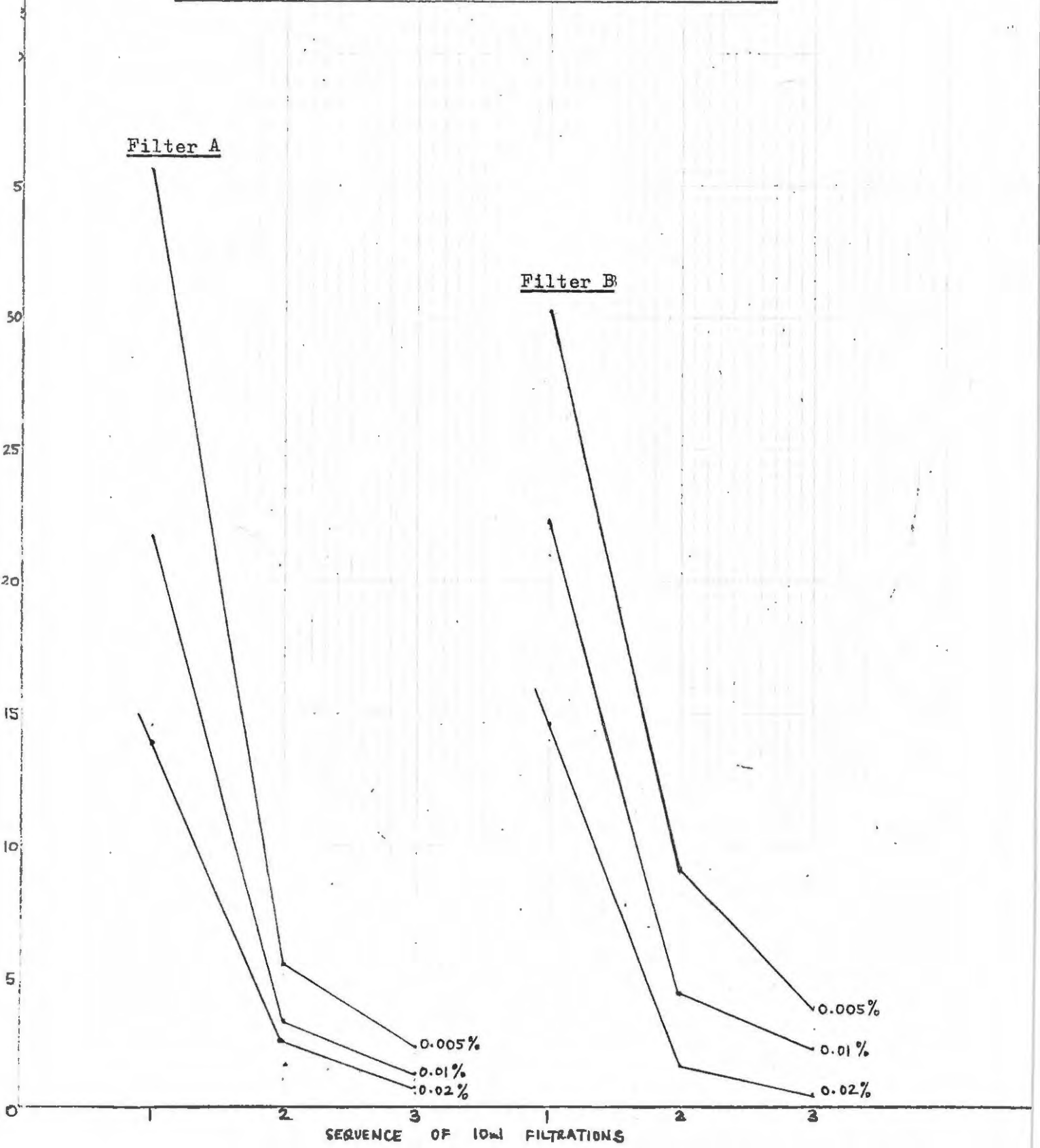


TABLE 6.2

EFFECT OF CONCENTRATION ON THE ADSORPTION OF CHLORHEXIDINE ACETATE

Concentration of Chlorhexidine Acetate	10ml Aliquots filtered at a vacuum of 25 ins. of mercury	Percentage Loss	
		Sintered Glass Filter A	Sintered Glass Filter B
0.02% w/v	1st	13.97	14.6
	2nd	2.5	1.5
	3rd	0.65	0.43
0.01% w/v	1st	21.7	22.15
	2nd	3.23	4.3
	3rd	1.07	2.15
0.005% w/v	1st	35.5	30.10
	2nd	5.37	9.03
	3rd	2.15	3.66

The concentration had no observable influence on the rates of filtration, as the rates were observed to vary randomly during the different filtrations e.g. the time taken to filter 10ml of solution of different concentrations at a vacuum of 25 ins of mercury is given in Table 6.3.

TABLE 6.3

Concentration of Chlorhexidine Acetate	Time taken for filtration of 10ml	
	A	B
0.02% w/v	45 secs	48 secs
0.01% w/v	78 secs	55 secs
0.005% w/v	51 secs	45 secs

The volume of Chlorhexidine Acetate 0.01% w/v required to saturate the filters may be determined from Table 6.4. It can be seen that the loss occurring from 10ml, on filtration through filter B had dropped to 10.75% as compared to 22.15% on a previous occasion, the rates of filtration on both occasions being similar. Filter A became saturated after 50ml of solution had been filtered, and filter B after 20ml. However, on a further occasion filter A seemed to behave differently. Loss from 10ml had dropped to 15.2% and this filter now became saturated after filtering 20ml of Chlorhexidine Acetate 0.01% w/v. (See Table 6.5)

TABLE 6.4

ADSORPTION OF CHLORHEXIDINE ACETATE ONTO SINTERED GLASS FILTERS A AND B,
TO DETERMINE THE VOLUME OF SOLUTION REQUIRED TO SATURATE THE FILTERS.

Filter A 10ml aliquots	Time (secs) for filtration	Percentage loss	Filter B 10ml aliquots	Time (secs) for filtration	Percentage loss
1	65	19.87	1	55	10.75
2	66	4.9	2	58	2.15
3	68	3.4	3	60	nil
4	70	1.7	4	60	nil
5	70	0.64	5	60	nil
6	72	nil	6	60	nil
7	72	nil	7	60	nil
8	75	nil	8	60	nil
9	80	nil	9	60	nil
10	83	nil	10	60	nil

From Table 6.5 it may also be seen that the rates of filtration for filter A became steady after repeated filtration (10ml/60 secs).

TABLE 6.5
ADSORPTION OF CHLORHEXIDINE ACETATE ONTO
SINTERED GLASS FILTER A

10ml aliquot	Time (Secs) for filtration	Percentage Loss
1	55	15.21
2	60	2.17
3	60	nil
4	60	nil
5	60	nil

Thus filter A caused decreasing losses on each of three occasions (21.7%, 19.87% and 15.21%). (See Table 6.6). This could probably be due to the repetitive cleaning process (using hot concentrated sulphuric acid and Sodium Nitrate) which could affect the porosity of the sinter. This was indicated by the increasing rates of filtration on each successive occasion. According to Sykes⁷¹ harsh cleaning methods could to some extent alter the pore size of the 5-layer.

Since repetitive cleaning of the filter increases the surface area of the filter, one would have expected a higher amount of adsorption to occur. However from the results obtained it

TABLE 6.6

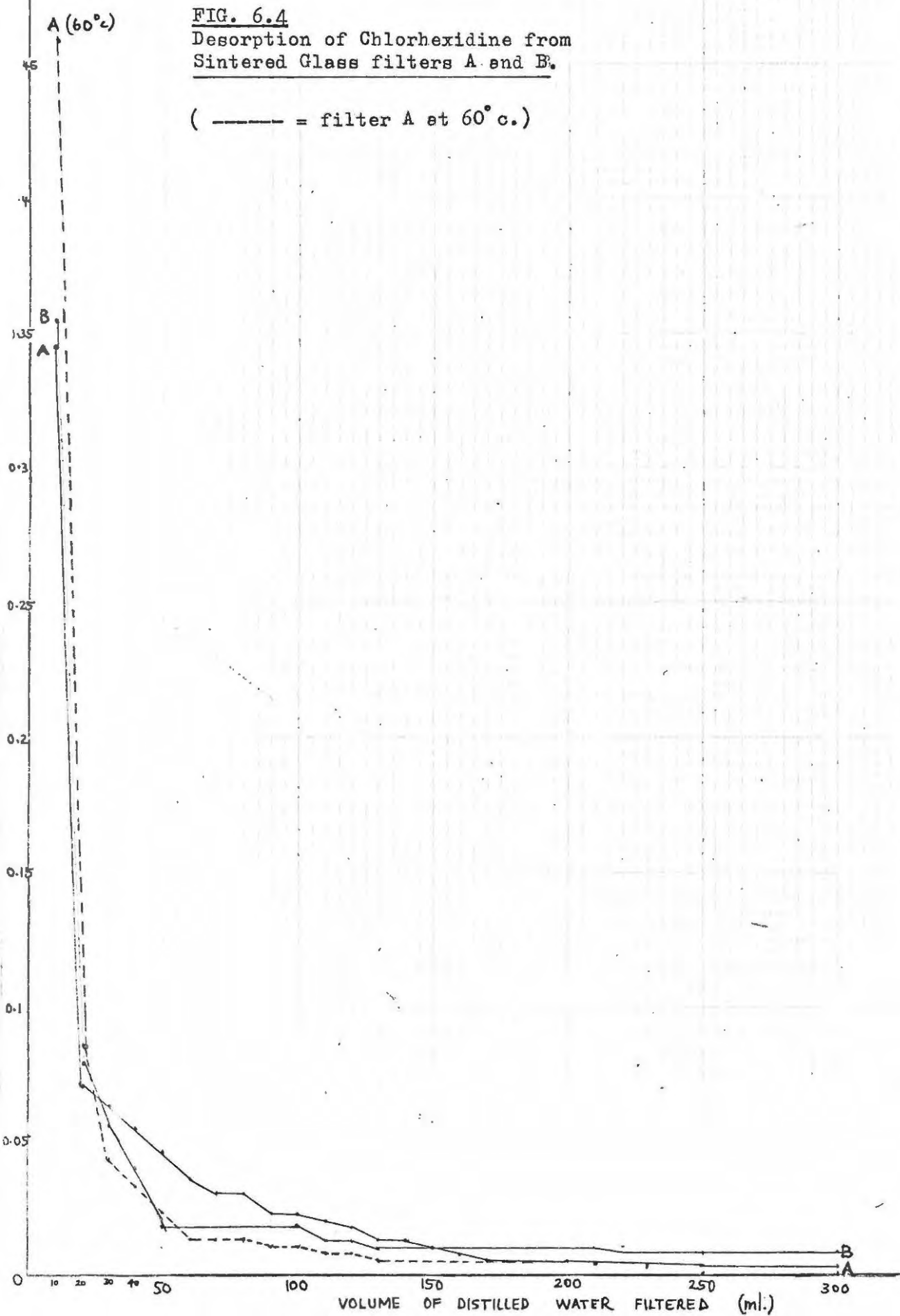
VARIATIONS OF THE FILTRATION RATES AND THE QUANTITY OF CHLORHEXIDINE ACETATE
ADSORBED ONTO SINTERED GLASS FILTER A ON DIFFERENT OCCASIONS

10ml aliquots	Time	Percent Loss	Time	Percent Loss	Time	Percent Loss
1	2m. 45 secs	21.7%	65 secs	19.87%	56 secs	15.2%
2	4m. 30 secs	3.23%	66 secs	4.9%	60 secs	2.17%
3	14m. 30 secs	1.07%	68 secs	3.4%	60 secs	nil
			(saturation after 50ml)		(saturation after 20ml)	

at 254 m.μ

FIG. 6.4
Desorption of Chlorhexidine from
Sintered Glass filters A and B.

(- - - - = filter A at 60° c.)



seems more likely that the porosities of the filters had been affected.

Desorption Studies.

It was found that about half (assuming 15% absorbed (150 micrograms)) of the absorbed Chlorhexidine was washed off with 10ml of distilled water, and after a 160ml of distilled water had passed through, a trace of Chlorhexidine was detected in the filtrate, this trace amount persisting after 300ml had been washed through. (See Table 6.7)

TABLE 6.7

DESORPTION OF CHLORHEXIDINE FROM FILTER A.

10ml aliquot of distilled water	Absorbance at 254 n.m.	Percent Chlorhexidine in filtrate
1st	0.345	0.00075%
2nd	0.070	0.00016%
16th	0.0075	0.00002%
30th	0.005	0.00001%

From Fig. 6.4 it was observed that the desorption pattern for filter B, resembled that of A in the initial stages, but appeared to deviate slightly as desorption proceeded.

Temperature effects.

Adsorption studies at elevated temperatures indicated that at higher temperatures adsorption occurred to a lesser extent. It was observed that

the absorbance reading of the first 10ml filtrate at 60°C was higher than the readings obtained when filtration was performed at room temperature. From Table 6.8 it may be seen that on repetitive filtration, the filtrates had a higher absorbance value than that of a similar dilution of the original unfiltered solution.

The original unfiltered solution on dilution (1-10) had an absorbance value of 0.465. The higher values obtained after filtration of 10ml of solution, could probably be due to a desorption effect on the previously adsorbed Chlorhexidine by the hot Chlorhexidine solution, until equilibrium was attained, or it could be due to a concentrating effect which resulted from evaporation of the hot solution on filtration.

At 90°C the loss from the first 10ml aliquot filtered was found to be 3.3%. Again, the successive filtrates were found to have higher absorbance values and after 60ml, the filtrate was found to have an absorbance value of 0.710.

The rates of filtration in both of the above cases (60°C and 90°C) were found to increase to 10ml/33 secs and 10ml/25 secs respectively.

It is a well known fact⁷⁴ that adsorption decreases with an increase in temperature. This explains the decreased losses occurring from the first 10ml aliquot filtered at 60°C and also a further decrease at 90°C. Filtration of hot solutions could also at the time, affect the

TABLE 6.8

ADSORPTION OF CHLORHEXIDINE ACETATE ONTO SINTERED GLASS FILTER A AT 60°C

10ml aliquot Chlorhexidine Acetate 0.01% w/v	Time for filtration (secs)	Absorbance at 254 n.m.	Percent Loss
1	30	0.410	11.82%
2	33	0.475	-
3	33	0.480	-
4	33	0.4875	-
5	33	0.4925	-
6	33	0.500	-
7	33	0.505	-
8	33	0.520	-
9	33	0.520	-

porosity of the filter medium and hence the rate of filtration of the solution being filtered. An increase in the porosity could thus also result in diminished adsorption. Table 6.9 indicates the losses occurring from 10ml at various temperatures.

TABLE 6.9
LOSS OF CHLORHEXIDINE DUE TO ADSORPTION ONTO
SINTERED GLASS FILTER A AT
DIFFERENT TEMPERATURES

Temperature	Percent loss from first 10ml aliquot filtered
25°C (ambient)	21.7, 19.87, 15.21
60°C	11.82
90°C	3.3

Desorption studies at elevated temperatures, on filter A which was previously saturated at room temperature, revealed that desorption was more accomplished than that at room temperature, about $\frac{3}{4}$ of the previously adsorbed quantity being desorbed with 10ml of distilled water at 60°C. However, desorption at 90°C was more or less similar to that at 60°C (Fig. 6.4).

Effect of Sodium Chloride and Sodium Acetate Buffer.

Sodium Chloride was found to affect the adsorption of Chlorhexidine considerably. A diminished loss occurred, and saturation of filter A occurred after filtration of 20ml of solution. The concentration

of the added Sodium Chloride appeared to have no effect, (except that containing Sodium Chloride 0.01%) and all solutions were found to lose the same amount of Chlorhexidine (Table 6.10).

TABLE 6.10
ADSORPTION OF CHLORHEXIDINE ACETATE ONTO SINTERED
GLASS FILTER A IN THE PRESENCE OF
SODIUM CHLORIDE

Percentage Sodium Chloride used with Chlorhexidine Acetate 0.01% w/v	Percent loss of Chlorhexidine Acetate when filtered in 10ml aliquots		
	1st	2nd	3rd
0.9% w/v	2.15	1.5	nil (saturation)
0.05%	2.15	1.09	nil
0.001%	2.10	0.63	nil

In the presence of Sodium Chloride 0.01% w/v, the filtrates on dilution (1-10) had absorbance values which were higher than that of the original solution, e.g. with the first 10ml aliquot filtered, the filtrate had an absorbance value of 0.485 (original absorbance value = 0.465). This value was found to increase when the filtrate was allowed to stand. The original unfiltered solution was also found to increase in value. Both the filtrates and unfiltered solution were also found to have a slight turbidity, which could be explained by the slow incompatibility between Chlorhexidine and

Sodium Chloride. (Equimolar quantities of each being present.)

Sodium Acetate buffer appeared to have a similar effect on the adsorption of Chlorhexidine. It was found that a loss of 2.1% occurred from the first 10ml filtered, followed by 1.0% from the next, after which saturation of the filter occurred.

In the presence of Sodium Chloride and Sodium Acetate, the filtration rates were observed to be steady (10mls/60-65 secs), without any appreciable slowing down, as filtration progressed.

Desorption in both cases was effected with 10ml of distilled water after which no Chlorhexidine was detected in the filtrate.

According to the Gibbs Equation⁷⁴ a particular substance will be adsorbed from solution by a solid adsorbent (filter medium), if that substance reduces the surface tension of the solvent. Water has a high surface tension, and most solutes (e.g. Chlorhexidine Acetate) reduce its value and are thus positively adsorbed from aqueous solution.

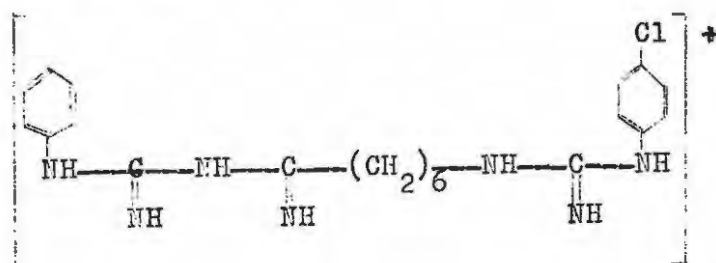
The effect of Sodium Chloride and Sodium Acetate could be explained in terms of the Gibbs adsorption equation, which states that for a dilute solution of concentration C;

$$S = -\frac{c}{RT} \cdot \frac{d\gamma}{dc}$$

where S is the excess concentration of solute per sq.cm. of surface, as compared with that in the

bulk of the solution; $\frac{d\gamma}{dc}$ is the rate of increase of the surface tension of the solution with the concentration of the solute; R is the familiar gas constant, and T is the absolute temperature. According to this equation any solute which causes the surface tension of the solvent to decrease, i.e. $\frac{d\gamma}{dc}$ is negative, will have a higher concentration in the surface than in the bulk of the solution, since S will be positive. In other words, a substance which decreases the tension at an interface will concentrate at that interface. If $\frac{d\gamma}{dc}$ is positive, the dissolved substance raising the surface tension, then S will have a negative value; the concentration of the solute will thus be lower in the surface than in the body of the solution. This behaviour, known as "negative adsorption" has been observed with some electrolytes. Thus in the presence of Sodium Chloride and Sodium Acetate the surface tension of the aqueous solution would be expected to be raised, thus diminishing the amount of Chlorhexidine adsorbed from the solution.

The adsorption phenomenon of Chlorhexidine Acetate could also be explained in terms of its cationic nature and its long-chain structure (Fig. 6.5).



Glass is known to carry a nett negative charge, thus attracting the Chlorhexidine cation. Sodium Acetate or Sodium Chloride results in the solution having positively charged Sodium ions, which probably tend to neutralize the negative charge on the glass, literally letting the Chlorhexidine cation pass through the pores of the sintered glass disc. Thus the attraction of the preservative to the electro-negative glass sinters could probably have been minimised by the preferential adsorption of Sodium ions.

Effect of Porosity.

The effect of using a filter with a larger porosity was found to be as expected. Filters A and B of porosity No. 4 were observed to adsorb 14.5% and 13.4% respectively from 10ml of Chlorhexidine Acetate 0.01% w/v. Both caused a loss of 1.7% from the second 10ml aliquot filtered, after which they became saturated. The filtration rates were high (10ml/5 secs), the vacuum being maintained at about 10ins of mercury.

(b) Adsorption onto Doulton-Pasteur Unglazed Porcelain Candles.

Two similar Pasteur Candles, A and B were cleaned by washing through 30ml of dilute Hydrochloric Acid, followed by several washes of hot distilled water (three litres), until the filtrate was acid-free, and had a zero absorbance value at 254 n.m. The filters were dried at 150°C for an hour, and when cool and required for use, a filter was attached to the receiver, which was incorporated into the filtration unit (Fig. 5.6b).

All filtrations were performed under a vacuum of 20 ins of mercury at room temperature (20°C) using 10ml or 30ml aliquots of solution. The filtrates of each aliquot were collected individually in the receiver (Fig. 5.4) then run off into large Pyrex test tubes and collected for assay, the receiver being rinsed and dried between the filtration of each aliquot. After every determination, the filter was desorbed of the adsorbed Chlorhexidine, and dried at 150°C for 1 hour, the weight of the filter being checked after drying.

10ml aliquots of Chlorhexidine Acetate 0.01% w/v were filtered through filter A until 90ml had been filtered altogether, in each case the solution being poured carefully into the reservoir of the candle without touching the sides, to ensure that the solution was exposed to the same surface area each time. The adsorbed Chlorhexidine was desorbed

using distilled water, dilute Hydrochloric Acid followed by several washes with distilled water and finally 30ml Sodium Chloride 0.1% w/v. (See Results and Discussion).

The process was repeated with filters A and B using three 30ml aliquots of Chlorhexidine Acetate 0.01% w/v, after which filter A was desorbed with 30ml aliquots, comprising a total volume of 120ml distilled water, 300ml Sodium Chloride 0.1% w/v, followed by several washes with distilled water until the filtrates were free of chlorides (tested with Silver Nitrate) and Chlorhexidine. Desorption of filter B was performed in a similar manner, except that 600ml of Sodium Acetate buffer was used instead of Sodium Chloride.

The effect of the concentration of the solution being filtered, was determined by filtering three 30ml aliquots of Chlorhexidine Acetate 0.02% w/v, through filter A, followed by desorption with 120ml of distilled water, 500ml Sodium Acetate buffer and, finally 500ml of distilled water.

Chlorhexidine Acetate 0.01% w/v was incorporated with Sodium Acetate buffer at a pH of 5.89, and the effect of the latter on the adsorption of Chlorhexidine, was determined by filtering three 30ml aliquots of this solution through filters A and B. Desorption of the filters was carried out with 30ml aliquots of, distilled water (120ml), Sodium Acetate buffer (500ml), followed by several washes with distilled water (500ml).

The volume of Chlorhexidine Acetate 0.01% w/v required to saturate filter A, was determined by filtering six 30ml aliquots. The process was repeated on a further occasion using three 30ml aliquots, to see whether any variations in results would be obtained. In each case desorption was carried out as before, using distilled water and Sodium Acetate buffer.

RESULTS AND DISCUSSION.

Adsorption of Chlorhexidine from 10ml of Chlorhexidine Acetate 0.01% w/v was found to be considerably high, the initial loss being 90.3% (Table 6.11). The filter continued adsorbing (3.6%) after 90ml had been filtered.

The adsorbed Chlorhexidine was found to adhere very strongly to the filter. On desorbing with 30ml aliquots of distilled water, the filtrate of the first aliquot was found to have an absorbance value of 0.475 at 254 n.m. After 400ml of distilled water had been filtered the absorbance value of the filtrate, dropped to 0.05. This value persisted until one litre of hot distilled water, 100ml of hot dilute Hydrochloric Acid, followed by three litres of hot distilled to render the filtrate acid-free, had been washed through. On washing through 30ml of Sodium Chloride 0.1% w/v, the absorbance value of the filtrate was found to drop to 0.01, and after the filter was washed several times with distilled water, until the filtrate when tested with silver nitrate was found

TABLE 6.11

ADSORPTION OF CHLORHEXIDINE ONTO PASTEUR CANDLE A.

10ml aliquot of Chlorhexidine Acetate 0.01% w/v	Time taken for filtration	Absorbance of a 1-10 dilution of filtrate at 254 n.m.	Percent loss
1	40 secs	0.045	90.3
2	45 secs	0.185	60.0
3	45 secs	0.3325	28.4
4	45 secs	0.390	16.12
5	45 secs	0.405	12.9
6	45 secs	0.410	11.82
7	45 secs	0.430	7.52
8	45 secs	0.445	4.3
9	45 secs	0.448	3.66
		1-10 dilution of original unfiltered solution = 0.465	

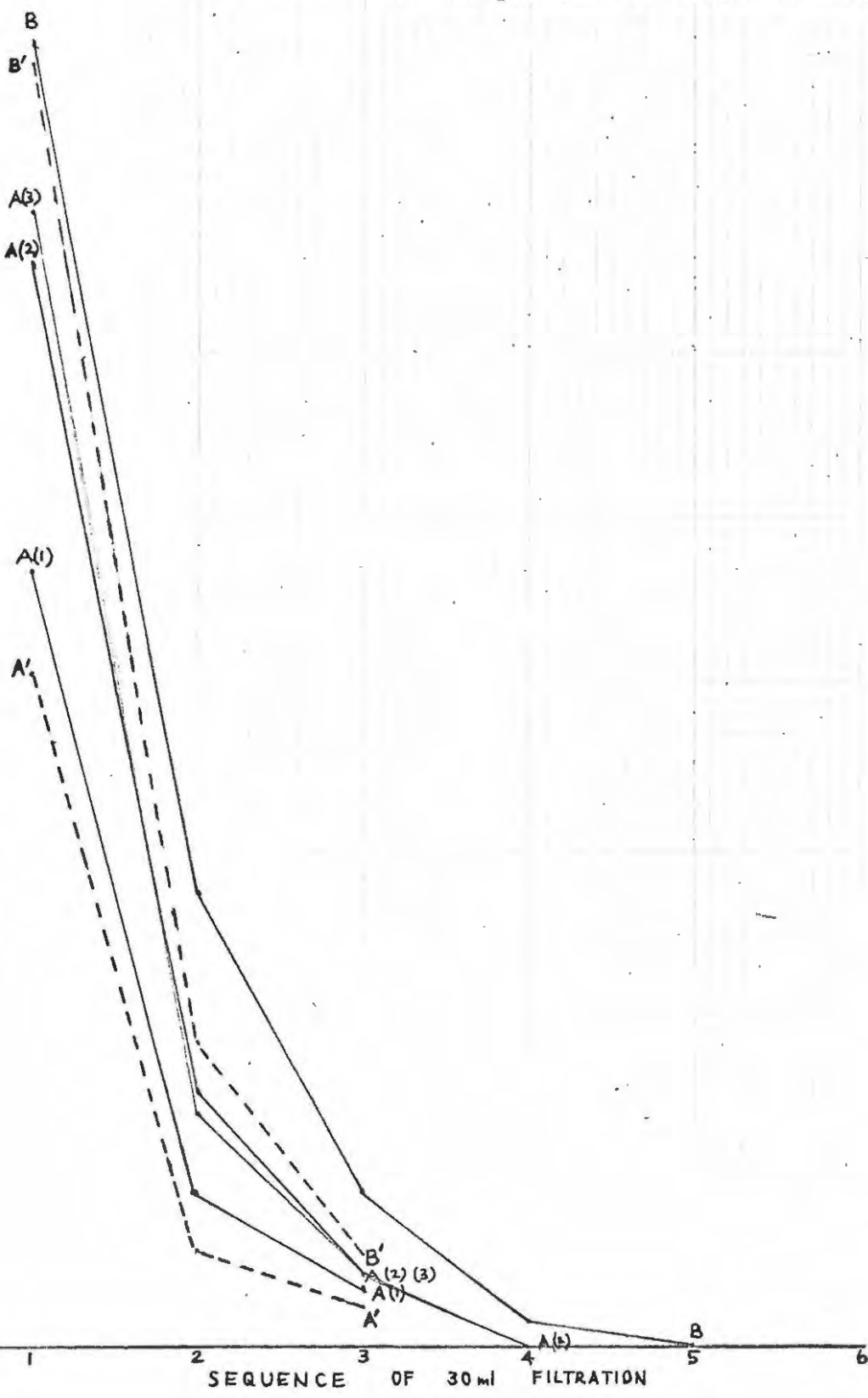
FIG. 6.6.

Adsorption of Chlorhexidine from a solution of Chlorhexidine Acetate 0.01% w/v, onto Doulton-Pasteur unglazed porcelain candles A and B, during repetitive filtration of 30 aliquots at a vacuum of 20ins. of mercury.

A(1), A(2), A(3) = Adsorption onto filter A, on three different occasions.

A' and B' = Adsorption onto filters A and B, in the presence of Sodium Acetate buffer.

PERCENT ADSORPTION



to be chloride-free, the absorbance value for the filtrate finally dropped to zero.

The overall loss occurring from 30ml of Chlorhexidine Acetate 0.01% w/v when filtered through filters A and B, was found to be 47% and 78.4% respectively. (Table 6.12) (See Fig. 6.6).

On desorbing filter A with 30ml aliquots of distilled water (120ml) the absorbance value of the filtrate had dropped from 0.610 to 0.05. The filter which was then washed through with Sodium Chloride 0.1% w/v was found to yield a filtrate with an absorbance value of 2.00, which dropped to 0.4 and 0.1 on successive filtrations of 30ml aliquots of Sodium Chloride 0.1% w/v. After 300ml of the Sodium Chloride had been washed through, the filtrate of the last 30ml aliquot which was filtered had an absorbance value of 0.01. After being washed through several times with distilled water, the filter yielded a filtrate free of chlorides and Chlorhexidine.

The desorption pattern of filter B appeared to follow a similar pattern but the amounts of Chlorhexidine desorbed differed to some extent. The absorbance value of the filtrate from the first 30ml aliquots of distilled water being 0.09, dropping to 0.04 after 300ml of distilled water had been washed through. On further washing through with Sodium Acetate buffer, the filtrate of the first 30ml aliquot had an absorbance value of 0.710 which dropped to 0.025 after 600ml and, finally zero, after 500ml of distilled water was washed through.

Because of the large surface area and irregularities within the filter candles, it seems

TABLE 6.12

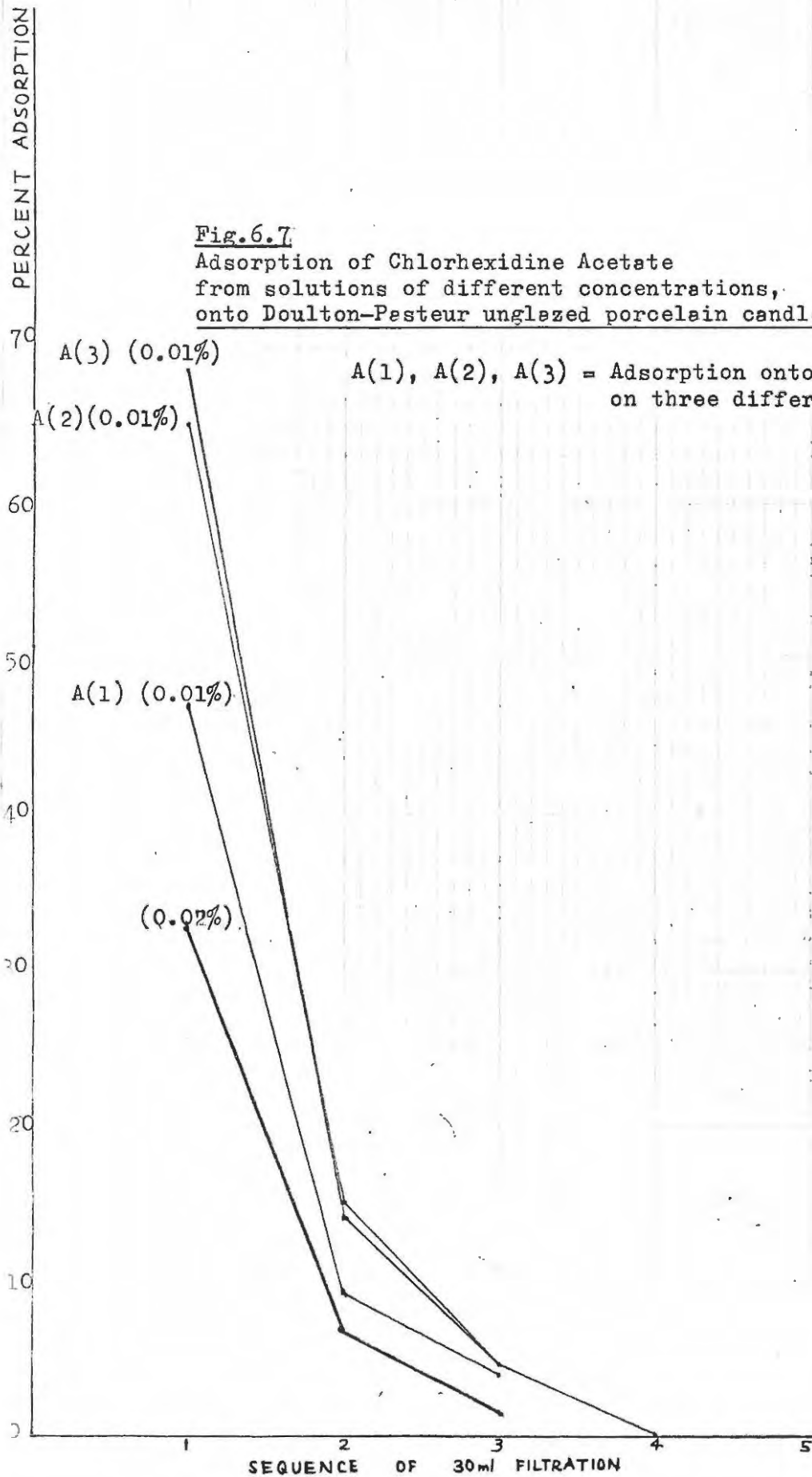
ADSORPTION OF CHLORHEXIDINE ONTO PASTEUR CANDLES A AND B FROM
CHLORHEXIDINE ACETATE 0.01% w/v.

