Formulation of a Stable Pilocarpine Hydrochloride Solution

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Abstract □ A stable 1% pilocarpine hydrochloride solution was formulated without added buffers which requires a minimum amount of alkali for neutralization. The solution has an equilibrium pH of about 3.8, contains about 93% of the original pilocarpine used in the formulation, and is self-buffered by a pilocarpine–pilocarpic acid system formed at equilibrium. A solution of pilocarpine hydrochloride is adjusted to about pH 5, and then equilibrium conditions are attained involving a slow, acid-catalyzed resynthesis of pilocarpine from its hydrolysis products.

Keyphrases □ Pilocarpine hydrochloride—formulation in stable solution without added buffers □ Ophthalmic formulations—stable pilocarpine hydrochloride solution without added buffers

Pilocarpine, as the hydrochloride or nitrate salt, enjoys a prominent position in ophthalmic therapeutics because of efficacy in the treatment of chronic wide-angle glaucoma (1–4). Although alkaloids, such as pilocarpine, are thought to be best absorbed through the cornea from an alkaline solution (5–7), the marked chemical instability of these drugs at pH values above 7 precludes formulation of a basic solution for distribution and use over a long period.

Unlike the decomposition of alkaloids containing an ester functional group, the hydrolytic decomposition of pilocarpine, a lactone, is unique in that ring closure of the base-catalyzed hydrolysis product takes place on acidification (8–10). This reversibility of the hydrolysis of pilocarpine was overlooked by several authors writing about pilocarpine instability (11–14).

Baeschlin and Etter (15) performed a kinetic study on the reversible decomposition of pilocarpine in buffered solutions. Using a published assay method (16), they determined that the hydrolysis of pilocarpine hydrochloride is a pseudo-first-order reaction at pH 6.0 in the presence of phosphate buffer. The equilibrium concentration of pilocarpine hydrochloride was found to be dependent on the pH value in solution but independent of temperature between 50 and 70°.

The kinetics of pilocarpine hydrolysis in aqueous solution utilizing pH-stat titrimetry and polarimetry were also studied (17). A cyclic mechanism for the hydrogen-ion- and hydroxyl-ion-catalyzed hydrolysis was proposed. Rate constants, equilibrium constants, and the energy of activation for the hydroxyl-ion-catalyzed hydrolysis were calculated.

This research was designed to study the stability of 1% (4.09×10^{-2} *M*) pilocarpine hydrochloride in unbuffered aqueous solutions stored in glass (Pyrex) and polyethylene containers at elevated temperatures utilizing an optimal ferric hydroxamate assay (18) for the determination of intact pilocarpine. Solutions found to exhibit suitable stability were compared with commercially available solutions using an *in vitro* model of expected irritation upon ocular instillation.

EXPERIMENTAL[1]

Stability Study Procedures—One percent (4.09×10^{-2} *M*) pilocarpine hydrochloride solutions were prepared by dissolving pilocarpine hydrochloride USP in water and adjusting the pH to the desired value with 70% perchloric acid, 4 *M* NaOH solution, or 18 *M* sulfuric acid. After sterilization by bacteriological filtration, aliquots of the solutions were transferred into previously sterilized and weighed 100-ml volumetric flasks (Pyrex) or 120-ml (4-oz) polyethylene[2] bottles. The filled containers were weighed and stored at 45 ± 2 and 65 ± 2°.

From the unused filtered solution in each batch, samples were taken for the initial pilocarpine assay by the method of Gibbs and Tuckerman (18), performed in triplicate, and for measurement of the pH value. Additional samples of each batch were placed in thioglycollate medium[3] for testing sterility. One sample solution in each batch at each temperature was maintained as a weight control. This container was weighed periodically, and the percentage of water lost by evaporation was calculated. This percentage factor, when larger than 1%, was used to determine the proportional volume of solvent necessary to reconstitute the other solutions of the batch at a given temperature based upon their weight at the time at reconstitution. The solvent employed for reconstitution was water adjusted to the pH value exhibited by the solution on the previous sampling by the addition of sodium hydroxide or perchloric acid. This solvent was sterilized by bacteriological filtration.

