# Application of Ultrasound for Increasing Alkaloid Yield from *Datura stramonium*

By A. E. DEMAGGIO and J. A. LOTT*

**A new approach is presented for the evaluation of ultrasonic energy when applied to the isolation of alkaloids from *Datura stramonium*. The method utilized made it possible to study the influence of ultrasound on the maceration process as well as during the actual extraction process. The application of ultrasound during short periods of maceration was effective in producing a greater yield of alkaloids than obtainable by conventional procedures. When applied during the process of continuous solvent extraction, ultrasound proved to be slightly more efficient in liberating the desired alkaloids from the drug. The utilization of ultrasound in commercial extraction procedures and for theoretical experimentation is discussed briefly.**

A NUMBER OF investigators have demonstrated that ultrasonic energy can be utilized productively in various extraction processes. The application of ultrasound to the extraction of enzymes, nucleic acids, and other biochemically important constituents from unicellular organisms and multicellular tissues is common practice. In addition, ultrasonic energy has been employed for the extraction of oil from peanuts (1) and fish tissue (2), bitter principles from hops (3, 4), poisonous substances from animal tissue homogenates (5) and in studies of immiscible liquid-liquid extraction (6, 7). Results of these studies have supported the expectation that a greater yield of the desired materials would be obtained in a shorter time period using ultrasound than by utilizing conventional extraction procedures alone. Moreover, these selected examples also serve to illustrate the potential application of ultrasound especially for the extraction of desired materials from both plant and animal sources.

In the last decade, several interesting studies have been conducted to determine if the more efficient extractions observed when ultrasound was applied to the diverse systems indicated above could be noted in the extraction of alkaloids from medicinal plants (8–12). To determine the effects of ultrasound on the extraction of alkaloids from *Cinchona succirubra*, Schultz and Klotz (8) employed a high frequency of ultrasound (2.4 Mcy[1]) and a frequency of sound in the audible range (less than 20 Kcy[2]). An improved yield of alkaloids was obtained from samples of drug treated with the audible frequency of sound and not from those samples exposed to high frequency ultrasound. In contrast, Head, Beal, and Lauter (9) employed frequencies of 20 Kcy and 450 Kcy ultrasound to extract cinchona alkaloids and obtained an increased yield of alkaloids from ultrasonically treated samples of the drug. It should be noted that at the higher frequency of ultrasound used by Schultz and Klotz, little gaseous cavitation probably was occurring since the minimum power necessary to initiate cavitation at this frequency would be very great. The majority of ultrasonic effects, *e.g.*, disruption of cells, which would influence extraction processes, generally are attributed to the occurrence of cavitation and the accompanying energy. In view of present knowledge concerning the mechanism of ultrasonic action, it is reasonable to assume that in the absence of cavitation little or no beneficial effect of ultrasound on the extraction of alkaloids would be noted.

During the extraction of alkaloids from *Atropa belladonna*, Wray and Small (10) observed that the insonation of a maceration mixture of solvents and ground drug for 20 minutes produced the same yield of alkaloids as a conventional maceration of 8 hours when both were followed by Soxhlet extraction. Bose, Sen, and Ray (11) have reported that the maceration time for the complete extraction of alkaloids from roots of

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[1] Megacycles per second.
[2] Kilocycles per second.

*Rauwolfia serpentina* can be reduced from the conventional 8-hour period to 15 minutes when ultrasound at a frequency of 25 Kcy is employed. Similar results have been reported by Colian and Tomas (12), who propose that the total energy accompanying cavitation in an ultrasonic field is considerably superior to mechanical mixing in facilitating extraction.

The present study originally was initiated to determine the influence of two frequencies of ultrasound (20 Kcy and 40 Kcy) on the isolation of alkaloids from *D. stramonium*. However, early in our work the need for characterizing the effect of ultrasonic treatment during the maceration process, opposed to the effect of ultrasonic treatment during the extraction process, became evident. In none of the previously published reports has a clear distinction been made between the maceration and extraction procedures involved in the isolation of alkaloids. The maceration procedure usually consists of the soaking and swelling of the drug in a suitable solvent or mixture of solvents and is followed by the extraction procedure which consists of the physical separation of the alkaloids from the drug, commonly by percolation or continuous extraction in a Soxhlet or similar apparatus. In published reports concerned with ultrasonic extraction, ultrasound has been applied to the macerating mixture and "true" ultrasonic extraction, in the sense of repeated exposure of the drug to fresh supplies of solvent during continuous ultrasonic treatment, has not been performed previously. The construction of a special extraction apparatus made it possible for us to analyze separately ultrasonic treatment when applied during the maceration process or when applied during the extraction process. The experiments reported here were designed to determine to which phase of the isolation procedure ultrasound can be utilized most efficiently for increasing alkaloid yield or decreasing maceration or extraction time.

