Controlled Crystallization of Hydrocortisone by Ultrasonic Irradiation

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A procedure has been developed to prepare low micron particles of hydrocortisone by direct crystallization of suitably prepared supersaturated solutions in an ultrasonic field. The solvent system employed for the preparation of the saturated solutions was observed to be a factor in controlling particle size distribution, while the effects of tenure of ultrasonic treatment and the extent of cooling the saturated solution were not as significant. A concurrent study was undertaken to determine the variables concerned with affecting crystal yields. The degree of crystallization was increased with ultrasonic treatment and with lower coolant bath temperatures. A method was developed to evaluate the effect of decreased cavitation on particle size distribution.

As a result of a series of investigations by several authors (1–16) it has been established that ultrasonic treatment increases the rate and extent of nucleation occurring from supersaturated solutions. Furthermore, it has been shown that increased nucleation results in the formation of smaller crystals.

The primary objective of this investigation was to determine the factors involved in the preparation of low micron particles of hydrocortisone[1] by direct crystallization of suitably prepared supersaturated solutions in an ultrasonic field.

EXPERIMENTAL

Since part of this study was an evaluation of the effects of solvents on crystallization induced by ultrasonics, one of the first problems was to select several solvents with satisfactory, yet slightly different solubility characteristics. The solubility of hydrocortisone in a series of solvents over a wide temperature range was studied. Isopropyl alcohol, ethyl alcohol, and a 50% blend of isopropyl alcohol and ethyl alcohol possessed all of the desired characteristics for this study and were selected as solvents to be used throughout. Representative solubility curves are shown in Fig. 1.

For the preparation of the saturated solutions of hydrocortisone in the blended solvents a standard taper three-holed flask was used. This was fitted with a water-cooled reflux condenser, a stirrer, and removable glass stopper to allow the withdrawal of samples. This procedure insured maintenance of the specific concentration of the mixed solvents in the blend.

Ultrasonic treatment of various saturated solutions of hydrocortisone in the three solvents described above was accomplished in the following manner with a schematic representation shown in Fig. 2. The Sonifier[2] was activated 10 minutes prior to beginning a run to allow sufficient warm up of its electrical and mechanical systems. The treatment vessel, a 20-ml. beaker, was placed into the coolant bath approximately 30 seconds prior to the addition of the saturated solution. The tip of the Sonifier was positioned relative to the base of the treatment vessel with the aid of a 2.3-mm. deep template. Ten milliliters of saturated solution were poured into the treatment vessel while, simultaneously, the stopwatch and Sonifier were activated. Samples were treated for 1, 2, 4, or 16-minute periods of time. Following treatment, portions of the samples were withdrawn for particle size and weight distribution analyses.

In all the test runs that were performed to evaluate the selected variables, three coolant temperatures were employed. These three temperatures, 25, 0, and −65° were chosen to allow analysis of the effect of reducing the temperature of the saturated solutions over an extremely wide range.

The effect of solute concentration on particle size distribution was studied to determine the relative

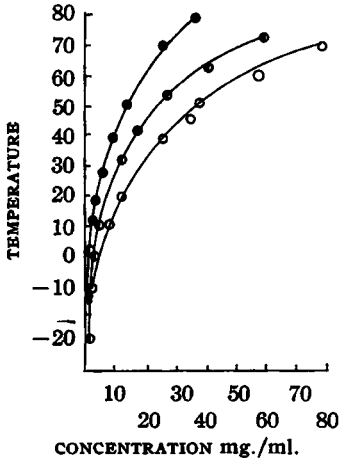

Fig. 1.—Solubility curves of hydrocortisone in the selected solvents. Key: ●, isopropyl alcohol; ○, ethyl alcohol; ◐, blend.

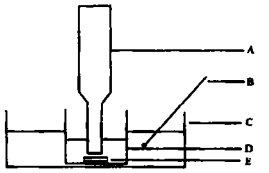

Fig. 2.—Diagram of the insonation system. Key: A, Sonifer; B, thermometer; C, coolant bath; D, treatment vessel; E, template.