From the other solutions held at a given temperature, samples of approximately 8 ml each were removed on days subsequent to preparation. After allowing the sample to cool at room temperature, one 2.00-ml aliquot from each sample was assayed and the pH value of the residual sample was measured. Concomitant with removal of samples for assay and pH measurement, samples for sterility testing were aseptically taken from each container.

All solutions were maintained in a sterile state throughout the study.

The slope and absorbance intercept (ordinate) for the set of standards run each day were calculated by linear regression analysis. The pilocarpine content of the samples assayed on that day was obtained from the linear equation.

Graphical representations of typical concentration–pH profiles obtained in glass (Pyrex) and polyethylene containers are shown in Figs. 1 and 2. On each day of sampling, three samples of each batch were assayed. Where a range of assay or pH values was obtained, the range is represented by a vertical line with an intersecting horizontal line at the average value.

Titration with Sodium Hydroxide Solution as a Measure of Expected Ocular Irritation upon Instillation of Pilocarpine Hydrochloride Solutions—The amount of alkali needed to ti-

[1] The equipment used consisted of a Gilford Instrument Laboratories model 2000 automatic spectrophotometer with a Beckman Instruments model DUR monochromator, a Cary Instruments model 15 recording spectrophotometer, and a Fisher Scientific Co. model 210 pH meter with a Beckman Instruments combination electrode, No. 39013.

[2] Alathon 20, Dupont.

[3] Bacto Fluid Thioglycollate Medium, Difco Laboratories.

Table I—Pilocarpine Hydrochloride Content at Time of Last Assay of Titrated Solutions, Initial pH Values, and Volume of Titrant (0.1 *N* Sodium Hydroxide Solution) Necessary to Attain pH 7.40 in the Solutions

Solution	Mean Pilocarpine[a] Hydrochloride Concentration in Titrate, moles liter^{-1} × 10^2	Mean Initial pH Value before Titration	Mean Volume of Titrant to Attain pH 7.40 in Mixture, ml
A	3.54 ± 0.07	4.77 ± 0.03	3.81 ± 0.09
M	3.82 ± 0.02	4.71 ± 0.02	3.96 ± 0.04
I	—	4.87 ± 0.00	3.48 ± 0.04
R	3.78 ± 0.01	3.91 ± 0.01	2.97 ± 0.03
S	3.71 ± 0.01	3.90 ± 0.01	2.91 ± 0.01
T	3.88 ± 0.05	3.50 ± 0.01	3.11 ± 0.04
U	3.88 ± 0.02	3.48 ± 0.01	3.04 ± 0.01

[a] Obtained by ferric hydroxamate assay procedure.

trate the stable solutions to the pH of lacrimal fluid (pH 7.4) was compared with that needed for commercial preparations. The solutions titrated and their compositions are: Solution A, a sterile buffered solution of 1% pilocarpine hydrochloride with 0.01% benzalkonium chloride, boric acid, potassium chloride, and sodium carbonate; Solution M, a sterile buffered solution of 1% pilocarpine hydrochloride with 0.01% benzalkonium chloride, 0.01% disodium edetate, sodium phosphate, sodium biphosphate, and sodium chloride; Solution I, a sterile buffered solution of 1% pilocarpine hydrochloride with 0.5% hydroxypropyl methylcellulose (4000 cps), benzalkonium chloride (1:25,000), phenylmercuric nitrate (1:75,000), boric acid, sodium chloride, and sodium citrate; Solution R, 1% pilocarpine hydrochloride solutions prepared at pH 5.11 and stored at 65° in flasks (Pyrex); Solution S, 1% pilocarpine hydrochloride solutions prepared at pH 5.08 and stored at 45° in flasks (Pyrex); Solution T, 1% pilocarpine hydrochloride solutions prepared at pH 4.15 and stored at 65° in flasks (Pyrex); and Solution U, 1% pilocarpine hydrochloride solutions prepared at pH 4.08 and stored at 45° in flasks (Pyrex).

