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— *Review Article* —

Some Pharmaceutical Applications of Ultrasonics

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IT IS DIFFICULT to imagine the scope of ultrasonics until one searches through the recent literature. On gathering material for this review the author has covered approximately 9000 references, a great many of which are allied to pharmacy. It is obvious that a complete review of the literature is impossible in this journal. References were selected which were, in the opinion of the author, most likely to stimulate interest and to illustrate the broad scope of ultrasonic technology.

It is apparent that much less attention has been paid to ultrasonics in pharmacy in the United States than in biology, medicine, chemistry, and engineering. This is brought out in the relatively few research papers in this field which have been published in the past two decades. It is also evident that many more research reports have appeared in foreign literature and in particular in the Russian literature than in our own country.

It would appear that part of the reluctance on the part of the pharmaceutical researcher to use ultrasonics in solving some of his problems has been the lack of standardized equipment or satisfactory standardizing techniques. It is now possible to obtain equipment which will indicate output on a meter and thus aid in standardizing procedures in so far as energy requirements are concerned. With the development of several color-

metric standardizing techniques, it is also possible to standardize procedures even though meters are not supplied with the equipment.

In addition, modern equipment in the ultrasonic field has kept pace with power requirements and actually has much more power than is presently needed in the pharmaceutical field.

For the purpose of this review, the author has divided the references into more or less distinct interest areas. In certain instances, there seems to be some overlapping but it does not appear to be very extensive.

EMULSIFICATION

One of the earliest reports of the use of ultrasound for dispersions of oil and water and mercury in water was by Wood and Loomis (1) in 1927. Richards (2) studied the emulsifying action of ultrasound shortly thereafter and came to the conclusion that the emulsification is due largely to the action of transverse waves in the walls of the glass vessel. He observed clouds of emulsion coming from the sides of the container when he used low intensity sound. He concluded from this that compressional waves within the liquid were not primarily responsible for the emulsifying action.

Bondy and Sollner (3-6) performed a series of experiments to clarify the mechanism of emulsification by ultrasonic waves. It was their conclusion that a so-called "cavitation" at the interface of water and oil caused emulsification. They found (3) that emulsions were obtained when con-

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ditions were such that cavities were formed and collapsed. Liquids irradiated in vacuum formed cavities, but the cavities did not collapse because the vapor pressure inside the cavities was practically in equilibrium with the outside pressure. If liquids were sealed under a gas and exposed to ultrasonic vibration, they were easily emulsified.

Bondy and Sollner (4) concluded from their observations that the mechanism of emulsification of mercury was quite different from that of oils and did not involve cavitation. They confirmed Richards' (2) mechanism, in which it was thought that droplets of water are thrown into the mercury, thin films of mercury form around them, and on bursting, a gray cloud of emulsion is driven into the water. Since this happened even *in vacuo*, cavitation was ruled out as the cause of emulsification.

Campbell and Long (7) were unable to form emulsions of mineral oil, peanut oil, and light liquid petrolatum at concentrations much above 10% with or without emulsifying agents at frequencies of 1000 and 500 kc. From this they concluded that the higher ultrasonic frequencies do not favor the formation of emulsions other than the dilute ones. When they used a generator at a 15-kc. frequency they were able to make a 50% peanut oil-in-water emulsion using triethanolamine stearate as the emulsifying agent. It was found that the globule size of this emulsion was smaller than the same emulsion prepared by hand shaking or by homogenization.

Audouin and Levavasseur (8) experimented with emulsification of peanut, linseed, olive, and mineral oils in an effort to determine the effect of frequency on results. They found that frequency was a predominant factor with regard to the possibility of formation and orientation of an emulsion in a certain direction (w/o or o/w) and only in this direction. The presence of an emulsifying agent normally thought to influence emulsification in the opposite direction did not change the type of emulsion. At a frequency of 960 kc., orientation occurred in the direction of water-in-oil even in the presence of sodium oleate, which normally orients in an oil-in-water direction. At 187 kc. and 320 kc., orientation occurred in the oil-in-water direction in spite of the presence of barium oleate, normally a water-in-oil director. They pointed out that an emulsion begun in a direction with a certain frequency continued to be formed in this direction, even if submitted to another frequency which normally gives an emulsion in the opposite direction. Frequencies of 576 kc. and 720 kc. seemed to have little tendency to form emulsions with most oils. Using a frequency of 25 kc. they first obtained an

oil-in-water emulsion which was abruptly converted to a very concentrated water-in-oil emulsion after 15 min. insonation.

Audouin and Levavasseur (8) found that an elevation in temperature is unfavorable to emulsification and favorable to coagulation, due to lessened cavitation. With a small rise in temperature, there was a reduction in viscosity which favored emulsification. Beal and Skauen (9) came to the same conclusion since a temperature rise did not hinder emulsification; on the contrary, it rendered viscous oils more easily emulsified.

Esche and Wenke (10) state that the formation of an emulsion in an ultrasonic field occurs in cases where the viscosity of the dispersing medium is low—less than 120 cps. As the viscosity of the medium increases, there is greater absorption of acoustic energy with diminished cavitation.

While studying emulsifying agents under the influence of ultrasound, Tamas (11) found that the viscosities of tragacanth and polyethylene glycol 400 solutions were diminished by 50% and 20%, respectively. The particle size of emulsions was first reduced and then increased. They presumed that every kind of oil has an optimum frequency at which emulsions form. In addition, as ultrasonic intensity increases, cavitation forces increase, and the rate of emulsification increases.