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[2] Branson Instruments Inc., Stamford, Conn.

importance of this factor in sonic-induced crystallization. Two saturated solutions at different temperatures were prepared (hence different amounts of solute per milliliter of solution), crystallized, and evaluated.

One of the most important phases of this work dealt with determining the effect that ultrasonics has upon the crystallization procedure. Supersaturated solutions are extremely metastable and, thus, are very sensitive to mechanical shock or vibrations.

Vigorous sonic agitation is one of the most prominent manifestations of ultrasonic treatment of liquids. This is caused by the rarefaction and compression of ultrasonic waves passing into the solution. Consequently, it can be assumed that improvements in the crystallization procedures were not caused by cavitation, which is the primary effector in ultrasonic systems, but simply by the vibrational effects (stirring) produced by ultrasonics.

It was thought necessary to establish a suitable modification in the original system to reduce or, if possible, eliminate cavitation. Then, a comparison of the size frequency and weight distributions of the crystals prepared in this manner with those prepared by the normal procedures would serve to define the effect that cavitation produces in these systems. That is, if particle size and weight distribution data obtained in these modified systems were substantially inferior to the data obtained in the basic systems, this would indicate that it is sonics (cavitation) and not simple stirring that is the primary effector.

Investigators (17–19) have reported that cavitation can be substantially decreased by treating samples under pressures which have been reduced to less than 100 mm. Hg. They have observed that the ability of an ultrasonic field to induce emulsion formation, increase the rate of sonochemical reaction, destroy microorganisms, and denature proteins is suppressed by subjecting the systems to either high or low pressures.

This phenomenon can be explained in the following manner. Liquids when exposed to ultrasonics are slowly degassed; however, if this treatment is initiated while the system is being exposed to reduced pressure, degassing occurs quickly and almost completely.

Cavitation is initiated by the expansion and contraction of gas-filled nuclei within a liquid (20); therefore, since the concentration of these nuclei is significantly reduced in degassed liquids, the commencement of the catastrophic phase of cavitation is substantially hindered.

In addition, it has been observed that the catastrophic phase of cavitation occurs when the total pressures within the cavities exceed the cohesive forces of the liquid and the ambient pressure above the liquid. In reduced-pressure systems, the cavities collapse, but, because the ambient pressures have been reduced, much less energy is imparted to the system, and thus characteristic effects of sonics do not occur.

Samples of saturated solutions in isopropanol at 70° were evaluated in the usual manner regarding treatment times and coolant bath temperatures. The samples were evaluated at 114–120 mm. Hg and at 15–20 mm. Hg. Thus, data were obtained at these pressure levels for the evaluation of the effect of reduced pressure on sonic efficacy.

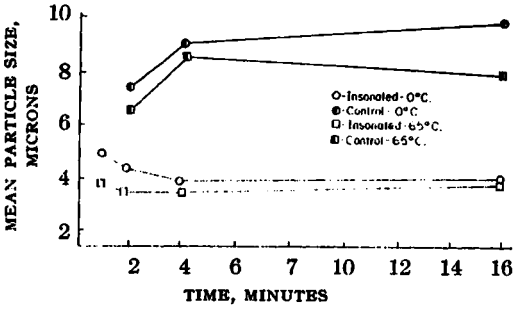

Fig. 3.—Comparison of particle size data of insonated and uninsonated samples of hydrocortisone in a 70° saturated solution in isopropyl alcohol at 0 and −65°.

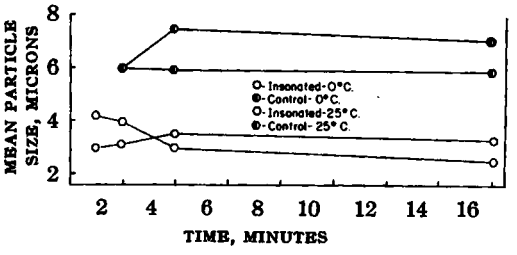

Fig. 4.—Comparison of particle size data of insonated and uninsonated samples of hydrocortisone in a 73° saturated solution in ethyl alcohol at 0 and 25°.

