then subjected to vacuum sublimation. The sublimate was crystallized from anhydrous ether until a constant melting point was obtained at 195° C. (uncorr.). The 3-hydroxyphthalic acid anhydride thus obtained was very pale yellow in color and gave a deep purple-red color with ferric chloride. It was soluble in water or alcohol.

3-Hydroxyphthalic Acid.—Hydrolysis of a sample of the 3-hydroxyphthalic acid anhydride yielded upon crystallization from anhydrous ether and petroleum ether very pale yellow crystals of 3-hydroxyphthalic acid that melted at 154° C. (uncorr.). This compound was soluble in water or alcohol and gave a deep purple-red color with ferric chloride.

The Influence of Carbohydrates and Salines upon the Separation of Chloral Alcoholate*

By Roland T. Lakey and Carl C. Pfeiffer

The first to record chloral alcoholate incompatibility was G. F. H. Markoe (1), who on June 4, 1885, read a paper entitled "On the Incompatibility of Chloral Hydrate in the Presence of Potassium Bromide and Alcohol" before the Massachusetts State Pharmaceutical Association. He stated that the following prescription was sent to him to determine whether the pharmacist had made a mistake in compounding, because it had subsequently separated into two layers:

℞

Potassium Bromide Chloral Hydrate, āā	ʒiij
Tr. Opii et Camp. Syr. Zingiber, āā	ʒiss

After experimenting with this prescription, he came to the conclusion that chloral alcoholate was formed and separated as a top layer. He also observed that potassium bromide, sodium bromide, sodium chloride and magnesium sulfate aided in causing the separation. From his studies he also claimed that calcium bromide, ammonium chloride, ammonium bromide and potassium nitrate did not produce any separation.

G. W. Hargreaves (2) published a study of this type of incompatibility in the June, 1932, issue of the JOURNAL of the AMERICAN PHARMACEUTICAL ASSOCIATION. By analysis, Hargreaves found the oily layer to consist of alcohol, chloral, chloral alcoholate and a small amount of saline. He also found that sucrose in the presence of potassium bromide would cause the separation of chloral alcoholate. These two pertinent papers seem to be the only ones in the English literature on this subject.

This study was prompted by the renewed popularity of chloral hydrate in prescriptions and also by the fact that it has not always been easy to demonstrate this incompatibility to our classes. The purpose, then, of this investigation was to determine the "critical concentration" (a term used by Hargreaves) of each of the ingredients necessary to cause the separation of chloral alcoholate in a chosen basic formula containing chloral hydrate, alcohol, potassium bromide and sucrose.

EXPERIMENTAL

The basic formula chosen for this study is the same as the Elixir of Chloral and Potassium Bromide, Compound, N. F. VI (3):

℞

Chloral Hydrate	12.5 Gm.
Potassium Bromide	12.5 Gm.
Sucrose	10.5 Gm.
Alcohol	...
Water, *q. s. ad.*	50 cc.

One drop of a 1% solution of the fat-soluble dye Sudan III was added to each 50 cc. of the prescription. This serves to delineate any separated chloral alcoholate, since the red-colored dye concentrates in this layer. Kodachrome pictures were taken at the critical point in each experiment. The surface tensions of some of the separated and non-separated solutions were determined by the Du Noüy apparatus to ascertain whether marked changes in surface tension were involved in the separation of chloral alcoholate. All experiments were conducted at room temperature and no heat was used to effect solution of any of the solutes. All of the critical experiments were checked by repetitition. The results of alterations made in the basic formula are given in tabular form.

From Table I it will be observed that separation into two layers occurred with 1% less alcohol when the chloral hydrate was first dissolved in alcohol. This

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Presented to the Scientific Section of the A. PH. A., Detroit meeting, 1941.

is shown in the table by comparing experiment 3 and 4 with experiments 8 and 9.

TABLE I.—ALCOHOL SERIES

Expt. No.	Alcohol, Cc.	Results
PART 1. ALCOHOL ADDED LAST		
1	15	Separates
2	10	Separates
3	6	No separation
4	5	No separation
PART 2. CHLORAL FIRST DISSOLVED IN ALCOHOL		
5	15	Separates
6	7.5	Separates
7	6	Separates
8	5.5	Separates
9	5	No separation

For the rest of the experiments, the formula was made up of 5 cc. of alcohol, and the other ingredients were systematically varied.

TABLE II.—SUCROSE SERIES (CHLORAL HYDRATE FIRST DISSOLVED IN 5 CC. OF ALCOHOL

Expt. No.	Sucrose, Gm.	Results
1	21	Separates
2	15.75	Separates
3	13.13	Separates
4	12.5	Separates
5	11	Separates
6	10.75	Slight separation
7	10.5	No separation

The data of Table II reveal that it requires only a very slight change of sucrose concentration in the base formula to cause separation to take place—1%, *w/v*. This is shown by experiments 5 and 6.

TABLE III.—POTASSIUM BROMIDE SERIES

Expt. No.	Potassium Bromide, Gm.	Results
1	15.75	Separates
2	13.75	Separates
3	13.00	Separates
4 (Check)	13.00	Separates
5	12.6 ($1^1/_{125} \times B\frac{1}{2}$)	Separates

The data of Table III indicate that separations occur even when there is as little as 0.2% increase in potassium bromide over the base formula.