There is some disagreement in relation to the relative importance of high- versus low-frequency equipment for emulsification purposes. Relative intensity of insonation has likewise been discussed by numerous investigators. Mounier *et al.* (12) prepared emulsions at frequencies from 958 kc. to 288 kc. and state that a stable oil-in-water emulsion is always formed. An emulsion prepared at one frequency is not destroyed at another frequency regardless of the oil.

According to Lifshits (13), homogeneous emulsions were formed at a sonic frequency of 7.6 kc. The dispersion of the emulsions increased with increase in the duration of irradiation. There was a limit to the decrease in mean globule size.

Neduzhii (14-16) studied the relationship of sound intensity to emulsification. At a frequency of 22 kc. with a water-dibutylphthalate system he found that a threshold intensity of sound was required to initiate emulsification and was different depending upon whether it was a water-in-oil or an oil-in-water emulsion. As the sound intensity was raised, the rate of emulsification increased. He suggests that the particle size of the dispersed phase is independent of the sound intensity. Neduzhii (16) also indicates that on

continued insonation there is a tendency for the particles to begin to coalesce.

Beal (9) investigated the effect of exposure time on a liquid petrolatum emulsion and reported that up to 30 min. there was a continued decrease in globule size.

While reports that highly dispersed and concentrated emulsions have been produced by ultrasonic means without emulsifiers (17), there is general agreement that the presence of emulsifying agents improves the stability and quality of emulsion systems.

Beal and Skauen (9) showed that hard soap was the emulsifier of choice for a mineral oil emulsion, with polyethylene glycol 400 monostearate and polysorbate 80 following in that order. This study proves the necessity to examine the effect of ultrasonics on various components of emulsions, since tragacanth when used as an emulsifier appeared to suffer degradation. Tamas (11) reported a 50% loss in viscosity with tragacanth and a 20% loss with polyethylene glycol 400 monostearate. Haavisto and Hagner (18) attempted to prepare emulsions of peanut oil and water without the aid of emulsifiers and found them to be nonhomogeneous and unstable. Using a 5% total sorbitan monoleate-polyoxyethylene 20 sorbitan monopalmitate¹ (1:1) emulsifier, they found that superior emulsions could be made with 5, 20, and 40% peanut oil and an ultrasonic step-horn transducer.

Emulsions of mineral oil were prepared by Myers and Goodman (19) using a liquid whistle type of apparatus called a Rapisonic.² Liquids are pumped through an orifice at high pressures and velocities against a steel blade, causing the blade to vibrate at ultrasonic frequencies. When the system reaches a steady state, a cavitation field is produced. Results reported by the authors indicated that on microscopic examination the diameter of the globules was generally in the range of 1-3 μ .

Singiser and Beal (20) carried out a study comparing the efficiency of several ultrasonic generators and a colloid mill for the production of an emulsion. Their evaluation covered emulsions of heavy mineral oil, peanut oil, and safflower oil, and four emulsifiers—acacia, polyoxyethylene 20 sorbitan monopalmitate-sorbitan monooleate, poloxalkol³-sorbitan monooleate, and sodium lauryl sulfate. The Minisonic Mark 2⁴ hydrodynamic generator produced emulsions superior to those made by any other apparatus. It would

appear that the hydrodynamic apparatus is the best of the ultrasonic generators for large volume liquid processing. Major advantages, including faster through-put, no premix, faster emulsification, and reduced temperature requirements, are suggested by Marshall (21) and McCarthy (22) for this type of generator.

With the recent development of the step-horn type of ultrasonic transducer, it is possible to study various emulsion systems where small batches are involved and where power output is important. One of the advantages of this type of transducer is that the ultrasonic horn is in direct contact with the liquid being processed with the net transfer of maximum energy. Another advantage considered important for such devices is the availability of a variety of transducers with different power output. It is even possible for instruments to be made to match requirements.

Kann and Tester (23) used a step-horn transducer called the Sonifier⁵ to produce an emulsion for a parenteral contrast medium for lymphography. It was their desire to prepare a 50% emulsion with a particle size less than 0.5 μ with a viscosity of 15-20 cps. at 30°. They reported satisfactory results with both the Sonifier and the Mini-Mill.

EXTRACTION

El'piner (24) has reviewed the literature very thoroughly in regard to viral and bacterial extraction and associated action of ultrasonic waves. He has, in addition, reported on the action of ultrasonic waves on unicellular and multicellular organisms.

This review therefore includes only those references which seem to be most significant with respect to the field of pharmacy.

Much of the early work in extraction was accomplished with equipment of low power output and relatively high frequency. With the recent development of the step-horn transducers with low frequency and high power output, it is expected that many more studies on effects of ultrasound on cells of pharmaceutical interest will appear.

Yamada and Ishida (25) studied the mechanism of fish oil extraction by means of ultrasonic waves. They reported that the muscle protein absorbed the ultrasonic energy selectively, for the temperature rise of the protein always preceded that of the oil layer by about 10°. The protein was coagulated by heat and water was released from

¹ Marketed as Span 80 and Tween 40, respectively, by Atlas Chemical Industries, Wilmington, Del.

² Ultrasonics Ltd., Westgate, Otley, York, England.

³ Pluronic F-68.

⁴ Sonic Engineering Corp., Norwalk, Conn.

⁵ Branson Sonic Power Co., Danbury, Conn.

the muscle. The lipids of the connective tissue within the muscle were emulsified along with the water and flowed out.

Gomez and Matas (26) applied ultrasonic waves to seeds of *Strophanthus gratus* that had lost 50% of its strophanthin on storage. The yield of alkaloid was not improved and was actually diminished in alcoholic solution. The same authors (27) studied the observed loss of activity of extracts of *S. gratus* in terms of alkaloidal degradation and suggested hydrolytic effects due to ultrasonic stimulation.