Commercial Solutions A and M and all experimental solutions titrated were assayed by the ferric hydroxamate method within 5 days of the titrimetric studies. Commercial Solution I was not assayed because of the presence of the thickening agent, hydroxypropyl methylcellulose. The effect of this cellulose derivative upon the results of the assay procedure is not known.

From each sample of experimental or commercial solutions employed, a 10.00-ml aliquot was removed and placed in a small beaker. A combination electrode was inserted into the solution, the pH value of the solution was measured, and the solution was titrated with 0.1 *N* sodium hydroxide. The pH value of the titrated solution was measured after the addition of each 0.20- or 0.40-ml volume increment of titrant. The solution was stirred by hand while adding titrant and between the addition of incremental volumes of titrant. Titrant was added until the pH value of the titrated solution was slightly above pH 7.40.

The volume of 0.1 *N* sodium hydroxide necessary to attain pH 7.40 was calculated using interpolation of the titrant volume and pH value data where the addition of an exact 0.20-ml increment of titrant did not yield the pH value 7.40. Table I shows the mean values together with the standard deviations for the pilocarpine content of the solutions employed, the initial pH value of the solutions before addition of titrant, and the volume of titrant necessary to raise the pH value of each solution to 7.40.

RESULTS

Only those solutions prepared at pH 6.80 and stored in polyethylene bottles at 45 and 65° showed significant degradation with time (Figs. 3 and 4). Actual assay values are given in Tables II and III. In all cases, however, the pH values decreased and, in glass (Pyrex), finally approximated a stable value. The rate of approach to equilibrium was increased by increasing the temper-

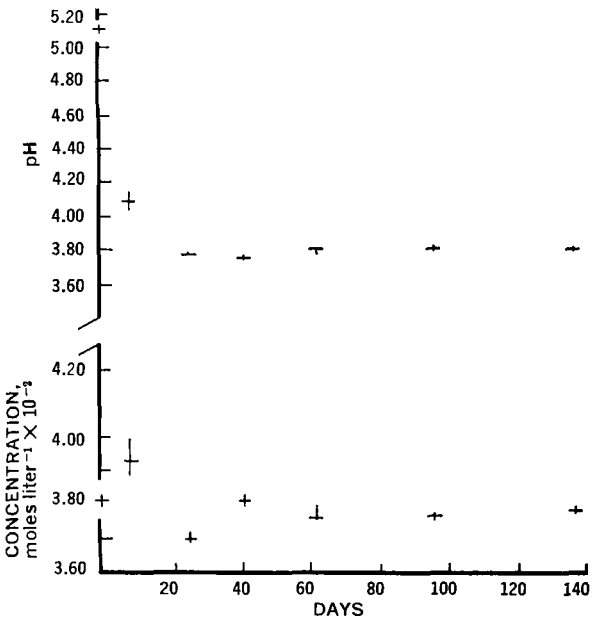

Figure 1—*Concentration–pH profile for pilocarpine hydrochloride solution prepared at pH 5.11 and stored at 65° in flasks (Pyrex).*