TABLE IV.—CHLORAL HYDRATE SERIES

Expt. No.	Chloral Hydrate, Gm.	Results
1	13	Separates first, then dissolves
2	12.6	Separates first, then dissolves
3	14	Separates first, then dissolves until only a film remains; finally dissolves
4	14	Separates, layer top and bottom; finally rises to top

By changing the concentration of chloral hydrate (Table IV), we find that it requires an addition of only 0.2% of chloral hydrate beyond that of the base formula to produce separation. This is shown by a comparison of experiment 4 with the base formula.

TABLE V

Expt. No.	Potassium Bromide, %, *w/v*	Sucrose, %, *w/v*	Results
1	23	...	Separates
2	22.5	...	Separates
3	...	...	No separation
4	...	56.26	No separation

The data of Table V show that the potassium bromide causes a separation in amounts less than that of the base formula when there is no sucrose present. In this case the chloral alcoholate sinks to the bottom of the bottle. Experiment 3, with no sucrose and no potassium bromide, shows no separation. Experiment 4 shows that even an increase of 56.2%, *w/v*, of sucrose with no potassium bromide present fails to cause a separation. No more sucrose could be dissolved even with several hours of constant agitation and long standing.

Surface tension determinations by the Du Noüy apparatus at 25° C. gave the following: 64.8 dynes/sq. cm. for distilled water, and 44.9 dynes/sq. cm. for the unseparated formula. When separation was effected by the addition of 1% more of alcohol (increased from 11% to 12%), the surface tensions of the layers were, respectively, as follows: chloral alcoholate, 45.3 dynes/sq. cm.; and the water layer, 47.2 dynes/sq. cm.

DISCUSSION

Because of the inability to present the data graphically, it is difficult to visualize the separation of this five- or six-phase system composed of sugar, KBr, water, alcohol, chloral hydrate and chloral alcoholate. That surface tension and interfacial tensions play an important role in the separation of chloral alcoholate cannot be doubted, but here again it is extremely difficult to obtain valid data because altering one constituent such as alcohol is apt to alter the surface tension. Once the separation has occurred, however, a definite change in the surface tension of the two layers has occurred.

The specific gravity of the vehicle also has a role, in that when no sugar is present the chloral alcoholate sinks to the bottom, but when the specific gravity is raised by the addition of sugar the chloral alcoholate then floats to the top. This represents the only danger of this incompatibility, in that the patient might pour the top layer into a glass and obtain all of the chloral at one dose. The work of Adams (7) showing that chloral alcoholate is no more toxic than chloral hydrate has been confirmed in this laboratory by intraperitoneal toxicity studies on the rat.

SUMMARY

Chloral alcoholate will separate from the N. F. prescription when alcohol is added in excess of 10%. In an alcoholic prescription, increasing the sugar beyond 21%, or the KBr beyond 25%, or the chloral hydrate beyond 29% will result in separation. In the absence of sugar the alcoholate will separate when 23% of KBr is dissolved. In the absence of both sugar and KBr, no separation occurs. In the absence of KBr, sugar alone, even if added to the point of saturation, will not cause separation of the alcoholate. The addition of an alcohol-soluble dye to the prescription promptly delineates any separation.

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A Fast Method of Dry, Low-Heat Sterilization*

By P. Goedrich and W. Schmidl

Sterilization, as we all know, is destruction of every form of life, harmful or innocuous. Bacteriologists now agree that any distinction between disease-producing and other microörganisms is vague, and that apparently there is no organism which might not cause disease (1). Even the ordinarily harmless *Bacillus subtilis* (hay bacillus) has been known to cause serious infection in the human eye (2) and occasionally to invade the blood stream (3). It is therefore essential in sterilization to destroy not only all vegetative forms of bacilli, but also all spores. Unfortunately, there are wide discrepancies in the results reported concerning the thermal resistance of spores. But all authorities agree that no period of exposure to boiling water has been found to be completely adequate. In the opinion of leading bacteriologists, boiling water never constitutes adequate sterilization for surgical instruments (4).

Robert Koch (5), who established that all vegetative forms of bacteria were killed by a temperature just over 100° C. in 1½ hrs., overestimated the value of boiling water against spores. Falcioni (6) found spores of tetanus to resist destruction for 2½ hrs. in live steam. Theobald Smith (7) showed that tetanus spores occasionally were able to survive 70 min. of steaming. Von Hibler (8) studied some strains of *Clostridium tetani* that required 3 hrs. of boiling to destroy them. Bigelow and Esty (9) found some microörganisms remaining virulent for 22 hrs. at 100° C., while at 110° C. nearly 4 hrs. was necessary to destroy them. Becker (10) reported the death point of two strains of tetanus as 2 hrs. and 3 hrs., respectively, in boiling water. Murray and Headler (11) found strains of *Clostr. welchii* to be resistant to boiling for 90 min. Esty and Meyer (12) found the heat resistance of tetanus spores to vary at 100° C. from 15 to 90 min., the average survival time being 25 min. Dried spores of *B. anthracis* were found to withstand boiling temperature for hours (13).

In general it is recognized that a temperature of 150° C. continued for at least an hour will destroy any bacterial spores. Nevertheless, glassware in the bacteriological laboratory is usually sterilized at 160° C. for 2 to 3 hrs. (14). By using steam

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Presented to the Scientific Section of the A. PH. A., Detroit meeting, 1941.