Head *et al.* (28) studied the effect of ultrasonic waves on the extraction of *Cinchona succirubra*. Ultrasonic extractions at 20 kc. and 450 kc. were compared with conventional Soxhlet extraction under similar conditions. The ultrasonic extraction was more efficient than Soxhlet extraction, and there was no destructive action by the ultrasound on the alkaloids.

Adamski *et al.* (29) also treated cinchona species and showed an increase of 50% in the extraction yield when the bark was treated with ultrasound prior to maceration or percolation. They mention that the keeping qualities of the extracts were good but that decomposition of quinine was noticed with treatment lasting more than 30 min.

Using the Sonifier, Ovadia and Skauen (30) were able to increase not only the rate but the extent of extraction of cinchona bark. No destruction of alkaloids was noted even after 3 hr. insonation.

Colian and Tomas (31) reported that ultrasonic extraction of *Atropa belladonna* at 800 kc. produced 36.7% more alkaloids than mechanical agitation. The yield was only 2.4% superior to solvent extraction but extraction time was shorter. The amount of alkaloids did not increase after 15 min. insonation nor was decomposition observed. Wray and Small (32) also studied the effect of ultrasonic treatment on the extraction of belladonna leaf. Samples of maceration mixtures were put in surface treatment vessels and were subjected to ultrasonic treatment at 500 kc. for 20 min. Then Soxhlet extraction and ultrasonic extraction in a submersion treatment vessel were effected. The ultrasonic maceration shortened the maceration time from 8 hr. to 20 min., and the ultrasonic maceration followed by ultrasonic extraction resulted in considerable saving of time. Analysis of the extract showed no decomposition products of the alkaloids.

El'piner and Stekol'nikov (33) subjected various alkaloids to ultrasonic treatment, among them atropine and quinine. These alkaloids, they state, are attacked by ultrasound. Alka-

loidal salts were treated for 15–90 min. at 800 kc. under saturated air, hydrogen, argon, or helium. The pH of all solutions decreased, with the greatest change in contact with air. Atropine lost its activity by such treatment under air but retained it in a hydrogen, helium, or argon atmosphere. Quinine also showed little change after exposures except in those cases where it was heated for 60–90 min. in contact with air.

Rauwolfia serpentina roots were extracted under ultrasonic irradiation by Bose *et al.* (34). Samples were subjected to ultrasonic maceration at 25 kc. for 5, 10, and 15 min. It was found that the maceration time was reduced from 8 hr. to 15 min. for the complete extraction of the alkaloids.

DeMaggio *et al.* (35) examined the effect of change in frequency on extraction yield of *Datura stramonium*. They state that a 15% increase in alkaloidal yield results when the frequency is increased during a 1–2 hr. insonation period.

DeMaggio and Lott (36) studied the influence of ultrasound on the maceration process as well as during the extraction of *D. stramonium*. The application of ultrasound during short periods of maceration was effective in producing a greater yield of alkaloids than conventional methods. When ultrasound was applied during the process of continuous solvent extraction, it proved to be slightly more efficient in liberating the desired alkaloids from the drug.

Shinyans'kii and his co-workers (37) exposed the dry leaves, seeds, seed pods, and stalks of the poppy plant to ultrasonic waves at 500 kc. for 15–17 min. in an aqueous medium. The extent of extraction of morphine was equivalent to 24 hr. of conventional liquid extraction. A similar study was reported (38) using 22, 300, and 750 kc. on pods of opium poppies in an aqueous environment. Approximately 60% of the morphine was extracted into the aqueous phase during the first 10 min. At each frequency studied, there seemed to be no change in the amount extracted. Length of exposure to ultrasound was an important factor, since the yield decreased with increased exposure time because of decomposition of morphine. Changing the solvent system effected a corresponding increase in morphine yields.

Paradissis and Maney (39) irradiated ergot at 400 kc. and found that ergotoxin was evidently destroyed. When cationic surfactants and polyethoxylated aliphatic amine⁶ were used extraction was improved. They also found that a frequency of 400 kc. was superior to 10 kc. for extraction of ergot.

⁶ Ethomeen C-25, Armour Industrial Chemical Co., Chicago, Ill.

Pivnenko and Sotnikova (40) used a 500-kc. generator for the extraction of ergot alkaloids and found that extraction time was three times more rapid than by the USSR Pharmacopoeia method.

The efficiency of extraction of jaborandi leaf and ipecac root with the Sonifier was investigated by Ovidia and Skauen (30). The amount of alkaloids obtained by ultrasonic extraction of ipecac root was higher than that obtained by the U.S.P. XVI method, while the amount obtained by ultrasonic extraction of jaborandi under temperature controlled conditions was also greater than the B.P.C. 1949 extraction method. The amount of alkaloids extracted from ipecac root in 30 sec. was greater than the amount produced by Soxhlet extraction in 5 hr. Fifteen seconds of ultrasonic treatment resulted in greater yields of from jaborandi leaf than 5 hr. of Soxhlet extraction. After 0.5-min. extraction, jaborandi leaf alkaloids showed degradation, with almost complete degradation after 15 min. Kusano (41) reports no such loss of pharmacological activity of pilocarpine or atropine after ultrasonic irradiation.

Kazarnovskii (42) used ultrasonics as part of an extraction technique for obtaining epinephrine from adrenal glands. Ultrasound was helpful in obtaining complete extraction of all the adrenaline present.