The Sonifier model S-75, was the ultrasonic instrument utilized in this study. This instrument is of the low-frequency (20 kc. per second) piezoelectric type and is capable of yielding high-intensity power outputs. Throughout the entire investigation, maximum power was employed because it was noted that at peak power levels the operating efficiency of the instrument was at a maximum.

For the collection of crystalline cake used in particle size and weight distribution analysis a Millipore[3] filter apparatus containing Whatman No. 42 filter paper was used. Volumetric portions of the treated samples were poured into the funnel, vacuum was applied, and the dried cake was obtained. However, filtration rates were dependent upon the particle size of the crystals formed. To prevent any change in the particle size distribution, a maximum filtration time was established. This maximum time, during which no significant particle size changes occurred, was approximately 6–8 minutes. To obtain a significant safety factor, a 2-minute filtration limit was established. Thus, depending on the estimated particle size of crystals, various sized volumetric portions (1–5 ml.) of the treated samples were placed into the Millipore funnel. The cakes of hydrocortisone after separation of solvent were weighed on an analytical balance.

The term "mean particle size" which is used throughout the paper, refers to an arithmetic mean obtained by measuring the longest diameter of the observed crystals in a manner similar to that described by Singiser (21). The following is a short synopsis of the procedures employed by this earlier worker. Photographs were taken through the barrel of a microscope fitted with a camera adapter.

[3] Millipore Filter Corp., Bedford, Mass.

A 35-mm. black and white film was utilized for recording the observations. The film was developed and projected onto a cardboard grid so prepared that the dimensions of each square of the grid were exactly the same. The projector was then placed at a specific distance from the cardboard grid. This distance was selected so that the calibrated index, when projected onto the board, aligned itself with the grid in such a manner as to be exactly superimposed upon it. This calibrated index was prepared in such a fashion that the distance between each line would represent a length of 5 μ. Thus, since this index was superimposed on the grid, each grid represented a square of 5 μ.

A single exposure was taken of each slide prepared in the following manner. A small sample of the crystalline cake was taken and placed onto the slide. The crystals were distributed uniformly with the aid of a few drops of a 2% methylcellulose 400 solution. The slide was then examined, and a picture was taken of what appeared to be a representative sample of the crystal population on that slide. Following this, a second slide of the same material was prepared, examined, and photographed in the exact manner. Thus, for each sample two different exposures were obtained. This method was employed to reduce the hazards in measuring only a small per cent of the total population.

Approximately 150 particles were counted from each exposure to give a total of 300 measured particles per sample. With this many particles and with the usual standard deviations (2–4 μ) and the usual means (2–7 μ), a 95% confidence level could be obtained between means of 0.5 μ. For example, 95% of the time observed, means of 4.5 and 5.0 μ will be significantly different.

DISCUSSION

The evaluation of the factors involved in the crystallization of hydrocortisone in an ultrasonic field established a number of extremely important

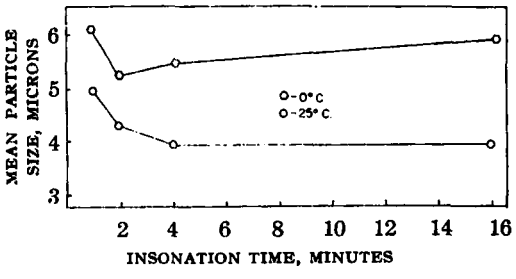

Fig. 5.—Relationship of particle size data to insonation times of samples of hydrocortisone in a 70° saturated solution in isopropyl alcohol at 0 and 25°.

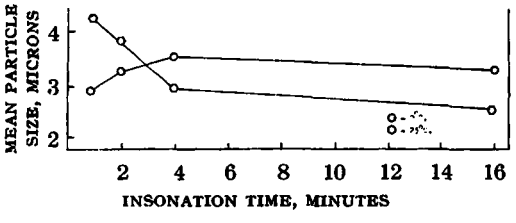

Fig. 6.—Relationship of particle size data to insonation times at 0 and 25° of samples of hydrocortisone in a 73° saturated solution in ethyl alcohol.

