1966

Stability of Penicillin G in Oleaginous Formulations Containing Colloidal Silica

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STABILITY OF PENICILLIN G IN OLEAGINOUS FORMULATIONS CONTAINING COLLOIDAL SILICA

BY

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

PHARMACY

UNIVERSITY OF RHODE ISLAND

1966

Title Abstract

PENICILLIN G STABILITY WITH SILICA

MASTER OF SCIENCE THESIS

OF

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UNIVERSITY OF RHODE ISLAND

1966

ABSTRACT

Stability of procaine and potassium salts of penicillin G in oleaginous materials containing colloidal silica was studied. The instability of penicillin G in these bases seemed to be due primarily to the surface acidity of the silica in conjunction with the ester constituents of oils. The stability of the peanut oil/silica/penicillin G preparations could be improved by reducing the surface acidity of the silica by esterification. Dehydration of the silica also gave formulations with improved stability, but with unacceptable rheological properties. The color reactions of procaine penicillin G/silica/oleaginous materials appeared to be unrelated to the stability of the drug and might be due to interactions of procaine with traces of aldehydes present in the oily materials.

ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks and appreciation to Dr. James C. Price, under whose supervision and direction this work was done, for his valuable advice, guidance and counsel; to Dr. Robert J. Gerraughty, for his wise counsel and support at every stage of the work; to Dr. George E. Osborne and Dr. Leonard R. Worthen, for their helpful suggestions and advice.

The author also gratefully acknowledges the support of the Masti-Kure Product Company, and wishes to express his special thanks to Mr. Russell E. Rhodes, for performing the microbiological assays for this project.

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I. INTRODUCTION

The discovery of penicillin (1), the first of the antibiotics, is considered to be one of the great landmarks in the history of pharmacy and medicine. Probably no other drug has received more attention nor has been studied more intensively. The original widespread use of penicillin in medicine can be attributed to its high degree of antibacterial activity and to its low toxicity. Unfortunately, the inability of penicillin to counteract staphylococcal infections caused by penicillin resistant strains, coupled with certain other disadvantages in preparation and use of the drug, resulted in a decreased use of penicillin during the years, 1948-1958 (2). Further progress in the penicillin field was minimal until 1958, when Doyle et al. (3) isolated from fermentation medium pure 6-Amino-penicillanic-Acid, the so-called "nucleus" of the penicillin molecule. The discovery of this nucleus gave renewed impetus to penicillin research and caused a rapid development in its semi-synthesis. All these achievements, including production of new penicillins, served to restore the initial popularity of the drug. Unfortunately, penicillin in many pharmaceutical preparations is not stable, and this problem still plagues the practicing pharmacist and the manufacturer alike.

The stability of penicillin has been extensively studied by many workers in the field of pharmacy and allied

areas. As early as 1947 Brodersen (4) studied the degradation of penicillin in aqueous medium at pH ranges from 1 to 13 and found the optimum pH for stability of the drug to be 6.5. In 1949, Woodward (5) explained that inactivation of penicillin in neutral or alkaline mediums is a result of the hydrolysis of the β-lactam ring of the penicillin molecule to penicilloic acid. Later, in 1956 Krejci (6) determined rate constants for both the overall rate of loss of penicillin and the rate of formation of penicillenic acid (a degradation product) using polarographic techniques. In 1960, Levine (7) obtained some evidence from ultraviolet absorption data that decomposition of penicillin in neutral medium proceeds through the formation of penicillenic acid. Recently, Schwartz (8) has suggested possible degradation routes in acidic aqueous medium based on the analysis of kinetic data. His proposed routes appear to be more feasible than those of other investigators, namely, Woodward, Krejci and Levine, who have suggested several degradation routes in an acidic medium.

Brunner, Swintosky, et al. (9,10) have studied the stability of amine salts of penicillin in aqueous mediums. The rate of loss of penicillin from a suspension in which the two phases were kept in equilibrium at constant temperature and pH, was found to follow a pseudo zero order rate. The addition of procaine hydrochloride to the system increased the stability of sparingly soluble procaine

penicillin G in aqueous suspension. This increase in stability resulted through the common ion effect, which was responsible for the decrease in solubility of procaine penicillin.

Stability studies of penicillin in oleaginous materials have also been reported by many investigators. In 1948, Gulter (11) published a paper on the stability of various penicillin ointments. He reported that crystalline penicillins had greater stability than amorphous ones and that the presence of moisture or of additives such as polyethylene glycol, zinc stearate, or dried aluminum hydroxide gel hastened penicillin decomposition. In contrast, benzocaine and adrenalin had no effect.

During the same year Gradnich (12) reported that an oily suspension of calcium penicillin was stable at room temperature for at least one year. In this preparation, peanut oil and white wax were heated separately, filtered through sterile gauze, and sterilized at 120°C for 20 minutes. Ninety-six parts of the oil were then mixed with four parts of the wax to form a base into which calcium penicillin was incorporated.