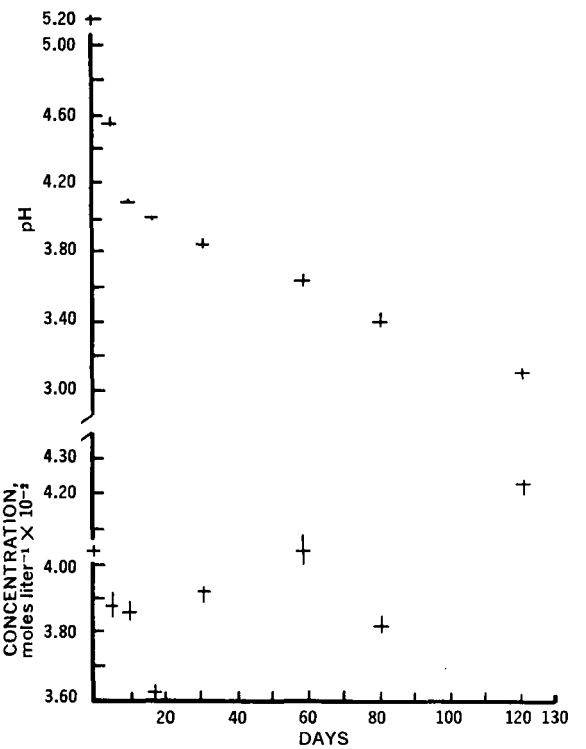

Figure 2—*Concentration–pH profile for pilocarpine hydrochloride solution prepared at pH 5.15 and stored at 65° in polyethylene bottles.*

Table II—Mean Assay Values for Unbuffered Pilocarpine Hydrochloride Solutions Stored in Flasks (Pyrex) at 45 and 65° and Percent Recovery Based on Initial Concentration (1% w/v, 4.09×10^{-2} mole liter^{-1})

Solution							
Initial pH	Final (Equilibrium) pH	Storage Temperature	Time of Study, days	Total Number of Assays	Mean Assay Value, moles liter$^{-1} \times 10^{2}$	Standard Deviation	Percent Recovery Based on Mean Assay Value and Initial Concentration of 4.09×10^{-2} mole liter^{-1}
5.11	3.80	65°	137	21	3.79	0.07	92.7
5.08	3.80	45°	118	18	3.80	0.13	92.9
4.15	3.40	65°	137	21	3.86	0.09	94.4
4.08	3.40	45°	118	18	3.92	0.13	95.8

Table III—Mean Assay Values for Unbuffered Pilocarpine Hydrochloride Solutions Stored in Polyethylene Containers at 45 and 65°

Solution						
Initial pH	Final pH	Storage Temperature	Time of Study, days	Total Number of Assays	Mean Assay Value, moles liter$^{-1} \times 10^{2}$	Standard Deviation
5.15	3.15	65°	121	24	3.93	0.17
5.15	3.85	45°	131	24	3.89	0.10
4.00	3.00	65°	111	18	3.93	0.13
4.00	3.32	45°	104	17	3.96	0.10
5.17	3.14	65°	148	15	3.75	0.12
5.17	3.77	45°	148	15	3.84	0.12
4.10	3.00	65°	147	15	4.00	0.11
4.10	3.40	45°	147	15	3.99	0.09
3.72	2.95	65°	147	15	3.94	0.18
3.72	3.24	45°	147	15	3.94	0.16

ature, but the final value obtained depended only on the initial pH at preparation, not on the temperature of storage.

The solution prepared at pH 5.11 had a final pH of 3.80 and an intact pilocarpine content of 92.7% of the original value. The solution prepared at pH 4.15 had a final pH of 3.40 and an intact pilocarpine content of 94.4% of the original value.

The solutions stored in polyethylene, although showing the decrease in pH, did not approach a constant value; the final pH was below the equilibrium value found in glass (Pyrex). In addition, the mean final pH value for solutions held at 65° was uniformly lower than for those held at 45°.