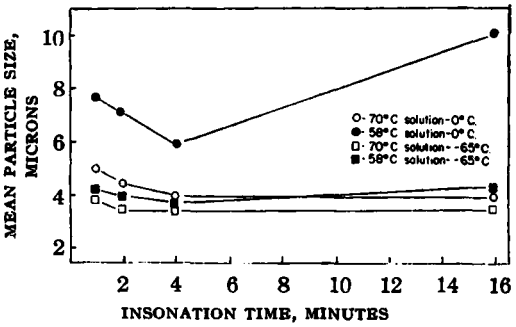

Fig. 7.—Relationship of particle size data to coolant bath temperatures of 0 and −65° for insonated samples of hydrocortisone in 70 and 58° saturated solutions in isopropyl alcohol.

facts. The results indicated that ultrasonic treatment played an extremely important part in the crystallization of hydrocortisone. It was observed that ultrasonics affected not only the particle size distributions, but also the amount of hydrocortisone which was induced to crystallize.

The discussion of the effects of ultrasonic treatment on the crystallization of hydrocortisone has been divided into three broad areas. Particle size effects are discussed first, followed by a presentation of the weight distribution analyses and, finally, a discussion of the effects of reduced pressure on sonic efficacy.

Before entering into a discussion of the specific effects of ultrasonics on the crystallization process, it was necessary to substantiate the effects of ultrasonic treatment by comparing data of treated and untreated samples.

Throughout this experiment, controls were run under the exact conditions as the treated samples. Data were thus obtained which could be used to evaluate the effectiveness of ultrasonic treatment during crystallization. The data indicate two significant facts; first, that under almost all treatment conditions, the rate of the crystallization of the controls was substantially lower than the treated samples. Occasionally, in the controls in which crystals did form, the amount of crystallate approximated that of the treated samples. In the 72 controls that were processed, 23 exhibited some crystallization and, of these 23 samples, only 10 yielded approximately the same amount of crystallization as treated samples. Thus, it is reasonably safe to say that sonics does play a significant part in enhancing the rate of crystallization.

The particle size distributions of the controls were usually larger by a factor of 2 or 3. Figures 3 and 4 illustrate these particle size effects. Hence, with regard to the two most significant factors that were investigated in this problem, reduction in particle size distribution and increased yield of crystals, ultrasonics definitely played a significant role.

Particle size effects elicited by ultrasonic treatment of saturated solutions of hydrocortisone can themselves be subdivided. Since this problem has been investigated from many different facets, the results obtained are more illustrative by analyzing each section separately.

The effects which the length of ultrasonic treatment had on the particle size distributions of the crystallates were almost negligible. Particle size

data, obtained throughout the study, indicated that there were very few cases of actual modifications in the particle size distribution of samples which had been treated for varying periods of time. A perusal of Figs. 5 and 6 clearly indicates this occurrence.

This effect can probably be explained by consideration of the treated system. In the treatment vessel there is, in addition to the crystals, a supersaturated solution containing excess molecules of solute. Normally, if the system is not disturbed, these crystals grow until they reach a finite size. This can be observed in the control experiments. However, because sonics itself has the ability to cause attrition of crystals, the growth effect is hampered. In addition, since sonics has been shown to increase the extent of nucleation, the degree of supersaturation of solution is markedly reduced and, thus, there is less of a tendency for the growth of crystals to occur.

The effect of the different coolant temperatures on particle size distribution can be observed in Figs. 7 and 8. There is very little difference in the particle size distributions obtained at the different temperatures. Except for a few samples prepared with isopropanol, particle size distributions were relatively unaffected by the differential in temperature of the saturated solutions.

These data serve to enhance further the importance of ultrasonic treatment of saturated solutions in invoking fine particle crystallizations. Usually, as a supersaturated solution is cooled, the crystals become smaller. Hence, when the −65° coolant bath was employed, smaller crystals should have been formed. Since this was not observed, this indicated that sonics was the primary influence in the preparation of these low micron crystals and not the extent of cooling.

Three solvent systems were employed in this study to determine what effect, if any, the solvent might have on the formation of low micron crystals.