In 1949, Floyd (13) studied one of the most satisfactory of the oleaginous preparations of penicillin, a product still in use and now official in the United States Pharmacopeia XVII. This product consists of procaine penicillin in an aluminum monostearate-peanut oil gel; it

provides a highly stable drug in addition to a slow rate of release. The slow release mechanism is said to be probably due to the formation of a protective film of procaine stearate at the penicillin surface.

Penicillin-polyethylene glycol ointments have been studied by Culter, Sherwood and Coates, et al. (14,15). Although these workers have suggested some possible explanations for the inactivation of penicillin in these bases (such as peroxide content of polyethylene glycol) the real cause of decomposition is not well understood.

In 1953, Buckwalter (16) reported a series of stability studies on various penicillin products. He found that procaine penicillin ointment made with a base consisting of peanut oil, beeswax and petrolatum was stable for four years at room temperature.

The stability of penicillin in different ointment bases has been reported by Corubolo and Tralle-Lassen (17, 18). These studies indicate that stability depends chiefly on the water content of the bases. In anhydrous bases, penicillin was relatively stable, whereas, in emulsion bases the opposite was true. Furthermore, addition of water-absorbing compounds such as dried potato starch, anhydrous lactose, and an anhydrous buffering mixture (pH 6.5), have been shown to increase the stability of penicillin in bases containing not more than 0.1% water.

Stanciu, et al. (19) found that an ointment base consisting of bentonite, glycerol, lanolin and water had thixotropic characteristics and, when combined with penicillin, afforded a high degree of absorption of the drug into the body. However, because penicillin is unstable in this base, the drug could not be incorporated into the base until just before actual use.

It was obvious from these studies that neither a solution nor a suspension of penicillin in aqueous mediums could be made stable for more than a few weeks, even if stored at refrigerator temperatures. On the other hand, penicillin has been found to be relatively stable in a limited number of oleaginous preparations. These preparations, including certain ointments, have found widespread clinical use but may not, in all cases, have ideal rheological characteristics and may have other undesirable features.

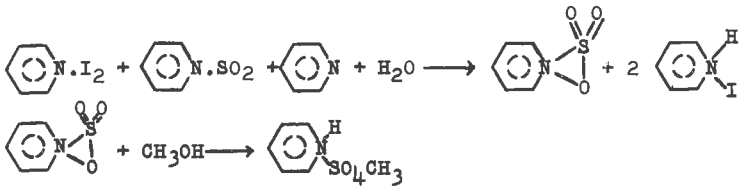
When it is combined with liquid material in sufficient concentration, colloidal silica has the property of causing gel formation (20). Because of this property, and because it is relatively inert physiologically, colloidal silica has been suggested as a material for use in the formulations of superior ointment-like preparations. Such formulations would be composed of colloidal silica and inert oleaginous substances and might prove to be of value in the development of acceptable ointment preparations of penicillin.

Thus, the purpose of this study was to evaluate the stability of penicillin G in oleaginous preparations containing colloidal silica and to elucidate, if possible, some of the mechanisms of penicillin degradation in this system.

II. EXPERIMENTAL

Characterization of Formulation Materials

Because of the reported importance of moisture on the degradation of penicillin, all formulation materials were analyzed for moisture content by the Karl Fischer method (21) using a Beckman Model KF-2 Aquameter.[1] The principle involved in this method is based on a quantitative reaction between the Karl Fischer reagent (a mixture of pyridine-sulfur dioxide solution and iodine-methanol solution) and water to give a sharp electrometric end point which can be detected with the aquameter. The following equations indicate the reactions that probably take place:



A direct titration was performed for all formulation materials, using Karl Fischer reagent, which was standardized with sodium tartrate dihydrate. About 6 Gm. of each oily material and about 0.250 Gm. of each formulation powder were

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[1]Beckman Instruments, Inc., Fullerton, California.

used for the determinations. The process was repeated twice for each of the formulation materials. A summary of the results is given in Table I.

In addition, the oleaginous materials were analyzed twice each for acid value, iodine value, and saponification value, according to methods outlined in the United States Pharmacopeia. The refractive indexes of oily materials also were determined by means of a Bausch & Lomb Model 3-L Abbe Refractometer.[2] The refractive index determinations were performed at 25°C ± 0.5. Summary of all these data is given in Table II.

Preparation of Unmodified Cab-O-Sil[3] Formulations

The ointment base of colloidal Cab-O-Sil in oleaginous material was made by mixing 2.5 Gms of Cab-O-Sil in 100 Gms of oily material. The mixture was slowly heated to 120°C with the aid of a hot-plate, and then was allowed to cool to room temperature.