Grochowska (43) reports on the use of ultrasound to aid in removal of barbiturates from human tissue. He states that the time of extraction is considerably shortened.

CRYSTALLIZATION

The use of ultrasonic energy during crystallization of supercooled melts is well documented. Danilov (44) found that the appearance of the first crystallization center of *o*-chloronitrobenzene was accelerated by ultrasound. When salol, naphthalene, and camphor were treated, an increase in rate of crystallization resulted (45). Danilov further reported a large increase in the number of nuclei in piperine after insonation (46). Ultrasonics has been applied to the crystallization of betol (47), thymol (48), benzophenone (48), and piperonal (48).

Supersaturated solutions have been treated with high-frequency sound resulting in smaller crystals and more uniform size distributions. The crystallization of tartaric acid was accelerated by ultrasound, and the crystals were found to be more finely dispersed and homogeneous (49). Thorium oxalate, when prepared by precipita-

tion under ultrasonic treatment, yielded more uniform crystals with smaller particle size (50).

The production and growth of crystals of thymol, potassium alum, salol, Rochelle salt, and hyposulfite from supersaturated solutions treated with ultrasound was investigated by Chormonov (51). He stated that spontaneous crystallization was accelerated by ultrasound.

One of the most energetic workers in the field of ultrasonic crystallization of sugars has been Van Hook (52). He has been able to improve the crystal size distribution of the final crop as well as induce crystallization in such normally refractory supersaturated solutions as arabinose, fructose, sorbitol, glucose, and melibiose.

Progesterone was prepared as microcrystals by insonating a saturated solution of the hormone (53). Cohn (54) investigated the ultrasonic crystallization of hydrocortisone. He developed a procedure which allowed him to prepare low micron particles of hydrocortisone by direct insonation of a saturated solution. Fincher (55) insonated a supersaturated solution of sulfisoxazole and successfully prepared 1.7- μ crystals.

It has been stated (56) that the effect of ultrasound is due to the acoustic wind and to the mechanical vibrations of the crystal. This was based on the observations that the growth rate of a crystal in an ultrasonic field increases with the ultrasonic intensity and that the velocity of growth increases with decreasing supersaturation.

Berlaga (57, 58) reported that ultrasound increased the number of nuclei in betol, which caused a great increase in crystallization rate. He explained this by assuming that the field separates the fresh nuclei from ones formed previously. Levin (45) uses the same theory to explain the effect of ultrasound on the crystallization of salol, naphthalene, and camphor. Smaller crystals were obtained with increased intensity of vibration. It was stated that the decrease in size of primary crystals and the increased rate of crystallization are caused by the cavitation bubbles which separated the growing crystals at the crystal solution interface and carried them into solution. Kudryavtsev (59) suggested that the increased crystallization rate in ultrasonic crystallization is due to an increase in the number of crystallization centers as a result of ultrasonic scattering of the microcrystals forming in the melt.

Kapustin (48) and Polotskii (60) have felt that ultrasound increased the number of crystal nuclei by shattering or eroding the nuclei that occur in a normal manner. Sokolov (61) states that the mechanism is associated with the initiation of the cavitation process which disrupts the

growing crystal. After studying the effect of ultrasound on crystallization of potassium bitartrate, it was concluded that ultrasound augmented the formation of crystalline centers in the beginning stages of the process (62). Kapustin (63) indicates that ultrasonics breaks off microcrystals which act as fresh nuclei. Teumin (64) explains that the mechanism is due to "the formation of cavitation processes, which disintegrate the growing crystals, to radiation pressure, which transports the crystal fragments to the body of the melt where they form fresh crystallization centers, and to agitation, which results in temperature equalization throughout the entire volume, thereby assisting bulk crystallization." Babikov (65) in general agrees with Teumin but also includes the possible effect of ultrasound on the origination of primary crystallization centers, while Meleshko (66) attributes the crystallization effects to the dispersing action of the ultrasonic field.

Kapustin (67, 68) reported that ultrasound effects crystallization by producing many fresh nuclei from any seed. A small crystal of thymol fixed in a wooden holder was immersed in water in a tube. Liquid thymol was carefully layered on the water. No crystals arose on standing, but crystallization began when ultrasonics was applied to the bottom of the tube. He observed the field to break off minute particles from the seed which were carried to the interface. Identical tests without the seed did not initiate crystallization. Sokolov (69) also found that insonation produced more rapid crystallization when solutions of beet sugar and zinc sulfate were seeded prior to insonation.

Kapustin (70) studied the process of relieving supersaturation in an aqueous solution of potassium alum by ultrasonics and reports desaturation occurs in the presence and absence of nucleation.

Polotskii (71) presented photographic evidence to support the belief that ultrasound acts by dispersing nuclei. He observed cavitation at the crystal-melt interface of supercooled, chemically pure salol to cause strong dispersion of the newly formed crystals. In addition Polotskii (72) photographed the shattering of acicular crystallites of salol and a naphthalene-camphor eutectic shortly after their formation. Without ultrasound, this did not occur.

Van Hook (73) found that the number of crystallization centers increases with the duration of insonation in an autocatalytic way. Due to the limiting effect of prolonged treatment, it was suggested that ultrasound acted chiefly on newly formed crystallites. Nickus (74) makes a sim-

ilar observation, stating that insonation at 25-50 kc. accelerated the formation of crystallization centers as well as increasing the crystallization rate of molten menthol. Kapustin (75) stated that ultrasonics brought about the formation of new crystallization centers only when a crystal reaches a certain size.