A comparison of Figs. 5, 6, and 9 graphically illustrates this effect. It can be seen readily from an analysis of these data that a relationship exists between the three solvent systems. The lowest particle size distributions are obtained when ethyl alcohol is used as a solvent. Crystals obtained from isopropyl alcohol are generally the largest, while the blend yields crystals intermediate in size between the two pure solvents.

This relationship can be explained on the basis of solubility data. Hydrocortisone is most soluble in ethyl alcohol, least soluble in isopropyl alcohol, and intermediately soluble in the blend. This is the exact relationship of the particle size data of the solvents. Hence, the particle size distribution of

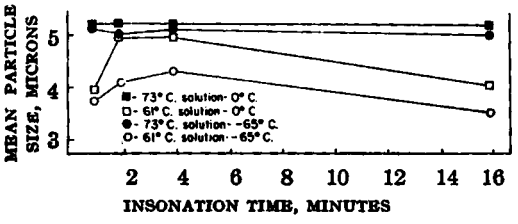

Fig. 8.—Relationship of particle size data to coolant bath temperatures of 0 and −65° for insonated samples of hydrocortisone in 73 and 61° saturated solutions in the blend.

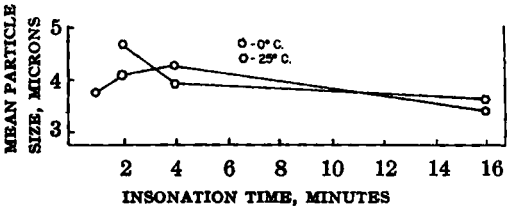

Fig. 9.—Effect of the solvent system on the particle size distributions of insonated samples of hydrocortisone in a 73° saturated solution in the blend.

hydrocortisone can be described as being a function of the extent of its solubility in each of the solvents.

This effect is further substantiated by an analysis of Figs. 7 and 8, which graphically illustrate particle size data as a function of the temperature at which the saturated solutions were prepared. It can be observed that smaller crystals occurred when the higher temperature saturated solution was treated.

This relationship can be explained best on the basis of known solubility and supersolubility theories. Since hydrocortisone is most soluble in ethyl alcohol, it is obvious that when these saturated solutions are cooled to approximately the same degree as the other solvents, they will exist in a higher unstable state. Thus, saturated solutions prepared from ethyl alcohol would be most prone to nucleate and to form smaller crystals.

There appears to be a disagreement in the data derived from this series of experiments when compared to the data obtained from the effects of different coolant temperatures on particle size distributions. As previously reported, the different coolant temperatures did not seem to affect the particle size data significantly; but a careful analysis of the problem indicates that this is probably not normally the case.

This occurrence can possibly be explained in the following manner. If one considers that a saturated solution is prepared with X molecules of solute, then there are X number of molecules which, under suitable conditions, can be caused to nucleate and form crystals. However, no matter how low the temperature is reduced, there are still only X number of molecules in the solution to invoke nucleation. If a saturated solution is prepared composed of $X + Y$ molecules, then, under the same conditions, $X + Y$ molecules are available to nucleate.

It has been observed by the authors that nuclei formation is strongly enhanced by ultrasonic treatment. In addition, it has been reported (22) that maximum nuclei formation has been demonstrated to be advantageous for the crystallization of low micron particles. Thus, the solution that nucleates to a greater extent most probably would yield smaller crystals. If we assume, as is reasonable in the light of the established data, that sonics is the primary factor in inducing nucleation, then, even though the saturated solutions are exposed to different coolant temperatures, a stimulation effect on crystallization would occur. Since the systems are induced to nucleate by sonics to the same degree, the saturated solutions, prepared with more solute (a higher-temperature saturated solution)—for example, the $X + Y$ saturated solutions—would be more prone to nucleate and, therefore, form smaller crystals.

Ultrasonics has been shown to increase the rate of

crystallization of hydrocortisone from saturated solutions.