Formulation then proceeded by incorporating 100,000 units of penicillin G into every 12 Gms of the above base in a mortar and pestle and the entire mass was passed several times through a hand homogenizer until a uniform, homogeneous, product was obtained. One hundred grams of each of these preparations was then poured into three different one ounce ointment jars, so that stability studies could be performed

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[2]Bausch & Lomb Optical Co., Rochester, New York.

[3]Cabot Corporation, Boston, Massachusetts.

at room temperature and under accelerated conditions.

Preparation of Dehydrated Cab-O-Sil Formulations

One objective of this project was to determine the cause of degradation of penicillin G in an oleaginous-colloidal silica system. Hence, to evaluate the combined effect of physisorbed and chemisorbed water, Cab-O-Sil was dehydrated by a method reported by De Boer and his co-workers (22), who studied the effect of temperature on surface hydroxyl or silanol groups. They found that physisorbed water could be removed from the surface by heating in air at 120°C or by evacuation at room temperature, and that the remaining chemisorbed water (hydroxyl groups) could be progressively removed by heating in air to higher and higher temperatures. The chemisorbed water finally being completely removed at about 900°C.

Based on these studies, the dehydration of Cab-O-Sil was carried out by heating it in a muffle furnace[4] at 1000°C for 24 hours.

The reaction that occurs during dehydration is generally accepted to be the elimination of water from pairs of hydroxyls attached to the surface of the adjacent silicon atoms, resulting in the formation of strained siloxane bridge systems as shown in reaction below:

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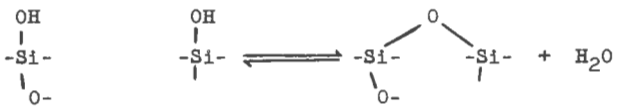
[4]Thermolyne Corporation, Dubuque, Iowa.



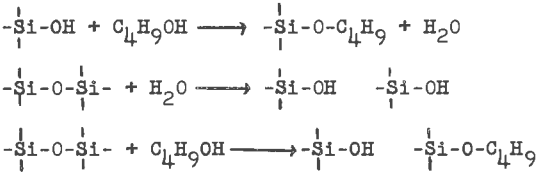
The procedure used to incorporate the dehydrated Cab-O-Sil was the same as that used with the unmodified Cab-O-Sil.

Preparation of Esterified Cab-O-Sil Formulations

An alternative method for the evaluation of the effect of surface hydroxyl groups on penicillin stability was the modification of the groups by esterification of the silica. This reaction was extensively studied by Iler (23), whose methods consisted of: (1) suspending the silica in an alcohol and removing the free water from the system by azeotropic distillation and then heating the anhydrous mixture obtained, either in a stainless steel autoclave or in sealed glass tubes; or (2) by first partially dehydrating the silica at 450-500°C in air, and then refluxing it with the alcohol.

The latter method was chosen in this work, since the reaction is known to take place at a much faster rate. The Cab-O-Sil was heated at 500°C for 10 hours in a muffle furnace and then was placed in an excess of n-butanol and refluxed for 15 hours with a Dean Stark Distilling Receiver attached for trapping the water. The esterified silica was

recovered by filtration, washed with acetone, and then dried under vacuum. The reactions occurring during this process may be shown as follows:



The esterified silica so formed is said to be fairly stable to hydrolysis, since the bulky alkyl groups provide considerable steric protection to the surface ester linkage; in addition, its apolar surface is water repellant and has an organophilic character. Formulations of penicillin G were prepared with the esterified Cab-O-Sil by the same method as mentioned for unmodified Cab-O-Sil.

Rheology Studies of Cab-O-Sil and Modified Cab-O-Sil Formulations

Suspensions of both esterified and non-esterified Cab-O-Sil were made by incorporating 4 per cent of each into peanut oil. Comparative studies of the flow characteristics of the two systems were made, using a Stormer Viscometer,[5] wherein different weights were applied to the instrument and the rate of rotation of the bob determined for each weight. The cup and bob used for the determinations were

[5]Arthur H. Thomas Co., Philadelphia 5, Pennsylvania.

those supplied with the instrument. The results of these studies are reported in Table III, and a plot of the rate of rotation in revolutions per minute against weight applied to the instrument is shown in Figure 1.

In order to determine the viscosity change of dehydrated and esterified Cab-O-Sil preparations and to compare these viscosities with those formulations containing unmodified Cab-O-Sil, suspensions of different concentrations of modified and unmodified Cab-O-Sil in peanut oil were prepared. The viscosities of these formulations were measured by means of a Brookfield Viscometer, Model RVF,[6] using spindle number 2 and a speed of 20 revolutions per minute, except, in the case of unmodified Cab-O-Sil preparations where the spindle had to be changed (to spindle number 3) when the concentration of the Cab-O-Sil in suspension exceeded 4 per cent. A summary of these results is given in Table IV, and a plot of the logarithm of the viscosities against corresponding concentrations is shown in Figure 2.