## EXPERIMENTAL

**Materials and Equipment.**—Commercially available *Datura* leaf, granular (S. B. Penick); U.S.P. grade reagents and solvents; polyethylene wide mouth containers, 300 ml.; Soxhlet extractors; and a newly designed extracting apparatus (13) for ultrasonic extraction were employed. In addition, three commercial ultrasonic generators and cleaning tanks were utilized: 20 Kcy magnetostriction unit (Bendix Aviation Corp., model No. UC-4X8, 140 w.); 40 Kcy piezoelectric unit (National Ultrasonic Corp., Model No. 100, 60 w.); 40 Kcy piezoelectric unit (Circo Ultrasonic Corp., model No. 125T, 125 w.).

**Procedure for Ultrasonic Maceration.**—The influence of 20 Kcy and 40 Kcy ultrasonic energy when applied during the maceration of the drug was studied employing the three generators previously listed. Ten-gram samples of granular *Datura* leaf were placed in 300-ml. polyethylene containers and 80 ml. of a macerating solvent (ether, 20; U.S.P. alcohol, 12; and ammonia, 8 parts by volume) added. The recommended U.S.P. volume of macerating solvent was increased to cover the drug in the containers completely. The containers were then immersed to the neck in the water of the ultrasonic bath and the drug samples macerated with ultrasonic energy for periods of 30 minutes or 1 hour as indicated in the data. To prevent vaporization of the solvent during ultrasonic treatment, water-cooled reflux condensers were attached to the polyethylene containers, and the water in the ultrasonic bath was maintained 8–10° below the boiling point of ether (34.6°). This was accomplished by pumping the water through coils immersed in a constant temperature bath maintained at 25 ± 2°. After maceration was completed, the samples were transferred to extraction thimbles and extracted for 3 hours in a Soxhlet.

**Procedure for Ultrasonic Extraction.**—Ultrasonic extraction was studied employing the 20 Kcy Bendix and the 40 Kcy Circo ultrasonic generators. The 40 Kcy National ultrasonic generator was excluded from this phase of the investigation since results of the maceration studies showed it to be less efficient than its counterpart, the Circo unit. Both the National and Circo generators are piezoelectric units.

Maceration of the drug was performed in essentially the same manner indicated in the preceding section. However, in this phase of the study, ultrasound was not employed in macerating the samples. Samples of the drug were macerated by the conventional method for either a varied period of time or for a fixed period, depending on the design of the experiment. Following maceration, all samples were extracted in the newly designed extractor, while continually subjected to ultrasonic energy for various time periods.

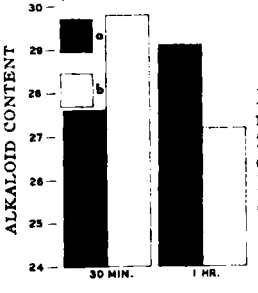


Fig. 1.—Results of 20 Kcy (Bendix) ultrasonic maceration of the drug, followed by 3 hours of conventional extraction. Key: *a*, control samples; *b*, insonated samples.

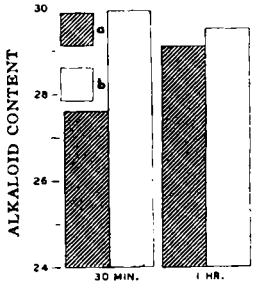


Fig. 2.—Results of 40 Kcy (National) ultrasonic maceration of the drug, followed by 3 hours of conventional extraction. Key: *a*, control samples; *b*, insonated samples.

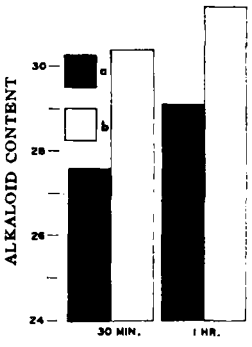


Fig. 3.—Results of 40 Kcy (Circo) ultrasonic maceration of the drug, followed by 3 hours of conventional extraction. Key: *a*, control samples; *b*, insonated samples.