Since ultrasonic treatment imparts energy to the system in the form of heat, this effect is even more significant. It is well known that yields from the usual crystallization procedure are primarily dependent upon the extent of reduction in temperature. The larger the temperature differential, the more pronounced are the yield differentials. However, in almost all of the treated samples, the yields were greater than in the untreated samples, even though the differential in the temperature of the treated samples was less.

The effect of the length of ultrasonic treatment on the amount of the crystallates formed is illustrated in Figs. 10 and 11. These data indicate that for ethyl alcohol and the blended solvent, increased treatment times somewhat increased the amounts of the crystallate. For isopropanol, increased yield occurred only for the −65° run. It is interesting to note the agreement of these data with the known solubility data of the three solvents. Hydrocortisone is most soluble in ethyl alcohol, least soluble in isopropyl alcohol, and intermediately soluble in the blend. A review of the data indicates that this is the same relationship as the yields of the crystallate.

The various coolant temperatures employed in this study markedly affected the yields of the crystallate. In almost every treated sample, the yields of the crystallate were directly related to the coolant temperature. That is, when −65° was utilized as the coolant temperature, maximum yields occurred, and when 25° was employed, minimum yields were obtained. This effect was expected because once sufficient nuclei are formed, the yields of crystals obtained in a crystallization procedure are primarily dependent upon the temperature effects.

The amount of crystallate formed was a function of the solvent system employed for the crystallization. Hydrocortisone is soluble to its maximum extent in ethyl alcohol. Hence, when the temperature of this saturated solution is reduced to the same extent as for example isopropanol (lower solubility) more crystals would be expected to form. This occurrence was observed throughout this series of experiments. The same effect was noted when a comparison of data obtained from the high and low saturated solutions of the same solvents was evaluated. This series of experiments, dealing with the yields of crystals obtained from saturated solutions which had been treated with sonics, agrees quite well with established crystallization principles.

The final area investigated dealt with the determination of the effects produced by reducing the external pressure of the system. As previously noted (17–19), a reduction in external pressure brings about a reduction in sonic efficacy. Employment of this procedure should offer information relative to the actual effect of ultrasonics on the crystallization procedures. That is, if the particle size data and the yields of crystallates obtained in this series of experiments are inferior to the data obtained in the normal treated samples, this would indicate that sonics effects (cavitation) are primarily responsible for the superior results obtained in the treated samples.

A series of experiments clearly defined the effect of reduced pressure on particle size distributions. The data indicate that, as the external pressure is

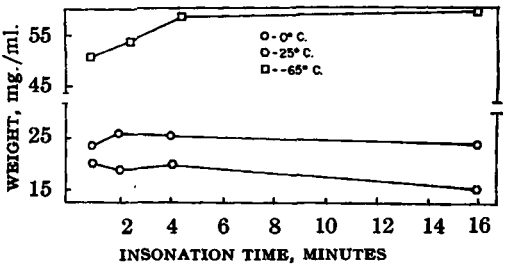

Fig. 10.—Relationships of crystal yields to insonation times at 0, −65, and 25° of samples of hydrocortisone in a 70° saturated solution in isopropyl alcohol.

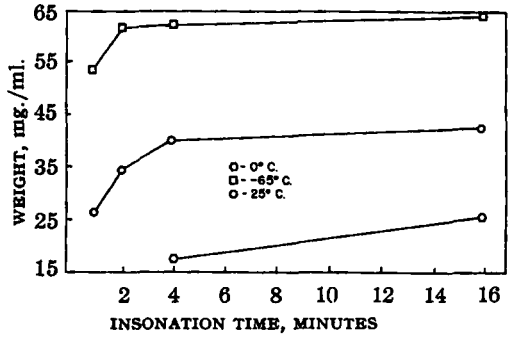

Fig. 11.—Relationship of crystal yields to insonation times at 0, −65, and 25° of samples of hydrocortisone in a 73° saturated solution in the blend.

reduced, the particle size distributions increase. Figures 12 and 13 denote this increase in particle size distribution as a function of the reduced external pressure.

The application of a vacuum to the system to effect a reduction in pressure caused an increase in the yield of the crystallate. This effect occurred even though cavitation was substantially reduced.