Color Reactions

An interesting yellow color reaction was observed when procaine penicillin G was being mixed with peanut oil/Cab-O-Sil base. The intensity of this yellow color increased and

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[6]Brookfield Engineering Laboratories, Inc., Stoughton, Massachusetts.

turned orange on storage of this preparation at an elevated temperature. Since the procaine salt of penicillin G in these formulations showed less stability than the potassium salt, and since no such color reaction appeared for the potassium salt, the question arose as to whether this color reaction had any relation with the instability of procaine penicillin formulations. It might be mentioned here that none of the potassium salt preparations gave any color reaction, but the same color became apparent when a formulation of procaine penicillin in 1-octadecene/Cab-O-Sil was made.

In an attempt to determine the cause of color reaction in the peanut oil/procaine penicillin/Cab-O-Sil formulation, the Schiff's test was applied to all of the oleaginous materials. The results of these tests are reported in Chapter III.

III. RESULTS

Stability Studies of Reference Formulations

Formulations of potassium penicillin G and procaine penicillin G in peanut oil plus aluminum monostearate, in mineral oil plus Cab-O-Sil, and in peanut oil plus Cab-O-Sil, were prepared according to the method mentioned in the experimental chapter and submitted for accelerated stability tests (45°C for three weeks and at 56°C for one week).[1] The results of these tests are shown in Table V.

It can be seen from Table V, that the Cab-O-Sil had a marked influence on the stability of procaine penicillin G bases. Preparations with peanut oil/Cab-O-Sil/procaine penicillin G and with mineral oil/Cab-O-Sil/procaine penicillin G, lost almost half their original potency after 3 weeks at 45°C, as compared to the aluminum monostearate/peanut oil/procaine penicillin G base. However, the effect of Cab-O-Sil on the stability of potassium penicillin G with peanut oil base was considerably less in comparison with the procaine salt. The stability of potassium penicillin/Cab-O-Sil/mineral oil base was good as compared with the peanut oil base. In all cases, the stability of penicillin in mineral oil bases was much better than in peanut oil bases.

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[1]Microbiological assays of penicillin were performed by Masti-Kure Products Co.

TABLE I

MOISTURE CONTENT OF FORMULATION MATERIALS

Formulation Material	Moisture %
Mineral Oil	0.0020
Peanut Oil	0.0045
1-Octadecene	0.0043
Butyl Stearate	0.0377
Isopropyl Myristate	0.0455
Cab-O-Sil at 25°C	2.170
Dehydrated Cab-O-Sil	0.175
Esterified Cab-O-Sil	0.676
Aluminum Monostearate	1.224
Procaine-Penicillin G	3.042
Potassium-Penicillin G	0.261

TABLE II

CHARACTERISTICS OF OLEAGINOUS MATERIALS

Formulation Material	Acid Value	Iodine Value	Saponification Value	Refractive Index @ 25°C
Peanut Oil	0.146	93.4	188.1	1.4695
Mineral Oil	0.000	0.0	--	1.4770
1-Octadecene	0.052	96.9	--	1.4429
Butyl Stearate	0.456	--	185.6	1.4413
Isopropyl Myristate	0.107	--	211.3	1.4330

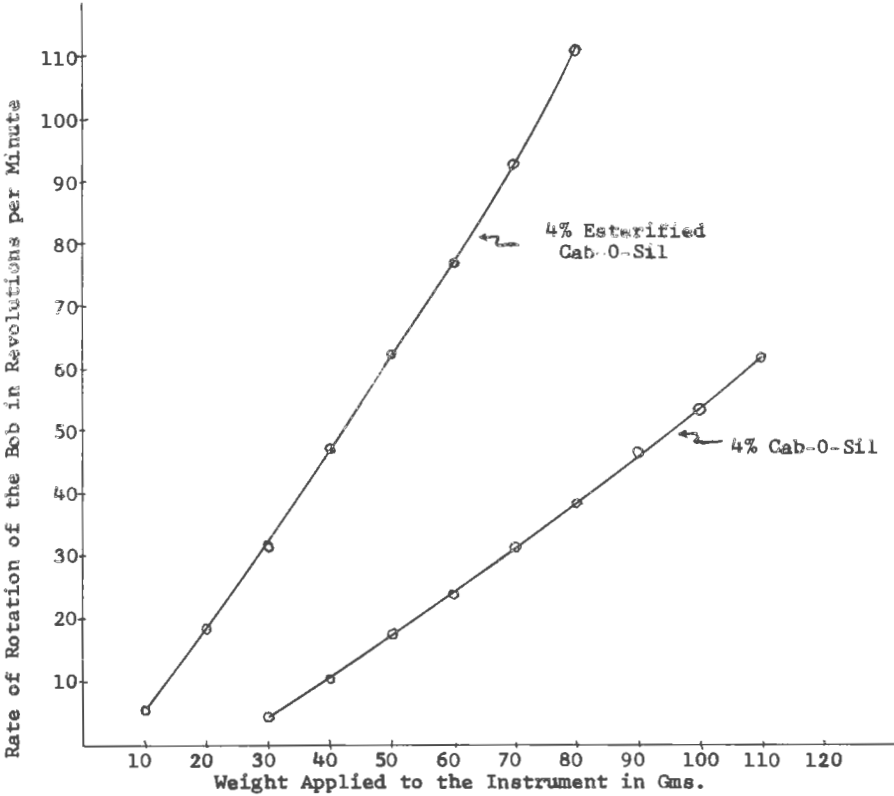


Figure 1. Effect of Cab-O-Sil and Esterified Cab-O-Sil on Flow Characteristics of the Peanut Oil.