**Assay.**—Samples were assayed for alkaloid content according to the U.S.P. XVI assay for belladonna leaf. Experiments were performed in triplicate; the assay results were averaged and expressed as milligrams of alkaloid per 10-Gm. sample of treated drug.

Results of assaying the maceration mixture for total alkaloids prior to Soxhlet extraction are not included in this report. Preliminary studies have shown that it is more satisfactory to compare results from maceration and extraction studies when similar treatments are employed—in this case, a period of continuous solvent extraction is included as a routine part of both procedures.

## RESULTS AND DISCUSSION

**Ultrasonic Maceration.**—For the initial studies, the influence of ultrasound on the macerating mixture was observed for intervals of 30 minutes and 1 hour. Using the 20 Kcy Bendix generator, the alkaloid content of samples insonated for 30 minutes was about 9% greater than that of control or non-insonated samples (Fig. 1). When the insonation time was doubled, no increase in alkaloid yield was noted; the yield from the control samples, in this case, was slightly greater than the yield from insonated samples. A similar situation did not exist, however, in the maceration studies using the two 40 Kcy ultrasonic generators (Figs. 2 and 3).

With these 40-Kcy units there was a consistent increase in alkaloid yield when samples were macerated with ultrasound. Figure 2 shows the results obtained when samples were macerated 30 minutes and 1 hour with ultrasonic energy supplied by the National unit. A 9% increase in the yield of alkaloids was obtained when samples were exposed to ultrasound for 30 minutes, while a smaller increase (approximately 3%) was noted for samples macerated during 1 hour. Using the Circo ultrasonic generator (Fig. 3), a 10% increase in alkaloid yield was observed for samples macerated with ultrasound for 30 minutes. When samples were macerated for 1 hour with ultrasound, the increased yield of alkaloids was approximately 9% compared to control samples.

Conventional maceration without ultrasound when carried out for 1 hour was effective in causing an increase of 3% in alkaloid yield compared to samples macerated for only 30 minutes. It is interesting to note that only with the 40 Kcy Circo ultrasonic generator did samples macerated for 1 hour yield greater quantities of alkaloids than samples macerated for 30 minutes. An increase of 4% in total alkaloids was noted when samples insonated with the Circo unit for 30 minutes and 1 hour were compared. While no reason is readily apparent to account for the increase in alkaloids obtained using one ultrasonic generator and not the other two, there is some indication that after prolonged maceration, the difference in yield of alkaloids from insonated and control samples diminishes. This difference cannot be attributed to variations in the power output of the ultrasonic generators used.

From the preceding data, it is evident that the difference in the amount of alkaloids recovered between control and insonated samples is greatest during the first 30 minutes of ultrasonic maceration. Moreover, these data indicate that the 40 Kcy Circo generator is more effective than the other units employed in releasing the alkaloids from the drug during the short maceration periods used.

Short periods of ultrasonic maceration also have been effective in liberating the desired alkaloids from cinchona, belladonna, and rauwolfia. Head, Beal, and Lauter (9), employing 15 minutes of ultrasonic maceration, noted considerable increase in alkaloid yield from *C. succirubra*. A similar period of ultrasonic maceration was beneficial in extracting alkaloids from *R. serpentina* (11); while Wray and Small (10) macerated *A. belladonna* leaf with ultrasound for periods of 5 to 20 minutes and found 20-minute maceration times most effective. The similarity of results from these investigations, utilizing different ultrasonic generators and different species of medicinal plants, demonstrates that the effect of ultrasound is beneficial for increasing alkaloid yields when employed in conjunction with short periods (less than 1 hour) of maceration.

**Ultrasonic Extraction.**—The extraction studies to be described here differ fundamentally from previous reports concerned with ultrasonic extraction because a distinct separation of the maceration process from the actual extraction process has been achieved. For the present work, an extraction apparatus was utilized which made it possible to subject the drug sample to ultrasonic energy while, at the same time, performing continuous extraction of the liberated alkaloids. From previous data obtained during the maceration studies, it appears that a 10% increase in alkaloid yield is the maximum which can be obtained utilizing ultrasound in the manner described. The present series of experiments were undertaken to determine whether a greater yield of alkaloids could be obtained when ultrasound was applied during the extraction process rather than when applied during the maceration process.