An explanation of this occurrence is based upon the fact that the solvent is removed from the system because of the reduced pressure, and because of the temperature of the treatment vessel. Vacuum crystallizations have been successfully employed for the preparation of numerous crystalline materials. However, as the data indicate, to achieve increased yields of crystallizations, the particle size frequency of the crystallate must be sacrificed to some extent.

SUMMARY AND CONCLUSIONS

The purpose of this investigation was to evaluate the factors involved in the preparation of low micron particulates of hydrocortisone by the application of ultra sound. A concurrent study was carried forth to determine the variables concerned with increasing crystal yields.

(*a*) Ultrasonic treatment of saturated solutions of hydrocortisone in isopropyl alcohol, ethyl alcohol, and a 50% v/v blend of these two solvents indicated that, under suitable conditions, low micron crystals can be obtained.

(*b*) The length of ultrasonic treatment of the samples has been observed not to be a factor in affecting the particle size distributions of the crystals.

(*c*) The particle size distributions of samples, sub-

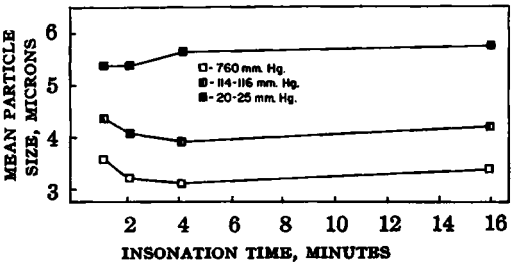

Fig. 12.—The effect of reduced pressure on the particle size distribution of insonated samples of hydrocortisone in a 70° saturated solution in isopropyl alcohol at −65°.

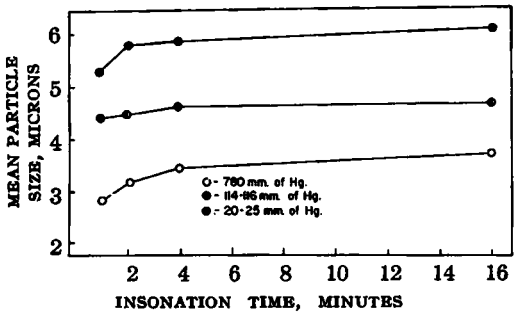

Fig. 13.—The effect of reduced pressure on the particle size distributions of insonated samples of hydrocortisone in a 73° saturated solution in ethyl alcohol at 0°.

jected to ultrasonic treatment at the various coolant temperatures, were quite similar. Except for a few samples prepared with isopropanol, particle size distributions were relatively unaffected by the differential in temperature of the saturated solutions.

(*d*) The solvent system employed for the preparation of the saturated solutions was observed to be a factor in controlling particle size distributions. Crystals obtained from treated saturated solutions utilizing ethyl alcohol as the solvent were consistently smaller in size than those obtained from saturated solutions in either the blend or isopropanol.

(*e*) The degree of saturation of the solvents was observed to affect particle size distribution; the higher the level of saturation, generally, the smaller the crystals.

(*f*) The yields of crystals obtained from samples treated with sonics were in almost all cases larger than untreated samples.

(*g*) The length of ultrasonic treatment was observed to affect slightly the yields of crystals from saturated solutions in isopropanol. However, the amount of crystallate obtained from saturated solutions in both ethyl alcohol and the blend was enhanced with increased treatment time.

(*h*) The amount of hydrocortisone, which crystallized from the solvents when subjected to ultrasonic treatment was shown to be directly related to the temperature of the coolant bath employed. The lower the temperature of the coolant bath, the greater the extent of crystallization.

(*i*) The degree of saturation of the solvents was observed to affect the amount of hydrocortisone obtained from treated samples. Greater yields of crystals were obtained from more saturated solutions in the same solvent than from lower ones.

(*j*) Decreased cavitation, caused by a reduction in the external pressure of the system, was observed to yield crystals of larger particle size distribution than those obtained from the usual sonic procedures.

(*k*) Enhanced yields of crystals were obtained from samples treated under reduced pressure. The increased amounts of the crystallate were due to the removal of the solvents by the applied vacuum.

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