TABLE III

EFFECT OF CAB-O-SIL AND ESTERIFIED CAB-O-SIL ON FLOW PROPERTIES OF THE PEANUT OIL

Weight Applied to the Instrument in Gms.	RPM* of 4% Cab-O-Sil in Peanut Oil	RPM of 4% Esterified Cab-O-Sil in Peanut Oil
10	0.0	5.6
20	0.0	18.5
30	4.0	31.8
40	10.2	47.3
50	17.4	62.6
60	23.9	77.0
70	31.1	92.5
80	38.2	111.0
90	46.2	128.5
100	53.5	147.0
110	61.5	161.0

* Rate of rotation of the bob in revolutions per minute.

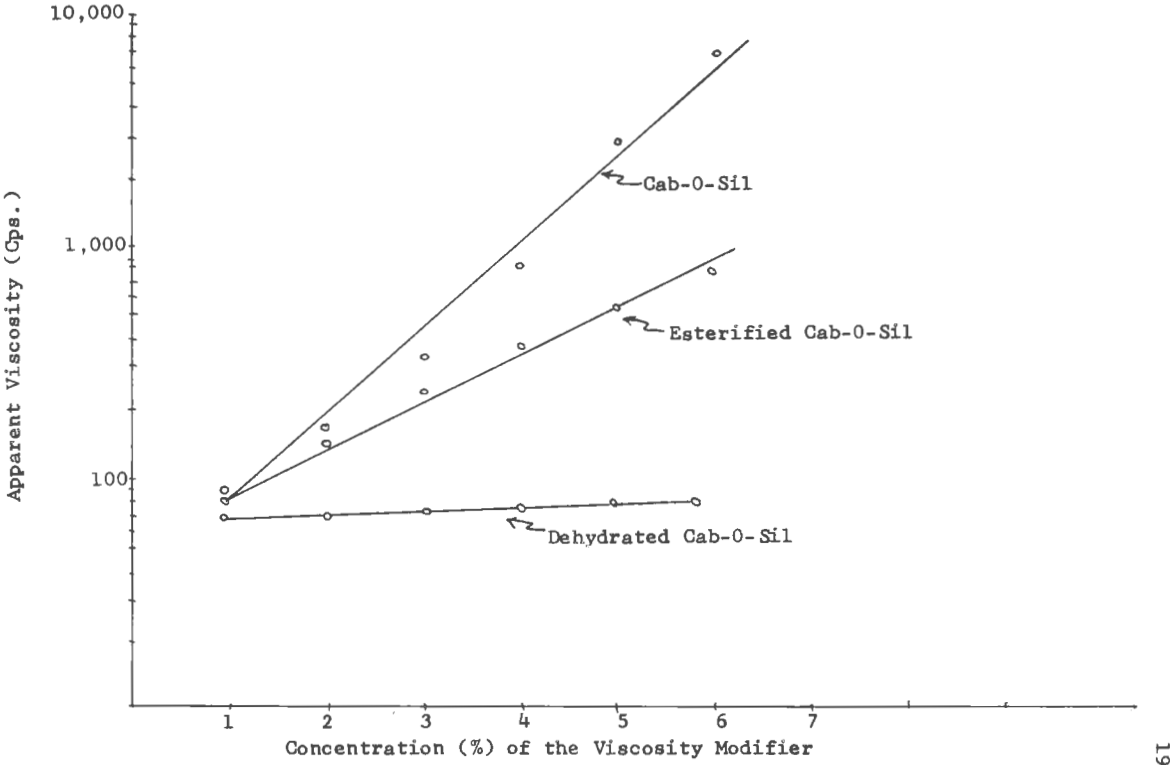


Figure 2.

Effect of Cab-O-Sil and Modified Cab-O-Sil on Viscosity of the Peanut Oil

TABLE IV

EFFECT OF CAB-O-SIL AND MODIFIED CAB-O-SIL ON VISCOSITY OF THE PEANUT OIL

Viscosity Modifier	Concentration 0%	1%	2%	3%	4%	5%	6%
Cab-O-Sil	63	84	166	330	840	2850	6700
Esterified Cab-O-Sil	63	80	144	242	372	560	790
Dehydrated Cab-O-Sil	63	69	70	70	73	76	78

All viscosities were determined at room temperature and are given in centipoise units.