A number of workers have felt that ultrasound generates crystal nuclei. An investigation of the effect of ultrasound on supersaturated solutions of thymol, Rochelle salt, salol, and hyposulfite proved that ultrasound significantly accelerates the process of spontaneous crystallization and plays an essential role during the formation of nuclei (51).

Kapustin (76) stated that ultrasound affects the production of the first crystallization center. This was based on the action of ultrasound on *o*-chloronitrobenzene. The compound was heated to 100°, slowly cooled, and exposed to 10 sec. of 700 kc. ultrasound of varying intensities. The field did not affect the production of the first crystallization center above 20° but caused "spontaneous crystallization" at 18 to 20°. He explained that a system which is macroscopically homogeneous has a certain micro nonhomogeneity which varies little with time. Thus ultrasound could cause a redistribution of the micro nuclei and alter the probability of spontaneous production of crystals.

Brown (77) states that "the effect of ultrasonic irradiation on the crystallization process is similar to its effect on precipitation, *i.e.*, the cavitation produced offers nucleation centers for the crystals to initially form around, and it also breaks up any large crystals." Mazhul (78) studied ultrasonic crystallization and especially the electric fields associated with cavitation. His experiments led him to conclude that nucleation in the melt cannot be explained by the electric fields associated with cavitation. Rather the sites of cavitation themselves act directly as nuclei. He adds that cavitation cannot be taken as the sole cause of the effects, for weak fields which do not cause cavitation still increase the nucleation rate. After experimenting with betol, Mazhul (47) later stated that cavitation ruptures may themselves become crystallization centers under varying external pressures. Glinkskii (79) agrees in reporting that the effect of ultrasound on primary crystallization is associated with a decrease of grain size in the melt under pressure. He shows on a theoretical basis that equal compression and rarefaction cause the same effect as to the number of crystal nuclei arising. The effect of ultrasound increases with lower supercoolings.

During the ultrasonic crystallization of sulfisoxazole (80) it was explained that ultrasonics aids crystallization "by supplying cavitation bubbles, which act as the site of nucleation."

In his work on the ultrasonic crystallization of sugar, Van Hook (81) proposed both a heterogeneous and a homogeneous mechanism. He appeared to agree with Mazhul and Brown when he stated that "if one irradiates a strong syrup with a bar transducer, cavitation loci appear at definite points according to the size, shape, and arrangement of the system. The crystal nuclei are generated, presumably within these small volumes. They may then be dispersed and grow to visible size." He also reported that irradiation of a "sterile, isolated solution" gave poorer results or no improvement in grain at all. This suggested to him a heterogeneous mechanism rather than the spontaneous generation of nuclei by the ultrasonic field.

Hem (82) reported reproducible reduced crystal size distributions from insonating a supersaturated solution, whose background particle count was controlled, at a fixed temperature. The crystallization temperature of both insonated and uninsonated samples was dependent on the number of heterogeneous nuclei present.

DISPERSION

The application of ultrasonic waves for the dispersion of coherent solid materials dates back to the classical work of Richards (2) in 1929. He reported unsuccessful attempts to disperse such solids as glass and quartz in liquids. Sollner (83) commenting on this work says that only brittle and soft substances of high cleavage seem to offer promise. He irradiated mica, gypsum, steatite, and hematite in water, producing colloidal or semicolloidal solutions.

Most of the early work was done with coherent solid bodies, but it has been shown that suspended particles can be dispersed. In all systems containing preformed particles the latter may be separated from one another by insonation. Sound-dispersed preparations do not show any special features, the sound-induced cavitation being merely an efficient mechanical dispersing agent (83).

The sonic peptization of gels and gel-like substances has been investigated (84-86). Using a liquid whistle, Ramaszeder (87) dispersed potato starch and suggests that the particle size of the starch granules can be reduced by increased time of insonation. He suggests the use of ultrasonic dispersion of starch granules as an alternative to mechanical homogenization (88).

The dispersion of dyestuffs and pigments by ultrasonics has been reported in the literature (89-91). Fischer (92) stated that the disintegrating effects of ultrasound is negligible, and the changes produced cannot be distinguished from those caused simply by heating and stirring of the pigment. However, since more powerful equipment has become available, other workers have reported some success in dye dispersion. Lifshits (93) compared dye dispersion using piezo-quartz, magnetostrictor, and hydrodynamic ultrasonic devices and a mechanical mixer. He states that a higher degree of dispersion was obtained by ultrasound than by mechanical mixing. Prakash (94-97) has studied the fading of colors of various dyes such as Congo red, rhodamine B, bromocresol green, and safranin O. He explains the decolorization on the basis that the high cavitation energy is responsible for a molecular breakdown of the dye.

Chambers (98) reported the preparation of microcrystals of sulfathiazole using ultrasound. Direct sonic vibration of suspensions of the preformed crystals produced no measurable effect. An indirect method was developed consisting of the neutralization of a solution of the sodium salt of the drug while the reaction mixture was kept in violent agitation by sonic vibrations. The size of the resulting crystals was regulated by controlling the speed of admixture or the temperature of the reactants or both. The microcrystals produced were fairly uniform in size. Gartner (99) investigated the action of ultrasound at frequencies of 500, 800, and 1500 kc. on aqueous suspensions of magnesium carbonate, lead carbonate, and barium sulfate. Best results were obtained at a frequency of 500 kc. and at suspension concentrations of 0.002 Gm./ml. Misek (100) studied the suitability of ultrasonic energy for dispersing progesterone in suspensions. He used a surface-active agent to obtain a satisfactory dispersion and states that the degree of dispersion increased as the ultrasonic intensity increased. Several other parameters were also studied (101). It was found that the extent of dispersion decreased as the viscosity of the suspending liquid increased. The application of 15 lb. of pressure upon the suspension during insonation rendered dispersion most complete. Such dispersions showed that 85% of the suspended material was 10μ or less in size. At the frequencies studied, 300, 500, and 750 kc., the frequency of the ultrasonic vibrations had no effect on the dispersion. Araujo (102) insonated suspensions of zinc oxide, bismuth subcarbonate, sulfathiazole, and procaine penicillin G at 400 kc. In all the cases studied, they report that ultrasonic energy