30ml aliquot of Chlorhexidine Acetate 0.01% w/v	Time taken for filtration		Percent Loss	
	A	B	A	B
1	60 secs	80 secs	47.0	78.4
2	60 secs	80 secs	9.1	27.3
3	60 secs	80 secs	3.68	9.03
4	-	80 secs	-	1.3
5	-	80 secs	-	0
6	-	80 secs	-	0

Fig.6.7

Adsorption of Chlorhexidine Acetate
from solutions of different concentrations,
onto Doulton-Pasteur unglazed porcelain candle A

A(1), A(2), A(3) = Adsorption onto filter A,
on three different occasions.



that the Chlorhexidine which becomes adsorbed in the superficial layers of the candle's matrix, is easily washed off with distilled water, but that which is more strongly adsorbed in deeper layers requires a stronger desorbing solution containing Sodium Chloride or Sodium Acetate, these having either a surface tension or electrokinetic effect on the desorption process.

Filter A appeared to adsorb a lesser amount of Chlorhexidine from a higher concentration of Chlorhexidine Acetate 0.02% w/v (Fig. 6.7), the rate of filtration remaining unaffected (10ml/60 secs) (Table 6.13).

It would appear that Sodium Acetate would affect adsorption as it does desorption, but the effect of Sodium Acetate buffer on the adsorption of Chlorhexidine, onto filters A and B, was however found to be slight. The filtration rate of filter A was found to decrease slightly, becoming very similar, to that of filter B (Table 6.14)

From Table 6.14 and Fig. 6.6, it may be seen that Sodium Acetate appears to affect adsorption onto filter B from the 2nd and 3rd aliquots. Desorption of filter A in this instance required 120ml of distilled water, the absorbance of the filtrates at 254 n.m. dropped from an initial value of 0.510 to 0.03; followed by 500ml of Sodium Acetate buffer, the absorbance value rose to 0.980 and dropped to 0.03; and finally 500ml of distilled water.

On determining the volume of Chlorhexidine

TABLE 6.13

EFFECT OF THE CONCENTRATION OF CHLORHEXIDINE ACETATE, ON ITS
ADSORPTION ONTO PASTEUR CANDLE A

30ml aliquot filtered	Time taken for filtration		Percent Loss	
	0.01% Chlorhex. Ac.	0.02% Chlorhex. Ac.	0.01% Chlorhex. Ac.	0.02% Chlorhex. Ac.
1	60 secs	60 secs	47.0	32.9
2	60 secs	60 secs	9.1	6.9
3	60 secs	60 secs	3.68	1.5

TABLE 6.14

THE EFFECT OF SODIUM ACETATE BUFFER (pH 5.89) ON THE ADSORPTION
OF CHLORHEXIDINE FROM CHLORHEXIDINE ACETATE 0.01% w/v, ONTO
PASTEUR CANDLES A AND B.

30ml aliquot filtered	Time taken for filtration (seconds)				Percent Loss			
	A		B		A		B	
	without NaOAc	with NaOAc	without NaOAc	with NaOAc	without NaOAc	with NaOAc	without NaOAc	with NaOAc
1st	60	90	80	80	47.0	40.47	78.4	77.4
2nd	60	90	80	90	9.1	5.85	27.3	18.3
3rd	60	90	80	90	3.68	2.17	9.03	5.38

Acetate 0.01% w/v required to saturate filter A, the amount of Chlorhexidine adsorbed from the first 30ml aliquot was found to increase to 65%, the filter requiring about 100-120ml for saturation (Table 6.15), after which no further loss occurred.

The filtration rate of filter A may be seen to have decreased, as compared to previous rates (Tables 6.12, 6.14). However the rate is equal to that obtained in the presence of the Sodium Acetate buffer (Table 6.14), the losses through adsorption, being markedly different (65.05% and 40.47%).

From table 6.12 it may be seen that filter B required about 120-150mls of Chlorhexidine Acetate 0.01% w/v for saturation.

On a further occasion, on repeating the process of adsorption, with filter A, the amount of Chlorhexidine adsorbed from solution was found to increase even further, to 68%, the rate of filtration decreasing even further (Table 6.16).

After the third occasion it seemed that the amount of adsorption occurring onto filter A, was approaching that quantity adsorbed onto filter B, but the behaviour of filter A and B cannot be paralleled, because of their different filtration rates (30ml/150 secs and 30ml/80 secs respectively), that of A being almost half that of B.

It may be noted however, that as the experimental determinations proceeded the filtration rate of filter A, slowed down gradually (Table 6.16),

TABLE 6.15

ADSORPTION OF CHLORHEXIDINE FROM CHLORHEXIDINE ACETATE

0.01% w/v ONTO PASTEUR CANDLE A.

30ml aliquot Chlorhexidine Acetate. 0.01% w/v	Time taken for filtration	Percent Loss
1	90 secs	65.05
2	90 secs	15.05
3	90 secs	4.3
4	90 secs	nil
5	90 secs	nil
6	90 secs	nil

TABLE 6.16

ADSORPTION OF CHLORHEXIDINE ONTO PASTEUR CANDLE A ON
EACH OF THREE DIFFERENT OCCASIONS

30ml aliquot of Chlorhexidine Acetate 0.01% w/v	Time taken for filtration (seconds)			Percent Loss		
	1	2	3	1	2	3
1st	60	90	150	47.0	65.05	68.3
2nd	60	90	160	9.09	15.05	14.0
3rd	60	90	210	3.68	4.3	4.3

despite thorough cleaning and drying between determinations. Thus it seems that the adsorptive properties of the candle varies, these variations probably being due to irregularities within the candle, clogging by particulate matter, changes in porosity as a result of repeated cleaning, and, being a characteristic depth-type of filter, absorption of a fair proportion of the bulk of the liquid being filtered, resulting in a particular retention volume which should not be overlooked.

(c) Adsorption onto Asbestos Filter Pads.

The asbestos pads used were 3.6cm in diameter and of the G.S. grade. The later determinations were done on the S.10 grade, both grades being equivalent in porosity, the latter now being manufactured by Carlson-Ford in place of the previously designated G.S. grade.

The pad was clamped into the metal holder (Fig. 5.5) when required for use. A large pyrex test tube was attached to the unit as a receiver, and all filtrates were collected individually for assay. All filtrations were carried out under a vacuum of 20ins of mercury, and a new pad was used for every determination.

On filtration, the asbestos pads impart a considerable amount of alkaline water-soluble extractives, and loose fibres to the filtrate. This could affect the true spectral absorbance of the filtrate being assayed, and hence the final assay of the solution. Therefore, control experiments were performed, every filtration being accompanied by a corresponding blank (distilled water, or an appropriate aqueous vehicle e.g. Sodium Acetate buffer) which was filtered through another similar pad. The extent of adsorption was estimated after making blank corrections, by using the blanks prepared, in the reference cuvette (diluting 1-10 if necessary), when assaying spectrophotometrically, the dilution of the corresponding filtrate, containing the Chlorhexidine.

Effect of the metal holder on Chlorhexidine Acetate.

To determine the effect of the silver-plated metal holder of the filter unit, the stem of the holder was closed with a glass plug and the reservoir filled with Chlorhexidine Acetate 0.01% w/v. This was allowed to stand for ten minutes, after which the solution in the reservoir was assayed. The glass plug was then released and the solution obtained, assayed as well.

Water-soluble extractives.

This investigation does not attempt to determine the exact nature of the chemical impurities extracted from the asbestos pads, but only to what extent they are present, so as to allow blank corrections to be made when assaying the filtrates containing Chlorhexidine. To determine this, 10ml aliquots of distilled water were filtered through each of three pads A, B and C, and the spectral absorbance of the filtrates measured without dilution at 254 n.m. The process was repeated, using pads D, E and F to filter five 30ml aliquots of distilled water, the pH value of the first aliquot being measured on a Metrohm pH meter.

The filtrates obtained from one of the pads (D) were kept for later use as corresponding blanks when determining the adsorption of Chlorhexidine onto the asbestos pads.

The extent of adsorption of Chlorhexidine onto the filter pads was determined by filtering five

30ml aliquots of Chlorhexidine Acetate 0.01% w/v through each of two pads G and H. The filtrates of both the test and control (obtained from pad D) were diluted (1-10) and their spectral absorbance measured at 254 n.m., using the control dilution in the reference cuvette. (The blank filtrates obtained from pad D were used in the determination of both pads G and H.)

Desorption studies were performed on pads G and H containing the adsorbed Chlorhexidine; (the filter holder having been previously rinsed and dried) 30ml aliquots of distilled water were washed through and the absorbances of the undiluted filtrates were measured at 254 n.m., using the blank filtrate undiluted, it being originally obtained from the 5th aliquot filtered through pad D.

The effect of Sodium Acetate buffer on the adsorption of Chlorhexidine was determined by incorporating Chlorhexidine Acetate 0.01% w/v with the buffer, the final solution having a pH value of 5.89. Five 30ml aliquots were filtered through filter pad I; corresponding blanks having been prepared by filtering five 30ml aliquots of Sodium Acetate buffer through another filter pad J, these being used as described earlier.

Desorption of filter pad I was carried out by using three 30ml aliquots of distilled water, followed by 30ml of the Sodium Acetate buffer solution.

Effect on the adsorptive properties of the asbestos pads, after removal of the alkaline water-soluble extractives.

To determine whether the alkaline water-soluble extractives from the pads exerted any influence on the adsorption of Chlorhexidine, two filter pads (grade S,10) K and L were washed through with 30ml of N/10 Hydrochloric acid, in order to neutralize the alkaline extractives. These pads were then washed through with distilled water (about 50ml) until the filtrates obtained were acid-free. The pads were then dried at 70°C for two hours. When dry, 30ml of Chlorhexidine Acetate 0.01% w/v was filtered through pad K, and 30ml of distilled water through pad L (blank control) which provided the corresponding blank filtrate. The filtrate from pad K was then assayed for Chlorhexidine (1-10 dilution of each of the filtrates from pads K and L having been prepared).

Adsorption of Chlorhexidine onto an untreated filter pad M (S.10 grade) from 30ml of Chlorhexidine Acetate 0.01% w/v was also determined and compared with adsorption onto the acid-treated filter pad. (The blank control was prepared by filtering 30ml of distilled through a similar pad N.)

The changes in pH value of the filtrates from the treated and untreated filter pads, were compared to that of the original unfiltered solution; all pH values being measured on a pH meter.

The effect of autoclaving on adsorption.

To prepare sterile eye-drops, the solution should be filtered through a sterile filter. The effect of steam sterilization on the adsorptive properties of the asbestos filter pads, was determined by autoclaving two filter pads O and P (grade S.10) at a press of 15lbs per sq. in. gauge, for 15 minutes. After autoclaving, the pads were dried in an oven at about 60° - 70°C for two hours. 30ml of Chlorhexidine Acetate 0.01% w/v was then filtered through pad O, the blank control having been prepared by filtering 30ml of distilled water through filter pad P; both filtrates being diluted (1-10) for assaying spectrophotometrically. The pH values of all filtrates were compared to that of the original unfiltered solution. The amount of adsorption which occurred was compared to that occurring onto the unsterilized filter pad M.

RESULTS and DISCUSSION.

Effect of the metal-holder.

The solutions of Chlorhexidine Acetate from the reservoir of the holder and that obtained by unplugging the stem were found on dilution (1-10), to have an absorbance value of 0.450, indicating a loss of 3.23% (1-10 dilution of the original solution having an absorbance value of 0.465 at 254 n.m.). Thus the losses obtained in the following determinations also account for the loss occurring either through adsorption onto, or inactivation by, the metal holder.

Water-soluble extractives.

The filtrates obtained after filtering 10ml aliquots of distilled water, were found to have slight differences in their absorbance values, possibly because the filter pads vary in the amount of water-soluble extractives that each have present. Table 6.17 shows the absorbance values at 254 n.m. of the filtrates obtained after filtering 10ml aliquots of distilled water through filter pads A, B and C.

On filtering 30ml aliquots of distilled water through filter pads D, E and F, lower absorbance values were obtained; the absorbance of filtrates decreasing as the volume of distilled water filtered, increased. This was expected, as there is only a maximum number of impurities present in each filter. Each pad yielded filtrates with slight differences in absorbance values. (Table 6.18). The filtration rates were also found to differ slightly. The pH value of the distilled water filtered through pad D was found to be 6.0 before filtration; the pH value of filtrate obtained from the first 30ml aliquot was found to increase to 9.0, indicating the alkalinity of the water-soluble extractives obtained from the pad.

Thus it may be seen that the estimations which follow are general approximations, as the water-soluble extractives vary from pad to pad; the same blank control from one pad being used for the estimations on the other filter pads.

TABLE 6.17

FILTRATION OF 10ml ALIQUOTS OF DISTILLED WATER THROUGH ASBESTOS PADS A, B AND C.

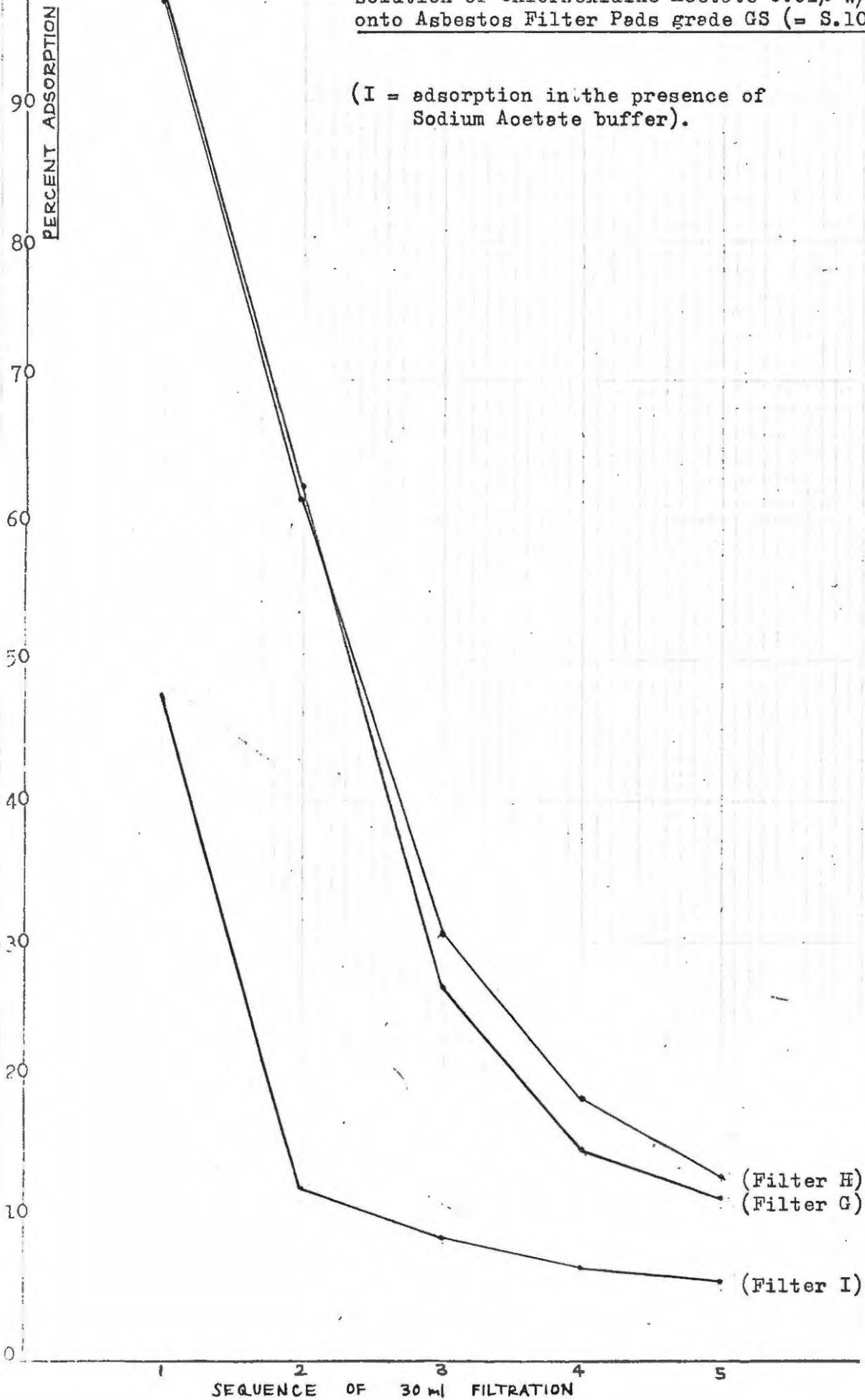
10ml aliquots of distilled water	Time taken for filtration (seconds)			Absorbance at 254 n.m.		
	A	B	C	A	B	C
1	75	100	80	.210	.208	.202
2	150	100	-	.110	.08	-
3	90	100	-	.035	.055	-
4	120	100	-	.022	.03	-
5	170	-	-	.0175	.025	-
6	90	-	-	.015	-	-
7	90	-	-	.010	-	-
8	90	-	-	.015	-	-
9	90	-	-	.0125	-	-

TABLE 6.18
FILTRATION OF 30ml ALIQUOTS OF DISTILLED WATER THROUGH
ASBESTOS PADS D, E AND F.

30ml aliquots of distilled water	Time taken for filtration			Absorbance at 254 n.m.		
	D	E	F	D	E	F
1	5m. 20 secs	5m. 20 secs	5m. 40 secs	0.095	0.093	0.130
2	6m. 30 secs	7m. 30 secs	6m. 50 secs	0.005	0.0225	0.030
3	6m.	5m. 20 secs	7m. 50 secs	0.005	0.015	0.0125
4	5m. 20 secs	6m. 30 secs	-	0.005	0.0075	-
5	8m.	5m. 30 secs	-	0.005	0.0075	-

FIG. 6.8 Adsorption of Chlorhexidine from a solution of Chlorhexidine Acetate 0.01% w/v, onto Asbestos Filter Pads grade GS (= S.10).

(I = adsorption in the presence of Sodium Acetate buffer).



The extent of adsorption of Chlorhexidine which occurred onto filter pads G and H from 30ml of solution was found to be more than 90%. Both filters were found to adsorb to a similar extent (Pad G adsorbing slightly more than pad H). After filtration of 60ml, pad H was found to adsorb slightly more than pad G, adsorption continuing after 150ml of Chlorhexidine Acetate 0.01% w/v had been filtered. (Table 6.19 and Fig. 6.8). The rates of filtration for the two pads were found to be slightly different, filter G having a slightly lower rate.

The desorption patterns for pads G and H were found to be fairly similar, about 50% of the adsorbed Chlorhexidine, being desorbed by 30ml of distilled water; the initial absorbance value at 254 n.m., after desorbing with 30ml of distilled water, being 1.8 and 1.5 for pads G and H respectively, dropping to 0.450 after 90ml had been filtered through both filters.

The effect of Sodium Acetate buffer on the adsorption of Chlorhexidine onto filter pads I, was found to be notable; the extent of adsorption being more or less halved in its presence. (Table 6.20 and Fig. 6.8). The filtration rates were found to be lower than previous determinations. The water-soluble extractives from pad J were observed to be slightly higher than that obtained when preparing the blanks (control) with distilled water.

TABLE 6.19

ADSORPTION OF CHLORHEXIDINE ONTO ASBESTOS PADS G AND H FROM
CHLORHEXIDINE ACETATE 0.01% w/v.

30ml aliquot of Chlorhexidine Acetate 0.01% w/v	Time taken for filtration		Absorbance at 254 n.m.		Percent Loss	
	G	H	G	H	G	H
1	5m. 20 secs	5m. 15 secs	0.010	.0126	97.84	97.3
2	6m. 45 secs	5m. 10 secs	0.175	.179	62.36	61.5
3	5m. 50 secs	5m. 20 secs	0.340	.323	26.88	30.53
4	8m. 30 secs	5m.	0.395	.378	15.05	18.93
5	9m.	6m. 10 secs	0.410	.404	11.83	13.12
			(original solution (1-10) = .465)			

TABLE 6.20

ADSORPTION OF CHLORHEXIDINE ONTO ASBESTOS PAD I, FROM CHLORHEXIDINE
ACETATE 0.01% w/v BUFFERED TO pH 5.89 WITH
SODIUM ACETATE.

30ml aliquot	Time taken for filtration	Absorbance at 254 n.m.	Percent Loss
1	6m. 20 secs	0.245 (blank J) 0.190	47.33
2	7m. 5 secs	0.409 0.055	12.04
3	10m. 10 secs	0.424 0.038	8.82
4	11m. 15 secs	0.434 0.0275	6.67
5	11m. 30 secs	0.439 0.025	5.6

On desorbing filter pad I with 30ml of distilled water an absorbance value of 0.245 was obtained, followed by 0.0025 for the filtrate obtained from a second 30ml aliquot of distilled water, filtered through the pad. On desorbing the pad further, with 30ml of the Sodium Acetate buffer, the absorbance value of the filtrate obtained, was found to increase to more than 2.0.

The effects of Sodium Acetate on the adsorption and desorption of Chlorhexidine seems to indicate that the filter medium acts as a cation exchanger, the sodium ions in solution being preferentially absorbed to the asbestos fibres which presumably carry a negative charge, thus preventing an excessive amount of Chlorhexidine cations from being adsorbed onto the asbestos fibres; or on being preferentially adsorbed, the sodium ions allow the adsorbed Chlorhexidine cation to be easily desorbed.

Washing filter pad K with N/10 Hydrochloric Acid to neutralize the alkaline-water-soluble extractives, was found to have no effect on the adsorption of Chlorhexidine; the amount of adsorption occurring, being the same as that occurring onto the untreated filter pad M, i.e. 98.9% loss occurs from 30ml of Chlorhexidine Acetate 0.01% w/v. The pH value of the filtrate obtained from filter K was found to be 6.55, (pH of the original unfiltered solution being 6.5), whilst that obtained from filter M, was found to

be 9.4. It thus appears that the pH value of the filtrate obtained has no influence on the extent of adsorption that occurs. The water-soluble extractives from the acid-treated filter pad, were found to have an absorbance value of 0.03 at 254 n.m., whilst that from the untreated filter had an absorbance of 0.09.

Effect of autoclaving.

Autoclaving the filter pads was found to have no effect on the adsorption of Chlorhexidine, and only a very slight effect on the pH value of the blank filtrate. Filter pad O was found to adsorb 98.9% of Chlorhexidine from 30ml of Chlorhexidine Acetate 0.01% w/v, an amount similar to that adsorbed onto the unsterilized pad, M. (M and O having similar filtration rates.) The pH value of the blank filtrate obtained from the sterilized filter pad P, being 8.98 and that from the unsterilized pad M, 9.4, whilst the pH value of the Chlorhexidine filtrate obtained from sterilized filter pad O, was found to increase to 8.85 (the original unfiltered Chlorhexidine Acetate solution having a pH value of 6.5).

(d) Adsorption onto Sartorius Membrane Filters.

The Sartorius membrane filter may be attached to two types of filter units, viz. a polycarbonate plastic, or an all glass type. (Chapter 5.d.) The plastic unit has its own self-contained receiver, which screws onto the base of the reservoir and filter support. Buchner tubes (50ml) with side arms were used as receivers for the glass unit.

Before every determination the units were cleaned thoroughly with distilled water until the filtrates of distilled water that were washed through had zero absorbance at 254 n.m. Before attaching the filters, the units were allowed to dry under mild heat.

The filtrate obtained, from filtering 10ml of distilled water through two filters was found to have zero absorbance at 254 n.m.; thus the use of blanks was found to be unnecessary. All filtrations were carried out under a vacuum of 8 - 10 ins of mercury, as the membranes were found to be incapable of maintaining a higher vacuum especially, when filtering small volumes of solution, through the filter attached to the plastic units.

Effect of the plastic unit on Chlorhexidine Acetate.

To determine whether the plastic material interacted with Chlorhexidine Acetate, 10ml of preservative at a concentration of 0.01% w/v was run through the unit in the absence of the membrane.

The solution was allowed to stand for 15 minutes and then assayed.

i) Sartorius Plastic filter unit.

Five 10ml aliquots of Chlorhexidine Acetate 0.01% w/v were filtered through each of membrane filters A and B, the receiver being rinsed and dried before each filtration. The filtrates were collected individually in large Pyrex test tubes and then assayed spectrophotometrically at 254 n.m. using a 1-10 dilution of each.

The influence of Sodium Acetate buffer on the adsorption of Chlorhexidine was determined by, filtering five 10ml aliquots of a solution containing Chlorhexidine Acetate 0.01% w/v incorporated with the buffer at a pH value of 5.89, through membrane filter C.

The effect of steam sterilization on the adsorptive properties of membrane filters was determined by autoclaving filter D, which was attached to the unit, at a pressure of 10lbs per sq. in. gauge for 30 minutes. According to Van Ooteghem and Herbots,²⁷ membrane filters should not be sterilized at temperatures greater than 120°C, as this causes the filter material to deteriorate with possible shrinkage.³² After being autoclaved, the entire unit was dried under mild heat and allowed to cool. 10ml of Chlorhexidine Acetate 0.01% w/v was then filtered and the loss determined and, compared to the extent of adsorption occurring onto filters A and B.

ii) Sartorius Glass Filter Unit.

Three 10ml aliquots of Chlorhexidine Acetate 0.01% w/v were filtered through membrane filter B, and the filtrates collected individually, for assay.

RESULTS and DISCUSSION.

Effect of the Plastic Unit.

The plastic unit was found not to interact with the Chlorhexidine Acetate solution, the pre-filter absorbance reading of a 1-10 dilution being 0.465, was observed after the solution was run through the unit and allowed to stand for 15 minutes.

The filtration rates of filters A to B in all the determinations, were found to high (10ml/5 secs) at a vacuum of about 10 ins of mercury, there being no significant difference, when using the plastic or glass units. The membranes showed no signs of slowing down the filtration rates, as filtration proceeded.

i) Sartorius Plastic Filter Unit.

Loss of Chlorhexidine, through adsorption onto membrane filters A and B from 10ml of Chlorhexidine Acetate 0.01% w/v, was found to be 4.3%. From Table 6.21 it may be seen that both membranes A and B adsorbed to the same extent, saturation occurring after filtration of 20ml. (See Fig. 6.9).

FIG. 6.9 Adsorption of Chlorhexidine from a solution of Chlorhexidine Acetate 0.01%w/v, onto Sartorius Membrane Filters.

(E)
(C)
(A)

A = Adsorption onto Filter A attached to the plastic unit.
C = Adsorption onto Filter C in the presence of Sodium Acetate buffer. (plastic unit)
E = Adsorption onto Filter E attached to the glass unit.

PERCENT ADSORPTION
15
10
5
0

1 2 3 4
SEQUENCE OF 10ml FILTRATION

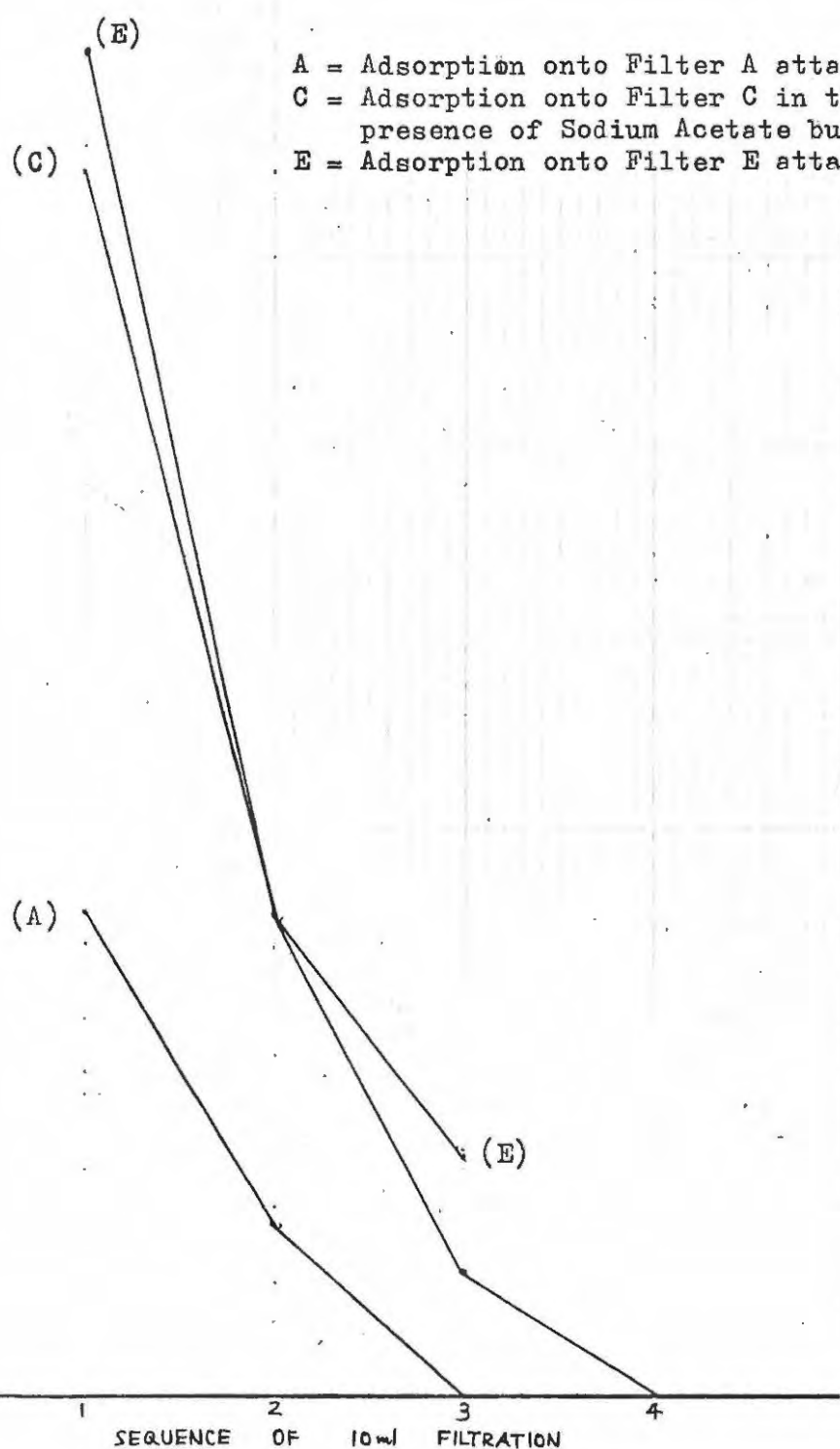


TABLE 6.21
ADSORPTION OF CHLORHEXIDINE ONTO
SARTORIUS MEMBRANE FILTERS

	10ml aliquot of Chlorhexidine Acetate 0.01% w/v	Percent Loss		
		A	B	C (NaOAc buffer)
1		4.3	4.3	10.75
2		1.5	1.5	4.3
3		nil	nil	1.08
4		nil	nil	nil
5		nil	nil	nil

In an investigation concerning the adsorption of Chlorhexidine Gluconate onto Sartorius membrane filters, Van Ooteghem and Herbots²⁷ found that 1.9% and 2.3% adsorption occurred onto filters of diameters, 1.3cm and 2.5cm respectively, from 10ml of Chlorhexidine Gluconate 0.02% w/v, in the presence of Boric Acid and Sodium Borate.