TABLE V

STABILITY STUDIES OF REFERENCE FORMULATIONS

Base*	Penicillin Salt	Original Penicillin Concentration Units x 10^{-3}/12 Gm.	Penicillin Concentration After 3 Weeks at 45°C Units x 10^{-3}/12 Gm.	Penicillin Concentration After 1 Week at 56°C Units x 10^{-3}/12 Gm.
A#	Potassium	43	43	44
B	Potassium	103	80	69
C	Potassium	133	115	77
A	Procaine	109	109	87
B	Procaine	106	42	20
C	Procaine	107	57	51

* Base A contained peanut oil plus 2% Aluminum Monostearate.
Base B contained peanut oil plus 2.5% Cab-O-Sil.
Base C contained mineral oil plus 2.5% Cab-O-Sil.

\# Stability results with this base are in question.

Effect of Modification of Cab-O-Sil on the Stability of Penicillin G

In order to determine the effect of physisorbed and chemisorbed water, Cab-O-Sil was dehydrated according to the method outlined in the experimental section, and formulations of potassium and procaine penicillin G in peanut oil containing dehydrated Cab-O-Sil were prepared. The results of these accelerated stability tests (Table VI) indicate that the formulations containing dehydrated Cab-O-Sil and procaine penicillin were comparable in stability to those containing aluminum monostearate as the viscosity modifier (see Table V). Unfortunately, the flow properties of the dehydrated Cab-O-Sil formulations were about the same as those of peanut oil with no viscosity modifier present (see Table III). However, a comparison of the potassium penicillin G/dehydrated Cab-O-Sil formulation with the aluminum monostearate base cannot be made because of the questionable results with the latter base.

The increased stability of the penicillin G in formulations containing dehydrated Cab-O-Sil is thought to be due to the reduction of surface acidity of the Cab-O-Sil as well as to the removal of available water.

In order to test the possibility that the surface acidity of the Cab-O-Sil to some degree accelerated the degradation of the penicillin, a method for reducing the acidity other than by dehydration was sought. The method

TABLE VI

EFFECT OF MODIFICATION OF CAB-O-SIL ON PENICILLIN G STABILITY

Formulation	Original Penicillin Concentration Units x 10^{-3}/ 12 Gm.	Penicillin Concentration After 3 Weeks at 45°C Units x 10^{-3}/12 Gm.	Penicillin Concentration After 1 Week at 56°C Units x 10^{-3}/12 Gm.
Procaine Penicillin in Peanut Oil with Unmodified Cab-O-Sil	106	42	20
Potassium Penicillin in Peanut Oil with Unmodified Cab-O-Sil	103	80	69
Procaine Penicillin in Peanut Oil with Dehydrated Cab-O-Sil	110	95	69
Potassium Penicillin in Peanut Oil with Dehydrated Cab-O-Sil	114	86	88
Procaine Penicillin in Peanut Oil with Esterified Cab-O-Sil	101	68	47
Potassium Penicillin in Peanut Oil with Esterified Cab-O-Sil	104	95	112

chosen consisted in esterification of the Cab-O-Sil by the method reported in Chapter II.

When stability studies with formulations containing this esterified Cab-O-Sil were carried out, there was a significant increase in stability over those containing hydrated or unaltered Cab-O-Sil. Table VI shows that potassium penicillin G stability was excellent, and that procaine penicillin G stability was considerably improved over unmodified Cab-O-Sil procaine penicillin preparations, but was not as good as that of potassium penicillin.

The results obtained with the dehydrated and esterified Cab-O-Sil preparations would almost certainly indicate that the acidic surface hydroxyl groups on the Cab-O-Sil exert a considerable effect on the degradation of penicillin in these systems. The reason for the greater stability of the penicillin G in the mineral oil/Cab-O-Sil formulation was not clear from the above results.

Effect of Pure Compounds Similar to Peanut Oil Constituents on Penicillin G Stability

In an effort to determine what components or groups in peanut oil are responsible for the decreased penicillin G stability (as compared to the mineral oil formulations), preparations of potassium and procaine penicillin G in 1-octadecene, butyl stearate, and isopropyl myristate, with Cab-O-Sil were prepared. The results of accelerated stability studies of these formulations are summarized in Table VII.