reduces the particle size of the suspended medication and retards its rate of sedimentation, providing a more stable preparation. The dispersive action of ultrasound on crystals of potassium dichromate in ethanol was observed by Kosolapov (103). Sheikh (104) suspended polyethylene spheres in water and subjected them to the action of ultrasonics at 100 kc. He found that there was a significant reduction in particle size only if a surfactant was present. Increasing insonation time brought about reduction up to 2 hr.

When dealing with the action of ultrasound on suspended material, one must be concerned with the fact that dispersion is not the only effect that can be obtained. Coagulation can also be brought about by ultrasonic irradiation of suspensions. Bondy and Sollner (6) have explained the action of ultrasound on dispersions. They suggest that dispersion requires an energy level exceeding a certain limit, and coagulation may therefore result below this energy limit. Dispersion may also be precluded by prevention of cavitation.

Thompson and Vilbrandt (105) investigated the effect of ultrasonic energy on the coagulation of clay particles and reported that mild intensity insonation (below cavitation threshold) increased the rate of coagulation, while intense insonation (above cavitation threshold) retarded settling of the clay due to dispersion and disruption of the aggregates.

Kruglitskii (106) obtained a more uniform disperse phase when montmorillonite was insonated at 19.5 kc. He reports cavitation breaking of bonds between the disperse phase and the dispersion medium. After 8.5 min., dispersion is maximum, and the clay structure formed has maximum bond energy and stability. Further treatment caused coagulation with the coagulated particles so stable that further treatment does not destroy them. Kruglitskii (107) reports that more satisfactory thixotropic properties and greater stabilities can be obtained by treating clay suspensions with ultrasound, colloids like carboxymethylcellulose, and sodium chloride or calcium chloride than by using any single one or combination of any two of these means.

OTHER PHYSICAL-CHEMICAL AND CHEMICAL EFFECTS

For a very thorough and instructive discourse the interested person is urged to read the text of El'piner (180).

The effect of ultrasonic waves on the degradation of natural and synthetic polymer solutions has been well established.

In 1949, Prudhomme and Graber (108) claimed

that the rupture of high polymer chains was due entirely to cavitation. Time required for depolymerization was inversely proportional to the intensity of sound and directly proportional to the concentration, but the final degree (limiting degree) of depolymerization was independent of these factors. They also claimed that polymers of the same composition but different molecular weights approach the same limiting chain length with longer periods of insonation. Later, Prudhomme (109) confirmed his claim that the rupture of the polymers was due entirely to cavitation by insonating 1% aqueous solutions of carboxymethylcellulose at 960 kc. under vacuum, air, and CO₂. He showed that the forces of friction between solvent and polymer molecules are not the basis of the degradation process. Weissler (110) observed that the depolymerization of polystyrene in toluene occurred only in the presence of cavitation. A sample degassed by boiling under vacuum showed no cavitation and no appreciable molecular weight change. This study revealed that oxidants produced by ultrasonic waves cannot be responsible for degradation. Jellinek and White (111) tried to study the number and weight-average molecular weight distributions and number and weight-average chain lengths of polystyrene after insonation and found satisfactory agreement between calculated values for an assumed degradation mechanism and experimental results.

Head and Lauter (112) investigated the ultrasonic depolymerization of a number of natural and semisynthetic polymers. By relating viscosity measurements to molecular weights, they showed that most of the polymers followed a first-order rate equation for degradation. Okuyama (113) explained polymer rupture by ultrasound by stating that at the contracting step of a violently vibrating bubble, the polymer molecule takes a linearly extended configuration owing to the suction force of the bubble occurring by slipping and friction between solvent molecules and the extended part of the polymer. The partial chain is subject to a tensile force arising from friction, which is maximum at the middle carbon atom of the chain. If this force is greater than the critical tensile strength of the C—C bond, the polymer degrades. Zorina and El'piner (114) exposed an aqueous solution of dextran (mol. wt. 35,000) to 840 kc. ultrasound in atmospheres of oxygen, nitrogen, and argon. After 3–4 hr. of insonation the molecular weight decreased to a minimum value of about 20,000. When irradiated in an alkaline solution (pH 13) dialyzable particles were formed. They were also able to produce low molecular weight substances by ir-

radiating solutions of mono-, di-, and trisaccharides at pH 13. El'piner and Pyshkina (115) exposed a 5% solution of sodium carboxymethyl-cellulose solution to 740 kc. ultrasonics and found that in the presence of oxygen a decrease of molecular weight and viscosity occurred, while in the presence of hydrogen the molecular weight increased and the degree of hydration and viscosity decreased.

Kukoz (116) discussed some of the literature on the chemical action of ultrasonics. The results of experimental studies confirming the cavitation-photoelectrochemical theory of chemical action of ultrasound and experiments demonstrating the need of improving or modifying this theory are given. He mentions that free radicals, ions, and excited molecules of the medium can all play a definite role in ultrasonic action. Information is presented showing the contradictory view that chemical action of ultrasound is explainable by local increase in temperature or the mechanical-chemical effects. He makes the important point that the chemical action of ultrasound on an aqueous medium may be due to many factors, and the role of each of these factors must be analyzed separately in each concrete case.