The use of Sodium Acetate buffer, resulted in an increased amount of adsorption occurring onto membrane filter C (Table 6.21 and Fig. 6.9).

Autoclaving filter D, appeared to have no effect on its adsorptive properties, the same amount of adsorption (4.3%) occurring from 10ml of solution, as that obtained when using filters A and B.

ii) Sartorius Glass Filter Unit.

As anticipated higher loss from solution occurred after filtration through membrane filter B, which was attached to the glass unit. This was expected because of the filter support disc, which being composed of sintered glass, also caused a certain amount of adsorption of the preservative from the solution being filtered. (Table 6.22).

TABLE 6.22

ADSORPTION OF CHLORHEXIDINE ONTO MEMBRANE FILTERS, A AND E,
ATTACHED TO THE PLASTIC AND GLASS UNITS RESPECTIVELY.

10ml aliquot of Chlorhexidine Acetate 0.01% w/v	Percent Loss	
	Plastic Unit with filter A	Glass Unit with filter E
1	4.3	11.83
2	1.5	4.3
3	nil	2.15

SUMMARY (Table 6.23)

(a) Sintered Glass Filters.

Adsorption and desorption experiments performed indicated that from 10ml of Chlorhexidine Acetate 0.01% w/v the amount lost through adsorption varied from 15% - 21%, the filters becoming saturated after 20 - 50ml had been filtered. The effect of varying the vacuum required for filtration could not be related to the extent of adsorption which occurred. Higher amounts of adsorption occurred from solutions of lower concentrations.

About 50% of the adsorbed Chlorhexidine was desorbed with 10ml of distilled water, a total volume of more than 300ml being required for complete desorption.

Adsorption at elevated temperatures was found to be diminished, and desorption, more accomplished.

Sodium Chloride and Sodium Acetate minimised the extent of adsorption; this probably being due to either a surface tension or, electrokinetic effect.

Filters with larger porosities were observed to adsorb less than those with smaller porosities e.g. a filter of porosity No. 4 adsorbed 14% from 10ml of solution.

(b) Doulton-Pasteur unglazed porcelain candles.

Adsorption of Chlorhexidine from 30ml of Chlorhexidine Acetate 0.01% w/v was found to vary respectively between 47% to 68% and 78% for two similar filters; saturation being effected after

90 - 120ml and 120 - 150ml had been filtered, respectively. Desorption of the filters could not be effected with distilled water alone, and required Sodium Chloride or Sodium Acetate solutions.

Adsorption from solutions of a higher concentration, was found to be diminished. The effect of Sodium Acetate buffer was found to be slight, despite it having a pronounced effect on desorption.

(c) Asbestos Pads.

The metal holder for the filter pads was found to inactivate or adsorb Chlorhexidine on contact, causing a loss of 3.23% from solution. The extent to which water-soluble extractives were present in the filters pads was determined. Adsorption of Chlorhexidine from 30ml of Chlorhexidine Acetate 0.01% w/v was found to be greater than 90% in each of two cases.

The effect of Sodium Acetate buffer was most pronounced, minimising the extent of adsorption by about 50%.

Neutralization and removal of the alkaline water-soluble extractives, was found to have no effect on the extent of adsorption, neither did autoclaving.

(d) Sartorius Membrane Filters.

Adsorption of Chlorhexidine from 10ml of Chlorhexidine Acetate 0.01% w/v was found to be 4.3% for each of two filters, the amount adsorbed

increasing to 11.83% when using the glass unit. Sodium Acetate buffer caused an increase in the amount of Chlorhexidine adsorbed. Autoclaving the membranes was found to have no effect on their adsorptive properties.

TABLE 6.23

ADSORPTION OF CHLORHEXIDINE ONTO DIFFERENT FILTER TYPES FROM
10ml CHLORHEXIDINE ACETATE 0.01% w/v.

Sintered Glass Porosity No. 5	Sintered Glass Porosity No. 4	Doulton-Pasteur Candles No. 1	Asbestos Pads Grade GS(S.10)	Sartorius Membranes GmbH	
				(Plastic Unit)	(Glass Unit)
Ca. 15-21%	Ca. 14%	Ca. 90%	Ca. 98% (from 30ml)	Ca. 4%	Ca. 11%

CHAPTER SEVEN

ADSORPTION OF PHENYLMERCURIC NITRATE

Phenylmercuric nitrate (P.M.N.) of molecular formula $C_6H_5Hg.OH$, $C_6H_5.Hg.NO_3$ and molecular weight 634.4 occurs as odourless white plates or crystalline powder with a weakly metallic and astringent taste. A solution in water has a pH of about 4.2. It is soluble 1 in 1500 of water, 1 in 160 of boiling water and 1 in 1000 of alcohol, and is incompatible with halides with which it forms less soluble halogen compounds.

Phenylmercuric nitrate was incorporated into solution at a concentration of 0.002% w/v, which was prepared by diluting a stock solution containing Phenylmercuric nitrate 0.02% w/v in distilled water, this solution being kept in an incubator at 25°C to prevent it precipitating out at room temperature (18°C - 20°C). Solutions containing Phenylmercuric nitrate 0.002% w/v, Phenylmercuric nitrate 0.002% w/v in combination with Sodium Edetate 0.01% w/v which is, the di-sodium salt of ethylene diamino-tetra-acetic acid (E.D.T.A.) and, Phenylmercuric nitrate 0.002% w/v buffered with Sodium Acetate, were filtered through the various media and, all filtrates assayed for their Phenylmercuric nitrate content, the original solutions being pre-assayed before every determination.

The content of Phenylmercuric nitrate in the filtrates was determined according to a method

used by Christensen and Dauv,⁷⁵ but with a slight modification of the acidity of the solution. The method of assay was based on the reaction⁷⁶ that mercury salts in acid solution give violet to blue insoluble inner complex salts with diphenylcarbohydrazide (Fig. 7.1a) and its oxidation product diphenylcarbazone (Fig. 7.1b), the salt of the carbazone having the structure in Fig. 7.1c.

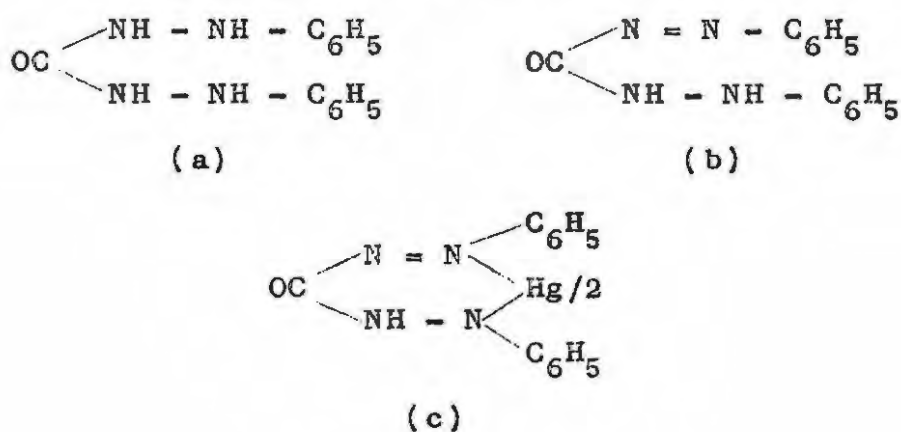


Fig. 7.1

The sensitivity of this reaction with diphenylcarbazone depends on the acidity (pH) of the solution being assayed, and decreases with decreasing acidity.

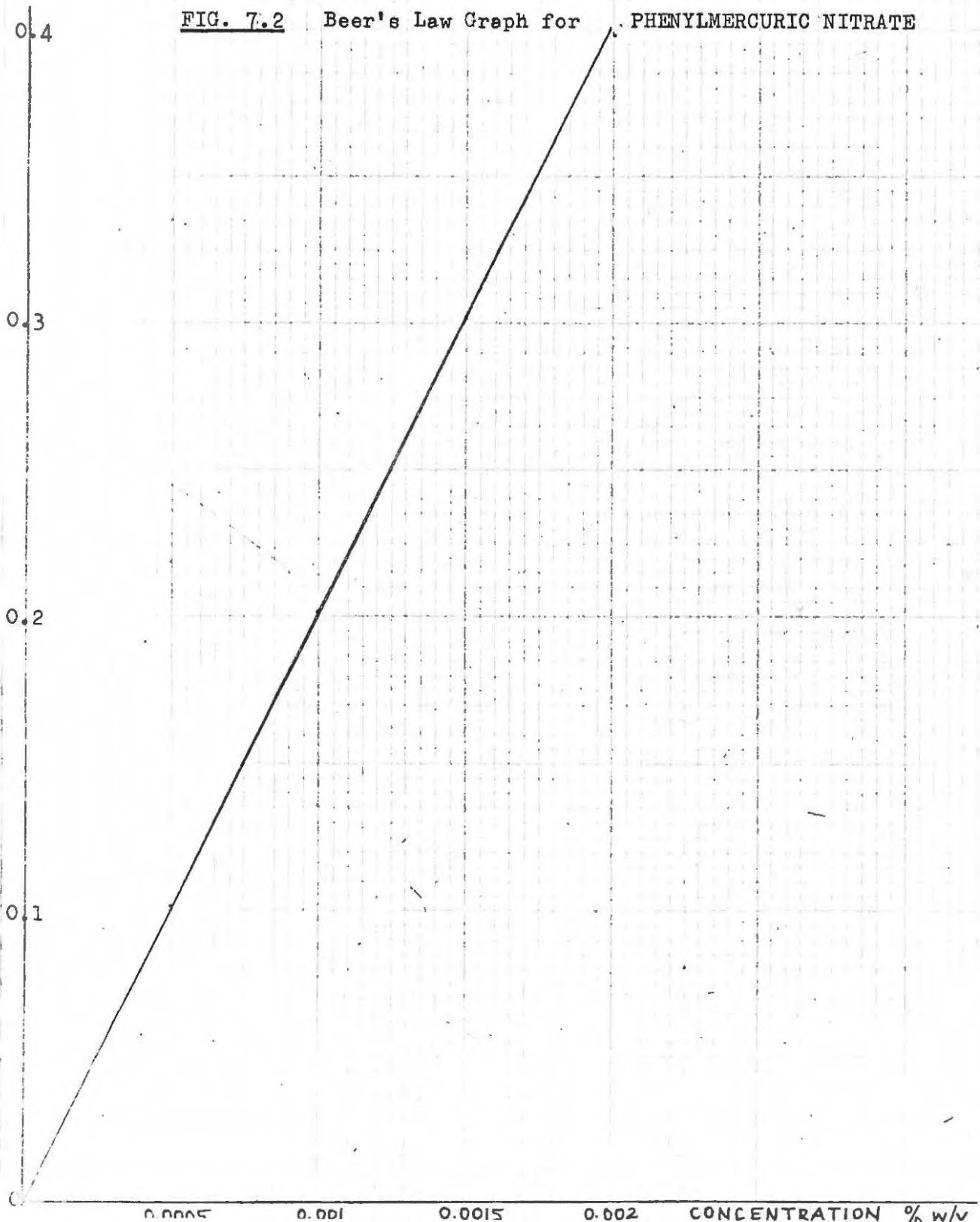
Preparation of Standard Beer's Law Curve.

Phenylmercuric nitrate when assayed by the complexometric method, was found to obey Beer's Law within the range 0.0005% - 0.002% w/v.

Solutions containing 0.002% w/v, 0.0015% w/v, 0.001% w/v and 0.0005% w/v of Phenylmercuric nitrate, were prepared by appropriately diluting a stock solution containing 0.02% w/v. In each case, 3ml of the respective dilution was pipetted into a

ABSORBANCE at 481 m.μ.

FIG. 7.2 Beer's Law Graph for PHENYLMERCURIC NITRATE



100ml conical separating flask containing 20ml of Nitric Acid 0.05% w/v. 10ml of a 0.001% w/v solution of Dithizone (Diphenylthiocarbazone) in Chloroform (freshly prepared for every filter determination) were then pipetted into the flask, and the latter shaken for one minute. The organic phase was allowed to separate from the aqueous phase and the spectral absorbance of the Chloroform layer was then measured at a wavelength of 481 nanometer (n.m.) (visible) (on a Beckman D.B. spectrophotometer) against a blank (consisting of distilled water) prepared in a similar manner, and the results plotted graphically (Fig. 7.2).

The quantity of Phenylmercuric nitrate present in the filtrate was determined by diluting the filtrate (1 - 2), 2.5ml being made up to 5.0ml with distilled water in a volumetric flask (\approx to 0.001% w/v). 3ml of this dilution was then pipetted into the separating flask containing the Nitric Acid, the rest of the assay being performed as mentioned above, and finally the absorbance of the chloroform layer being measured at 481 n.m.

The percentage loss (percent adsorption) was thus determined relative to a concentration of 0.001% w/v (all solutions being pre-assayed before filtration) which had an absorbance reading of 0.202 at 481 n.m. (Fig. 7.2).

(a) Adsorption onto Sintered Glass Filters.

Two sintered glass filters, A and B, of porosity No. 5 were cleaned before every determination by filtering 30ml of hot concentrated sulphuric acid, and finally with hot distilled water until the filtrates obtained were acid free and, on assaying had a zero absorbance value at 481 n.m. The filters were then dried in an oven at 150°C for 1 hour, and allowed to cool to room temperature before use.

All solutions were filtered at room temperature (Ca 20°C) under a vacuum of 20 ins of mercury, the filtrates being collected in large Pyrex test tubes and the rates of filtration reckoned with a stop-clock.

The extent of adsorption of Phenylmercuric nitrate onto the sintered glass filters was determined by filtering five 10ml aliquots of Phenylmercuric nitrate 0.002% w/v through each of filters A and B. This was followed by desorption of the adsorbed preservative, with 10ml aliquots of distilled water, the filtrates obtained, being assayed undiluted.

The determination was repeated using 30ml aliquots of Phenylmercuric nitrate 0.002% w/v, desorption being performed with two 30ml aliquots of dist water, followed by 30ml of Sodium Acetate buffer (pH = 5.89).

The influence of Sodium Edetate 0.01% w/v, on the adsorption of Phenylmercuric nitrate was determined by incorporating Sodium Edetate with

Phenylmercuric nitrate 0.002% w/v (the final solution having a pH value of 5.0) and, filtering five 30ml aliquots through filter A. The filtrates were assayed using a corresponding dilution of Sodium Edetate 0.01% w/v for the blank determination. (Sodium Edetate was previously found not to interfere with the complexation of Dithizone and Phenylmercuric nitrate in the assay process.) Finally, the filter was desorbed with 30ml of distilled water.

Phenylmercuric nitrate 0.002% w/v was incorporated with Sodium Acetate buffer, the final solution having a pH value of 5.9. The effect of the buffer on adsorption, was determined by filtering five 30ml aliquots through filter A. The appropriate dilution of the buffer, was used for the blank determination during the assay process. (Sodium Acetate buffer was previously found not to interfere with the assay process.) The filter was then desorbed with 30ml of distilled water.

The effect of the porosity of the filter on the adsorption of Phenylmercuric nitrate, was estimated by filtering five 30ml aliquots of Phenylmercuric nitrate 0.002% w/v through each of filters A and B of porosity No. 4 (Pyrex G.4), the extent of adsorption being compared with that occurring, onto the filters of porosity No. 5.

RESULTS and DISCUSSION

On filtering 10ml aliquots of Phenylmercuric

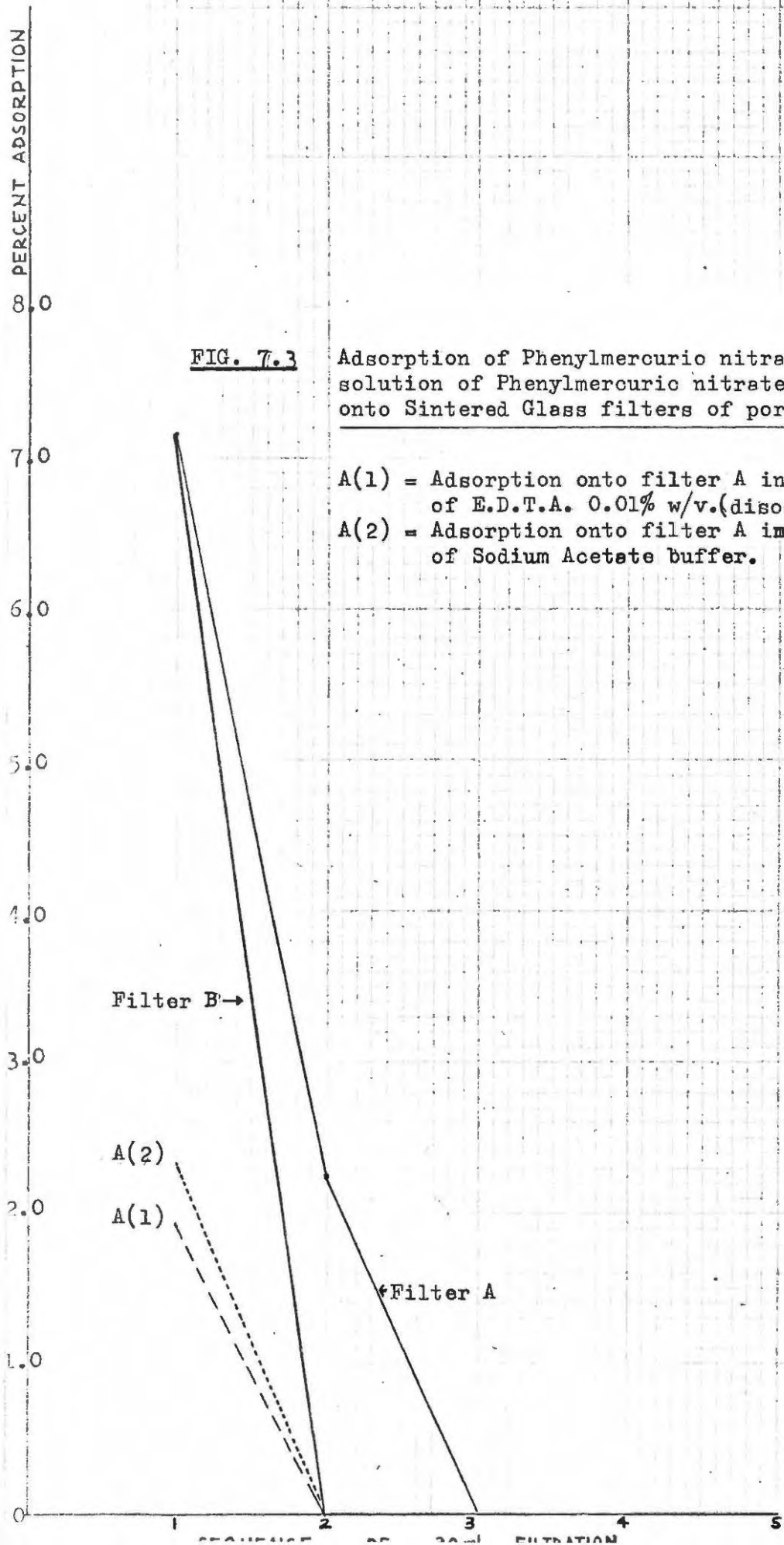


FIG. 7.3

Adsorption of Phenylmercuric nitrate from a solution of Phenylmercuric nitrate 0.002% w/v, onto Sintered Glass filters of porosity No.5.

A(1) = Adsorption onto filter A in the presence of E.D.T.A. 0.01% w/v. (disodium salt).
 A(2) = Adsorption onto filter A in the presence of Sodium Acetate buffer.

Filter B →

A(2)

A(1)

← Filter A

SEQUENCE OF FILTRATION

nitrate 0.002% w/v through sintered glass filters A and B. Filters A and B became saturated after filtration of 20ml of solution. From Table 7.1 it may be seen that the extent of adsorption from 10ml of the solution, onto filters A and B was 14% and 16.8% respectively. The filtration rates of filters A and B were found to be closely similar.

Desorption studies revealed that almost all of the adsorbed Phenylmercuric nitrate was desorbed from sinters A and B, with 30ml of distilled water, after which no further Phenylmercuric nitrate was detected in the filtrate.

Both filters A and B adsorbed similar amounts of Phenylmercuric nitrate from the first 30ml aliquot of solution filtered, however filter A became saturated after 60ml of Phenylmercuric nitrate 0.002% w/v was filtered and, filter B after 30ml. (Table 7.2. and Fig. 7.3). The filtration rates of filters A and B were observed to differ slightly, that of B being slightly higher than A.

Both filters A and B appeared to become desorbed similarly; after washing through with 60ml of distilled water the absorbance of the filtrate on assay, at 481 n.m. became zero, however on further desorption with 30ml of Sodium Acetate buffer, the absorbance of the filtrate was observed to become 0.01, indicating that the buffer solution exerted some influence on the desorption of Phenylmercuric nitrate.

In the presence of Sodium Edetate the amount

TABLE 7.1

ADSORPTION OF PHENYLMERCURIC NITRATE ONTO SINTERED GLASS

FILTERS A AND B

10ml aliquot of Phenylmercuric nitrate 0.002% w/v	Time taken for filtration		Percent Loss	
	A	B	A	B
1	70 secs	65 secs	14.0	16.8
2	70 secs	65 secs	4.5	5.94
3	70 secs	70 secs	nil	nil
4	73 secs	70 secs	nil	nil
5	75 secs	70 secs	nil	nil

TABLE 7.2

ADSORPTION OF PHENYLMERCURIC NITRATE ONTO SINTERED GLASS

FILTERS A AND B

	30ml aliquot of Phenylmercuric nitrate 0.002% w/v		Time taken for filtration		Percent Loss	
	A	B	A	B	A	B
1	4 minutes	4 minutes	7.18	7.20		
2	5 minutes	4m. 15 secs	2.23	nil		
3	5m. 30 secs	4m. 40 secs	nil	nil		
4	7 minutes	5m. 10 secs	nil	nil		
5	7m. 45 secs	6m. 30 secs	nil	nil		

Phenylmercuric nitrate adsorbed from 30ml of solution was found to decrease, Filter A became saturated after filtration of 30ml of solution (Table 7.3). The filtration rates were observed to decrease when compared to the rates obtained previously.

Complete desorption appeared to be effected with 30ml of distilled water.

Sodium Acetate buffer also resulted in the amount of adsorption being diminished, but not to the same extent as that which occurred in the presence of Sodium Edetate (Fig. 7.3). The filter became saturated after filtering 30ml of Phenylmercuric nitrate 0.002% w/v, the filtration rates however, being higher than that obtained when using Sodium Edetate (Table 7.3). Desorption of the filter also appeared to be effected with, 30ml of distilled water.

The overall effect of Sodium Edetate and Sodium Acetate buffer could be considered as being similar, but the rates of filtration (Table 7.3 : b and c) will be observed as being markedly different. Sodium Edetate and Sodium Acetate could exert a surface tension (Chapter 6a) or electrokinetic effect on the adsorption process, which results in diminished adsorption. The electrokinetic effect could be explained on the assumption that Phenylmercuric nitrate ionizes in solution to yield the Phenylmercuric cation⁷⁷ (Ph.Hg⁺) which becomes adsorbed to the negatively charged glass

TABLE 7.3

ADSORPTION OF PHENYLMERCURIC NITRATE ONTO SINTERED GLASS

FILTER A IN THE PRESENCE OF SODIUM EDETATE 0.01% w/v;

AND SODIUM ACETATE BUFFER.

30ml aliquot filtered	Time taken for filtration			Percent Loss		
	a	b	c	a	b	c
1	4 minutes	5m. 20 secs	3m. 50 secs	7.18	1.92	2.32
2	5 minutes	6m. 40 secs	4 minutes	2.32	nil	nil
3	5 m. 30 secs	7m. 45 secs	4m. 10 secs	nil	nil	nil
4	7 minutes	10m. 10 secs	4m. 45 secs	nil	nil	nil
5	7m. 45 secs	11m. 30 secs	5m. 30 secs	nil	nil	nil

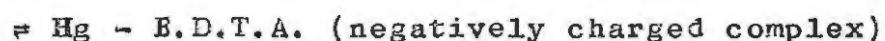
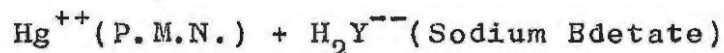
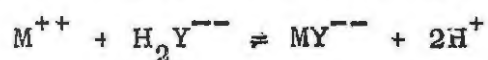
a = Phenylmercuric nitrate 0.002% w/v (P.M.N.)

b = P.M.N./Sodium Edetate 0.01% w/v (pH 5.0)

c = P.M.N./Sodium Acetate buffer (pH 5.9)

surface; the presence of Sodium ions in solution (Disodium Salt - E.D.T.A. or, Sodium Acetate) could have the same effect as in the case of Chlorhexidine, of being preferentially adsorbed, so as to allow the Phenylmercuric cation to pass through the glass pores and, into the filtrates.

On the other hand Sodium Edetate complexes^{73,77} with divalent metal ions to form complexes which are stable in basic or slightly acidic solution. The disodium salt of Ethylenediamine tetra-acetic acid (H_4Y), being Na_2H_2Y , affords the complex forming ion, H_2Y^{--} in aqueous solution which complexes with metal cations (mercury) e.g.



The Hg - E.D.T.A. complex which is formed may thus be repelled by the negatively charged glass surface, thus resulting in diminished adsorption onto the filter.

The effect of Sodium Edetate in minimising adsorption could also be as a result of both complex formation and the preferential adsorption of sodium ions from the ionization of the disodium salt of E.D.T.A.

On filtration of Phenylmercuric nitrate 0.002% w/v through sintered glass filters A and B of porosity No. 4, the amount of adsorption which occurred from 30ml of solution was 4.0% and 3.45% respectively. Both filters became saturated

after filtration of 30ml. Thus the extent of adsorption is reduced by almost 50% when compared to that occurring onto filters of porosity No. 5.

(b) Adsorption onto Doulton-Pasteur Unglazed Porcelain Candles.

Two similar Pasteur candle filters, A and B were cleaned by washing through 30ml of dilute Hydrochloric Acid, followed by several washes of hot distilled water until the filtrates obtained, were acid-free, and on assaying had a zero absorbance value at 481 n.m. The filters were dried at 150°C for one hour and allowed to cool before use.

All solutions were filtered at room temperature (Ca. 20°C) under a vacuum of 20ins of mercury. The filtrates were collected individually in the receiver (Fig. 5.4) (the receiver being rinsed and dried before every filtration), and then run off into large Pyrex test tubes and collected for assay. In the case of determinations with Sodium Edetate and Sodium Acetate buffer, blank determinations containing these salts respectively, in their appropriate dilutions, were used for the assays of Phenylmercuric nitrate, present in the filtrates.

After each determination, desorption studies were performed on the filters with distilled water, and Sodium Acetate buffer, after which the candles were cleaned as above with dil. Hydrochloric Acid etc. and the absorbances of the filtrates checked for freedom from Phenylmercuric nitrate. The filtrates obtained from desorption studies were

assayed undiluted.

The amount of adsorption occurring from 10ml of Phenylmercuric nitrate solution, onto filter A, was determined by filtering ten 10ml aliquots of Phenylmercuric nitrate 0.002% w/v. This was followed by desorption of the adsorbed Phenylmercuric nitrate with 30ml aliquots of distilled water.

The amount of adsorption occurring from 30ml of solution, was determined by filtering four 30ml aliquots Phenylmercuric nitrate 0.002% w/v through each of filters A, and B. On a further occasion this process was repeated with filter B, to see whether any variations occurred, in the extent of adsorption.

The effect of Sodium Edetate on the adsorption of Phenylmercuric nitrate, onto filter B, was determined by filtering four 30ml aliquots of a solution containing Phenylmercuric nitrate 0.002% w/v and Sodium Edetate 0.01% w/v. After filtration the filter was desorbed, using 30ml aliquots of distilled water.

Phenylmercuric nitrate 0.002% w/v was incorporated with Sodium Acetate buffer at a pH value of 5.9. The effect of the buffer on adsorption of Phenylmercuric nitrate, was determined by filtering four 30ml aliquots through filter B. Desorption of the filter was carried out using four 30ml aliquots of distilled water, followed by 30ml of Sodium Acetate buffer, and a further 500ml of distilled water, to wash out the buffer.

RESULTS and DISCUSSION

On filtration of 10ml aliquots of Phenylmercuric nitrate 0.002% w/v through filter A, the extent of adsorption from solution was found to be considerably high, (Table 7.4) with a loss of 100% of the preservative from the first 10ml aliquot of solution filtered, the loss gradually diminished with slight, irregular increases and decreases. The filter continued to adsorb the preservative from solution after 100ml had been filtered. The irregularity in the adsorption pattern could be attributed to a variation in the surface area exposed to the 10ml of solution being filtered, despite measures being taken to ensure that, the solution was poured carefully into the reservoir of the candle with a minimum amount of random contact with the inside walls. The filtration rate appeared to slow down as filtration proceeded, taking up to 10 minutes for the filtration of 10ml (with a slight retention volume).

On assaying, initial absorbance value at 481 n.m. of the filtrate, obtained from washing through with 30ml of distilled water, was found to be 0.132, gradually diminishing to 0.050 after 150ml of distilled water had passed through the filter.

Adsorption of Phenylmercuric nitrate from 30ml was found to be considerable as well, both filters A and B adsorbing 100% of the preservative from the first 30ml aliquot of Phenylmercuric nitrate 0.002% w/v which was filtered. Loss from solution

TABLE 7.4

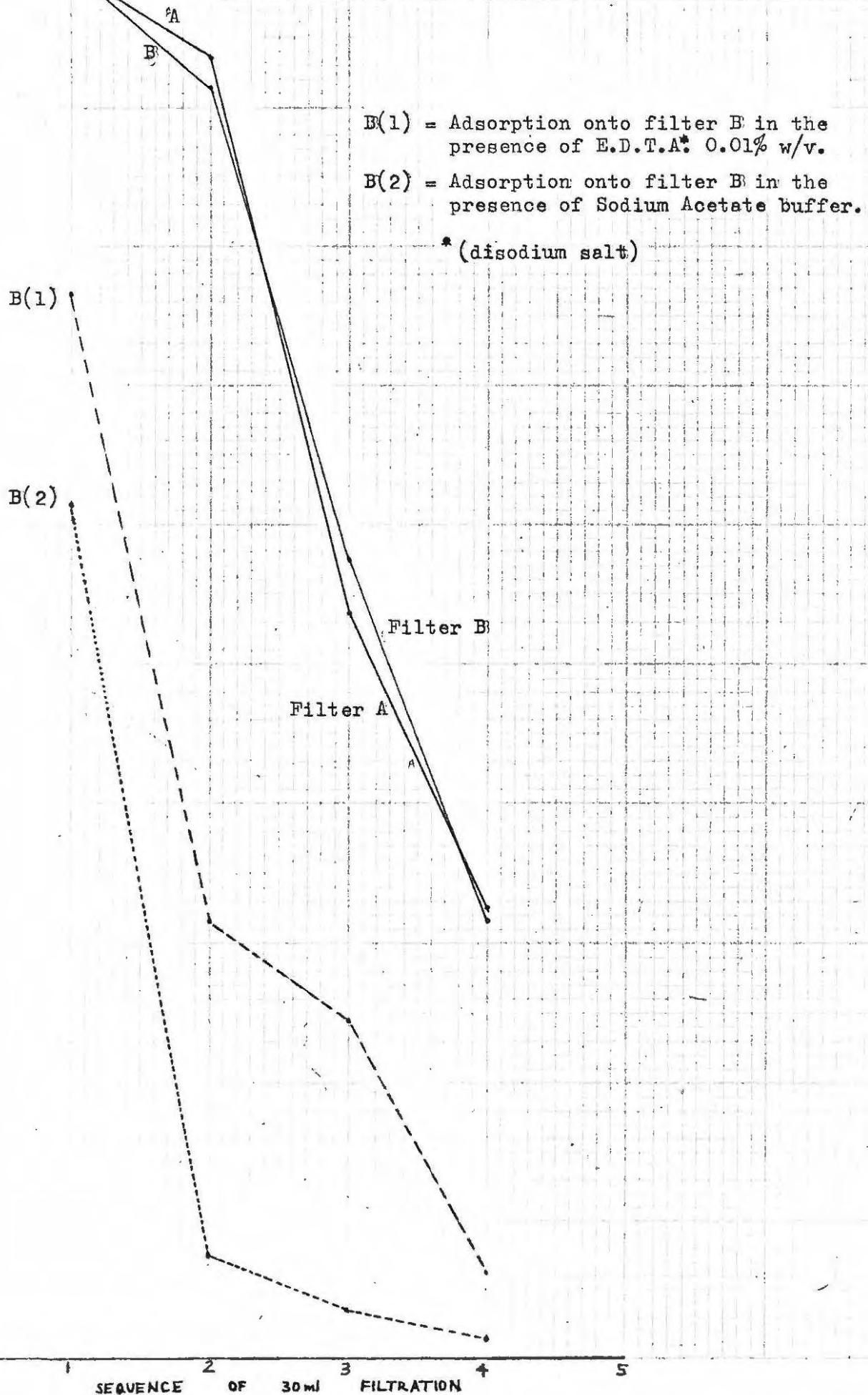
ADSORPTION OF PHENYLMERCURIC NITRATE ONTO PASTEUR

CANDLE FILTER A

10ml aliquot P.M.N. 0.002% w/v	Time taken for filtration	Percent Loss
1	2m. 15 seconds	100.0
2	2m. 45 seconds	98.7
3	4m. 15 seconds	74.4
4	4m. 30 seconds	50.0
5	4m. 35 seconds	20.5
6	8 minutes	24.4
7	8 minutes	32.3
8	8 minutes	39.7
9	10 minutes	36.0
10	10 minutes	28.2

PERCENT ADSORPTION

FIG. 7.4 Adsorption of Phenylmercuric nitrate from a solution of Phenylmercuric nitrate 0.002% w/v, onto Dowlton-Pasteur unglazed porcelain candle filters



was observed to continue after the filtration of 120ml (Table 7.5, Fig. 7.4).