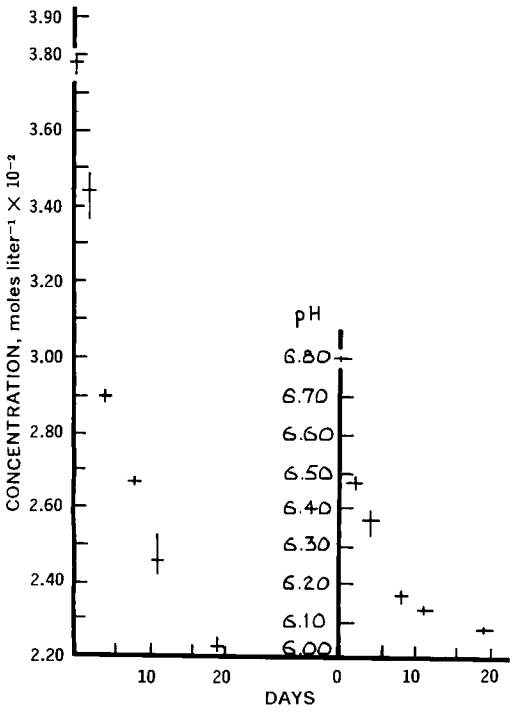

Figure 3—*Concentration–pH profile for pilocarpine hydrochloride solution prepared at pH 6.80 and stored at 65° in polyethylene bottles.*

The titrimetric studies (Table I) showed that Solutions R, S, T, and U, the solutions prepared without added buffer, require substantially less alkali for neutralization than the commercial preparations. If the amount of alkali consumed is taken as a measure of potential irritation upon ocular instillation, Solution S must be considered as an optimum formulation.

DISCUSSION

In the past, because of the use of lime-glass containers that leached alkali which catalyzed the hydrolysis of pilocarpine, it was desirable to buffer pilocarpine solutions. The availability of glass containers that leach very little alkali and, more recently, the availability of plastic containers[4] (20) for dispensing ophthalmic solutions obviate this need for a buffered formulation.

It was found (21) previously that there is little need to apply a buffered pilocarpine hydrochloride solution of near physiological pH and that a somewhat more acid, but unbuffered, solution will suffice. Furthermore, an isotonic solution of pH 3.0 hydrochloric acid is not irritating while isotonic phosphate and acetate buffers of approximately 0.1 *M* and pH 5.0 are distinctly irritating upon ocular instillation (22). Adjustment of the pH value of a solution to the acid range using a buffer may actually have the deleterious effect of providing resistance to normal change toward neutrality upon ocular instillation, thus resulting in a prolongation of irritation and decreased drug absorption (23).

The final pH values for the solutions prepared at pH 4.15 and 4.08 and stored in glass (Pyrex) at 45 and 68° agree within 0.1 pH unit with the values obtained (12) with unbuffered pilocarpine hydrochloride solutions containing sodium chloride and phenylmercuric nitrate stored for 12 months at room temperature. The decrease of pH in solutions stored in polyethylene containers to values below the equilibrium values in glass (Pyrex) is attributed by the authors to the leaching of oxidative degradation products

[4] Drop-Tainers.