In the first extraction experiment, the drug was extracted with ultrasound supplied by the 20 Kcy Bendix unit. To determine the interaction of maceration time and extraction time, experiments were conducted in which the drug was macerated for various time intervals (2 to 24 hours) and extracted for a fixed period of 2 hours. The 2-hour extraction period was chosen after preliminary results revealed that the maximum effect of ultrasound could be best observed during this time. A synopsis of the results is presented in Fig. 4.

A 10% increase in alkaloid yield was obtained from samples of drug macerated conventionally

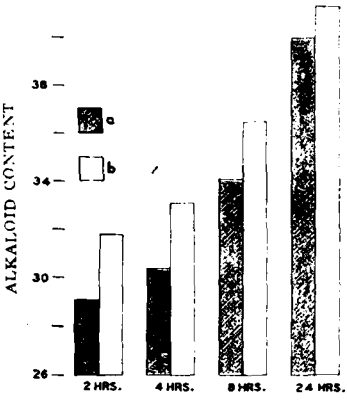


Fig. 4.—Results of 20 Kcy (Bendix) ultrasonic extraction of the drug for 2 hours, following various periods of conventional maceration. Key: *a*, control samples; *b*, insonated samples.

for 2 hours and for 4 hours and extracted with ultrasound for 2 hours. As the maceration time was increased, the difference in alkaloid content between control and insonated samples diminished; only a 3% increase was noted in samples macerated for 24 hours. These data suggest that ultrasonic extraction is most efficient when employed in conjunction with short periods of conventional maceration. However, this effect possibly could be due to further liberation of the alkaloids, such as that which occurs during maceration, rather than a direct effect of ultrasound on extraction. Therefore, a somewhat different approach was utilized in evaluating the influence of 40 Kcy ultrasonic energy.

In this case, the macerating time was fixed at 24 hours to insure complete liberation of the alkaloids, and the drug was extracted with the 40 Kcy Circo ultrasonic generator for 1 hour or for 3 hours (Fig. 5). Samples of drug extracted with ultrasound for 1 hour displayed a 15% increase in alkaloid yield when compared with control samples. This marked increase was not observed in samples extracted with ultrasound for 3 hours. In these samples little difference in yield was noted between insonated and control samples.

When a comparison is made between samples of the drug continually macerated for 24 hours and extracted for 3 hours with the 20 Kcy or 40 Kcy generator, the yield of alkaloids is greater with the 20 Kcy unit (Fig. 6). Assuming that complete liberation of the alkaloids had occurred during the 24-hour maceration period, it appears that the lower

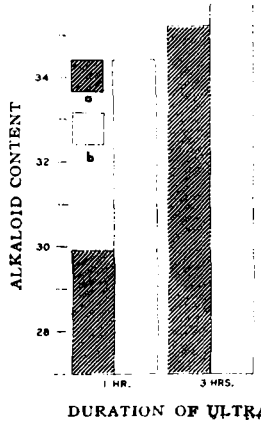


Fig. 5.—Results of 40 Kcy (Circo) ultrasonic extraction of the drug following a 24-hour conventional maceration period. Key: *a*, control samples; *b*, insonated samples.

frequency of ultrasound is more effective than the higher frequency during the extraction process.

It should be mentioned that complete exhaustion of the drug was not of primary concern during this investigation. Rather, the intent was to show the effect produced by ultrasound. This explains the relatively low yield of alkaloids obtained in certain studies. One should note also that ultrasonic action causes no detectable degradation of the active alkaloids (9, 10).

Comparisons between this study and similar but very limited work on alkaloid extraction reported in the literature are difficult to make. A great variety of ultrasonic generators, ranging from ultrasonic diathermy machines (8) to oil immersed piezoelectric crystals (9) and magnetostriction generators, have been used. However, one major similarity is evident in the increased yield of alkaloids obtained from drug samples ultrasonically treated for short periods of time (9, 10).

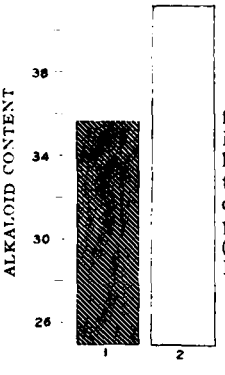


Fig. 6.—Comparative efficiency of 20 Kcy and 40 Kcy generators during 3 hours of ultrasonic extraction, following a 24-hour conventional maceration period. Key: *1*, 40-Kcy (Circo) generator; *2*, 20-Kcy (Bendix) generator.