On repeating the process with filter B, increased losses were observed; 100% loss occurred from 60ml of Phenylmercuric nitrate 0.002% w/v and loss (52.5%) continued after 120ml had been filtered. (Table 7.5 B*). These increased losses could be attributed to the decreased rates in filtration, which were observed, the initial 30ml aliquot required 20 minutes and after 90ml had been filtered, a 30ml aliquot required 40 minutes for filtration. These decreased rates of filtration could be due to clogging by particulate matter, or probably to the high extent of adsorption of Phenylmercuric nitrate from solution.

Sodium Edetate was observed to minimize the extent of adsorption, however adsorption from 30ml was still fairly high (76.8%), but decreased to 6.1% after 120ml had been filtered (Table 7.6).

Desorption appeared to be effected with 120ml of distilled water, after which no further trace of phenylmercuric nitrate could be detected in the filtrate.

Sodium Acetate buffer was found to minimize the extent of adsorption onto filter B, to a slightly greater extent than Sodium Edetate (Table 7.6) (Fig. 7.4). The initial loss from 30ml was 61.5%, and decreased to 1.4% after 120ml, had been filtered, despite the filtration rates being slightly lower, than that obtained when filtering

TABLE 7.5

ADSORPTION OF PHENYLMERCURIC NITRATE ONTO PASTEUR CANDLE FILTER

A AND B

	Time taken for filtration			Percent Loss		
	A	B	B*	A	B	B*
1	8 minutes	2m. 30 secs	20 minutes	100.0	100.0	100.0
2	8 minutes	3 minutes	25 minutes	93.6	91.46	100.0
3	10 minutes	3m. 30 secs	35 minutes	53.84	57.3	83.8
4	10 minutes	4 minutes	40 minutes	32.65	31.71	52.5

(* = filtration repeated with filter B on a further occasion)

TABLE 7.6

ADSORPTION OF PHENYLMERCURIC NITRATE ONTO PASTEUR CANDLE B
IN THE PRESENCE OF SODIUM EDETATE 0.01% w/v, AND SODIUM ACETATE BUFFER.

30ml aliquot filtered	Time taken for filtration			Percent Loss		
	a	b	c	a	b	c
1	2m 30 secs	3m 30 secs	7 minutes	100.0	76.82	61.53
2	3 minutes	4m 30 secs	8 minutes	91.46	31.7	7.45
3	3m 30 secs	5 minutes	10 minutes	57.3	24.4	3.85
4	4 minutes	5 minutes	12 minutes	31.71	6.1	1.4

a = Phenylmercuric nitrate 0.002% w/v (P.M.N.)

b = P.M.N./Sodium Edetate 0.01% w/v

c = P.M.N./Sodium Acetate buffer

Phenylmercuric nitrate in the presence of Sodium Edetate.

Desorption appeared to be effected with 120ml of distilled water, the filtrate obtained from the first 30ml aliquot of distilled water when assayed, had an absorbance value of 0.055, decreasing to 0.0075 after 120ml of distilled water had passed through the filter. However on further desorption with 30ml of Sodium Acetate buffer, the filtrate was observed to have an absorbance value of 0.120, thus indicating that Sodium Acetate exerted some influence on the adsorption and desorption of Phenylmercuric nitrate.

From Table 7.6 it will be observed that the effect of Sodium Acetate on the adsorption of Phenylmercuric nitrate is quite pronounced especially after filtration of the first 30ml aliquot, this probably being due to either surface tension or electrokinetic effects of the Sodium ions. It will be observed that the effect of Sodium Edetate is not as pronounced as that of the buffer, but nevertheless, the extent of adsorption does appear to be somewhat effected, either through the complexation of Sodium Edetate with mercury, or possibly as the result of an electrokinetic effect of the sodium ions from the disodium salt, or both.

(c) Adsorption onto Asbestos Filter Pads.

The filter pads used were 3.6cms in diameter and of the G.S. grade. They were used

as described previously, the filtrates being collected individually in large Pyrex test tubes.

Water-soluble extractions.

To determine whether the alkaline water-soluble extractives obtained from the asbestos filter pads, would in any way effect the assay process and hence the results, 10ml of distilled water was filtered through a filter pad, and 3ml of the filtrate used as the test solution in the assay process. The absorbance of the chloroform layer which was obtained finally, was read at 481 n.m. against a blank determination prepared with distilled water.

All filtrations were timed, and carried out under a vacuum of 20ins of mercury at room temperature (Ca 20°C).

The filtrates obtained from determinations in the presence of Sodium Edetate and Sodium Acetate buffer, were assayed, and read against a blank (control) which was prepared by filtering the corresponding volumes of Sodium Edetate or buffer solutions through a filter pad, the filtrates being used for the preparation of the blank determinations, against which the absorbance of the chloroform layer from the assay of the corresponding test solutions, was read at 481 n.m.

Effect of the metal holder on Phenylmercuric nitrate.

20ml of Phenylmercuric nitrate 0.002% w/v was allowed to remain in contact with the metal holder (as described in Chapter 6(c)) for 10 minutes.

The solutions obtained from the reservoir and from that run through the filter unit, were assayed and compared to the original solution containing Phenylmercuric nitrate 0.002% w/v. This determination was repeated using Phenylmercuric nitrate in combination with Sodium Edetate 0.01% w/v; and Sodium Acetate buffer.

The extent of adsorption of Phenylmercuric nitrate onto the filter pads, from 10ml of Phenylmercuric nitrate 0.002% w/v, was determined by filtering five 10ml aliquots of the solution, through filter pad A.

Before every desorption determination the filter pad was removed and, the filter holder washed free of Phenylmercuric nitrate and then dried. The filter pad to be desorbed was then resealed into the holder. The filtrates obtained from desorption experiments were assayed undiluted.

Desorption of filter pad A was carried out, using two 10ml aliquots of distilled water.

Adsorption from 30ml of solution was determined by filtering three 30ml aliquots of Phenylmercuric nitrate 0.002% w/v, through each of filter pads B and C. The pads were then desorbed with two 30ml aliquots of distilled water.

The effect of Sodium Edetate on the adsorption of Phenylmercuric nitrate, onto the filter pads, was determined by filtering three 30ml aliquots of a solution containing Phenylmercuric nitrate 0.002%

w/v and Sodium Edetate 0.01% w/v, through filter pad D. Desorption of the pad was carried out with two 30ml aliquots of distilled water, followed by 30ml of Sodium Edetate 0.01% w/v.

Phenylmercuric nitrate 0.002% w/v was incorporated with Sodium Acetate buffer to yield a solution with a pH value of 5.9. The effect of the buffer on the extent of adsorption was determined by filtering three 30ml aliquots through filter pad E. This was followed by desorption with two 30ml aliquots of distilled water, and 30ml of Sodium Acetate buffer.

RESULTS and DISCUSSION.

Water-soluble extractives.

On assaying the filtrate obtained from 10ml of distilled water, against a blank prepared from distilled water, a zero absorbance reading was obtained at 481 n.m. Thus the water-soluble extractives were found to have no effect on the assay process.

Effect of the metal holder.

The metal holder was observed to have no effect on the Phenylmercuric nitrate solution. The solutions obtained from the holder after being assayed were found to have an absorbance value similar to that of the original solution of Phenylmercuric nitrate 0.002% w/v (0.202 at 481 n.m.). The solutions containing Phenylmercuric nitrate and Sodium Edetate or Sodium Acetate were also found to retain their original concentration, reflecting no change or effect when in contact with the metal

The extent of adsorption onto filter pad A, from 10ml of Phenylmercuric nitrate was found to be 30%. After 90ml of the solution had been filtered, the amount of preservative adsorbed was found to decrease to 2.5% after which the filter became saturated, and ceased to adsorb further. (Table 7.7).

TABLE 7.7.
ADSORPTION OF PHENYLMERCURIC NITRATE ONTO
ASBESTOS FILTER PAD A.

10ml aliquot P.M.N. 0.002% w/v	Time taken for filtration	Percent Loss
1	68 secs	30.0
2	75 secs	8.75
3	85 secs	2.5
4	100 secs	nil
5	100 secs	nil

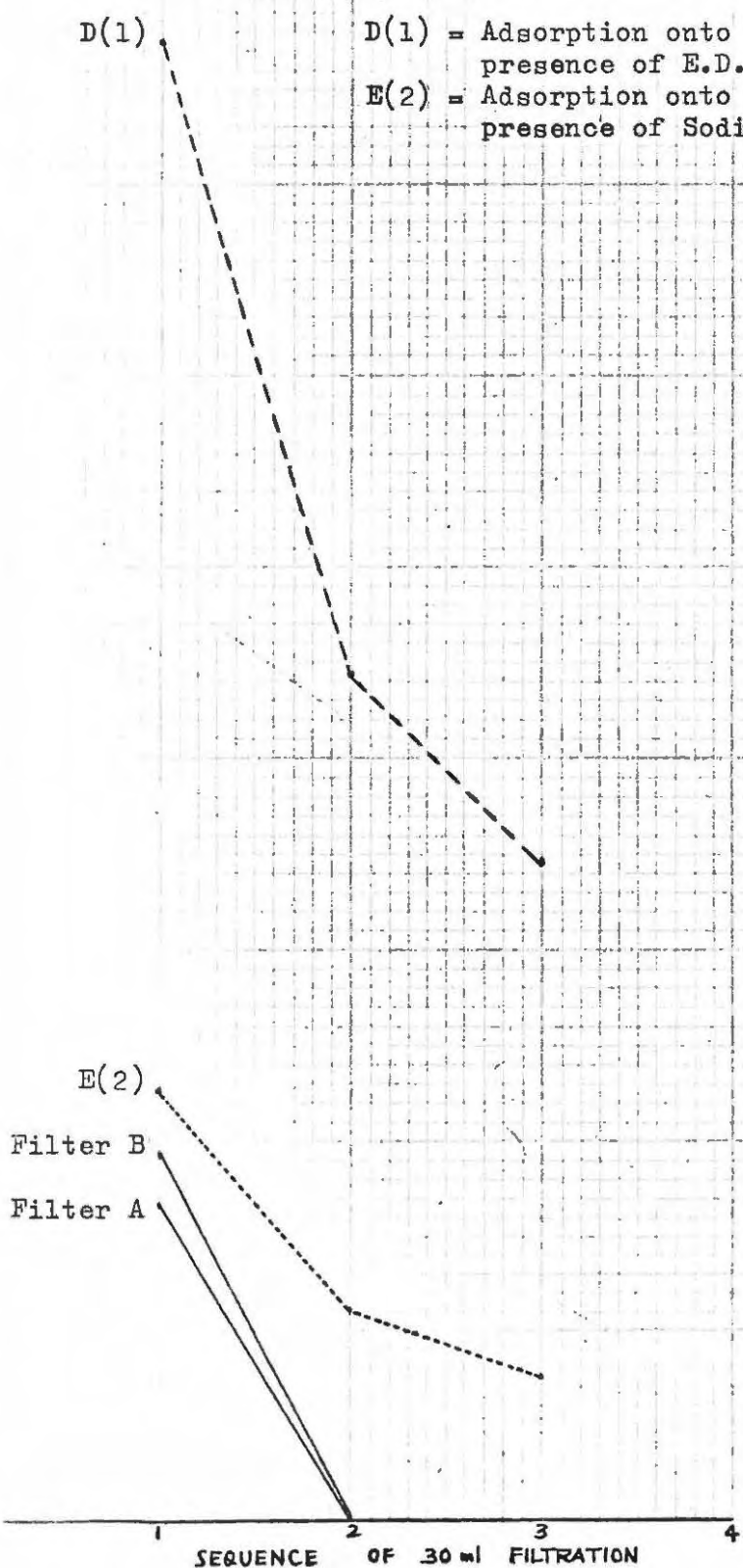
Desorption of filter pad A appeared to be effected with 20ml of distilled water, the absorbance of the filtrate from the first 10ml aliquot of distilled water, on assay was found to be 0.132 (about 80% - 90% desorption), after which no further Phenylmercuric nitrate was detected in the filtrate.

Filter B and C differed slightly in the amounts of Phenylmercuric nitrate, which each adsorbed from 30ml of Phenylmercuric nitrate 0.002% w/v. (Table

PERCENT ADSORPTION

FIG. 7.5 Adsorption of Phenylmercuric nitrate from a solution of Phenylmercuric nitrate 0.002% w/v, onto Asbestos Filter Pads grade GS.

D(1) = Adsorption onto filter pad D in the presence of E.D.T.A. 0.01% w/v. (disodium salt)
E(2) = Adsorption onto filter pad E in the presence of Sodium Acetate buffer.



7.8). Both filters however, became saturated after filtration of the first 30ml aliquot, (Fig. 7.5) after which no further loss was detected from the filtrate. The filtration rates of filters B and C were observed to be slightly different, that of C being slightly lower than that of B for the first 30ml aliquot which was filtered. This probably accounts for the slight differences which were observed in the extents of adsorption, onto filters B and C.

Both filter pads B and C, were found to become desorbed with 30ml of distilled water. The filtrates obtained from the first 30ml aliquot of distilled water, after being assayed, were found to have absorbance values of 0.07 and 0.08 respectively, this corresponding to about 90 - 100% desorption. The filtrates from the second 30ml aliquot of distilled water which were washed through filters B and C, on being assayed were found to have an absorbance value of zero.

Sodium Edetate was observed to cause an increase in the extent of adsorption, (38.7%) occurring from 30ml of Phenylmercuric nitrate 0.002% w/v, onto filter pad D. (Fig. 7.5) Adsorption continued after 90ml had been filtered (Table 7.9). The filtration rates appeared to be unaffected in the presence of Sodium Edetate.

Complete desorption of filter pad D was not accomplished with distilled water, the initial absorbance value of the filtrate of distilled

TABLE 7.8

ADSORPTION OF PHENYLMERCURIC NITRATE ONTO ASBESTOS FILTER PADS B AND C

30ml aliquot of P.M.N. 0.002% w/v	Time taken for filtration		Percent Loss	
	B	C	B	C
1	6 minutes	7m 30 secs	8.415	9.65
2	10 minutes	9m 30 secs	nil	nil
3	11 minutes	9m 50 secs	nil	nil

water on being assayed was found to be 0.095 and decreasing to 0.06 with the second 30ml aliquot of distilled water filtered. On further desorption with 30ml of Sodium Edetate 0.01% w/v the absorbance of the filtrate was found to increase slightly to 0.065, probably because of complexation of the Sodium Edetate with the adsorbed Phenylmercuric nitrate.

Sodium Acetate buffer also resulted in an increased amount of adsorption of Phenylmercuric nitrate from 30ml of Phenylmercuric nitrate 0.002% w/v, onto the filter pads, but not to the same extent as that which occurred in the presence of Sodium Edetate (Fig. 7.5). 11.47% adsorption was found to occur from solution, onto filter pad B (Table 7.9), and adsorption continued after 90ml of solution had been filtered.

On desorption of filter pad B with 30ml of distilled water, the filtrate after being assayed, was found to have an absorbance value of 0.0225, dropping to 0.008 after the second 30ml aliquot of distilled water had been washed through. On further desorption with 30ml of Sodium Acetate buffer, the absorbance value of the filtrate on being assayed, was found to have dropped further, to 0.0025.

The effect of increased adsorption in the presence of Sodium Edetate could probably be due to its complexation with metal ions within the pad itself. According to Vogel,³⁷ the asbestos pads

TABLE 7.9

ADSORPTION OF PHENYLMERCURIC NITRATE ONTO ASBESTOS FILTER PADS
IN THE PRESENCE OF SODIUM EDETATE 0.01% w/v AND SODIUM ACETATE BUFFER.

30ml aliquot filtered	Time taken for filtration			Percent Loss		
	C	D	E	C	D	E
1	9m. 30 secs	6m. 5 secs	7 minutes	9.65	38.7	11.47
2	9m. 30 secs	8m 50 secs	9 minutes	nil	22.17	5.5
3	9m. 50 secs	9m 55 secs	9m. 15 secs	nil	17.45	3.67

C = Phenylmercuric nitrate 0.002% w/v (P.M.N.)

D = P.M.N./Sodium Edetate 0.01% w/v

E = P.M.N./Sodium Acetate buffer

may contain small quantities of Calcium and Iron. These most probably complex with the Sodium Edetate in solution, giving rise to complexes which are easily washed through the pad, thus leaving behind an increased number of negatively charged adsorption sites, for the adsorption of the Phenylmercuric cation.

The effect of the Sodium Acetate buffer does not appear to be an electrokinetic one, as increased adsorption was observed to occur in its presence. From the results obtained (Table 7.9), it seems that the greater the alkalinity of the solution, the lower the extent of adsorption. The least adsorption occurred in the absence of Sodium Edetate and Sodium Acetate; these filtrates could have been expected to be fairly alkaline (pH 8-9) because of the water-soluble materials extracted from the filter pad. The filtrates obtained in the presence of Sodium Edetate were found to have a pH value of 5.3, and that from solutions containing the buffer, 5.9 - 6.0, the buffer resisting any changes in pH, which may be caused by the water-soluble-extractives. Thus it seems that the lower the pH value of the filtrate obtained, the greater was the extent of adsorption.

(d) Adsorption onto Sartorius Membrane Filters.

The determinations on the Sartorius membrane filters, were done with the membranes attached to the plastic, and glass filter units. Before each determination, the filter units were cleaned

thoroughly and rinsed with distilled water, so that the filtrate obtained on passing 10ml of distilled water through it, had a zero absorbance value at 481 n.m., when assayed. After cleaning, the units were allowed to dry under mild heat. In the case of the plastic unit, the receiver was rinsed and dried between filtrations.

All determinations were done at room temperature (Ca 20°C) under a vacuum of 20ins of mercury (when filtering aliquots of 30ml) and the filtration rates recorded.

The filtrate obtained by filtering 10ml of distilled water, through a membrane filter attached to the plastic unit, was assayed according to the method used for assay of the test solutions, and the absorbance of the chloroform layer read at 481 n.m. against a blank determination prepared with distilled water. This determination was repeated with another two membrane filters, using 30ml of Sodium Edetate 0.01% w/v, and Sodium Acetate buffer, respectively.

Effect of the plastic unit on Phenylmercuric Nitrate.

To determine whether the plastic unit interacted with the Phenylmercuric nitrate solution, 10ml of Phenylmercuric nitrate 0.002% w/v was passed through the unit, (without the membrane) and the solution allowed to remain in contact with the receiver of the unit, for one minute. The solution was then appropriately diluted, and 3ml assayed for its Phenylmercuric nitrate content. (The original

solution of Phenylmercuric nitrate 0.002% w/v, having been pre-assayed). This determination was repeated three more times, using 30ml of Phenyl-nitrate 0.002% w/v, 30ml of Phenylmercuric nitrate 0.002% w/v in combination with Sodium Edetate 0.01% w/v and, 30ml of Phenylmercuric nitrate buffered with Sodium Acetate at a pH value of 5.9.

(i) Sartorius Plastic Filter Unit.

The extent of adsorption which occurred from 10ml of Phenylmercuric nitrate 0.002% w/v, was determined by filtering five 10ml aliquots of the Phenylmercuric nitrate solution, through each of membrane filters A and B.

Before each desorption determination on the filter, the membrane was removed and the unit rinsed and dried, after which the membrane was refitted into its position. Membrane filter A was desorbed with 30ml of distilled water, and the filtrate assayed undiluted.

Adsorption from 30ml of solution was determined by filtering three 30ml aliquots of Phenylmercuric nitrate 0.002% w/v through membrane filter C.

The effect of Sodium Edetate on the adsorption of Phenylmercuric nitrate onto membrane filter D, was determined by filtering three 30ml aliquots of a solution containing Phenylmercuric nitrate 0.002% w/v and Sodium Edetate 0.01% w/v. This was followed by desorption of the membrane with 30ml of distilled water.

Phenylmercuric nitrate 0.002% w/v was incor-

porated with Sodium Acetate buffer at a pH value of 5.9, and the effect of the buffer on adsorption was determined by filtering three 30ml aliquots of the solution through membrane filter F. The membrane was then desorbed with 30ml of distilled water.

(ii) Sartorius Glass Filter Unit.

Three 30ml aliquots of Phenylmercuric nitrate 0.002% w/v were filtered through membrane filter F, which was attached to the glass unit.

The filter unit was then rinsed and dried with the membrane removed, after which membrane filter F was refitted, and washed through with 30ml of distilled water. All filtrates were collected individually in 50ml Buchner tubes.

RESULTS and DISCUSSION.

The filtrate obtained by passing 10ml of distilled water through a membrane filter attached to the plastic filter unit, was found to have a zero absorbance value at 481 n.m. when assayed. Similar results were obtained when the solutions of Sodium Edetate and Sodium Acetate buffer were filtered.

Effect of the Plastic filter unit on Phenylmercuric Nitrate.

The absorbance value of the original Phenylmercuric nitrate solution 0.002% w/v on dilution and assay, was found to be 0.202. The 10ml of Phenylmercuric nitrate solution, after remaining in contact with the receiver of the unit for one

minute, was found to have an absorbance value of 0.183 after being assayed, thus indicating a loss of 9.4%.

Loss from 30ml of Phenylmercuric nitrate 0.002% w/v was found to be 4.0%. The Phenylmercuric nitrate solutions containing Sodium Edetate and Sodium Acetate buffer, were found to lose 4.0% and 3.25% respectively, when allowed to remain in contact with the unit.

Thus in all the determinations using the Sartorius plastic filter unit, the total loss which occurs, accounts for adsorption onto the membrane filter, as well as inactivation by, or adsorption onto the plastic unit. Loss from solutions filtered through membrane filters attached to the glass unit, include adsorption onto the sintered glass support disc.

(1) Sartorius Plastic filter unit.

From Table 7.10, it may be seen that the losses which occurred from the 10ml aliquots of Phenylmercuric nitrate 0.002% w/v filtered through membrane filters A and B, remained constant after 30ml of solution had been filtered. This constant loss of 9.4% probably occurred as a result of the interaction between the filter unit and the preservative, as a loss of 9.4% was previously determined from 10ml of the Phenylmercuric nitrate solution, which remained in contact with the unit.

If it is assumed that 9.4% of Phenylmercuric nitrate is lost from solution through interaction

FIG. 7.6

Loss of Phenylmercuric nitrate occurring from Phenylmercuric nitrate 0.002% w/v, on filtration through Sartorius Membrane Filters C, D, E, and F.

- C = Loss on filtration through Filter C attached to the plastic filter unit.
- D = Loss on filtration through Filter D in the presence of Sodium Edetate 0.01% w/v.
- E = Loss on filtration through Filter E in the presence of Sodium Acetate buffer.
- F = Loss on filtration through Filter F attached to the glass filter unit.

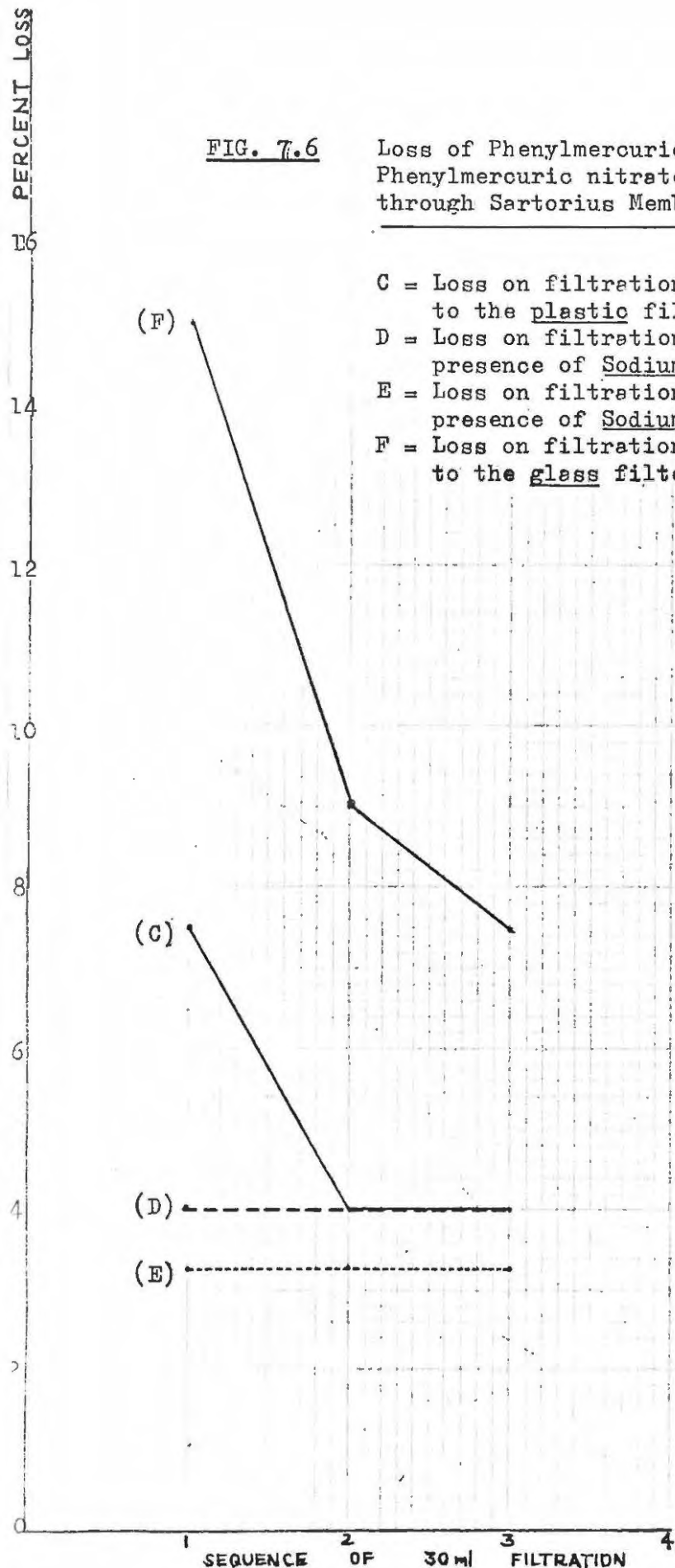


TABLE 7.10

LOSS OF PHENYLMERCURIC NITRATE, ON FILTRATION OF PHENYLMERCURIC NITRATE

0.002% w/v THROUGH MEMBRANE FILTERS A AND B

10ml aliquot filtered	Time taken for filtration		Percent Loss	
	A	B	A	B
1	5 seconds	5 seconds	18.57	18.60
2	5 seconds	5 seconds	11.88	11.85
3	5 seconds	10 seconds	9.9	10.0
4	10 seconds	10 seconds	9.4	9.4
5	10 seconds	10 seconds	9.4	9.4

with the unit, then it will appear that about 9.2% adsorption occurs onto the membrane from the first 10ml aliquot which is filtered, and about 2.4% and 0.5%, from the second and third aliquots respectively.

The filtration rates were observed to decrease slightly from 10ml/5 secs to 10ml/10 secs, probably because of gradual clogging of the membrane's pores.

The filtrate obtained from desorption of membrane A, was found to have a zero absorbance value on being assayed, indicating that the Phenylmercuric nitrate which was adsorbed to the membrane, was either adsorbed very strongly, or interacted chemically with it.

Loss from 30ml Phenylmercuric nitrate 0.002% w/v on filtration through membrane filter C was found to be 7.5% (Table 7.11), after which the loss remained constant at 4.0% (Fig. 7.6); this probably being due to interaction with the unit. Thus it seems that 3.5% adsorption occurred onto the membrane itself from the first 30ml aliquot filtered.

The filtration rates through filter C did not appear to follow the normal pattern; the first 30ml required 30 seconds and the third, 15 seconds.

In their investigation concerning the adsorption of Phenylmercuric Borate onto Sartorius membrane filters, Van Ooteghem and Herbots²⁷ found that 0.6% and 1.7% adsorption occurred onto filters of diameters 1.3cm and 2.5cm respectively. They found that with the filter of diameter 2.5cm, a

constant loss of 1.4% occurred, after filtration of 20ml of Phenylmercuric Borate 0.005% w/v, in the presence of Boric Acid and Sodium Borate.

In the presence of Sodium Edetate 0.01% w/v a constant loss of 4.0% was observed from the 30ml aliquots filtered, (Table 7.11 and Fig. 7.6); this probably being due to interaction with the plastic unit. Thus it seems that no adsorption occurred onto membrane filter D itself.

The filtrate obtained from desorption of the membrane, was found to have a zero absorbance value, on being assayed.

The effect of Sodium Acetate buffer appeared to be similar to that of Sodium Edetate (Table 7.11). A continuous loss of 3.25% was observed, from the 30ml aliquots of buffered Phenylmercuric nitrate solution, filtered through membrane filter E. (Fig. 7.6) This loss which occurred, was probably also as a result of the interaction between the preservative and the plastic unit, as a similar loss occurred when the buffered solution was allowed to remain in contact with the unit. The filtrate obtained from desorption of the membrane was also found to have a zero absorbance value, when assayed.

Thus it seems that in the presence of Sodium Edetate and Sodium Acetate buffer, no apparent loss occurs from solution, onto the membrane filters, the losses obtained being due to interaction with the plastic unit. The gradual slowing down of

TABLE 7.11

LOSS OF PHENYLMERCURIC NITRATE FROM SOLUTIONS OF PHENYLMERCURIC NITRATE

0.002% w/v, FILTERED THROUGH MEMBRANE FILTERS C, D AND E

30ml aliquot filtered	Time taken for filtration			Percent Loss		
	C	D	E	C	D	E
1	30 seconds	15 seconds	15 seconds	7.5	4.0	3.25
2	25 seconds	15 seconds	15 seconds	4.0	4.0	3.25
3	15 seconds	15 seconds	15 seconds	4.0	4.0	3.25

D = P.M.N. 0.002% w/v/Sodium Edetate 0.01% w/v

E = P.M.N. 0.002% w/v/Sodium Acetate buffer

the filtration rates, of solutions filtered through filter A and B, and the irregular filtration rates observed with filter C, seem to indicate that there is some chemical interaction between Phenylmercuric nitrate and the membrane, which clogs the membrane, or effects its porosity. This effect appears to be eliminated in the presence of Sodium Edetate and Sodium Acetate buffer.