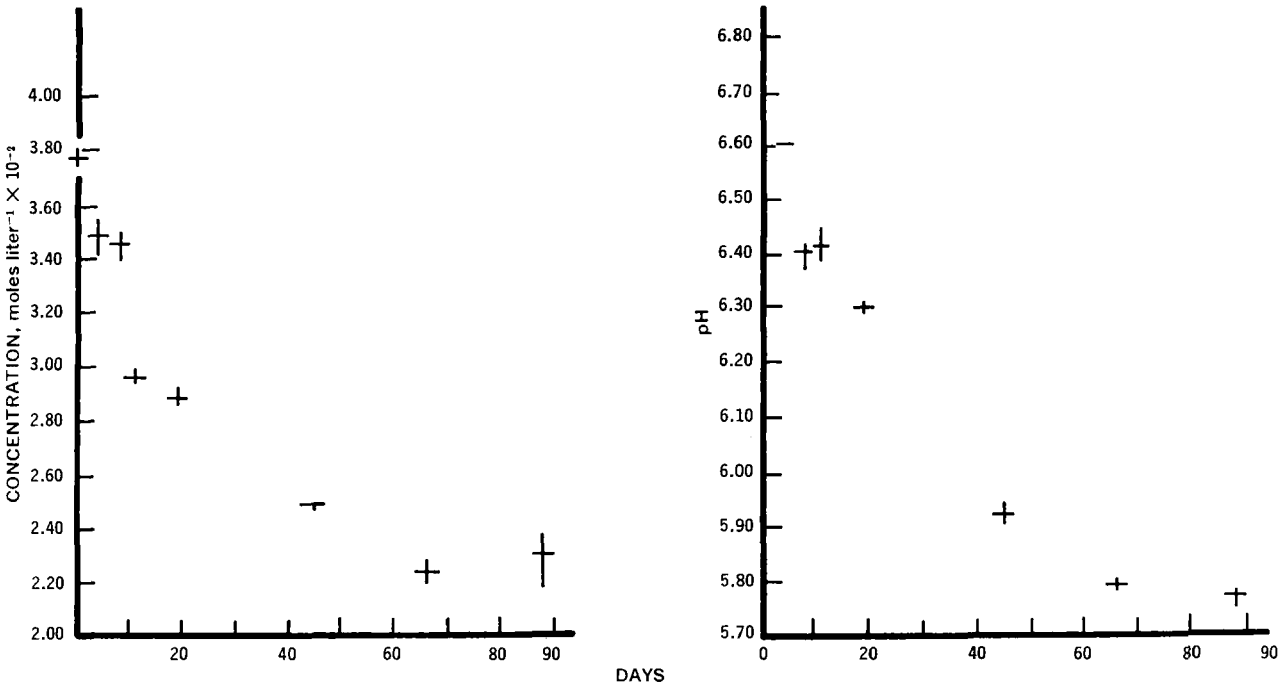

Figure 4—*Concentration–pH profile for pilocarpine hydrochloride solution prepared at pH 6.80 and stored at 45° in polyethylene bottles.*

formed in the polyethylene by the prolonged heating of the polyethylene at the elevated temperatures. The observation that the final mean pH value of solutions stored at 65° is lower than those stored at 45° is in accord with this explanation.

The amount of alkali needed to neutralize any of the commercial solutions is larger than that needed for any of the experimental solutions. The results are explainable since the commercial solutions contain additional buffer systems (observed as differences in the shape of the titration curves obtained for the commercial pilocarpine hydrochloride solutions and the experimental solutions) which account for the increased volume of titrant required to elevate the pH value of the solution to 7.40.

With the commercial solutions, the alkalimetric titration involves neutralization of the adjuvant buffer system, of any pilocarpic acid in solution, and of the less acidic protonated nitrogen of the imidazole moiety of the pilocarpine molecule (pKb = 7.15, pKa = 6.85). The alkalimetric titration of unbuffered Solutions R, S, T, and U, however, involves only neutralization of the pilocarpic acid and the protonated imidazole nitrogen.

CONCLUSION

An especially stable ophthalmic solution of 1% pilocarpine hydrochloride was prepared. This solution is expected to give minimal irritation and good drug absorption when instilled into the eye. This expectation is based upon the amount of alkali necessary to raise the pH value of the solution to the approximate pH value of the lacrimal fluid. The solution can be prepared by dissolving pilocarpine hydrochloride in distilled water, adjusting the pH value to about 5, sterilizing by bacteriological filtration or autoclaving, and packaging the solution in a sterile container made of an inert material.

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Microencapsulation of a Waxy Solid: Wall Thickness and Surface Appearance Studies

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Abstract □ Microencapsulation of solid stearyl alcohol particles by complex coacervation was studied. Spherical particles of solid stearyl alcohol were prepared by the vibrating capillary method. Various size fractions of these particles were encapsulated by a modified technique described in an earlier report. Particle concentration and particle-size studies revealed that only a small fraction of the total amount of colloid was used in the formation of the capsule wall. Wall thickness decreased with increasing ratios of solid particles and increased as the particle diameters increased. Scanning electron micrograph observations of the surface showed that acacia was retained on the surface of particles melted and congealed in acacia solution and that the final encapsulated particles had a scaly surface appearance. The studies tended to confirm indications from earlier work that gelatin molecules interact directly with acacia on the surface of the stearyl alcohol particles to form the capsule.