TABLE VII

EFFECT OF PURE MATERIALS SIMILAR TO PEANUT OIL CONSTITUENTS ON PENICILLIN G STABILITY

Formulation	Original Penicillin Concentration Units $\times 10^{-3}$/12 Gm.	Penicillin Concentration After 3 Weeks at 45°C Units $\times 10^{-3}$/12 Gm.	Penicillin Concentration After 1 Week at 56°C Units $\times 10^{-3}$/12 Gm.
Potassium Penicillin in Isopropyl Myristate c̄ Cab-O-Sil	99	58	20
Potassium Penicillin in Butyl Stearate c̄ Cab-O-Sil	105	49	20
Potassium Penicillin in 1-Octadecene c̄ Cab-O-Sil	108	117	86
Procaine Penicillin in Isopropyl Myristate c̄ Cab-O-Sil	102	17	20
Procaine Penicillin in Butyl Stearate c̄ Cab-O-Sil	72	22	20
Procaine Penicillin in 1-Octadecene c̄ Cab-O-Sil	140	114	111

It is evident from these data that the least stable penicillin G preparations were made from the pure esters, and that the stability of the penicillin in 1-octadecene was comparable to that obtained with mineral oil preparations (Table V).

Shelf Life Stability Studies of Penicillin G

For confirmation of accelerated stability studies, the data presently available on the shelf life of potassium and procaine penicillin G are given in Tables VIII and IX. It can be seen from these tables that the shelf life stability results are in fairly close agreement with those of accelerated ones. The more stable penicillin preparations were made up with mineral oil and 1-octadecene, and the less stable ones were those made up with the pure esters. Dehydrated Cab-O-Sil formulations show somewhat greater stability over esterified preparations.

Color Reactions

Positive Schiff's tests were obtained with peanut oil and 1-octadecene, while all other oleaginous materials gave negative results. Since the Schiff's test is a color reaction indicating aldehydes, it can be inferred that traces of aldehyde were present in the peanut oil and in the 1-octadecene. Significantly, procaine base (without penicillin) gave color reactions in the peanut oil/Cab-O-Sil and the 1-octadecene/Cab-O-Sil formulations, although this reaction was somewhat less intense than when penicillin was present.

TABLE VIII

SHELF LIFE STABILITY OF POTASSIUM PENICILLIN G FORMULATIONS

Base	Original Penicillin Concentration Units x 10^{-3}/12 Gm.	Penicillin Concentration After 4 Months at Room Temperature Units x 10^{-3}/12 Gm.	Penicillin Concentration After 6 Months at Room Temperature Units x 10^{-3}/12 Gm.
Peanut Oil/Dehydrated Cab-O-Sil	109	-	86
Peanut Oil/Esterified Cab-O-Sil	104	-	75
Mineral Oil/Cab-O-Sil	133	110	-
Isopropyl Myristate/Cab-O-Sil	99	52	-
1-Octadecene/Cab-O-Sil	108	120	-
Butyl Stearate/Cab-O-Sil	105	39	-

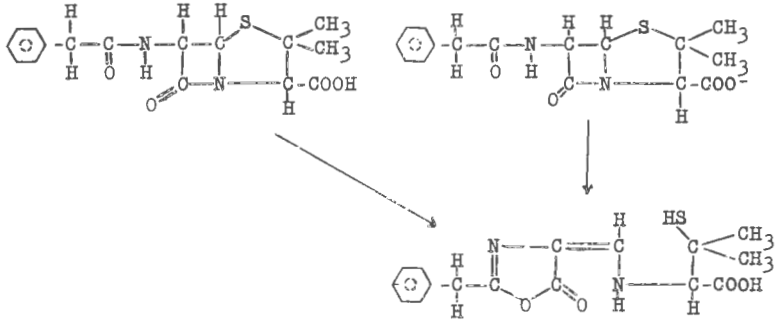
TABLE IX

SHELF LIFE STABILITY OF PROCAINE PENICILLIN G FORMULATIONS

Base	Original Penicillin Concentration Units x 10^{-3}/12 Gm.	Penicillin Concentration After 3 Months at Room Temperature, Units x 10^{-3}/12 Gm.	Penicillin Concentration After 6 Months at Room Temperature, Units x 10^{-3}/12 Gm.
Peanut Oil/Dehydrated Cab-O-Sil	100	-	84
Isopropyl Myristate/Cab-O-Sil	102	52	-
Peanut Oil/Esterified Cab-O-Sil	101	-	73
Mineral Oil/Cab-O-Sil	107	-	71
Peanut Oil/Aluminum Monostearate	109	113	-
1-Octadecene/Cab-O-Sil	140	135	-

IV. DISCUSSION

Schwartz (8) has suggested a mechanism for the degradation of penicillin G in acidic aqueous solution in which he claims that the formation of penicillenic acid comes about either by a rearrangement of the penicillin ion (P^-) following attack by a proton, or by the kinetically equivalent, uncatalyzed reaction of the non-ionized penicillin molecule



If the mechanism of degradation proposed by Schwartz can be applied to oleaginous formulations, then it can be postulated that the instability of penicillin could be primarily due to the surface acidity of the Cab-O-Sil, in conjunction with the slightly polar nature of the ester linkage constituents of the peanut oil. The first part of this hypothesis is supported by the stability results with formulations containing colloidal silica modified to reduce the surface acidity (see Table VI). The latter part of the hypothesis is supported by the results obtained with

formulations containing pure, saturated esters (isopropyl myristate and butyl stearate) as shown in Table VII.