(ii) Sartorius Glass filter unit.

Increased losses were observed from solutions filtered through membrane filter F attached to the glass filter unit, this being as a result of adsorption of Phenylmercuric nitrate onto the sintered glass support disc.

The loss of Phenylmercuric nitrate which occurred from solutions of Phenylmercuric nitrate 0.002% w/v, was 15.0% for the initial 30ml aliquot filtered, decreasing to 7.5% after filtration of 90ml (Table 7.12 and Fig. 7.6).

TABLE 7.12

LOSS OF PHENYLMERCURIC NITRATE FROM A SOLUTION OF PHENYLMERCURIC NITRATE 0.002%, ON FILTRATION THROUGH MEMBRANE FILTER F, ATTACHED TO THE GLASS FILTER UNIT.

30ml aliquot filtered	Time taken for filtration	Percent Loss
1	15 seconds	15%
2	15 seconds	9%
3	15 seconds	7.5%

If it is assumed that a loss of 3.5% from 30ml of solution occurred onto the membrane then 11.5% adsorption probably occurred from the first 30 ml aliquot filtered, onto the sintered glass support disc.

On desorption with 30ml of distilled water, the filtrate on being assayed, was observed to have an absorbance value of 0.025. This was probably due to the Phenylmercuric nitrate desorbed from the sintered glass support disc.

SUMMARY (Table 7.13).

(a) Sintered Glass Filters.

Adsorption from 30ml of Phenylmercuric nitrate solutions was found to be about 7.2%, filter A becoming saturated after filtration of 60ml, and filter B after 30ml. Most of the adsorbed preservative was easily desorbed with 30ml of distilled water.

Adsorption onto filters of porosity No. 4 was found to be 3.5 - 4.0%.

Sodium Edetate and Sodium Acetate minimised the extent of adsorption, the former to a slightly greater extent. The effect of Sodium Acetate was probably either a surface tension or, electrokinetic one; that of Sodium Edetate either as a result of complexation or electrostatic adsorption of Sodium ions, or possibly a combination of both.

(b) Doulton-Pasteur unglazed Porcelain Candles.

Adsorption of Phenylmercuric nitrate onto the candles was found to be considerable; from 30ml,

100% adsorption occurred onto both filters A and B. Adsorption was observed to continue after 120ml of solution had been filtered.

Sodium Edetate and Sodium Acetate buffer minimised adsorption, the effect of the latter being more pronounced. Complete desorption of the candles was not accomplished with distilled water alone; Sodium Acetate buffer was observed to desorb further amounts of adsorbed Phenylmercuric nitrate.

(c) Asbestos Filter Pads.

The extent of adsorption from 10ml and 30ml of Phenylmercuric nitrate solution was found to be about 30% and 9% respectively, the adsorbed preservative being easily desorbed with distilled water.

Sodium Edetate and Sodium Acetate buffer resulted in an increased extent of adsorption; that in the presence of Sodium Edetate being greater. It seemed that the greater the alkalinity of the solution on being filtered, the lower the extent of adsorption. Thus in an acidic environment, increased adsorption of Phenylmercuric nitrate could be anticipated.

(d) Sartorius Membrane Filters.

The Sartorius plastic filter unit seemed to interact with the Phenylmercuric nitrate solution; this interaction being observed in the presence of Sodium Edetate and Sodium Acetate buffer, as well.

Loss onto the membranes themselves, was found

to be about 9.0% and 3.5% from 10ml and 30ml of Phenylmercuric nitrate solution respectively; the total losses (including interaction with the unit) being 18.6% and 7.5% respectively.

In the presence of Sodium Edetate and Sodium Acetate buffer, there appeared to be no loss onto the membranes themselves, the only loss being observed, was that most probably due to interaction with the plastic unit.

All values obtained from desorption experiments were found to be zero (except that for the glass unit). This seems to suggest that some form of chemical interaction and not just physical adsorption, occurred between the Phenylmercuric nitrate solutions and the membranes through which they were filtered, this effect being eliminated in the presence of Sodium Edetate or Sodium Acetate.

Increased loss (15% from 30ml) occurred on filtration through the membrane attached to the Sartorius Glass Unit; this being due to some adsorption onto the sintered glass support disc.

TABLE 7.13

ADSORPTION (OR LOSS) OF PHENYLMERCURIC NITRATE ONTO DIFFERENT FILTER MEDIA,
FROM 30ml OF PHENYLMERCURIC NITRATE 0.002% w/v

Sintered Glass Porosity No. 5	Sintered Glass Porosity No. 4	Doulton-Pasteur Candles No. 1	Asbestos Pads Grade G.S.	Sartorius Membranes GmbH	
				Plastic Unit	Glass Unit
7.2%	4.0%	100%	Ca. 9.0%	7.5%	15%

CHAPTER EIGHT

ADSORPTION OF BENZALKONIUM CHLORIDE.

Benzalkonium Chloride is a quaternary ammonium compound with properties and uses similar to those of other cationic surface-active agents. It consists of a mixture of alkylbenzyl dimethyl ammonium chlorides, of the general formula $(C_6H_5 \cdot CH_2 \cdot N(CH_3)_2 \cdot R)Cl$, in which R represents a mixture of the alkyls from C_8H_{17} to $C_{18}H_{37}$.

A cationic surface-active agent such as Benzalkonium Chloride, dissociates in aqueous solution into a relatively large and complex cation which is responsible for the surface activity, and a smaller inactive anion. The cation usually contains a pentavalent Nitrogen atom, which is often present as a quaternary ammonium group, to which is attached the long chain alkyl group containing 8 to 18 carbon atoms (Fig. 8.1).

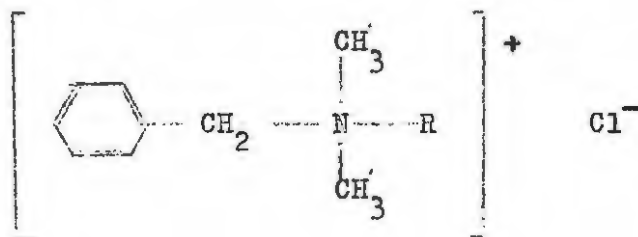


Fig. 8.1

Benzalkonium Chloride is very soluble in water, alcohol and acetone. A dilution in water is neutral or alkaline to litmus and foams strongly on shaking. Aqueous solutions of this preservative

are stable in acidic media and will withstand boiling and autoclaving. It is however, incompatible with anionic surface-active agents (since oppositely charged complex ions tend to neutralize each other, resulting in precipitation), and nitrates.

Benzalkonium Chloride solution B.P. (British Pharmacopoeia) which is an aqueous solution containing 50% w/v of Benzalkonium Chloride, was used to prepare solutions of Benzalkonium Chloride 0.02% w/v and 0.01% w/v. This was done by prediluting the 50% w/v solution to 1% w/v, from which the required dilutions were prepared in distilled water.

The combinations⁸⁰ of antibacterial agents with Sodium Edetate offer the possibilities of overcoming the problem of bacterial resistance to chemical preservatives. Resistant strains of Pseudomonas aeruginosa have been made sensitive to Benzalkonium Chloride by the inclusion of Sodium Edetate^{44, 68} 0.01% w/v to 0.1% w/v. According to Smith,⁸¹ the antibacterial activity of quaternary ammonium compounds, could be affected by hard water, and they should be protected by a sequestering agent such as Sodium Edetate, as the metal ions compete with the quaternaries for deposition on the bacteria.

The effect of Sodium Edetate, on the adsorption of Benzalkonium Chloride onto the various filter media, was determined by incorporating

Sodium Edetate 0.1% w/v into the preservative solutions which were filtered.

The effect of Sodium Chloride 0.1% w/v on the extent of adsorption was also determined.

According to Mueller⁸² the optimum pH value for full activity of Benzalkonium Chloride, should be between 5 and 8. The influence on adsorption, of buffers such as Sodium Acetate having pH value of 5.9 (Chapter 6 a) and Sodium Phosphate having a pH value of 6.8 (Sodium Acid Phosphate 0.4% w/v and Sodium Phosphate 0.473% w/v),⁶⁸ was observed by incorporating Benzalkonium Chloride 0.02% w/v into the buffers respectively, and filtering through the various filter media.

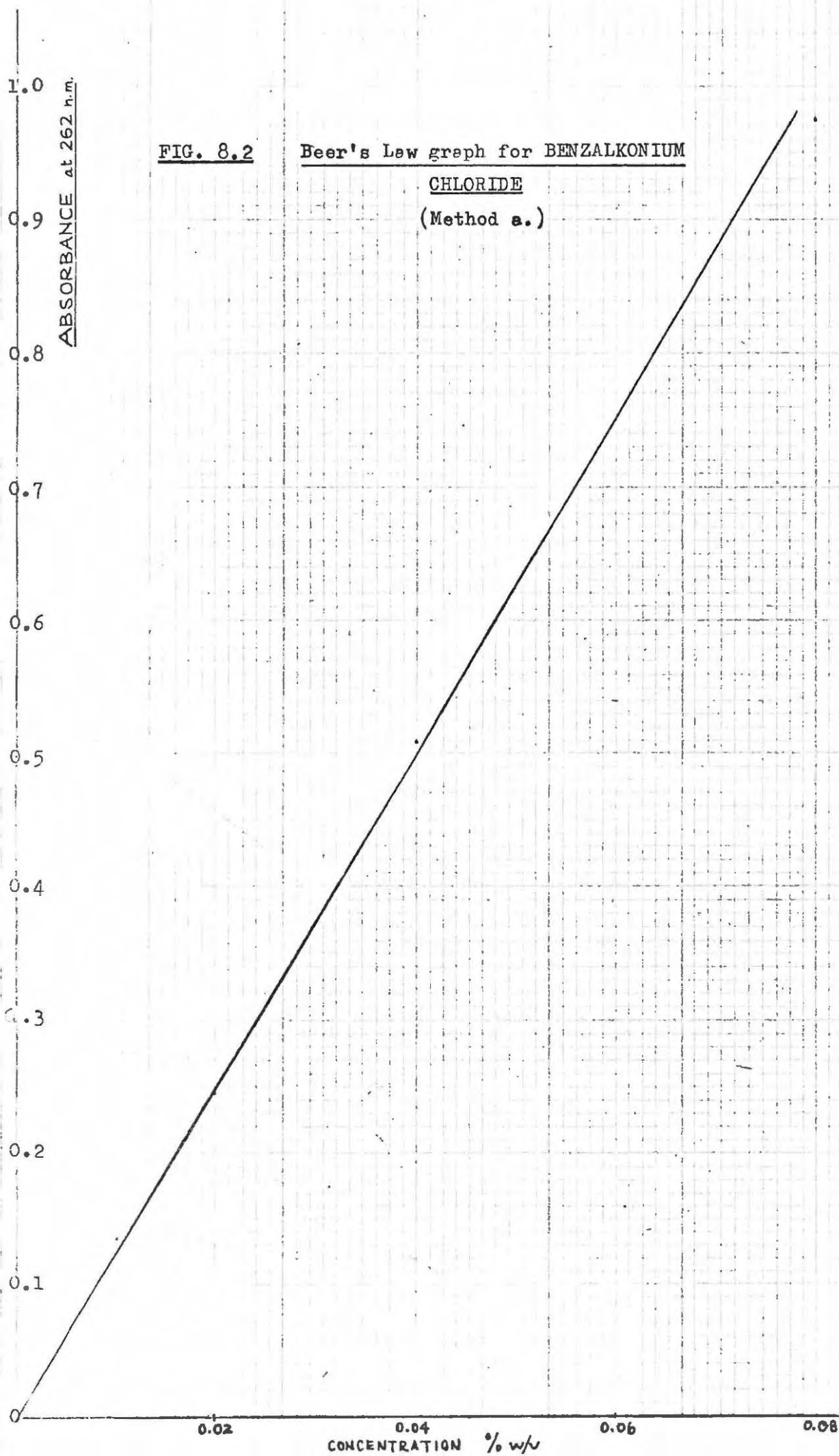
Benzalkonium chloride is sometimes used in conjunction with Phenylethanol.^{6, 68} The effect of Phenylethanol 0.3% v/v on the adsorption of Benzalkonium chloride, was determined by filtering the combined preservative solution through the various filter media.

Solutions were filtered through the various media under a vacuum of 20ins of mercury at room temperature, and the filtration rates recorded by stop-clock. All filtrates (except those obtained from later determinations on Asbestos filter pads and those concerning the adsorption of Benzalkonium in the presence of Phenylethanol) were assayed spectrophotometrically, by measuring the absorbance at a peak absorption wavelength of 262 n.m. (U.V.); Benzalkonium chloride obeying Beer's law within

FIG. 8.2

Beer's Law graph for BENZALKONIUM

CHLORIDE
(Method a.)



the range 0.01% w/v to 0.08% w/v (Method (a)).

The filtrates obtained when using the Asbestos filter pads, and those from the determinations in the presence of Phenylethanol, were assayed by a method⁷⁹(b), which was based on the complex formation of Benzalkonium chloride with Bromophenol blue, the complex being extracted with Dichloroethane, and the spectral absorbance of this extract measured at 603 n.m. (visible) on the Beckman D.B. spectrophotometer. According to Fokkens and Buurman⁷⁹ the precision of this method is $\pm 5\%$.

Preparation of Beer's Law Graphs (Standard Curves)

Method (a)

Solutions containing Benzalkonium chloride 0.01% w/v, 0.02% w/v, 0.04% w/v and 0.08% w/v were prepared by diluting a stock solution of Benzalkonium chloride 1% w/v, which was prepared from Benzalkonium chloride solution B.P. The spectral absorbance of each of these dilutions were measured at a wavelength of 262 n.m., against a blank of distilled water, and the results (taking an average between two determinations) plotted graphically (Fig. 8.2).

A solution of Benzalkonium chloride 0.02% w/v was found to have an absorbance value of 0.245 at 262 n.m. Thus the quantity of Benzalkonium chloride present in the filtrates obtained from the determinations, was determined by measuring the spectral absorbance of the undiluted filtrates at 262 n.m., and from this the quantity of Benzal-

konium chloride lost through interaction with the filter, was calculated. (All solutions were preassayed before filtration.)

The spectral absorbances of filtrates obtained from solutions filtered in the presence of Sodium Edetate, Sodium Chloride, Sodium Acetate buffer, and Sodium Phosphate buffer, were measured against blank solutions containing these salts and buffers respectively, in their appropriate concentrations.

Method (b) - Complexation of Benzalkonium chloride with Bromophenol blue.

A solution of Benzalkonium chloride 0.02% w/v (200 micrograms per ml) was prepared from Benzalkonium chloride solution B.P. This solution was diluted accordingly to yield solutions containing 2, 4, 6, 8 and 10 micrograms per ml respectively.

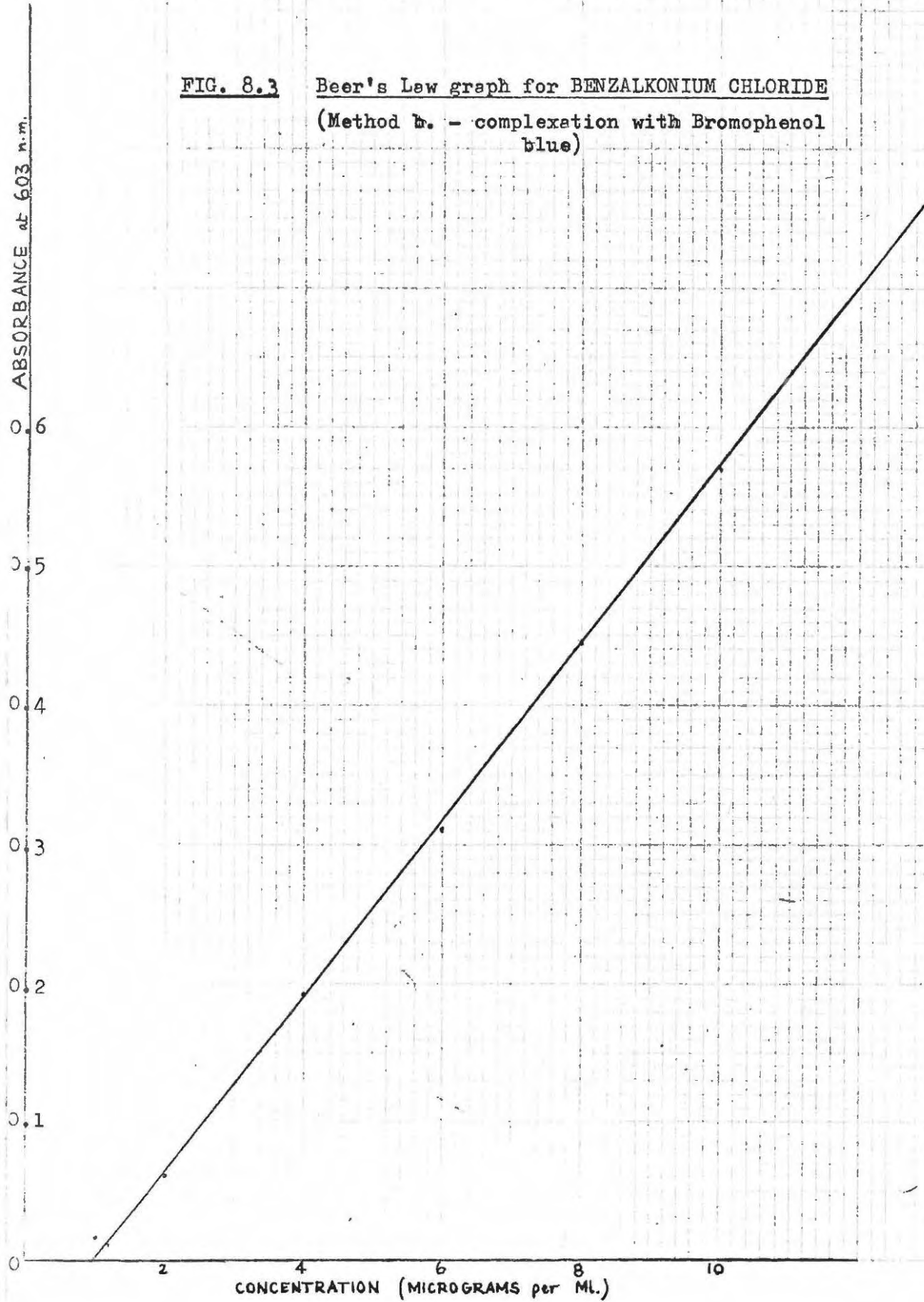
5ml of each dilution was pipetted into each of six 50ml round-bottom "quick-fit" flasks, fitted with glass stoppers.

The Bromophenol blue reagent was prepared by dissolving 17 mgms of Bromophenol blue in 100ml of Sodium Carbonate ($\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$) 10% w/v. 10ml of this solution was pipetted into a volumetric flask and, then made up to 50ml with anhydrous Sodium Phosphate 10% w/v to yield the final reagent.

The Bromophenol blue/Phosphate reagent had to be kept in an incubator at 25°C to prevent the precipitation of the Sodium Phosphate, and had to be used within 48 hours.

5ml of the Bromophenol blue reagent was then

FIG. 8.3 Beer's Law graph for BENZALKONIUM CHLORIDE
(Method B. - complexation with Bromophenol blue)



pipetted into each of the flasks containing the Benzalkonium chloride dilutions. To each of these, 10ml of 1,2-Dichloroethane (extra pure) was pipetted, and the flasks and their contents shaken fairly vigorously on a mechanical shaker for 3 minutes.

The contents from each flask were then transferred into each of six large-sized centrifuge tubes, and centrifuged at 3000 revs per minute for ten minutes. After this the lower organic layer, containing the extracted complex, was pipetted from each tube into each of six smaller centrifuge tubes, and the contents centrifuged at 3000 r.p.m. for a further two minutes.

The spectral absorbance of the Dichloroethane layer containing the complex-extract, was then measured against a blank containing Dichloroethane at 603 n.m. and the results plotted graphically (Fig. 8.3).

The results obtained, were found to deviate slightly from Beer's Law, as the curve could not be projected through the origin. This was also observed by Fokkens and Buurman⁷⁹. The precision of this method was found to depend to a great extent on the purity of the Dichloroethane reagent, as previous determinations employing a slightly lower grade of the reagent, were observed to yield extracts of lower absorbance values.

The solution containing Benzalkonium chloride 0.02% w/v at a dilution of 1 in 25 (8 micrograms per ml), was found to have an absorbance value of

0.445, on being assayed. Thus the filtrates obtained from the determinations, were assayed by using 5ml of a 1-25 dilution of each, the loss from solution being calculated relative to a concentration of 8 micrograms per ml, or to the amount contained in a similar dilution of the original unfiltered solution, which was preassayed before filtration.

Blank solutions containing Sodium Edetate, Sodium Chloride, Sodium Acetate buffer, Sodium Phosphate buffer and Phenylethanol, in their appropriate dilutions, were found to have a zero absorbance value at 603 n.m., on being assayed by this method, and were also found to have no effect on the complexation of Benzalkonium chloride with Bromophenol blue in the assay process.

(a) Adsorption onto Sintered Glass Filters.

The determinations were done on Sintered Glass filters (A and B) of Porosity No. 5, which were cleaned before every determination with sulphuric acid and distilled water as previously described; the spectral absorbances (at 262 n.m.) of the filtrates obtained from the distilled water were checked for freedom from impurities, so that the filtrate obtained from filtering 10ml of distilled water, had a zero absorbance value at 262 n.m. The filters were dried at 150°C for one hour, and allowed to cool before use.

The extent of adsorption from 10ml of Benzalkonium chloride solution, was determined by filtering

three 10ml aliquots of Benzalkonium chloride 0.02% w/v successively through filter A.

To determine the extent of adsorption from 30ml, the process was repeated using five 30ml aliquots, which were filtered through each of filters A and B respectively. This was followed by desorption of filter A, with two 30ml aliquots of distilled water, and 30ml of Sodium Chloride 0.1% w/v, after which, 30ml of distilled water was again washed through the filter; filter B being washed through with two 30ml aliquots of distilled water only. (Before desorption the stem and reservoir were rinsed and dried without affecting the sintered disc.)

The British Pharmaceutical Codex 1968 includes Benzalkonium chloride at a concentration of 0.01% w/v in a number of its formulations for eye drops. Thus the amount of adsorption occurring from 30ml of Benzalkonium chloride 0.01% w/v, was determined by filtering five 30ml aliquots of the preservative solution through filter A. This was followed by desorption of the adsorbed Benzalkonium chloride, with two 30ml aliquots of distilled water.

The effect of Sodium Chloride on the adsorption of Benzalkonium chloride, was determined by filtering five 30ml aliquots of Benzalkonium chloride 0.02% w/v containing Sodium Chloride 0.1% w/v, through filter A; this being followed by desorption with two 30ml aliquots of distilled water.

Sodium Edetate at a concentration of 0.1% w/v was combined with Benzalkonium chloride 0.02% w/v, and its effect on the adsorption of the preservative determined by filtering five 30ml aliquots of the solution through filter A, followed by desorption with two 30ml aliquots of distilled water.

Benzalkonium chloride 0.02% w/v was incorporated into a solution of Sodium Phosphate buffer having a pH value of 6.85. The influence of the buffer on the amount of loss occurring from the solution on filtration, was determined by filtering five 30ml aliquots of the buffered preservative solution, through filter A; this being followed by desorption with two 30ml aliquots of distilled water.

Benzalkonium chloride 0.02% w/v was also incorporated into a Sodium Acetate buffer solution having a pH value of 5.9, and the effect of the buffer on adsorption, determined by filtering five 30ml aliquots through filter A; followed by desorption.

The effect of the presence of Phenylethanol 0.3% v/v in a solution of Benzalkonium chloride 0.02% w/v, on the adsorption of the latter, was determined by filtering 10ml of the combined preservative solution through filter A. The filtrate obtained, was assayed according to Method (b), which comprises the complexation of Benzalkonium chloride with Bromophenol blue. The amount of Benzalkonium chloride lost from the solution filtered was compared to that lost from 10ml, in

the absence of Phenylethanol.

The effect of the porosity of the filter on its extent of adsorption, was determined by filtering five 30ml aliquots of Benzalkonium chloride 0.02% w/v through each of Pyrex G4 sintered glass filters A and B of Porosity No. 4, which were previously cleaned and dried,

RESULTS and DISCUSSION.

The amount of adsorption occurring from 10ml of Benzalkonium chloride 0.02% w/v, onto filter A, was found to be 19.2%. Adsorption was observed to continue after filtration of 30ml, and the loss from solution remained more or less constant, (Table 8.1) the filter showing no tendency to becoming saturated.

TABLE 8.1

ADSORPTION OF BENZALKONIUM CHLORIDE ONTO
SINTERED GLASS FILTER A FROM BENZALKONIUM
CHLORIDE 0.02% w/v

10ml aliquot filtered	Time taken for filtration	Percent Loss
1	80 seconds	19.2
2	95 seconds	16.33
3	120 seconds	16.33

Loss from 30ml was observed to be continuous and constant after 150ml of Benzalkonium chloride

solution had been filtered altogether through filter A. A slightly lower amount of adsorption was found to occur onto filter B, despite the filtration rates being lower than that observed with filter A. Filter B also appeared to become saturated after filtration of 30ml. (Table 8,2 and Fig. 8,4).

It seems that the porosity of filter B was somehow affected through cleaning or clogging, as filtration was observed to slow down. The lower extent of adsorption onto filter B could probably be due to the availability of fewer adsorption sites, as a result of contaminants within the matrix of the sintered disc.

Very little of the adsorbed preservative appeared to be desorbed on washing 60ml of distilled water through filters A and B, the filtrates each having an absorbance value of 0.005 at 262 n.m. However, on further washing through of filter A with 30ml of Sodium Chloride 0.1% w/v, the filtrate was observed to become foamy. The filtration rates were observed to increase to 30ml/4 minutes, as compared to the rates obtained when filtering the Benzalkonium chloride solution. On further desorption of filter A with 30ml of distilled water, the absorbance value of the filtrate was found to be 0.02, indicating some effect on desorption exerted by Sodium Chloride.

On repeating the filtration of Benzalkonium chloride solution through filter A, on two further

TABLE 8.2

ADSORPTION OF BENZALKONIUM CHLORIDE ONTO SINTERED GLASS FILTERS A AND B,
FROM BENZALKONIUM CHLORIDE 0.02% w/v

30ml aliquot filtered	Time taken for filtration		Percent Loss	
	A	B	A	B
1	6m. 20 secs	10 minutes	11.02	9.5
2	7 minutes	12 minutes	5.3	nil
3	7m. 40 secs	12m. 30 secs	2.32	nil
4	8m. 45 secs	13m. 15 secs	2.32	nil
5	9 minutes	14 minutes	2.32	nil

occasions, a slight increase (12.25%) in the extent of adsorption, occurred from 30ml of solution, but a similar pattern was observed in that, a continuous and constant loss of 6.95% occurred after filtration of the first 30ml aliquot. The filtration rates in these instances were found to be very similar to that observed during previous determinations. The amount of preservative desorbed from the filter also appeared similar in relation to the amount adsorbed; the filtration rates on desorption with distilled water, were also observed to increase as expected.

Increased adsorption of Benzalkonium chloride onto filter A, occurred from solutions containing 0.01% w/v (Table 8.3). Loss from solution was again observed to be continuous and constant, after the first 30ml aliquot had been filtered; the filter showed no signs of having become saturated.

An increase in the rate of filtration (30ml/4m) was observed on filtering distilled water through filter A in order to desorb the adsorbed preservative; only a small quantity appeared to be desorbed as the filtrate obtained from 30ml of distilled water, was found to have an absorbance value of 0.007, and dropped to zero after 60ml had been washed through.

Since most of the official eye-drop formulations contain Benzalkonium chloride at a concentration of

TABLE 8.3

EFFECT OF THE CONCENTRATION OF BENZALKONIUM CHLORIDE ON ITS
EXTENT OF ADSORPTION ONTO SINTERED GLASS FILTER A

30ml aliquot filtered	Time taken for filtration		Percent Loss	
	0.01% w/v	0.02% w/v	0.01% w/v	0.02% w/v
1	5m. 10 secs	6m. 20 secs	20.6	11.02
2	6m. 30 secs	7 minutes	17.3	5.3
3	6 minutes	7m. 40 secs	15.4	5.3
4	6m. 10 secs	8m. 45 secs	15.4	5.3
5	6 minutes	9 minutes	15.4	5.3

0.01% w/v, it should be borne in mind that in the determinations which follow, where a solution of Benzalkonium chloride 0.02% w/v has been used, increased adsorption would have occurred.

Sodium Chloride seemed to cause a slight increase in the amount of Benzalkonium chloride adsorbed from the first 30ml aliquot of solution filtered, but on further filtration a diminished loss which was continuous and constant, occurred from solution. (Table 8.4 and Fig. 8.4).

The filtration rates were observed to be similar to that in the absence of Sodium Chloride. On desorption, a larger amount of preservative appeared in the filtrate, which was found to have an absorbance value of 0.05 as compared to 0.005 which was previously obtained from the determination in the absence of Sodium Chloride.

The results obtained in the presence of Sodium Edetate were found to be similar to those obtained when Sodium Chloride was used (Table 8.4 and Fig. 8.4). The filtration rates however were found to be very slightly lower.

The inclusion of Benzalkonium chloride in the Sodium Phosphate buffer, resulted in an increased amount of adsorption from 30ml (Table 8.4), and loss was again observed to be continuous and constant, but slightly reduced, on further filtration. The filtration rates were found to have increased slightly as compared to previous determinations,

and the amount of preservative desorbed by 30ml of distilled water, was similar to that desorbed, after filtration of Benzalkonium chloride in the presence of Sodium Chloride and Sodium Edetate.

Sodium Acetate buffer was found to cause a slight increase in the amount of preservative lost from the first 30ml aliquot filtered, and on further filtration, a lower continuous constant loss was observed (Table 8.4). The absorbance value of the filtrate obtained after filtering 30ml of distilled water, was found to be similar to that obtained in the preceding determinations.

In all of the preceding desorption determinations the filtration rates were found to decrease when compared to that obtained from determinations in the absence of the salts or buffers.

In the presence of Phenylethanol, a loss of 23.35% of Benzalkonium chloride was found to occur from 10ml of solution as compared to a loss of 19.2% which occurred in its absence. Thus it appears that Phenylethanol does not in any way minimise adsorption, but being an alcohol it probably has an effect on the surface tension⁷² of the solution being filtered, thus affecting the amount of Benzalkonium chloride adsorbed.

Filtration through sintered glass filters A and B of porosity No. 4, resulted in decreased adsorption. Both filters showed similar behaviour and adsorbed 9.4% from the first 30ml, after which

FIG. 8.4 Loss of Benzalkonium chloride occurring from Benzalkonium chloride 0.02% w/v, on filtration through Sintered Glass Filters of porosity No.5.