Keyphrases □ Microencapsulation—acacia-coated solid stearyl alcohol particles with gelatin solution, wall thickness, surface appearance □ Encapsulation, micro—acacia-coated solid stearyl alcohol particles with gelatin solution, wall thickness, surface appearance □ Stearyl alcohol particles, acacia coated in gelatin solution—microencapsulation, wall thickness determined, surface appearance evaluated □ Gelatin—interaction with acacia on stearyl alcohol particle surface, microencapsulation, wall thickness, surface appearance □ Acacia—interaction with gelatin on stearyl alcohol particle surface, microencapsulation, wall thickness, surface appearance

There have been a number of reports concerning encapsulation of various substances but few concerning the extent or nature of encapsulation. Undoubtedly the characteristics of the product depend, not only on the extent of encapsulation, but also on the final thickness and integrity of the deposited encapsulating material. The gelatin–sulfamerazine ratio was used (1) as an indication of wall thickness in studying encapsulation of sulfamerazine by simple coacervation. Apparently few attempts have been made to evaluate the wall thickness of microcapsules made by complex coacervation. A recent report (2) considered wall thickness and diffusion from microcapsules containing a liquid organic base.

The purposes of this investigation were to: *(a)* encapsulate spherical solid particles by complex coacervation, *(b)* evaluate the wall thickness of the microcapsules, *(c)* study the effect of particle concentration and particle size on the wall thickness of microcapsules, and *(d)* study the surface appearance of the particles at several stages of the process.

EXPERIMENTAL

Materials—Acacia USP, stearyl alcohol USP, and pigskin gelatin[1] (isoelectric point at pH 8.0) were used.

Selection of Material to Be Encapsulated—To achieve uniformity of the particles to be encapsulated, it was desirable to produce spherical particles, preferably monodisperse. The selection of the material to be encapsulated was, therefore, quickly narrowed to materials that could be easily melted and rapidly congealed to fulfill requirements of available methods of spherical particle production. Wax-like materials most nearly fulfill these requirements; stearyl alcohol was chosen, although a number of other wax-like substances, *e.g.*, beeswax and paraffin, could have been used with little difficulty.

Selection of Particle Size of Core Material—The following reasons predetermined the use of particles larger than 250 μm in diameter: *(a)* convenience, ease in handling, and microscopic observation; *(b)* the discovery that the larger wax particles when encapsulated by a modified procedure seemed to give thin, uniform capsule walls (3) from which thicknesses could be more easily estimated; and *(c)* a desire to explain the formation of the thin, uniform walls around the waxy particles.

Preparation of Particles to Be Encapsulated—The vibrating capillary method of production of spherical particles, similar to that described previously (3, 4), was used. A nest of sieves was employed for the separation of particles in various size ranges. Particles passing through one sieve and retained on the next finer sieve were assigned the arithmetic mean size of the two screens. Sieves 60, 40, 30, and 20 were used to obtain particles having average diameters of 335, 505, 715, and greater than 840 μm, respectively. The particles obtained were spherical in shape and had a predictable size distribution.

Microencapsulation Procedure (Scheme I)—All experiments were carried out under identical experimental conditions. In all experiments, gelatin and acacia solutions were prepared by dissolving, separately, equal quantities of gelatin and acacia in 20 ml of distilled water. These solutions were allowed to hydrate for at least 12 hr before being used. Coacervation was carried out at 40° using a water bath maintained at 40 ± 1°.

A known weight of the solid stearyl alcohol particles was dispersed in acacia solution with gentle stirring, and the dispersion was heated to 60°. At this temperature, stearyl alcohol particles melted and apparently became coated with acacia. The dispersion was then cooled quickly to congeal the particles, and the acacia-coated particles were encapsulated by the procedure reported previously (3).

Particle Concentration Studies—Particle concentration studies were conducted to evaluate the effect of particle concentration

[1] American Agricultural Co., Detroit, Mich.