Vaisman (117) investigated the stability of some medicinal solutions toward ultrasound. The resistance of a series of inorganic and organic pharmaceuticals in aqueous solutions of different concentrations to the action of ultrasound was studied with respect to the visual changes of the solutions, the pH changes, and in some cases the decomposition products. At 800 kc. for 15 min. insonation, sodium chloride, potassium chloride, ascorbic acid, and phenol did not undergo a change. Potassium iodide, sodium salicylate, resorcinol, epinephrine, and procaine HCl were oxidized, and magnesium sulfate and zinc sulfate were partially hydrolyzed.

Maffei (118) treated pure expressed olive oil with ultrasonic waves. He noticed a shift of fluorescence from yellow to blue and attributes this to trace amounts of substances formed by decomposition of the glycerides of the oil. He concluded that the alteration of the visible spectrum is connected with a partial decomposition of carotenoids and chlorophyll.

Faust (119) used a 400-kc. generator to observe its effects on glutathione showing oxidation of the reduced glutathione. Nearly one half of the tripeptide is reported to have been lost after 41 min. exposure.

Examination of protein fragments produced by ultrasonic treatment of aqueous solutions of serum albumin or trypsin showed that the ultrasonic treatment did indeed produce protein fragments.

Such fragments are much more prevalent immediately following the irradiation than with the passage of time and disappear completely within 30–40 min. (120). Myosin and deoxyribonucleic acid specimens also showed this effect, yet gelatin failed to do so. Negative results were also obtained with substances such as glycine, alanine, cystine, glucose, and succinic acid. It was concluded that the fragmentation of these macromolecules caused by ultrasound produces macroradicals with half-life of the order of some minutes. A semiquantitative estimation showed that the molecules of serum albumin are cleaved into three parts, while those of trypsin are cleaved into two parts.

Miyagawa (121) reports acetates being hydrolyzed twice as rapidly as normal under the influence of 540 kc. ultrasonic waves. Mario (122) followed the rate of hydrolysis of aspirin with and without the influence of ultrasound. He states that there is an increase in the rate of hydrolysis using ultrasound that is comparable to the effect obtained by increasing the reaction temperature. On the other hand, Fenn (123) reported no significant difference in the rates of hydrolysis of procaine and butethamine hydrochlorides when insonated at 400 kc.

El'piner (124) followed the decomposition of sucrose in an ultrasonic field. The sucrose decomposed, as shown by the appearance in solution of reducing substances which increase in concentration with increased duration of exposure. The presence of carbon tetrachloride in buffered sucrose solutions enhances the rate of decomposition. Buchnea (125) subjected solutions of sucrose, glucose, and fructose to ultrasonic waves at various frequencies for times up to 100 hr. under various conditions. With increasing frequency the temperature of the solution increased, favoring splitting of sucrose. In the presence of oxygen, sucrose decomposed more rapidly with the formation of carbon dioxide and formic acid. With hydrogen, only slight inversion occurred without acid formation. The rate of inversion increased with sucrose concentration, but none resulted if the solution was cooled. The decomposition of the hexoses depended mainly on oxidation. Zapior (126) also reported an increase in rate of sucrose hydrolysis especially in strongly acid solutions but stresses the fact that the temperature was verified indirectly by use of a thermometer in the water bath.

El'piner (127–129) subjected insulin to ultrasonic irradiation and reported changes in the molecule which are influenced by the nature of the atmosphere in which treatment was performed. In the presence of oxygen or argon the

hormonal activity decreases, while in a hydrogen atmosphere the activity is increased. Chromatographic analysis showed that the hormone lost different substituent groups depending on the atmosphere used.

After ACTH in aqueous solution was exposed to 800 kc. ultrasound for 1 to 4 hr., a solution of yellow color and distinctive odor was produced (130). End-group analysis showed that methionine rather than serine became the end group. Longer exposure produced a solution which failed to respond to the usual end-group analysis.

Gramicidin-S was treated with ultrasound in ethyl alcohol by El'piner (131). This resulted in the formation of glyoxalic acid, but no great change in spectrophotometric analysis was noted in 8 hr. whether it was conducted in an atmosphere of oxygen or argon. However, the biological activity of the antibiotic was increased in these atmospheres. No such increase in activity was observed after treatment in a hydrogen atmosphere.

Gerraughty (132) explored the possibility of using the energy of ultrasound to effect the direct esterification of sterically hindered acids. The methyl, ethyl, *n*-propyl, and *n*-butyl esters of *o*-thymotic and ursolic acids were prepared. Branched chain esters of the acids, however, could not be made.

A number of investigators have studied the effect of ultrasonic irradiation on distilled water. Akiya (133) showed that irradiated water forms H_2O_2 , HNO_2 , and HNO_3 . The amount of these formed is related to the intensity of the ultrasonic waves. At maximum intensity the amount of these substances varies with the length of radiation. He also reported the liberation of iodine from an acid potassium iodide solution under ultrasonic activity. Grabar (134) also mentions the decomposition of water by supersonics into H_2 and O_2 . When water is subjected to ultrasonic waves at frequencies of 404 and 960 kc. in the dark, a nonuniform blue luminescence is produced which is independent of the pH or the presence of oxygen (135). Even in the absence of oxygen, water treated with ultrasonics develops oxidizing powers which are retained after treatment is stopped. A photochemical reaction probably occurs by which water is converted to H_2O_2 , H , and O .