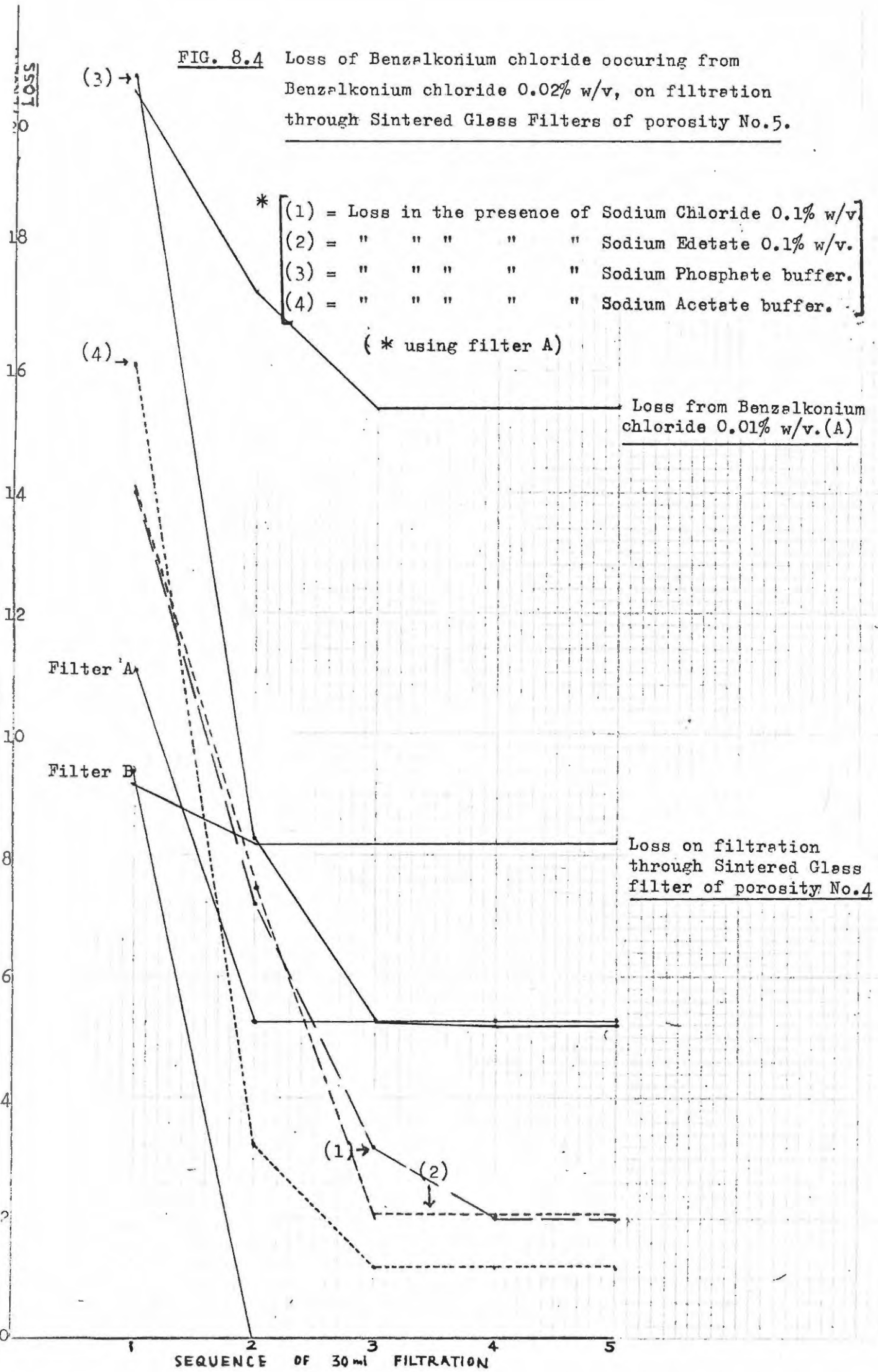


TABLE 8.4

ADSORPTION OF BENZALKONIUM CHLORIDE ONTO SINTERED GLASS FILTER A,
IN THE PRESENCE OF SODIUM CHLORIDE (1), SODIUM EDTATE (2),
SODIUM PHOSPHATE BUFFER (3) AND, SODIUM ACETATE BUFFER (4).

30ml aliquot filtered	Time taken for filtration				Percent Loss			
	1	2	3	4	1	2	3	4
1	6m. 45 secs	4m. 30 secs	3m. 45 secs	5m. 25 secs	14.0	14.05	20.8	16.1
2	8m. 50 secs	6m. 30 secs	4m. 20 secs	6 minutes	7.2	7.15	8.3	3.2
3	8m. 15 secs	7m. 15 secs	5 minutes	8m. 15 secs	3.2	2.06	5.2	1.2
4	7m. 50 secs	7 minutes	5m. 10 secs	8 minutes	2.0	2.06	5.0	1.2
5	8 minutes	7m. 30 secs	5m. 5 secs	7m. 45 secs	2.0	2.06	5.0	1.2

a constant loss of 8.2% was observed. Very little of the adsorbed preservative appeared to be desorbed from the filters A and B, the filtrates obtained from the first 30ml aliquots of distilled which were filtered had absorbance values of 0.008 in each instance, and decreased to zero after 60ml had been filtered.

The mechanism by means of which the preservative is adsorbed onto the glass surface, could probably be explained in terms of the electrostatic adsorption of the large cation of Benzalkonium chloride, onto the negatively charged glass surface of the sintered glass disc.

Minassian-Saraga⁸³ investigated the nature of the layer adsorbed on glass surfaces wetted with solutions of cationic surface-active agents, and suggested that the glass acts as a cation exchanger.

According to Folman,⁸⁴ two adsorption sites exist in the glass surface; these being a hydroxyl group and a Silica or oxygen atom. The reaction mechanism of quaternary ammonium compounds with surface hydroxyl groups was studied by Blackman,⁸⁵ and from pH and concentration effects, found that adsorption also occurred through a cation exchange mechanism.

(b) Adsorption onto Doulton-Pasteur Unglazed Porcelain Candles.

Two similar filter candles, A and B were

cleaned with hot dilute Hydrochloric Acid and distilled water, until the filtrates obtained, were acid free and had a zero absorbance value at 262 n.m. Before every determination the filter being used, was treated as above and then dried at 150°C for 1 hour, and allowed to cool before use. The receiver of the filter unit was thoroughly rinsed and dried before the filtration of each aliquot, for the adsorption and desorption determinations. All filtrates were assayed undiluted by measuring their spectral absorbance at 262 n.m. (except those obtained in the presence of Phenylethanol.)

The pH values of all solutions containing salts and buffers were measured on a Metrohm pH meter before and after filtration.

The amount of Benzalkonium chloride lost from 10ml of solution, was determined by filtering 60ml of Benzalkonium chloride 0.02% w/v in aliquots of 10ml, through filter A. The filter was then desorbed with three 30ml aliquots of distilled water.

The process was repeated using six 30ml aliquots of Benzalkonium chloride 0.02% w/v, which were filtered through each of filters A and B, followed by desorption with three 30ml aliquots of distilled water. The process was repeated with filter B on a further occasion, using eight 30ml aliquots of Benzalkonium chloride 0.02% w/v.

The effect of Sodium Chloride was determined

by filtering six 30ml aliquots of Benzalkonium chloride 0.02% w/v containing Sodium Chloride 0.1% w/v, through filter B. This was followed by desorption of the filter candle with four 30ml aliquots of distilled water.

The effect of Sodium Edetate was determined by filtering eight 30ml aliquots of Benzalkonium chloride 0.02% w/v containing Sodium Edetate 0.1% w/v, through filter B, which was then desorbed with three 30ml aliquots of distilled water.

Benzalkonium chloride 0.02% w/v was incorporated into a Sodium Phosphate buffer having a pH value of 6.9. Eight 30ml aliquots were filtered through filter B, followed by desorption with four 30ml aliquots of distilled water.

The effect of Sodium Acetate buffer was also determined by filtering eight 30ml aliquots of Benzalkonium chloride 0.02% w/v, buffered with Sodium Acetate at a pH value of 5.9, through filter B. This was followed by desorption with four 30ml aliquots of distilled water.

30ml of a solution containing Benzalkonium Chloride 0.02% w/v and Phenylethanol 0.3% v/v was filtered through filter B. The filtrate obtained was assayed according to Method (b), i.e. by complexation of Benzalkonium chloride with Bromophenol blue.

RESULTS and DISCUSSION.

The amount of loss of Benzalkonium chloride occurring from 10ml of solution on filtration through filter A was found to decrease in the usual way as filtration proceeded, but remained constant after 50ml had been filtered (Table 8.5). The filtration rates were found to be quite low, and dropped to 10ml/25 minutes after 60ml had been filtered.

TABLE 8.5

ADSORPTION OF BENZALKONIUM CHLORIDE ONTO
PASTEUR CANDLE A, FROM BENZALKONIUM CHLORIDE
0.02% w/v

10ml aliquot filtered	Time taken for filtration	Percent Loss
1	12 minutes	41.53
2	20 minutes	31.45
3	20 minutes	22.58
4	25 minutes	20.97
5	25 minutes	17.5
6	25 minutes	17.5

The filtrate obtained from 30ml of distilled water which was washed through the filter, was found to have a spectral absorbance of 0.05 at 262 n.m. This value decreased to 0.015 after 90ml of distilled water had been filtered.

On further washing with distilled water (200ml)

the filtrate was observed to become foamy and more Benzalkonium chloride was detected in it. Consequently after all determinations, the filter was washed through with about 1 litre of hot distilled water, until the absorbance value of the filtrate became zero at 262 n.m.

Loss of Benzalkonium chloride from 30ml of solution was found to be only slightly lower than that from 10ml. After 150ml of the preservative solution had been filtered through filters A and B, the amount of Benzalkonium chloride lost from solution was observed to remain constant (Table 8.6 and Fig. 8.5). The filtration rates between filters A and B were found to differ, that of filter A being lower than that of B.

The absorbance values of the filtrates obtained from desorption of filters A and B, were found to be similar to that obtained after 60ml (10ml aliquots) had been previously filtered through filter A.

On repeating the process with filter B on a further occasion, an increased loss of 57.7% was observed to occur from the first 30ml aliquot of Benzalkonium chloride 0.02% w/v filtered. After 120ml, the loss from solution dropped to 19.3%, after which similar losses (17.34%) which remained constant, were observed. The filtration rates however were much higher (10ml/3 minutes-9 minutes) than those indicated in Table 8.6. Thus, contrary

TABLE 8.6

ADSORPTION OF BENZALKONIUM CHLORIDE ONTO PASTEUR CANDLES A AND B,
FROM BENZALKONIUM CHLORIDE 0.02% w/v

30ml aliquot filtered	Time taken for filtration		Percent Loss	
	A	B	A	B
1	20 minutes	10 minutes	38.0	38.3
2	20 minutes	15 minutes	24.5	21.4
3	25 minutes	15 minutes	22.5	18.6
4	25 minutes	15 minutes	21.2	17.34
5	25 minutes	20 minutes	16.33	17.34
6	25 minutes	20 minutes	16.33	17.34

to theoretical expectations, it seems that lower losses occur when the filtrate has a lower filtration rate. This could be due to blocking of the filter by particulate matter, which probably minimises the number of adsorption sites available for the adsorption of the preservative, thus resulting in a lower extent of adsorption from the solution being filtered.

The pH value of the filtrates obtained on this occasion was found to vary from 3.6 to 5.45 after 240ml of solution had been filtered, the original unfiltered solution having had a pH value of 6.15. This decrease in the pH value after filtration could be due to an increase in the hydrogen ion concentration in the filtrates, these ions probably, having been exchanged for the adsorbed Benzalkonium cation, on filtration.

In the presence of Sodium Chloride 0.1% w/v an increased amount of Benzalkonium chloride was adsorbed from 30ml of solution, (cf. Table 8.6), however the losses decreased as filtration proceeded (Table 8.7 and Fig. 8.5). The filtration rates were also observed to increase. The original unfiltered solution of Benzalkonium chloride/Sodium chloride, was found to have a pH value of 5.8. The filtrates obtained from the first 30ml were found to have a low pH value (3.2) which gradually increased to 4.98 after filtration of 150ml of solution.

On desorption with distilled water (pH 5.65), a higher absorbance value of 0.245 at 262 m.μ. was obtained for the first 30ml filtered, and decreased to 0.10 after 120ml had been filtered. The pH values of these filtrates were observed to increase from 5.35 to 5.98.

Losses almost similar to that which occurred in the presence of Sodium Chloride, were obtained with Sodium Edetate 0.1% w/v. The filter was observed to adsorb a constant amount after 180ml of the solution had been filtered. (Table 8.7 and Fig. 8.5). The original unfiltered solution had a pH value of 4.72, and after filtration this was found to vary from 4.15 to 4.98.

The absorbance values of the filtrates obtained from the desorption of the filter candle, were found to be similar to those obtained when, desorption was carried out after filtration of Benzalkonium chloride in the presence of Sodium Chloride. The pH value of these filtrates were found to increase from 4.85 to 5.5. (pH value of the distilled water having been 5.6).

The use of Sodium Phosphate buffer resulted in an increased amount of adsorption from the initial 30ml aliquots filtered (Table 8.7 and Fig. 8.5). The pH values of the filtrates were found to differ very slightly from the unfiltered buffered solution, and varied from 6.9 to 6.95 after 240ml had been filtered.

The absorbance values of the filtrates obtained from the desorption determination were also found to be similar to those obtained in the determinations concerning Sodium Chloride and Sodium Edetate. The pH values of the filtrates obtained on desorption with distilled water, were found to vary from 7.0 to 7.1.

Increased loss was also found to occur in the presence of Sodium Acetate buffer, but to a slightly lesser extent than that which occurred with the Phosphate buffer. (Table 8.7 and Fig. 8.5). The pH value of the filtrates obtained from the solution, buffered at pH 5.9, were found to vary very slightly, from 5.85 to 5.92.

The absorbance values of the filtrates obtained from filtering distilled water (pH 5.95) through in order to desorb the candle, were found to be similar to those formerly obtained in the presence of Sodium Chloride, Sodium Edetate and Sodium Phosphate buffer. The pH value of these filtrates varied from 5.92 to 5.8.

In the presence of Phenylethanol, a loss of 53.0% of Benzalkonium Chloride occurred from filtration of 30ml of the combined preservative solution. If this loss is compared to the loss occurring onto filter B in the first determination (38.3%) then it seems that Phenylethanol causes an increase in the amount of adsorption, however on a further occasion a loss of 57.7% was observed

FIG. 8.5 Loss of Benzalkonium chloride occurring from Benzalkonium chloride 0.02% w/v, on filtration through Doulton-Pasteur unglazed porcelain candles.

- (1) = Loss in the presence of Sodium Chloride 0.1% w/v
 - (2) = " " " " " Sodium Edetate 0.1% w/v
 - (3) = " " " " " Sodium Phosphate buffer
 - (4) = " " " " " Sodium Acetate buffer
- B' = Filtration through filter B on a further occasion.

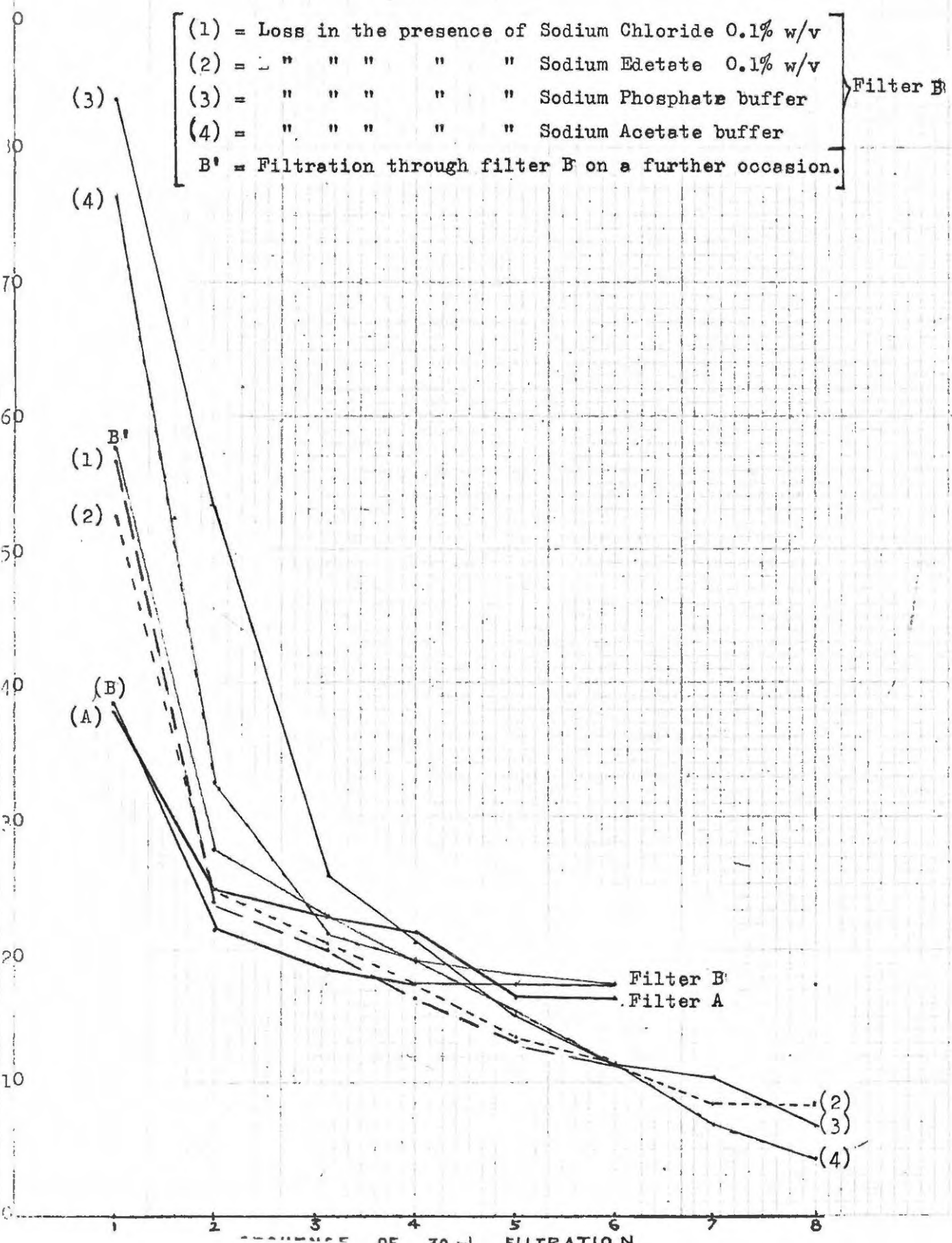


TABLE 8.7

ADSORPTION OF BENZALKONIUM CHLORIDE ONTO PASTEUR CANDLE B,
FROM BENZALKONIUM CHLORIDE 0.02% w/v CONTAINING, SODIUM CHLORIDE (1),
SODIUM EDETATE (2), SODIUM PHOSPHATE BUFFER (3), AND
SODIUM ACETATE BUFFER (4), RESPECTIVELY.

30ml aliquot filtered	Time taken for filtration				Percent Loss			
	1	2	3	4	1	2	3	4
pH value before filtration					5.8	4.72	6.9	5.9
pH value after filtration					3.2	4.15	6.9	5.85
1	1m. 30 secs	1m. 45 secs	1m. 40 secs	1m. 25 secs	56.73	52.24	83.7	76.32
2	1m. 40 secs	2 minutes	1m. 50 secs	1m. 45 secs	23.5	24.5	53.06	32.65
3	2 minutes	2m. 20 secs	2 minutes	2m. 10 secs	20.0	20.4	25.7	21.23
4	2m. 20 secs	2m. 40 secs	2m. 20 secs	2m. 25 secs	16.33	17.55	20.4	19.2
5	2m. 30 secs	2m. 45 secs	2m. 40 secs	2m. 50 secs	13.06	13.5	15.1	15.1
6	2m. 50 secs	3 minutes	2m. 50 secs	3 minutes	11.02	11.02	11.02	11.02
7	-	3m. 15 secs	3 minutes	3m. 15 secs	-	8.16	10.2	6.94
8	-	3m. 30 secs	3m. 15 secs	3m. 30 secs	-	8.16	6.94	4.08

when Benzalkonium chloride 0.02% w/v, was filtered through Candle B, thus almost nullifying the effect of Phenylethanol.

Thus of the buffers and salts used, it seems that Sodium Phosphate and Sodium Acetate increase the extent of adsorption onto the candle filter, the effect of the former being greater than that of the latter. Sodium Chloride and Sodium Edetate appear to have a similar effect with respect to each other, and do not seem to influence the amount of adsorption to the same extent as that obtained in the presence of the buffers.

From Table 8.7 it may be seen that solutions of Benzalkonium chloride having a low pH value before filtration lost the least amount of the preservative on filtration, and those with a high pH value lost a larger amount, but this correlation does not seem to hold when the loss occurring from solution at a pH value of 5.8, (56.73%) is compared with that occurring from a solution of pH value 5.9 (76.32%).

The decrease in pH value on filtration of the unbuffered solutions, seems to indicate a cation-exchange mechanism, in which the Benzalkonium cation on adsorption, is exchanged for hydrogen ions which pass into the filtrate, thus lowering its pH value.

(c) Adsorption onto Asbestos Filter Pads.

The filter pads used were of the grades SB(BKS) and S.10 (GS), the latter having a slightly larger porosity than the former. They were used as described previously (Chapter 6), the filtrates being collected in large Pyrex test tubes.

Water-soluble extractives:

To determine whether the alkaline water-soluble extractives would in any way affect the method of assay (Method (b)), which comprised the complexation of Benzalkonium chloride with Bromophenol blue, 10ml of distilled water was filtered through a filter pad of the GS grade, and 5ml of the filtrate obtained, assayed undiluted according to Method (b). This process was repeated on four other similar filter pads, using Sodium Chloride 0.1% w/v, Sodium Edetate 0.1% w/v, Sodium Phosphate buffer, Sodium Acetate buffer and Phenylethanol 0.3% v/v. The spectral absorbance of the Dichloroethane extract obtained in each case, was read against a blank prepared with the corresponding salt, buffer, or Phenylethanol respectively.

Effect of the metal holder on Benzalkonium chloride:

20ml of Benzalkonium chloride 0.02% w/v was allowed to remain in contact with the metal holder (as described in Chapter 6(a)) for 10 minutes. The solution obtained from the reservoir, and that run through the filter unit were assayed by measuring their spectral absorbance at 262 n.m.

(Method (a)). This determination was repeated using Benzalkonium chloride in combination with Sodium Chloride 0.1% w/v, Sodium Edetate 0.1% w/v, Sodium Phosphate buffer, Sodium Acetate buffer and Phenylethanol 0.3% w/v, respectively. (The latter being assayed according to method (b)).

Grade GB(EKS):

The extent of adsorption occurring onto grade SB asbestos filter pads from 10ml aliquots of Benzalkonium chloride was determined by filtering a total of 10ml of Benzalkonium chloride 0.02% w/v (in aliquots of 10ml) through filter pad A.

The filtrates obtained were assayed directly by measuring their spectral absorbance at 252 n.m., against corresponding blanks which were prepared previously, by filtering ten 10ml aliquots of distilled water through a similar filter pad, B.

The filter pad A, was then desorbed with three 10ml aliquots of distilled water. The absorbances of the filtrates obtained were read against the blank prepared from the 10th 10ml aliquot of distilled water, which was filtered through filter pad B. The pH values of all filtrates obtained, were measured and compared to that of the original unfiltered solution.

The amount of adsorption occurring from 30ml was determined by filtering five 30ml aliquots of Benzalkonium chloride 0.02% w/v through each of filter pads C and D. The filtrates were assayed

according to method (a), blank solutions having been prepared by filtering five 30ml aliquots of distilled water through a filter pad B.

Filters C and D were desorbed with three 30ml aliquots of distilled water, their absorbances being measured at 262 n.m. against the blank obtained from the 5th 10ml aliquot of distilled water filtered through filter pad B.

The filtrates obtained from filter pad D were also assayed according to method (b), and the results compared.

The pH values of all filtrates were recorded and compared to that of the original unfiltered solution.

Grade S.10 (GS):

To determine the effect of the porosity of the filter pads used, on their extent of adsorption, five 30ml aliquots of Benzalkonium chloride 0.02% w/v were filtered through filter pad A, which was then desorbed with two 30ml aliquots of distilled water. All filtrates obtained, including those from the desorption determination, were assayed according to Method (b). The pH values of all filtrates were measured and compared to that of the original unfiltered solution. This was repeated on further occasions when 30ml of Benzalkonium chloride 0.02% w/v, was filtered through filter pads B, C and D respectively; the filtrate obtained from filter B being assayed according to Method (b).

Effect of autoclaving:

Two asbestos pads B and F (grade S.10), were autoclaved at 15 lbs p.s.i.g. for 15 minutes. The pads were then dried in an oven at about 60°C for two hours. 30ml of Benzalkonium chloride 0.02% w/v was filtered through filter pad B and the spectral absorbance of the filtrate measured at 262 n.m., against a blank prepared by filtering 30ml of distilled water through filter pad F. The pH values of both filtrates were then measured.

Effect of the removal of the alkaline water-soluble extractives:

Two asbestos pads G and H (grade S.10) were washed through with 30ml of N/10 Hydrochloric Acid and distilled water, until the filtrates obtained were acid and alkali free. The pads were then oven dried at 60° for two hours. 30ml of Benzalkonium chloride 0.02% w/v was then filtered through filter pad G and the absorbance measured at 262 n.m. against a blank prepared, by filtering 30ml of distilled water through filter pad H.

Effect of Salts and Buffers on adsorption:

Five 30ml aliquots of solutions containing Benzalkonium chloride 0.02% w/v in combination with (1) Sodium Chloride 0.1% w/v, (2) Sodium Edetate 0.1% w/v, (3) Sodium Phosphate buffer and (4) Sodium Acetate buffer, were filtered through filter pads I, J, K and L (grade S.10), respectively. The pads were each desorbed with 30ml of distilled

water, and the pH values of all filtrates measured.

All filtrates were assayed according to method (b) i.e. by complexation of Benzalkonium chloride with Bromophenol blue. The spectral absorbance of the Dichloroethane extracts were read at 603 n.m. against that prepared from blank determinations containing the respective salts and buffers, which were filtered through other similar filter pads.

The effect of Phenylethanol on the adsorption of Benzalkonium chloride, was determined by filtering 30ml of Benzalkonium chloride 0.02% w/v containing Phenylethanol 0.3% v/v, through filter pad M (grade S.10). The pH value of the solutions were measured before and after filtration. The filtrate obtained, was assayed according to Method (b), and the absorbance of the Dichloroethane extract read at 603 n.m. against that obtained from a blank determination prepared from 30ml of Phenylethanol 0.3% v/v, which was filtered through a filter pad N.

RESULTS and DISCUSSION.

Water-soluble extractives:

The filtrates obtained from filtering distilled water, and solutions of Sodium Chloride, Sodium Bdetate, Sodium Phosphate buffer, Sodium Acetate buffer, and Phenylethanol, were found to have a zero absorbance value at 603 n.m., on being assayed according to method (b).

Effect of the metal holder on Benzalkonium chloride:

The metal holder of the filter unit was found to have no effect on any of the systems with which it was in contact with. All solutions were found to have the same absorbance value as that of their originals, after being placed in contact with the unit for 10 minutes.

Grade SB(BKS):

A loss of 100% was observed to occur from 10ml of Benzalkonium chloride solution filtered through the filter pad A, which continued to adsorb the preservative from the solution in decreasing amounts, after 100ml had been filtered (Table 8.8). The filtration rates through filter pad A were found to be low. (10ml/Ca. 7 minutes).

TABLE 8.8

ADSORPTION OF BENZALKONIUM CHLORIDE ONTO
ASBESTOS FILTER PAD A (GRADE S.B.) FROM
BENZALKONIUM CHLORIDE 0.02% w/v

10ml aliquot filtered	Time taken for filtration	Percent Loss
1	4 minutes	100.0
2	6m. 30 secs	55.1
3	7m. 20 secs	36.73
4	6 minutes	34.7
5	7m. 15 secs	49.0
6	8 minutes	30.6
7	6m. 30 secs	24.5
8	8 minutes	20.4
9	8 minutes	17.55
10	7 minutes	12.25

The filtrate obtained from desorption of filter pad A with 10ml of distilled water, was found to have an absorbance value of 0.140, and decreased to 0.03 after 30ml of distilled water had been washed through.

Adsorption from 30ml aliquots of Benzalkonium chloride 0.02% w/v, onto filter pads C and D were found to be similar, and continued after 150ml had been filtered. The filtration rates were observed to be very low (30ml/Ca. 25 minutes) (Table 8.9). The filtrates obtained were found to be alkaline, having pH values ranging from 9.5 to 9.1, and 9.3 to 8.95, for filter pads C and D respectively.

The filtrates obtained from the first 30ml aliquot of distilled water filtered through filters C and D in the desorption determinations, were found to have absorbance values of 0.065 and 0.075 respectively.

On assaying the filtrates obtained from filter pad D, according to Method (b), slightly different results were obtained. This could be due to errors in Method (a) introduced by the presence of the water-soluble extractives, and particulate matter, such as fibres in the filtrates. (See Table 8.10). Method (b) however should be more accurate than that of (a) with respect to filtrates obtained from asbestos pads, as the effect of the water soluble extractives and particulate matter is eliminated.

TABLE 8.9

ADSORPTION OF BENZALKONIUM CHLORIDE ONTO ASBESTOS FILTER PADS C AND D (GRADE S.B.),
FROM BENZALKONIUM 0.02% w/v HAVING A pH VALUE OF 6.7.

30ml aliquot filtered	Time taken for filtration		pH		Percent Loss	
	C	D	C	D	C	D
1	18 minutes	17m. 30 secs	9.5	9.3	46.9	47.0
2	24 minutes	19m. 30 secs	9.3	9.2	28.6	26.53
3	22 minutes	21 minutes	9.3	8.9	20.4	20.4
4	24 minutes	22 minutes	9.85	8.9	16.4	15.1
5	25 minutes	23 minutes	9.1	8.85	14.2	13.75

TABLE 8.10
ADSORPTION OF BENZALKONIUM CHLORIDE ONTO
ASBESTOS FILTER PAD D - COMPARISON BETWEEN
RESULTS OBTAINED BY ASSAY METHOD (a)
AND METHOD (b).

30ml aliquot filtered	Percent Loss	
	Method (a)	Method (b)
1	47.0	50.00
2	26.53	23.1
3	20.4	17.35
4	15.1	13.0
5	13.75	11.8

Grade S.10 (GS):

Contrary to theoretical expectations, the extent of adsorption onto the Asbestos filter pad A of grade S.10, was found to be slightly greater than that observed with the SB grade. (Table 8.11 and Fig. 8.6). As expected, the filtration rates were observed to be higher than that of Grade S.B. The filtration rates were also found to increase as filtration proceeded. However, on repeating the process with similar pads B, C, and D, losses of 49.4% (Method (b)), 56% and 65% (Method (a)) respectively, were observed to occur from 30ml of Benzalkonium chloride 0.02% w/v, as compared to 60.4% which was adsorbed onto filter pad A. The pH values of the filtrates obtained from filter pads B, C and D, were found

TABLE 8.11

ADSORPTION OF BENZALKONIUM CHLORIDE ONTO ASBESTOS FILTER PAD A (GRADE S.10),
FROM BENZALKONIUM CHLORIDE 0.02% w/v HAVING A pH VALUE OF 6.7.

30ml aliquot filtered	Time taken for filtration	pH	Percent Loss (Method b)
1	7m. 15 secs	9.5	60.4
2	14m. 20 secs	9.35	29.7
3	14 minutes	9.25	17.6
4	13m. 30 secs	9.25	17.2
5	11m. 50 secs	9.15	12.1

to be 9.55, 9.25 and 9.4 respectively, as compared to 8.5 for the filtrate obtained from pad A.

These differences in the extent of adsorption which occurred onto filter pads A, B, C, and D could be due to the variations of the water-soluble extractive content of these pads, and possibly to the differences in methods of assay.

On desorption of filter pad A with 30ml of distilled water, the filtrate was found to have an absorbance value of 0.085, and decreased to 0.04 after 60ml of distilled water had been filtered.

Effect of autoclaving:

A loss of 43% of Benzalkonium chloride was observed to occur from 30ml of Benzalkonium chloride 0.02% w/v, which was filtered through an autoclaved filter pad E. (Grade S.10). The pH values of the filtrates obtained from pads E and F(blank) were found to be 8.95 and 8.89 respectively; the original unfiltered preservative solution having had a pH value of 7.3.

The absorbance value at 262 n.m. of the water-soluble extractives obtained from filter pad F, was 0.07 as compared to 0.09 for the filtrates obtained from, pads used as corresponding blanks in the assay (method (a)) of the filtrates obtained from filter pads C and D. Thus some of the water-soluble extractives, are probably removed on autoclaving. This may possibly

result in a slightly lower extent of adsorption.

Removal of the water-soluble extractives:

On removal of the alkaline water-soluble extractives from filter pad G, the amount of adsorption which occurred from 30ml of Benzalkonium chloride was found to be 53.1%. The pH value of the filtrate was found to be 6.8. Thus it seems that the alkaline water-soluble extractives do not seem to influence the extent of adsorption in any appreciable way.

Effect of salts and buffers:

Sodium Chloride seemed to cause a slight decrease in the extent of adsorption occurring onto filter pad I (Table 8.12 and Fig. 8.6). The alkalinity of the filtrates, and the filtration rates were found to be more or less similar to that obtained in the determinations without Sodium Chloride. The filtrate obtained on desorption with 30ml of distilled water was found to have a pH value of 7.8, and an absorbance value of 0.07, on being assayed; this value being almost similar to that obtained in the previous determination without Sodium Chloride.