The decreased stability of the penicillin with the ester formulations may be due strictly to increased solubility of the penicillin, but could be due to an ability to dissociate the penicillin. If the latter is true, then according to Schwartz, the dissociated penicillin rearranges, and under the influence of the acidic surface of the silica, forms penicillenic acid. There was some evidence for the formation of penicillenic acid from the ultraviolet spectra studies. The degraded formulations gave a peak at approximately 322 mμ possibly indicating the presence of penicillenic acid as a break down product. However, confirmation was difficult because the absorbing substance was strongly adsorbed onto the colloidal silica and attempts to separate the Cab-O-Sil from the preparations were unsuccessful.

A question which remains unanswered is why should procaine penicillin be less stable than potassium penicillin? One possibility is that the procaine penicillin is adsorbed on the surface of the silica through the free amine group of the procaine, thus keeping the penicillin in the vicinity of the acidic surface of the silica. A more definite answer must await further experimental evidence.

A second cause of degradation of penicillin may be the traces of moisture in the formulation ingredients, although moisture content does not account for differences

in penicillin stability in the mineral oil-silica and peanut oil-silica formulations. It is clear from Table I that two of the components, procaine penicillin and Cab-O-Sil, have relatively high concentrations of moisture. Attempts to remove water from procaine penicillin were unsuccessful, probably because most of the water present was in the form of water of crystallization, and seemed to be essential to the integrity of the penicillin-procaine complex. It was found possible to remove physisorbed as well as chemisorbed water from the Cab-O-Sil by heating at 1000°C for 24 hours.

The color reaction of procaine penicillin-peanut oil, and procaine penicillin/1-octadecene formulations is apparently unrelated to penicillin stability and seems to be due to the interaction of traces of aldehydes with the amine group of procaine. This result was shown when both peanut oil and 1-octadecene gave positive results with the Schiff's test; in addition, the same color reaction with less intensity was observed when procaine base was used instead of procaine penicillin.

The traces of aldehydes possibly are produced by auto-oxidation of the 1-octadecene and of unsaturated components of the peanut oil. Farmer (24) has pointed out that the auto-oxidation of unsaturated oils consists of a series of steps which are not well known. However, hydroperoxides, epoxies, aldehydes, and acids are known to be the results of such oxidation.

V. SUMMARY AND CONCLUSIONS

Formulations of potassium and procaine salts of penicillin G in oleaginous mediums containing colloidal silica were prepared with peanut oil, mineral oil, 1-octadecene, butyl stearate, and isopropyl myristate, using Cab-O-Sil as the viscosity modifier. Other formulations of the different penicillin salts were also prepared from peanut oil, using dehydrated and esterified Cab-O-Sil. All formulations were submitted to stability studies under accelerated and room temperature conditions.

Those formulations containing mineral oil or 1-octadecene as the oleaginous ingredient were found to be the most stable. Peanut oil gave formulations which were considerably less stable than those made from hydrocarbon bases. Formulations containing the esters, butyl stearate and isopropyl myristate, were the least stable of all. In general, procaine penicillin G was less stable than potassium penicillin G in the oleaginous bases tested. When dehydrated silica was used as the viscosity modifier in the peanut oil formulations, stability of the penicillin salts was markedly increased, but the preparations had undesirable rheological properties. Esterified silica formulations also showed increased stability over the unmodified silica preparations; and in addition, these products had acceptable rheological properties, although a somewhat

higher concentration of esterified silica than unmodified silica was found necessary to maintain the same apparent viscosity.

The stability results from the ester formulations and from the modified silica formulations seem to indicate that penicillin instability in the peanut oil/silica preparations stems from the unfavorable influence of the acidic surface of the silica in conjunction with the environment of the slightly polar ester constituents of the peanut oil.

The lower stability of procaine penicillin G as compared to the potassium salt may be due to the higher moisture content of the procaine penicillin G; or it may be due to the adsorption on the surface of the silica through the free amine group, which maintains the penicillin in the vicinity of the acidic surface of the silica.

Color reactions noted with some of the procaine penicillin G formulations seem to be unrelated to stability and may be caused by interactions between the procaine and traces of aldehydes present in the oleaginous materials.

In order to clarify some other aspects of this problem, further work in the following areas is recommended.

1. Stability studies should be followed with polar side chain penicillins "so-called acid-stable penicillins," in order to find out whether or not these new penicillins are affected by surface acidity of the silica.

2. In order to modify the action of the amine group of procaine penicillin G and to point out the reason of its lesser stability as compared to the potassium salt, stability studies should be carried out with other amine salts such as benzathine penicillin G.

3. A technique should be worked out to separate silica from the systems in order to determine the break down products of penicillins.

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