Bhar (136) observed that when a solution of potassium iodide was insonated, decomposition resulted with the amount of iodine liberated being proportional to the strength of the ultrasonic field and inversely proportional to the strength of the iodide solution. Others (137-139) studied various aspects of the oxidation of

potassium iodide solutions, such as the effect of frequency, the kinetics of oxidation, and the mechanism of oxidation.

Weissler (140) developed a simple colorimetric procedure to measure cleaning efficiency of ultrasound. This was based on the liberation of chloride ion from carbon tetrachloride in an aqueous solution. Lur'e (141) indicates that the equation for the above reaction at a pH of 12 is as follows: $CCl_4 + H_2O \rightarrow Cl_2 + 2HCl + CO$. As the duration of insonation increased from 10-60 min., the degree of decomposition went from 60.3% to 100%. Liu (142) suggests the use of the reaction cited above as a technique to test for cavitation-activity measurements. He reports that chlorine is liberated from carbon tetrachloride in a linear relationship with time. A linear relationship to cavitation can also be plotted. Prakash (143) observed a similar result when he irradiated chloroethane in aqueous solution. He suggests that the reaction is governed by ultrasonic cavitation.

MISCELLANEOUS

Cleaning by high frequency sound is now a standard procedure in many industries. Central sterile supply departments are routinely washing glassware, surgical instruments, syringes, and needles in automatic ultrasonic equipment.

Clayton (144), Mamer (145), and McKay (146) discuss the use of ultrasound in the hospital environment. Beal and Skauen (147) were able to show that hypodermic syringes could be cleaned in a satisfactory manner by high-frequency sound. They later compared the cleaning efficiency of ultrasound with other methods used in hospital practice using radioactive iodide- ^{131}I soils (148).

Palmer and Skauen (149) compared various cleaning methods for catheters using an iodide- ^{131}I contaminated soil. Ultrasound cleaning was proven to be unfavorable in this study and was attributed to the "soft" nature of rubber catheters. Gligorijevic (150) found this to be a factor in a similar study, and he concludes that decontamination with ultrasound depends on the surface and the material.

Witekowa (151) felt that ultrasound might be used in various absorption phenomena. Accordingly, he treated a sample of charcoal with an aqueous solution of methylene blue and irradiated it with ultrasound. The suspension was treated with water, then filtered. The concentration of the methylene blue in the filtrate was determined colorimetrically. The quantity of absorbed methylene blue was 8-10% higher in the insonated sample than the control and increased

with power and frequency of ultrasound. He explains the increase on the basis of increased degree of fineness and a change of electrochemical properties of the charcoal.

Soloff (152) reviews the use of ultrasound for the sealing of various types of plastics. While this is a recent development, it would appear to offer certain benefits to the pharmaceutical industry.

Kuppusami (153) offers the suggestion that ultrasound can be used for determining the critical micelle concentration of surfactants. In his experiments he measured ultrasonic velocity in solutions of sodium dodecyl sulfate, cetyltrimethylammonium bromide, potassium laurate, and tetrahydroanacardol ammonium monosulfate. Each showed a reversal in slope of the velocity *versus* concentration curve in the region of the critical micelle concentration in agreement with the literature. This decrease in ultrasonic velocity he explains on the basis of internal pressure.

A list of reviews and books pertinent to the subject is included under *References* (154-185).

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Research Articles

Anti-Inflammatory Evaluation of Cryogenine

By HARVEY R. KAPLAN*, RICHARD E. WOLKE†, and MARVIN H. MALONE

Cryogenine was effective in limiting the development of artificially induced inflammatory responses in acute and chronic experiments in rats. Cryogenine altered the acute edematous reaction to plantar injection of carrageenin and inhibited development of increased foot thickness induced by plantar injection of nonviable mycobacterial adjuvant in chronic studies. Histopathologic examinations did not suggest actions that would interfere with the anti-inflammatory evaluations. Cryogenine demonstrated a low order of analgesic (rat tail flick) and antipyretic (peptone fever) activity, and was capable of partially reversing edema and pain produced by silver nitrate injections in the ankle joints of rats. Cryogenine blocked serotonin, bradykinin, and histamine responses in guinea pig ileum; and pretreatment limited serotonin-induced pedal edema in rats and blocked histamine-induced intradermal wheals in the rabbit. Cryogenine was ineffective as a fibrinolytic agent. Prototype nonsteroidal anti-inflammatory compounds as well as agents with structural similarities to cryogenine were also investigated. Cryogenine may, like aspirin, manifest its anti-inflammatory activity through a combination of selective central and non-specific peripheral mechanisms.

CRYOGENINE (vertine) was first isolated from *Heimia salicifolia* Link and Otto in 1963 by Blomster *et al.* (1), and its chemical structure has been proposed (2) and confirmed (3). The folklore concerning this Mexican plant is interesting and includes mention of its abilities to induce bizarre central nervous system reactions (4-6). The initial pharmacologic studies of Robichaud *et al.* (7) on both plant extracts and purified cryo-

genine alkaloid revealed qualitative and quantitative similarities between the two with the most interesting activity being the production of a selective central nervous system depression in unanesthetized animals. The general pharmacodynamics of cryogenine and other of the naturally occurring alkaloids have been reported in the literature (8, 9). Preliminary evaluation in this laboratory also indicated that cryogenine possessed activity in an acute anti-inflammatory screen in rats. These initial data suggested that cryogenine could be a new chemical prototype with nonsteroidal anti-inflammatory activity. Most recently Jiu has reported (10) that ethanol extractives from the plant showed depressant central nervous system activity, anti-atherogenic potential, and anti-inflammatory ability.

EXPERIMENTAL

General—During the investigations cryogenine was employed either as an aqueous suspension of

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