## CONCLUSIONS AND SUMMARY

As shown in the previous section, the effects of ultrasound on the liberation of alkaloids during maceration are greatest in the initial 30-minute maceration period. To explain this phenomenon, one might consider that in addition to the alkaloids present in the cell as salts of organic acids, some alkaloids may be present bound to essential cell constituents or precursors (14). The mechanism of the ultrasonic effect on maceration, then, would be to liberate the alkaloids and hasten their diffusion through the cell wall into the extracting solvent. In the absence of ultrasound, liberation of the bound alkaloids and their diffusion also occurs, but at a much slower rate. Thus, the action of ultrasonic energy would be to accelerate this rate controlling diffusional process.

If the maceration period is adequate to allow the slow diffusional process to go to completion (as in the U.S.P. overnight maceration), little difference in alkaloid yield between insonated and noninsonated samples would be observed. For this reason, it would be pointless and impractical to lengthen insonation periods if no effect of the applied ultrasound is noted during a short maceration time.

During maceration of the drug, diffusion of the alkaloids occurs as has been described. It is then necessary to separate physically the alkaloids from the drug. Ordinarily, this is accomplished during Soxhlet extraction where the residue is repeatedly

exposed to the solvent. During ultrasonic extraction, this physical separation is further enhanced by the localized stirring occurring as a consequence of cavitation. The combination of this stirring effect and the repeated washing of the drug with solvent is far superior to the simple washing procedure in Soxhlet extraction. Again, the effect of ultrasound is most noticeable during short-term extractions. For if the drug is washed often enough in the conventional procedure, all of the alkaloids eventually will be separated from the residue.

These results lead us to propose that at least two different mechanisms may be involved in the liberation of alkaloids during the isolation procedure. One of these mechanisms, occurring during the process of maceration, is stimulated or enhanced by the higher frequencies of ultrasonic energy. The other mechanism, active during the extraction process, is promoted by low frequencies of ultrasonic energy. Although the evidence supporting this hypothesis is only indirect, the experiments reported here have clearly demonstrated that it is possible to evaluate critically the effect of ultrasound on *both* phases of the procedure involved in alkaloid isolation—maceration and extraction. Furthermore, these exploratory studies indicate that the utilization of ultrasonic energy during the isolation procedure may yield valuable information concerning the exact mechanisms involved in the liberation of medicinal products from natural sources.

Ultrasonic energy definitely has utility in the extraction of alkaloid containing plants, providing that the ultrasound is of sufficient intensity and is correctly applied. Ultrasonic energy shortens considerably both the duration of the maceration and extraction process while simultaneously yielding extracts containing greater quantities of alkaloid than can be obtained by conventional procedures. Although quantitative differences in alkaloid yield have been noted, our experience has been that the maceration process lends itself most readily to the application of ultrasound. We suggest that ultrasonic treatment on a commercial scale could be utilized profitably and simply by applying ultrasound to the macerating mixture.

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# Development of a Simple Automated Film-Coating Procedure

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**Information obtained in a series of manually controlled coating experiments was applied in developing a simple automated procedure for film-coating tablets. The equipment consists of a group of interconnected timers which signal a solenoid valve controlling the hydraulic atomization of the coating solution. The film compositions were based on mixtures of hydroxypropyl methylcellulose and ethylcellulose and were applied to tablets automatically in both 18 and 42-in. coating pans. The apparatus and coating procedure offer advantages in both research and production film-coating operations.**

FILM-COATED TABLETS have gained increasing acceptance in the pharmaceutical industry; a growing number of tablet products coated in this way have reached the market. Numerous reports describing new and useful film-coating compositions have appeared in both the scientific and patent literature (1–5). The advantages of the film-coating technique include (among others) speed and convenience of operation and good control over tablet-to-tablet uniformity. A general review of the coating procedure has appeared in the reports by Wagner (6) and by Gross and Endicott (7). Recently, Lachman described a procedure for automating the film-coating operation (8). This technique was based on the use of punched-tape electronic instrumentation and may be overspecialized for general applications.

The present report describes the development of a simplified, inexpensive procedure for automatic film coating.

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## EXPERIMENTAL

**Materials.**—The coating agents used in this study were based on mixtures of hydroxypropyl methylcellulose and ethylcellulose (9, 10) as shown in Table I.

Coating solutions were prepared by dissolving the