The losses obtained in the presence of Sodium Edetate, were found to be higher from the initial 30ml aliquot filtered through filter pad J. (Table 8.12 and Fig. 8.6). The pH values of the filtrates were found to vary from 5.8 to 5.55, the original unfiltered solution having had a pH value of 5.3.

This could probably be due to complexation with alkali metals within the filter pad, with the formation of complexes which are not washed off the pad, or to the formation of water-soluble complexes, which are not alkaline in nature, hence the lowered pH values. On desorption, the filtrate obtained was found to have an absorbance value of 0.065 on being assayed, and a pH value of 6.05.

In the presence of Sodium Phosphate buffer the losses of Benzalkonium chloride which occurred from solution onto filter pad K, were found to be slightly lower than those observed in the presence of Sodium Edetate (Table 8.12 and Fig. 8.6). The pH value of the filtrates obtained varied slightly from that of the unfiltered solution, which had a pH value of 6.9. The filtration rates however, were found to be slightly higher than those observed in previous determinations. The filtrate obtained from desorption with 30ml of distilled water, was found to have an absorbance value of 0.055 on being assayed, and a pH value of 7.3.

Sodium Acetate buffer seemed to behave in an almost similar way to Sodium Chloride; the amount of adsorption occurring from 30ml aliquots, onto filter pad L may be seen from Table 8.12 and Fig. 8.6. The pH values of the filtrates seemed to vary slightly from the unfiltered buffered solution, which had a pH value of 5.9. The filtrate

FIG. 8.6 Loss of Benzalkonium chloride occurring from Benzalkonium chloride 0.02% w/v, on filtration through Asbestos Filter Pads

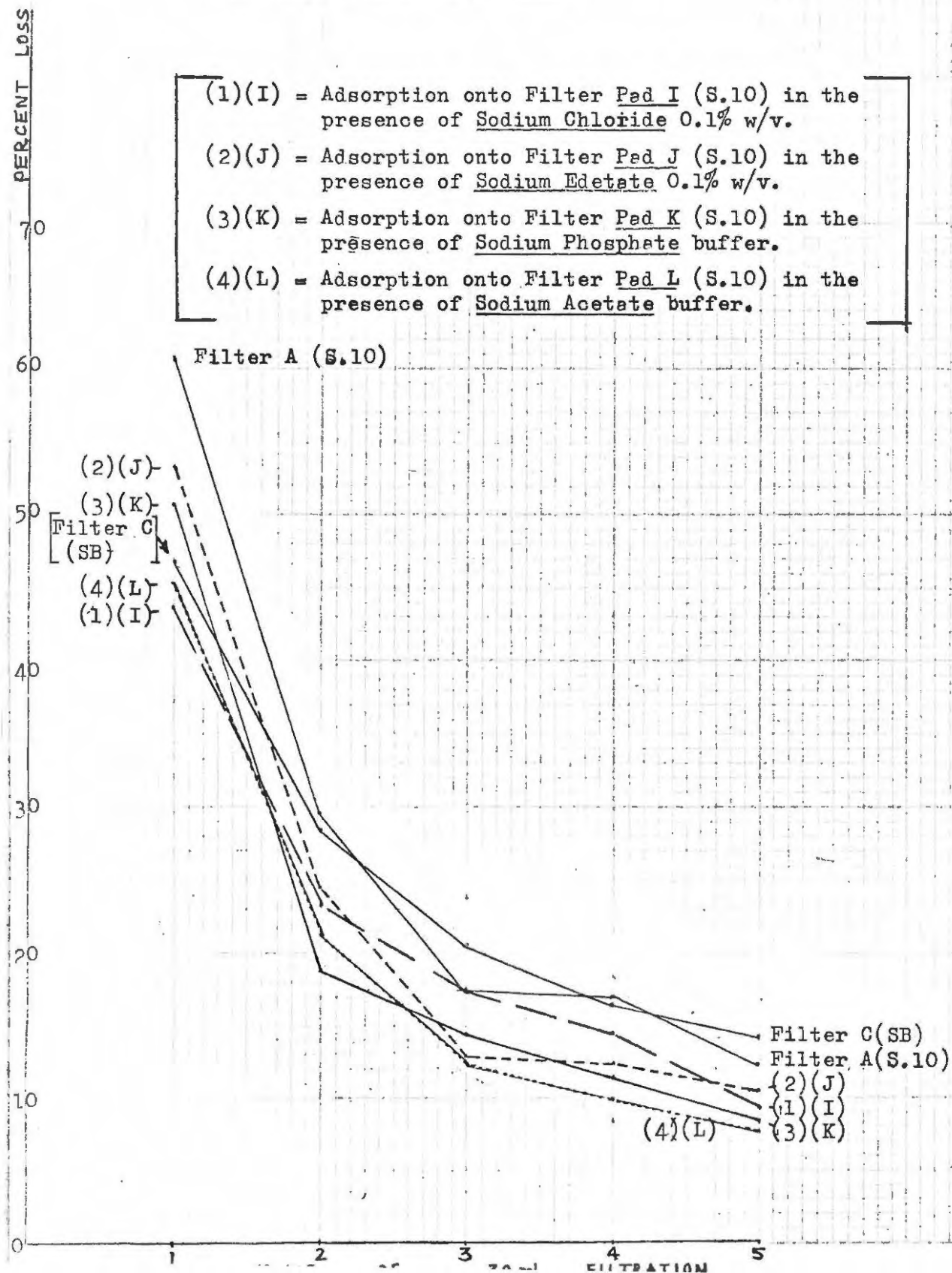


TABLE 8.12

ADSORPTION OF BENZALKONIUM CHLORIDE FROM SOLUTIONS CONTAINING BENZALKONIUM CHLORIDE 0.02% w/v IN COMBINATION WITH (1) SODIUM CHLORIDE 0.1% w/v (2) SODIUM EDETATE 0.1% w/v, (3) SODIUM PHOSPHATE BUFFER, AND (4) SODIUM ACETATE BUFFER, ONTO ASBESTOS FILTER PADS I, J, K, AND L, (GRADE S.10) RESPECTIVELY.

30ml aliquots filtered	Time taken for filtration				pH				Percent Loss			
					6.95*	5.3*	6.9*	5.9*				
	1(I)	2(J)	3(K)	4(L)	1(I)	2(J)	3(K)	4(L)	1(I)	2(J)	3(K)	4(L)
1	7m.50 secs	7m. 5 secs	6m.50 secs	8m.40 secs	9.35	5.6	6.95	6.02	43.9	53.1	50.7	45.1
2	12m.20 secs	10m.30 secs	9m.10 secs	13m.30 secs	9.65	5.65	6.95	5.93	23.1	24.2	18.96	21.1
3	13m.20 secs	11 minutes	9m.30 secs	14m.45 secs	9.55	5.60	6.92	5.93	17.42	12.8	14.22	12.2
4	13m.30 secs	11m.50 secs	9m.40 secs	13m.30 secs	9.15	5.55	6.92	5.93	14.48	12.6	11.2	10.0
5	14m.45 secs	12 minutes	9m.25 secs	15m.20 secs	9.05	5.55	6.92	5.93	9.1	10.46	8.1	7.8

(* pH values before filtration)

obtained from the desorption determination was found to have a pH value of 6.8 and an absorbance value of 0.07, on being assayed.

The amount of adsorption occurring in the presence of Phenylethanol was found to be slightly higher than that occurring in the presence of the aforementioned salts and buffers. A loss of 58.64% was observed to occur from 30ml of the combined preservative solution, on filtration through filter pad M of grade S.10; the filtrate having a pH value of 9.4 as compared to the original unfiltered solution which had a pH value of 6.92.

The effect of autoclaving, water-soluble extractives, salts, buffers and Phenylethanol cannot be generalized as the extent of adsorption occurring from standard solutions, onto similar untreated asbestos pads, was found to vary within the limits of adsorption observed with the autoclaved, and acid treated filter pads as well as those determinations in the presence of the various aforementioned additives. The results obtained from desorption determinations could not be correlated directly with the effects of these treatments and additives, but varied according to the amount of preservative adsorbed.

In the observations made from the determinations on the asbestos filter pads, it seems that the phenomenon of adsorption is associated with a surface tension effect, (Chapter 6) rather than a

cation-exchange mechanism, as the addition of salts did not appreciably minimise the amount of adsorption which occurred, to any great extent, as was observed with the determinations concerning the adsorption of Chlorhexidine Acetate, onto the asbestos filter pads.

(d) Adsorption onto Sartorius Membrane Filters.

The determinations were done on Sartorius membrane filters attached to the plastic filter unit. The glass filter unit was used on one occasion for comparative purposes. Before every determination, the units were thoroughly cleaned and rinsed with distilled water, so that the filtrates of distilled water obtained through them had a zero absorbance value at 262 n.m., after which they were allowed to dry under mild heat. The receiver of the unit was also rinsed and dried before each filtration.

The pH values of all solutions were measured before and after filtration, on a Metrohm pH meter. All filtrates, except those obtained in combination with Phenylethanol, were assayed according to Method (a), i.e. by measuring the spectral absorbance at 262 n.m. The spectral absorbance of the filtrates obtained from the determinations concerning the effects of salts and buffers, were read at 262 n.m. against a blank containing the salt or buffer, in appropriate quantities.

Water-soluble extractives:

10ml of distilled water was filtered through a membrane filter attached to the plastic filter unit, and the spectral absorbance of the filtrate measured at 262 n.m. against a distilled water blank. This determination was repeated using Sodium Chloride 0.1% w/v, Sodium Edetate 0.1% w/v, Sodium Phosphate buffer, Sodium Acetate buffer, and Phenylethanol 0.3% v/v (the latter being assayed according to method (b), i.e. complexation with Bromophenol blue.)

Effect of the Plastic filter unit, on Benzalkonium chloride:

To determine whether the plastic material of the filter unit interacted with Benzalkonium chloride, 10ml of Benzalkonium chloride 0.02% w/v was allowed to flow through the unit (without the membrane), and the solution allowed to remain in contact with the receiver for one minute, after which its spectral absorbance was measured at 262 n.m., and compared to the original solution.

This determination was repeated in the presence of Sodium Chloride 0.1% w/v, Sodium Edetate 0.1% w/v, Sodium Phosphate buffer, Sodium Acetate buffer, and Phenylethanol. (The Benzalkonium chloride in the latter being assayed according to method (b)).

(1) Sartorius Plastic Filter Unit:

The amount of adsorption occurring from 10ml aliquots of Benzalkonium chloride 0.02% w/v, was

determined by filtering five 10ml aliquots through membrane filter A, attached to the plastic filter unit.

This determination was repeated with membrane filters B and C using aliquots of 30ml.

Desorption of the filters was carried out by carefully removing the membrane from the filter unit, which was then rinsed and dried. The membrane was then refitted into the unit, and the filter desorbed with two 30ml aliquots of distilled water.

Effect of autoclaving:

Membrane filter D was attached to the plastic filter unit, and autoclaved at 10 lbs p.s.i.g. for 30 minutes. The unit with the membrane, was then allowed to dry under mild heat and when cool, 30ml of Benzalkonium chloride 0.02% w/v was filtered through it. The absorbance of the filtrate was then measured at 262 n.m., as well as the pH value.

Effect of salts and buffers:

Five 30ml aliquots of solutions containing Benzalkonium chloride 0.02% w/v in combination with, (1) Sodium Chloride 0.1% w/v, (2) Sodium Bdetate 0.1% w/v, (3) Sodium Phosphate buffer, and (4) Sodium Acetate buffer, were filtered through membrane filters B, F, G, and H, respectively. The filters were then desorbed with two 30ml aliquots of distilled water.

The effect of Phenylethanol on the adsorption

of Benzalkonium, was determined by filtering 30ml of a solution of Benzalkonium chloride 0.02% w/v containing Phenylethanol 0.3% v/v, through membrane filter I. The filtrates obtained were assayed according to Method (b). The loss which occurred, was compared with that occurring on filtration of Benzalkonium chloride 0.02% w/v, through membrane filter J; the filtrate being assayed according to Method (b) as well.

The process was repeated by filtering 30ml of the combined preservative solution (Benzalkonium chloride and Phenylethanol) in combination with, Sodium Chloride 0.1% w/v, Sodium Edetate 0.1% w/v Sodium Phosphate buffer, and Sodium Acetate buffer, through membrane filters K, L, M, and N respectively.

(ii) Sartorius Glass Filter Unit:

Six 30ml aliquots of Benzalkonium chloride 0.02% w/v were filtered through membrane filter O, attached to the glass filter unit. The membrane filter was then removed, and the unit rinsed and dried, after which the membrane was reattached and, desorbed with two 30ml aliquots of distilled water. All filtrates were collected in 50ml Buchner tubes.

RESULTS and DISCUSSION.

Water-soluble extractives.

The filtrates obtained from filtering distilled water, solutions of Sodium Chloride, Sodium Edetate,

Sodium Phosphate buffer, Sodium Acetate buffer and Phenylethanol, were found to have a zero absorbance value on being assayed.

Effect of the Plastic filter unit.

All solutions were found to lose between 4% and 5% when passed through the unit, and allowed to remain in contact with it for one minute.

(i) Sartorius Plastic filter unit.

The amount of loss which occurred from 10ml aliquots of Benzalkonium chloride 0.02% w/v, on filtration through membrane filter A, was found to remain constant (14.3%) after the first 10ml aliquot of Benzalkonium chloride was filtered (Table 8.13).

TABLE 8.13
LOSS OF BENZALKONIUM CHLORIDE ON FILTRATION
OF BENZALKONIUM CHLORIDE 0.02% w/v, THROUGH
MEMBRANE FILTER A (PLASTIC UNIT)

10ml aliquot filtered	Time taken for filtration	Percent Loss
1	6 seconds	19.2
2	7 seconds	14.3
3	7 seconds	14.3
4	7 seconds	14.3
5	7 seconds	14.3

The amount lost from 30ml of Benzalkonium

chloride 0.02% w/v on filtration through membrane filter B was found to be 14.3%, after which the loss remained constant at 12.24%, and continued after 150ml had been filtered. With membrane filter C, the loss remained constant at 14.3% (Table 8.14 and Fig. 8.7). The pH values of the filtrates obtained from filters B and C, were found to differ only very slightly from the unfiltered solution, which had a pH value of 7.0.

On desorption of filters B and C, an absorbance value of 0.003 and 0.002 respectively, was obtained from the first 30ml aliquot of distilled water filtered through, after which no further Benzalkonium chloride could be detected in the filtrate.

Van Ooteghem and Herbets²⁷ in their investigation concerning the adsorption of Benzalkonium chloride onto Sartorius membrane filters, from Benzalkonium chloride 0.01% w/v in combination with Boric Acid, found that 4.7% adsorption occurred from 30ml of the solution being filtered. They also determined the influence of the preservative concentration on the extent of adsorption, and observed that a larger amount of adsorption occurred from solutions of higher concentration.

The constant losses which were observed after filtering 10ml, and 30ml aliquots through membrane filters A, B, and C, could probably be due to interaction with the plastic unit, as well as the effects

TABLE 8.14

LOSS OF BENZALKONIUM CHLORIDE FROM BENZALKONIUM CHLORIDE 0.02% w/v,
ON FILTRATION THROUGH MEMBRANE FILTERS B AND C (PLASTIC UNIT).

30ml aliquot filtered	Time taken for filtration		pH		Percent Loss	
	B	C	B	C	B	C
1	20 seconds	15 seconds	7.0	6.9	14.3	14.3
2	20 seconds	15 seconds	6.9	6.9	12.24	14.3
3	20 seconds	15 seconds	6.9	7.0	12.24	14.3
4	20 seconds	20 seconds	7.0	7.0	12.24	14.3
5	20 seconds	20 seconds	7.0	7.0	12.24	14.3

of incompatibility between Benzalkonium chloride and the nitrates present in the filter medium. From the desorption determination, it seems that very little of the preservative is washed off the filter. This seems to suggest that the preservative is probably chemically inactivated or altered to some extent, by the membrane, and the filter unit.

Effect of autoclaving:

Autoclaving was found to have no effect on the adsorptive properties of the membrane filter, as a loss of 14.0% was observed to occur from 30ml of Benzalkonium chloride filtered through membrane filter D. The pH value of the filtrate was found to be more or less unchanged from that of the original unfiltered solution. Van Ooteghem and Herbots²⁷ also found that the sterilization of Sartorius membrane filters in an autoclave at 120°C, did not modify the adsorption properties.

Effect of Salts and Buffers:

The amount of loss occurring from Benzalkonium chloride solution, in the presence of Sodium Chloride, on filtration through membrane filter E, was found to decrease after the first 30ml aliquot was filtered. (Table 8.15 and Fig. 8.7). The pH value of the filtrates obtained were found to differ very slightly from that of the unfiltered solution.

On desorption, the filtrate obtained from the

first 30ml aliquot of distilled water filtered through membrane filter E, was found to have an absorbance value of 0.035, and decreased to 0.003 after 60ml of distilled water had been washed through.

In the presence of Sodium Edetate, a decreased loss occurred from the first 30ml aliquot filtered through membrane filter F, which was then observed to become saturated after 90ml of solution had been filtered (Table 8.15 and Fig. 8.7). The pH values of the filtrates were also observed to be more or less similar to that of the original unfiltered solution.

The filtrates obtained from the desorption determination, were found to have absorbance values of 0.018 and zero, after 30ml and 60ml of distilled water had been washed through respectively.

Sodium Phosphate buffer caused an increased loss from 30ml, on filtration through membrane filter G, but as filtration proceeded a lower loss which remained constant, was observed (Table 8.15 and Fig. 8.7). The pH values of the filtrates were found to remain unchanged from that of the original, unfiltered buffered solution.

On desorption, the absorbance of the filtrate obtained from the first 30ml aliquot of distilled water, was also found to be 0.018, and decreased to zero after 60ml of distilled water had been filtered.

FIG. 8.7 Loss of Benzalkonium chloride occurring from Benzalkonium chloride 0.02% w/v, on filtration through Sartorius Membrane Filters.

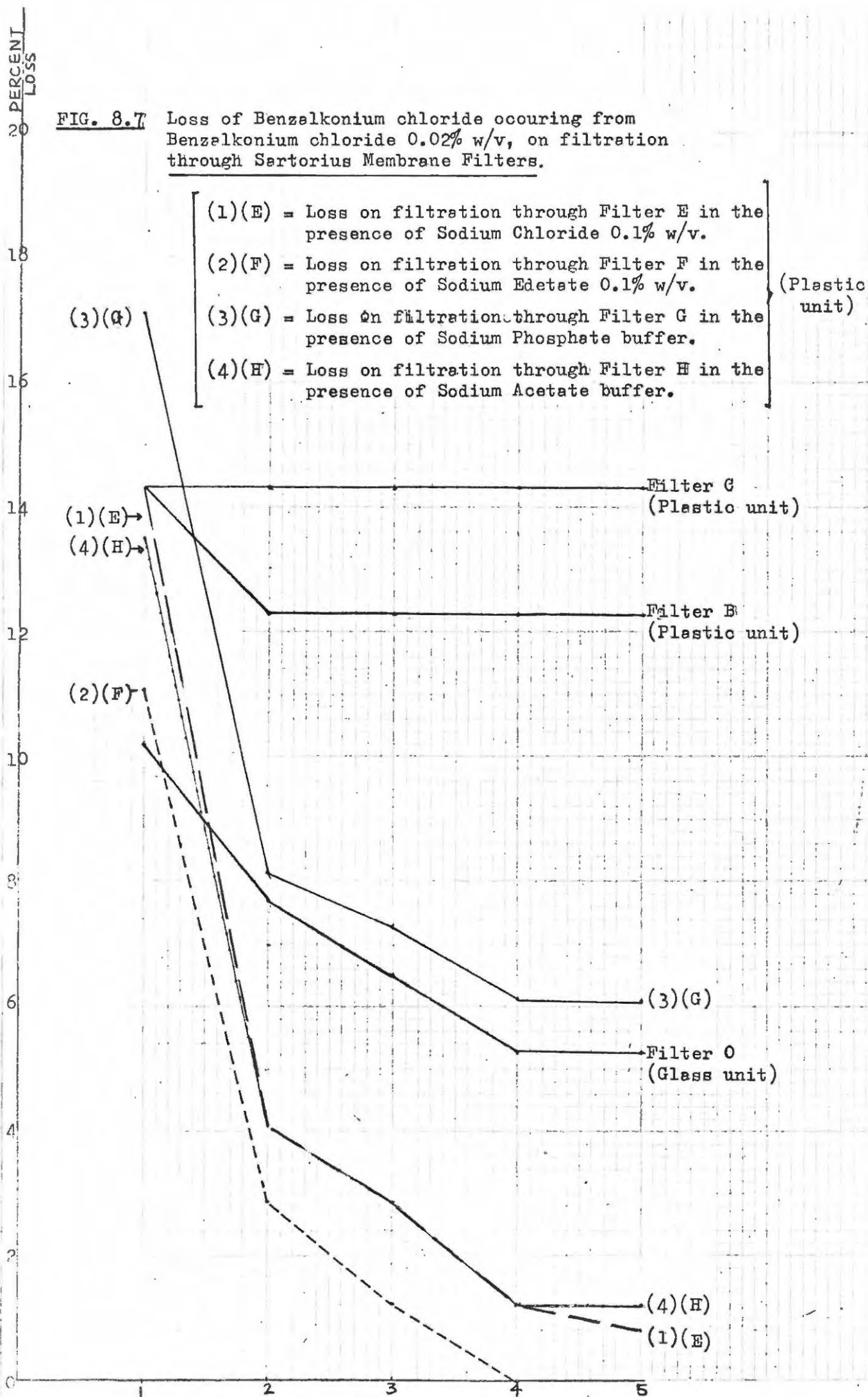


TABLE 8.15

LOSS OF BENZALKONIUM CHLORIDE FROM SOLUTIONS CONTAINING BENZALKONIUM CHLORIDE 0.02% w/v,
IN COMBINATION WITH (1) SODIUM CHLORIDE 0.1% w/v, (2) SODIUM EDETATE 0.1% w/v,
(3) SODIUM PHOSPHATE BUFFER, AND (4) SODIUM ACETATE BUFFER, ON FILTRATION
THROUGH MEMBRANE FILTERS E, F, G, AND H (PLASTIC UNIT).

30ml aliquot filtered	Time taken for filtration				pH				Percent Loss			
					6.9*	5.1*	6.9*	5.88*				
	1(E)	2(F)	3(G)	4(H)	1(F)	2(F)	3(G)	4(H)	1(E)	2(F)	3(G)	4(H)
1	10 secs	10 secs	10 secs	10 secs	6.75	5.05	6.9	5.88	14.3	11.0	17.2	13.5
2	12 secs	10 secs	10 secs	10 secs	6.85	5.05	6.9	5.88	4.1	2.9	8.2	4.1
3	12 secs	10 secs	10 secs	10 secs	6.9	5.1	6.9	5.88	2.9	1.2	7.35	2.9
4	12 secs	10 secs	10 secs	10 secs	6.9	5.1	6.9	5.88	1.2	nil	6.2	1.2
5	12 secs	10 secs	10 secs	10 secs	6.9	5.1	6.9	5.88	0.82	nil	6.2	1.2

(* pH values before filtration)

In the presence of Sodium Acetate buffer, 13.5% loss occurred from the first 30ml aliquot filtered. The loss which followed was observed to remain constant (1.2%) after 90ml had been filtered through membrane filter H. (Table 8.15 and Fig. 8.7). The pH values of the filtrates were also found to be unchanged from that of the original unfiltered solution. The absorbance values of the filtrate obtained after desorbing the filter with 30ml and 60ml of distilled water, was found to be 0.025 and zero, respectively.

In the determination concerning the effect of Phenylethanol on the adsorption or loss of Benzalkonium chloride from solution, a loss of 7.0% of Benzalkonium chloride, was observed to occur from 30ml of Benzalkonium chloride 0.02% w/v. (Assayed according to Method (b)), on filtration through membrane filter J, and a loss of 9.2% occurred from 30ml of the combined preservative solution containing Benzalkonium chloride 0.02% w/v and Phenylethanol 0.3% w/v, which was filtered through membrane filter I.

In the presence of Sodium Chloride an increased loss of Benzalkonium chloride was observed (19.8%) to occur on filtration of the combined preservative solution through membrane filter K. (Table 8.16). Sodium Bdetate and Sodium Phosphate buffer caused losses of 16.93% and 14.44%, on filtration of 30ml of each solution, through membrane filters L and

TABLE 8.16

LOSS OF BENZALKONIUM CHLORIDE FROM SOLUTIONS CONTAINING PHENYLETHANOL
IN COMBINATION WITH A SALT OR BUFFER.

Additive	Benzalkonium Chloride 0.02% w/v		Benzalkonium Chloride 0.02% w/v in combination with Phenylethanol 0.3% v/v	
	Membrane filter	Percent Loss	Membrane filter	Percent Loss
(1) Sodium Chloride	E	14.3	K	19.8
(2) Sodium Edetate	F	11.02	L	16.9
(3) Sodium Phosphate	G	17.2	M	14.44
(4) Sodium Acetate	H	13.5	N	9.0
None	B,C	12 - 14	I	9.0
	J	7.0 (Method b)		

M, respectively. The least loss (9.0%), was found to occur in the presence of Sodium Acetate buffer, on filtration through membrane filter N.

The increase in losses observed in the presence of Phenylethanol and the salts and buffers, could probably be caused through a further reduction in the surface tension of the solution, because of the presence of Phenylethanol. This however does not seem to apply in the case of Sodium Acetate.

(ii) Sartorius Glass Filter Unit.

The losses of Benzalkonium chloride which occurred on filtration of 30ml aliquots of Benzyl-konium chloride 0.02% w/v, through membrane filter O attached to the glass filter unit, were found to be slightly lower than that observed with the plastic unit. (Table 8.17 and Fig. 8.7). This could probably be due to there being no interaction with the unit, though a higher loss was expected because of adsorption onto the sintered glass support disc. After 120ml had been filtered, the loss which occurred was found to remain constant (5.3%). The pH values of the filtrates obtained, were found to differ very slightly from the original unfiltered solution, which had a pH value of 7.3.

The filtrates obtained from desorption of the filter with 30ml and 60ml of distilled water, were found to have absorbance values of 0.005 and zero, respectively.

With the glass unit, there was a decrease in loss, until the sintered glass support disc became

TABLE 8.17

LOSS OF BENZALKONIUM CHLORIDE FROM BENZALKONIUM CHLORIDE 0.02% w/v,
ON FILTRATION THROUGH MEMBRANE FILTER O, ATTACHED TO THE
SARTORIUS GLASS FILTER UNIT

30ml aliquot filtered	Time taken for filtration	pH	Percent Loss
1	10 seconds	7.2	10.2
2	10 seconds	7.25	7.8
3	10 seconds	7.3	6.5
4	10 seconds	7.3	5.3
5	10 seconds	7.3	5.3
6	10 seconds	7.3	5.3

saturated, whilst the membrane continued to adsorb or inactivate the Benzalkonium chloride, thus causing a constant loss.

With the plastic unit, it was seen that the loss which occurred after filtration of 30ml of Benzalkonium chloride 0.02% w/v was constant. This loss from the first 30ml aliquot could thus be due to interaction with the plastic unit and the membrane filter. After filtration of 30ml, the loss may be due to adsorption onto the filter only or both the unit and filter. The latter seems to be the case, when considering the loss which occurs when using the glass unit, where no saturation of the membrane was observed to occur, instead the amount lost, decreased probably because of saturation of the support disc, and then remained constant. The loss which remained constant was lower (5.3%) than that observed in the case of the plastic unit. This seems to indicate that the constant losses which were higher (Ca. 12%) when using the plastic unit, were probably due to interaction with the plastic unit and the membrane filter.

SUMMARY. (Table 8.18)

(a) Sintered Glass Filters.

Adsorption onto sintered glass filter A from 10ml and 30ml of Benzalkonium chloride 0.02% w/v, was found to be 19.2% and 11% respectively. In all the determinations, the extent of adsorption

onto filter A decreased after 30ml had been filtered, to a constant amount with the filter showing no signs of becoming saturated on continuous filtration.

Filter B however, adsorbed a slightly lower amount (9.4%) and became saturated after 30ml.

Increased adsorption (20%) was observed to occur from 30ml of Benzalkonium chloride 0.01% w/v.

The desorption determinations indicated that very little of the preservative was desorbed with distilled water.

In the presence of salts (Sodium Chloride, and Sodium Edetate) and buffers (Sodium Phosphate and Sodium Acetate), increased adsorption of Benzalkonium chloride occurred from 30ml of the preservative solution, but as filtration proceeded, decreased adsorption was observed to occur; the amount adsorbed however remained constant. The highest losses occurred in the presence of the buffers. Sodium Edetate and Sodium Chloride gave similar results.

The filtrates obtained from desorption of the filter, through which solutions containing the salts and buffers were filtered, were found to contain more Benzalkonium chloride, than that previously obtained in the absence of these salts or buffers.

A slightly lower loss (17.35%) of Benzalkonium chloride was found to occur from 10ml of Benzalkonium chloride 0.02% w/v in the presence of Phenylethanol, as compared to that which occurred

in its absence.

Lower losses (9.4%) from 30ml of Benzalkonium chloride 0.02% w/v, were observed on filtration through sintered glass filters of Porosity No. 4.

(b) Doulton-Pasteur Unglazed Porcelain Candles.

Pasteur candle filters, A and B were found to adsorb similar amounts (38%) of Benzalkonium chloride from 30ml of Benzalkonium chloride 0.02% w/v. Adsorption was observed to continue after 180ml had been filtered, both filters adsorbing constant amounts. On a further occasion filter B was found to adsorb a greater amount (57.7%).

The effect of Sodium Chloride and Sodium Bdetate, were found to be similar; Ca. 50% adsorption occurring from 30ml of the preservative solution. Sodium Acetate, and Sodium Phosphate buffer, both caused a greater increase in the extent of adsorption; 76% and 83% occurring from 30ml of solution, respectively.

In the presence of Phenylethanol a loss of 53% occurred from 30ml of solution. If this is compared to the losses observed in the initial determinations, then it can be assumed that it causes an increase in adsorption, but on a further occasion a loss of 57.7% was observed in the absence of Phenylethanol.

In all determinations, the adsorption process was found to be accompanied by a decrease in the pH value of the filtrates obtained (except in the

case of buffered solutions). This phenomenon was associated with a cation exchange mechanism, by which hydrogen ions of the filter were exchanged, for the Benzalkonium cations in solution.

(c) Asbestos Filter Pads.

Adsorption of Benzalkonium chloride onto the asbestos filter pads of grade S.B., from 10ml of Benzalkonium chloride 0.02% w/v was found to be considerable (100%). The extent of adsorption from 30ml of the preservative solution, onto filter pads of grade S.10, was found to be higher than that which occurred onto pads of grade S.B. of smaller porosity.

Adsorption from 30ml of solution onto different, but similar filter pads of grade S.10, was found to vary between 49% and 60%.

Autoclaving of the filter pad and the removal of the alkaline water-soluble extractives, seemed to have no influence on the extent of adsorption, neither did the salts, buffers and Phenylethanol, in later determinations.

The filtrates obtained were found to be reasonably alkaline, having pH values of Ca. 9.0. The buffered solutions however, were found to have very slight changes in their pH values after filtration, whilst solutions containing Sodium Bdetate were found to be more on the acidic side of neutrality after filtration.

It seemed that the overall adsorption

phenomenon, was associated with a surface tension effect, rather than a cation-exchange mechanism.

(d) Sartorius Membrane Filters.

The losses of Benzalkonium chloride which occurred from solution, on filtration through the membrane filters, was found to remain constant (Ca. 12%), as filtration proceeded. This was assumed to be due to interaction with the plastic unit and the nitrates present in the membrane, with which Benzalkonium chloride is incompatible. In the case of the glass unit, lower losses which remained constant (5.3%) were found to occur.

Autoclaving, was found to have no effect on the adsorption properties of the membrane filter.

In the presence of salts and buffers, the losses from the first 30ml aliquot filtered were higher in most cases than those observed in the absence of these additives. However as filtration proceeded the losses were found to decrease. (Sintered glass filters seemed to behave in a similar manner as well). The least loss was observed in the presence of Sodium Edetate. The effect of Sodium Chloride and Sodium Acetate buffer appeared to be almost similar, despite the pH values of the two solutions being different. The highest loss (17.2%) occurred in the presence of Sodium Phosphate buffer.

In the presence of Sodium Edetate, saturation of the filter was observed to occur, but with Sodium Acetate buffer and Sodium Chloride, a

constant loss of Ca. 1% occurred, and with Sodium Phosphate buffer, a constant loss of Ca. 6%.

All desorption determinations seemed to indicate that the preservative was either strongly adsorbed by the filter or inactivated by it. However in the presence of salts, and buffers, absorbance values which were slightly higher, were obtained from these determinations.

Phenylethanol seemed to cause a slight increase in the amount of Benzalkonium chloride adsorbed from solution, and in the presence of salts and buffers a further increase was observed, except in the case of Sodium Acetate, where less adsorption occurred.

TABLE 8.18

LOSS OF BENZALKONIUM CHLORIDE OCCURING FROM 30ml OF BENZALKONIUM
CHLORIDE 0.02% w/v, ON FILTRATION THROUGH VARIOUS FILTER MEDIA

Filter Type	without additive	with Sodium Chloride	with Sodium Edetate	with Sodium Phosphate buffer	with Sodium Acetate buffer	with Phenyl-ethanol
Sintered Glass Porosity No. 5 (Porosity No. 4)	Ca 11% (9.4%)	14%	14%	21%	16%	23%(10ml) (cf. 19%)
Doulton-Pasteur candles	Ca 38% - 57%	57%	52%	53%	33%	53%
Asbestos Pads S.10 (SB)	Ca 49% - 60% (Ca 47%)	44%	53%	51%	45%	58.6%
Sartorius Membrane filters - Plastic unit (- Glass unit)	Ca 12% - 14% - 7% (Method b) (Ca 10%)	14.3%	11%	17.2%	13.5%	9.2%

CHAPTER NINE

ADSORPTION OF PHENYLETHANOL

Phenylethanol (2-Phenylethylalcohol) is a primary aromatic alcohol of molecular formula $C_6H_5 \cdot CH_2 \cdot CH_2OH$, and molecular weight 122.2. It is a colourless liquid with a rose-like odour, and is soluble 1 in 50 of water, and very soluble in alcohol.

Phenylethanol was used in all determinations at a concentration of 0.5% v/v, which was prepared by diluting the original solution of Phenylethanol manufactured by the British Drug Houses Ltd. (B.D.H.).

Solutions of Phenylethanol 0.5% v/v in combination with Sodium Chloride 0.1% w/v, Sodium Edetate 0.1% w/v, Sodium Acetate buffer (pH 5.9) and, Sodium Phosphate buffer (pH 6.9), were also filtered through the various filter media, under a vacuum of 20 ins of mercury at room temperature (Ca 22°C).

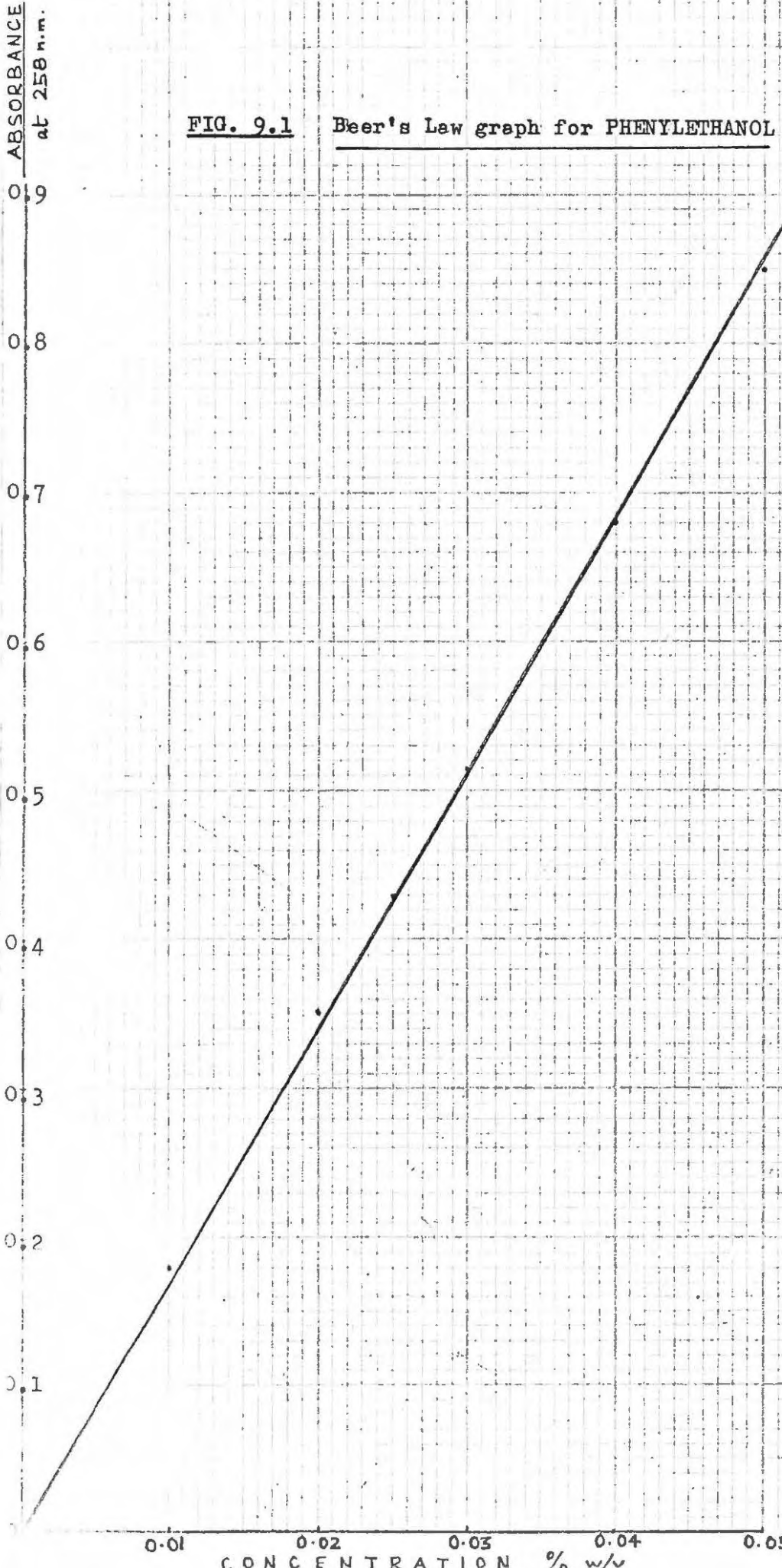
All filtrates were assayed spectrophotometrically by measuring the absorbance at a peak absorption wavelength of 258 n.m. (U.V.), Phenylethanol obeying Beer's Law within the range 0.05% v/v to 0.01% v/v.

Preparation of Beer's Law Graph:

Solutions containing Phenylethanol 0.05% v/v, 0.04% v/v, 0.03% v/v, 0.02% v/v and 0.01% v/v, were prepared by appropriately diluting the B.D.H.

FIG. 9.1

Beer's Law graph for PHENYLETHANOL



reagent with distilled water. The spectral absorbances of each of these dilutions were measured directly at a wavelength of 258 n.m., against a blank of distilled water, and the results (taking an average of two determinations), plotted graphically. (Fig. 9.1)

A solution containing Phenylethanol 0.5% v/v on dilution (1-20)(=0.025%), was found to have an absorbance value of 0.430 at 258 n.m. Thus the quantity of Phenylethanol present in the filtrates obtained from the determinations was assayed by, measuring the spectral absorbance of a 1-20 dilution of the filtrate at 258 n.m., and from this the quantity of Phenylethanol adsorbed from solution, was calculated. All solutions were preassayed before filtration, by measuring the spectral absorbance of a 1-20 dilution. Solutions containing salts or buffers, were assayed by measuring the spectral absorbance against a blank containing the respective salt or buffer in appropriate quantities.

(a) Adsorption onto Sintered Glass Filters

Two similar sintered glass filters A and B, of porosity No. 5 (5 on 3), were cleaned before every determination with hot sulphuric acid containing sodium nitrate 1% w/v, and distilled water, until the filtrates were acid free, and had a zero absorbance value at 258 n.m. The filters were dried at 150°C for an hour, and allowed to

cool before use. All filtrates were collected in Pyrex test tubes.

The extent of adsorption of Phenylethanol onto the filters, was determined by filtering five 10ml aliquots of Phenylethanol 0.5% v/v through each of filters A and B.

The effect of salts and buffers, was determined by filtering five 30ml aliquots of solutions, containing Phenylethanol 0.5% v/v in combination with Sodium Chloride 0.1% w/v, Sodium Bdetate 0.1% w/v, Sodium Phosphate buffer, and Sodium Acetate buffer, through filter A, respectively.

RESULTS and DISCUSSION

The absorbance values of a 1-20 dilution of the filtrates obtained from the filtration of 10ml aliquots of Phenylethanol through filters A and B, were found to be 0.430. Thus no loss of Phenylethanol was observed to occur on filtration through sintered glass filter A.

No loss of Phenylethanol was observed in the presence of the salts and buffers as well.

(b) Adsorption onto Doulton-Pasteur Unglazed Porcelain Candles.

Two similar Pasteur candle filters A and B, were cleaned before determinations, with dilute Hydrochloric acid and distilled water, until the filtrates were acid free and had a zero absorbance value at 258 n.m., after which they were allowed to dry at 150°C for an hour, and then cooled to room temperature. The filtrates were collected

individually, and then transferred from the receiver to large Pyrex test tubes, the receiver being rinsed and dried before each filtration.

Five 10ml aliquots of Phenylethanol 0.5% v/v, were filtered through Filter A. This was repeated using five 30ml aliquots, which were filtered through each of filters A and B.

The effect of salts and buffers was determined by filtering five 30ml aliquots of solutions containing Phenylethanol 0.5% v/v in combination with Sodium Chloride 0.1% w/v, Sodium Edetate 0.1% w/v, Sodium Phosphate buffer, and Sodium Acetate buffer, through filter B, respectively.

RESULTS and DISCUSSION.

No loss of Phenylethanol was observed on measuring the absorbance of a 1-20 dilution of all the filtrates obtained from filtering 10ml and 30ml aliquots through filters A and B.

In the presence of the salts and buffers no loss was observed to occur from the solutions, as well.

(c) Adsorption onto Asbestos Filter Pads.

The asbestos filter pads of the grade S.10 (GS), were used to determine whether Phenylethanol was adsorbed onto them during filtration. The pads were used as described earlier, and the filtrates collected in large Pyrex test tubes.

Effect of the metal holder:

To determine whether the metal holder of the

filter unit would interact with Phenylethanol, 10ml of Phenylethanol 0.5% v/v, was allowed to remain in contact with the holder for ten minutes, as described in Chapter 6(c). The solution obtained from the reservoir, and that run through the filter unit, were diluted (1-20) and their absorbance measured at 258 n.m. This determination was repeated, using 10ml of Sodium Chloride 0.1% w/v, Sodium Edetate 0.1% w/v, Sodium Phosphate buffer, and Sodium Acetate buffer, respectively.

The extent of adsorption onto the filter pads, was determined by filtering five 10ml aliquots through each of filter pads A and B. The absorbance values of the dilutions of the filtrates, were read at 258 n.m. against corresponding blank dilutions prepared from filtrates obtained by, filtering five 10ml aliquots of distilled water through filter pad C.

The effect of salts and buffers was determined by filtering five 30ml aliquots of Phenylethanol 0.5% v/v in combination with Sodium Chloride 0.1% w/v Sodium Edetate 0.1% w/v, Sodium Phosphate buffer and Sodium Acetate buffer, through each of filter pads D, E, F and G, respectively. The absorbances of the filtrate dilutions were read against corresponding blank dilutions, prepared from the filtrates obtained, by filtering five 30ml aliquots of Sodium Chloride 0.1% w/v, Sodium Edetate 0.1% w/v Sodium Phosphate buffer and Sodium Acetate buffer, through filter pads H, I, J and K, respectively.

RESULTS and DISCUSSION.

The metal holder of the filter unit, was found to have no effect on any of the systems with which it was in contact with. All dilutions prepared from the solutions in contact with the reservoir, and that run through the unit, were found to have similar absorbance values to those prepared from their originals.

The absorbance values of the dilutions prepared from the filtrates obtained by, filtering 10ml aliquots of Phenylethanol 0.5% v/v, through filter pads A and B, were found to be very close to, or slightly greater than 0.430 which was the absorbance value of a 1-20 dilution of the original unfiltered solutions. This could be accounted for by the presence of particulate matter, and the variation of the water-soluble extractive content of different filter pads.

In the presence of salts and buffers, no loss was observed from the solutions filtered through filter pads D, E, F, and G.

(d) Adsorption onto Sartorius Membrane Filters.

The determinations on the Sartorius membrane filters were done with the filter attached to the Sartorius plastic filter unit.

Water-soluble extractives.

10ml of distilled water was filtered through a membrane filter, and the absorbance of the filtrate measured directly at 258 n.m. This was

repeated using 10ml aliquots of Sodium Chloride 0.1% w/v, Sodium Edetate 0.1% w/v, Sodium Phosphate buffer, and Sodium Acetate buffer, which were filtered through four similar membrane filters respectively. The absorbance values of the filtrates were measured against blanks containing the corresponding salts and buffers in solution.

Effect of the Plastic Filter Unit on Phenylethanol.

To determine whether there was any interaction between the plastic filter unit and Phenylethanol, 10ml of Phenylethanol 0.5% v/v was passed through the unit, and allowed to remain in contact with the receiver for one minute, after which the spectral absorbance of a 1-20 dilution of the solution was measured.

The extent of adsorption onto the membrane filters, was determined by filtering five 10ml aliquots of Phenylethanol 0.5% v/v, through membrane filters A and B, which were attached to the plastic filter unit. The process was repeated using five 30ml aliquots of Phenylethanol 0.5% v/v, which were filtered through membrane filter C.

The effect of salts and buffers was determined by filtering five 10ml aliquots of solutions containing Phenylethanol 0.5% v/v in combination with, Sodium Chloride 0.1% w/v, Sodium Edetate 0.1% w/v, Sodium Phosphate buffer and Sodium Acetate buffer, through each of membrane filters D, E, F and G, respectively.

RESULTS and DISCUSSION.

Water-soluble extractives.

The absorbance value of the filtrates of distilled water, and solutions of Sodium Chloride, Sodium Edetate, Sodium Phosphate buffer, and Sodium Acetate buffer, were all found to be zero at 258 n.m.

Effect of the Plastic filter unit.

A 1-20 dilution of the solution obtained from the receiver of the plastic unit, was found to have an absorbance value of 0.430, indicating no interaction between the unit, and the Phenylethanol.

No loss was observed to occur from the solutions of Phenylethanol 0.5% v/v filtered through membrane filters A, B and C, and from the solutions in combination with the salts and buffers, which were filtered through membrane filters C, D, E, F and G.

The fact that Phenylethanol is not adsorbed onto any of the filter media, on filtration, may be explained by the following assumptions: Phenylethanol probably dissociates in aqueous solution to yield the carbonium ion, $^{86} \text{Ph} \cdot \text{CH}_2 \cdot \overset{\oplus}{\text{C}}\text{H}_2$. The carbonium ion supposedly involved, would not be markedly stabilised by the phenyl group on the β -carbon atom, for this is too far away to exert any marked inductive effect, and delocalisation via its 71 orbitals is prevented by the intervening saturated carbon atom. It has, therefore, been

suggested on this and other evidence that the carbonium ion involved is actually a bridged structure that can stabilise itself by delocalisation via the π orbitals of the aromatic nucleus. (Fig.9.2)

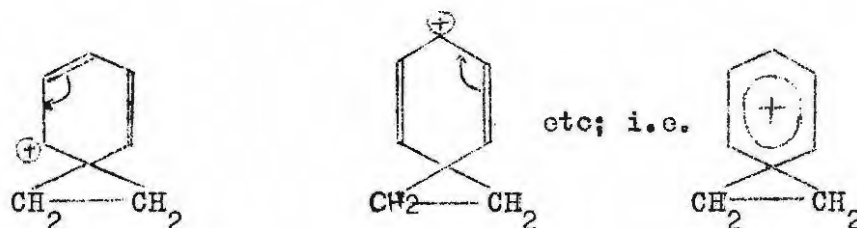


Fig. 9.2

It is probably this delocalisation of charge which occurs, that is responsible for the carbonium ion not becoming electrostatically adsorbed by the negatively charged filter surface.

The low molecular weight of Phenylethanol, as compared to the other preservatives, also accounts to a certain extent, for it being able to pass through the filters, without being withdrawn from solution.

SUMMARY

No loss of Phenylethanol from 10ml or 30ml aliquots of Phenylethanol 0.5% v/v as such, or in combination with salts or buffers, such as Sodium Chloride, Sodium Edetate, Sodium Phosphate buffer and Sodium Acetate buffer, was found to occur, on filtration through the various filter media such as, sintered glass filters of porosity No. 5,

Doulton-Pasteur unglazed porcelain candles, Asbestos filter pads, and Sartorius membranes, attached to the plastic filter unit.

PART III

CHAPTER TEN

CONCLUDING DISCUSSION

The loss of preservatives used in ophthalmic solutions, through interaction with various types of filter media, during the process of filtration sterilization has been determined. Table 10.1 indicates the loss occurring from 30ml of the preservative solution, on filtration through the various filter media.

The type of interaction which occurred between the preservatives in solution, and the filter media through which they are sterilized, seemed to be one that was associated with electrostatic and physical adsorption. In some cases, such as the filtration of solutions containing Phenylmercuric nitrate, or Benzalkonium chloride, through membrane filters, a certain degree of chemical interaction was assumed to have occurred, resulting in the loss of some of the preservative from the solution, on continuous filtration.

The extent of adsorption of the different preservatives, onto the various filter media, seemed to depend to a large degree on the nature of the preservative, with respect to its behaviour in solution, and its concentration; to the nature of the filter media with respect to its surface area, and the electrostatic charges prevalent at its surface at the time of filtration; to the pore

TABLE 10.1

	Loss from 30ml			
	Chlorhexidine Acetate 0.01% w/v	Phenylmercuric nitrate 0.002% w/v	Benzalkonium chloride 0.02% w/v	Phenylethanol 0.05% v/v
<u>Filter Medium</u>				
Sintered glass porosity No. 5	Ca 21%	7.2%	Ca 11%	nil
Pasteur unglazed porcelain candles	Ca 47% - 68%	100%	Ca 38%	nil
Asbestos Pads grade S.10	Ca 97%	Ca 9%	Ca 60%	nil
Sartorius Membranes	Ca 2%	Ca 13%	Ca 14%	nil
Solubility in distilled water	1 - 55	1 - 1500	very soluble	1 - 50
Molecular wt.	625.6	634.4	283 - 423	122.2

size of the filters used, and in most cases to the presence of various ionic substances in solution. The extent of adsorption however, seemed to depend to a lesser degree, on the solubility of the preservative being used, and its rate of filtration.

Although the experimental determinations were standardized as much as possible, variations in the extent of adsorption were obtained on a number of occasions, when using the same filter system and preservative solution. Depth filters with a large internal surface area are difficult to clean and although the filtrates obtained after several washings appeared to contain none of the previously filtered preservative, some of the more strongly adsorbed preservative is likely to remain behind, thus affecting the extent of adsorption of preservatives from the solutions to be filtered. Also, the porosities and hence the flow rates of certain filter media could vary with repetitive cleaning, thus introducing variables into the determinations.

The preparation of ophthalmic solutions on a bulk scale presents no real problem, as far as loss of certain preservatives from solution, during filtration sterilization, is concerned, since it is usual for that part of the filtrate which is obtained initially, to be discarded, thus allowing the filter to become saturated, so that no further loss occurs from solution.

However, this phenomenon of the interaction of the preservatives with the filter medium, poses a problem in a hospital or retail dispensary, where some eye drops may be prepared extemporaneously, or where one or other ingredients have to be incorporated into a sterile prepacked preparation, which then has to be reesterilized, if necessary, by filtration.

Aseptic incorporation of a small volume of the concentrated preservative solution, after bacterial filtration of the rest of the vehicle containing the active ingredients, would seem a way of preventing the loss through interaction with the filter system, but the method could prove tedious and risky, besides which, the preservative solution would have to be prefiltered to remove particulate matter.

Presaturation of the filter being used, with the respective preservative solution, could be carried out as a means of preventing further loss of the preservative through adsorption onto the filter medium. From Table 10.2, the volume of various preservative solutions required to saturate the different filter media may be seen. However, this cannot be totally relied upon as variations in the extent of adsorption and in certain characteristics of similar filter media, could occur.

TABLE 10.2
VOLUME OF PRESERVATIVE SOLUTION REQUIRED TO SATURATE VARIOUS
FILTER MEDIA

	Chlorhexidine Acetate 0.01% w/v	Phenylmercuric nitrate 0.002% w/v	Benzalkonium chloride 0.02% w/v
<u>Filter Medium</u> Sintered Glass porosity No. 5	30 - 50ml	Ca 60ml	Ca 120ml (constant loss obtained)
Unglazed porcelain candles	Ca 120ml	>150ml	Ca 200 - 250ml
Asbestos Pads grade S.10	>150ml	30ml	>300ml
Sartorius Membranes	20ml	>50ml (constant loss obtained)	constant loss obtained due to possible chemical interaction with the filter

Filtration of a small volume of the concentrated preservative solution through the filter being used, followed by washing through of the filter with the rest of the vehicle, in order to make the solution up to the finally required volume, could in a way overcome the loss caused by adsorption, but there are certain drawbacks in this respect; in some cases (filtration on Benzalkonium chloride solution through membrane filters²⁷) increased adsorption occurs at higher concentrations; some of the adsorbed preservative may not be easily desorbed from filter medium, i.e. the required concentration, would not be washed off by the vehicle; the presence of other ingredients in the vehicle, and their effects on desorption, would have to be considered.

Thus when the situation of the extemporaneous preparation of eye drops which have to be sterilized by filtration, arises, forethought should be given to the choice of the filter medium, and preservative to be used. (The choice of preservative also being governed by incompatibility effects.) The presence of various additives in solution, the method of cleaning of the filter and its efficiency, should not be overlooked as well.

In most cases, membrane filters would appear to be the most suitable as they are highly efficient, and do not impart particulate matter to the filtrates. They also tend to adsorb almost negligible quantities of preservative from solution, except where

incompatibilities and interaction with the filter and unit, exists.

Although not widely used, Phenylethanol it seems, would be the ideal preservative to be used in eye drops which are sterilized by filtration, as it was found not to become adsorbed onto any of the bacterial filters used in this investigation; besides, it is also effective against the more serious ocular pathogens, such as Pseudomonas aeruginosa.

The chief danger attached to the use of inadequately preserved ophthalmic solutions, is the risk of serious infections developing. The contamination of eye drops may also result from a failure of patient hygiene, rather than breakdown or loss of the preservatives. It may be mentioned that the recent innovation^{37,88} of packing sterile eye drops in single dose, disposable containers, seems to be an effective way of obviating the risks of infection and preservative loss. However, the potential risks and consequences of inadequate preservation during processing, should be considered, especially when sterilizing multidose ophthalmic solutions by filtration.

BIBLIOGRAPHY

- (1) Sykes, G. - "Disinfection and Sterilization" 157 Pitman (London) 1958.
- (2) British Pharmaceutical Codex - Pharmaceutical Press, London 1968.
- (3) Hugill, P.R. et al - Amer. J. Hosp. Pharm. 17 (1960) 535.
- (4) Crisafi, R.C. - J. Amer. Pharm. Assoc. NS4 (1964) 548-550.
- (5) Pfau, M. - Amer. J. Hosp. Pharm. 22 (1965) 454-457.
- (6) Gunn, G. and Carter, S.J. - Cooper and Gunn's Dispensing for Pharmaceutical Students, 11th Edition, Pitmans Medical, London 1965.
- (7) Hugo, W.B. - Pharm. J. 204 No. 5565 (1970) 714.
- (8) S.A. Pharm. J. 36 No. 12 (1970) 23.
- (9) Amihai, A. and Fisher, B. - Harokesch (Hebrew Pharmacist) 12 (1966) 8-9.
- (10) Smith, G. - Pharm. J. 198 (1967) 55.
- (11) Harris, M. - "Pharmaceutical Microbiology", Ballière Tindall and Cox, London 1964.
- (12) du Ban, G. - Boll. Chim. Farm. 103 (1964) 849-866.
- (13) Whittet, T.D., Hugo, W.B. and Wilkinson, G.R. - "Sterilization and Disinfection", Butler and Tanner, London 1965.
- (14) Berg, H.F., Guess, W.L. and Autian, J. - J. Pharm. Sci. 54 (1965) 79.
- (15) Levy, G. and Reuning, R.H. - J. Pharm. Sci. 53 (1964) 1471.
- (16) Levy, G. and Tatsuzaga, T. - *ibid.* 54 (1965) 1003.
- (17) Saad, H.Y. and Higuchi, W.I. - *ibid.* 54 (1965) 1205.
- (18) Goldberg, A.H., Gibaldi, M. and Kanig, J.L. - *ibid.* 55 (1966) 487.
- (19) Mansour, Z. and Guth, B.P. - *ibid.* 57 (1968) 404.

- (20) Mayersohn, M. and Gibaldi, M. - *ibid.* 55 (1966) 1323.
- (21) Lin, S.L., Menig, J. and Lachman, L. - *ibid.* 57 (1968) 2143.
- (22) Feldman, S. and Gibaldi, M. - *ibid.* 58 (1969) 425.
- (23) Anderson, R.A. and Morgan, K.J. - *J. Pharm. Pharmacol.* 18 (1965) 449.
- (24) Anderson, K. and Crompton, D. - *Lancet* vol. II No. 7523 (1967) 968.
- (25) Inscoc, May, N. - *Nature* 211 No. 5053 (1966) 1083-5.
- (26) Chiou, L. Win and Smith, D.L. - *J. Pharm. Sci.* 59 No. 6 (1970) 843.
- (27) Van Ooteghem, M. and Herbots, H. - *Pharm. Acta Helv.* No. 10 (1969) 602.
- (28) McCarthy, T.J. - *J. Mond. Pharm.* 4 (1969) 321.
- (29) McCarthy, T.J. - *S.A. Pharm. J.* 36 No. 10 (Supp.) (1970).
- (30) McBride, R.J., Murray, J.B. and Smith, G. - *Pharm. J.* 705 No. 5566 (1970) 26.
- (31) Mulvany, J.G. - *Soap Perf. Cosm.* XLIII No. 8 (1970) 489.
- (32) Nash, R.A., Haeger, B.B., Powers, J.T. and Personeus, G.R. - *Bull. Parent. Drug Assoc.* 21 No. 5 (1967) 165.
- (33) Haupt, H. - *Chem. Abstracts* 52 (1958) 9539h.
- (34) Stapowick, H.E. - *Chem. Abstracts* 59 (1963) 7316g.
- (35) Wendland, W. - *Chem. Abstracts* 67 (1967) 120153g.
- (36) Carlson-Ford - *Literature on "Filtration-Media"*.
- (37) Vogel, G.P. - *Chem. Abstracts* 61 (1964) 6871b.
- (38) Millipore Filter Corp. U.S.A. - *Millipore Catalogue MT-1* (1965).
- (39) Maier, K. - *Chem. Abst.* 54 (1957) 6219g.
- (40) Preusser, H.J. - *Chem. Abstracts* 67 (1967) 103111z.
- (41) Mirimanoff, A. - *Chem. Abstracts* 53 (1959) 17421d.

- (42) Smith, G. - J. Indian Pharm. Mufns. 6 (1967) 135-142.
- (43) Crompton, D.O. and Richards, M.E. - Lancet 1 (1964) 41-42.
- (44) Foster, J.H.S. - Mnfg. Chem. and Aerosol News 36 (1965) 43-46.
- (45) Montgomery, W.F. and Halsall, K.G. - Pharm. J. 138 (1964) 407.
- (46) Guven, K.C. - Chem. Abstracts 63 (1964) 439b.
- (47) Parker, M.S. - Soap Perf. Cosm. 39 (1966) 371-374.
- (48) Kohn, S.R. - Chem. Abstracts 60 (1963) 7875d.
- (49) Schmid, J. - Chem. Abstracts 52 (1957) 8462b.
- (50) Hugo, W.B. and Foster, J.H.S. - J. Pharm. Pharmacol. (Supp) 16 (1964) 124T-126T.
- (51) Richards, M.E. - Aust. J. Pharm. 48 (1967) 586-589.
- (52) Brown, M.R.W., Foster, J.H.S. and Norton - Pharm. J. 192 (1964) 8.
- (53) Anderson, K. and Crompton, D.O. - Pharm. J. 138 (1964) 593-594.
- (54) Anderson, K. and Clark, J. - Aust. J. Pharm. 48 (1967) 580-582.
- (55) Jechman, J. - Farm. Glasnik 20 (1964) 163-178 (Int. Ph. Abst. 494 (1965)).
- (56) Go, L.S. and Vrij, J. - Pharm. Weekblad 104 No. 26 (1969) 611.
- (57) Anderson, R.A. and Stock, B.H. - Aust. J. Pharm. 39 (1958) 1110-1112.
- (58) Chaikovs'ka, M.A. - Chem. Abstracts 67 (1967) 57258c.
- (59) Yousef, R.T. - Chem. Abstracts 65 (1966) 6092a.
- (60) Lawrence, C.A. - Chem. Abstracts 55 (1960) 4651h.
- (61) Humberstone, B. - Aust. J. Pharm. 42 (1961) 1030.
- (62) Mitchell, J.A. - Aust. J. Pharm. 42 (1961) 1284-5.

- (63) Jeffs, P.L. - Pharm. Digest 28 (1964) 242-244.
- (64) Polack, A.E. - Aust. J. Pharm. 48 (1967) 564.
- (65) Jaminet, F. - Chem. Abstracts 72 (1970) 59031a.
- (66) Adamski, R. - Chem. Abstracts 68 (1967) 16124h.
- (67) Smazynski, T. - Chem Abstracts 68 (1967) 43141t.
- (68) Martindale, W. - Extra Pharmacopoeia 25th edition Vol. 1 p. 772, The Pharm. Press (London) 1967.
- (69) Soehring, K. - Chem. Abstracts 53 (1959) 17432.
- (70) Dale, J.K. - Chem. Abstracts 53 (1959) 10661f.
- (71) Sykes, C.H. - M and B Pharm. Bulletin 7 No. 6 (1958) 62-64.
- (72) Glasstone, S. and Lewis, D. - Elements of Physical Chem. p. 566 Papermac 27 MacMillan (London) 1963.
- (73) Vogel, A.I. - Quant Inorg. Anal. 3rd Edition, Longmans (London) 1961.
- (74) Cooper, J.W. and Gunn, C. - Tutorial Pharmacy 5th Edition p. 182, Pitmans Medical 1964.
- (75) Christensen, K. and Dauv, E. - J. Mond. Pharm. 12 No. 1 (1969) 5.
- (76) Feigl, F. - Spot Tests in Inorg. Anal. 5th Edition p. 64, Elsevier 1958.
- (77) Petrosyan, V.S. and Reutov, O.A. - Chem. Abstracts 70 (1969) 7948w.
- (78) Belcher and Nutten - Quant. Inorg. Anal. 2nd Edition p. 289.
- (79) Fokkens, J. and Buurman, E. - Pharm. Weekblad 104 No. 9 (1969) 177-83.
- (80) Richards, M.E. - Aust. J. Pharm. 48 (1967) S96-S101.
- (81) Smith, R.L. - Sequestration of Metals 1st Edition p. 149 Chapman and Hall (London) 1959.
- (82) Mueller, F. - Chem. Abstracts 61 (1964) 5463d.
- (83) Minassian-Saraga, L.T. - Chem. Abstracts 54 (1960) 12725g.
- (84) Folman, M. and Yates, D.J.C. - Chem. Abstracts 52 (1958) 19403b.

- (85) Blackman, L.C.F. and Harrop, R. - Chem. Abstracts 68 (1968) 8103Op.
- (86) Sykes, F. - Mechanisms in Organic Chem. 4th Edition p. 79 Longmans, 1963.
- (87) Pharmaceutical Practitioner - Supp. to S.A. Pharm. J. 36 No. 12 (1970) iii.
- (88) Smith and Nephew - Medical Bulletin (Oct. 1970) 4.