# Effect of Surface-Active Agents on the Extraction of Belladonna Herb

By G. P. SRIVASTAVA and T. N. CHADHA

**The effect of two surface-active agents, Tween 20 and Tween 80, on the extraction of belladonna herb (*Atropa belladonna*) by percolation and mechanical agitation processes using 70% alcohol and water as solvents has been studied. The concentration of the surfactants in the solvents was uniformly 0.2%. These surfactants have been shown to potentiate the solvents leading to a more complete and expedient extraction of the drug. Seventy per cent alcohol and water containing 0.2% Tween 20 extracted the drug better than the unpotentiated solvents. Tween 80 gave better results than Tween 20 when used in the extraction by mechanical agitation. In the percolation process only Tween 20 was used.**

In recent years some novel methods of drug extraction have been suggested. Bay and Gisvold (1) worked out a method for extracting digitalis leaves by disintegrating them in a Waring Blendor in presence of water as menstruum. Greco and Dumez (2) used a pressure cooker for extracting vegetable drugs like nux vomica, belladonna, hyoscyamus, stramonium, cascara, and wild cherry. Changes in the use of solvents have also been suggested. Carkhuff and Gramling (3) used various furans for the extraction of belladonna. Campo and Gramling (4, 5) extended the use of furans to the extraction of defatted ergot and cinchona. Dean, *et al.* (6), made use of a colloid mill for preparing tinctures of belladonna and stramonium. Head, *et al.* (7), worked on the ultrasonic extraction of *Cinchona succirubra* using various solvents. Bose, *et al.* (8), also employed this method in the extraction of *Rauwolfia serpentina*. Different workers have shown advantages of their respective methods.

The use of surface-active agents in the extraction of vegetable drugs was suggested by Butler and Wiese (9), who successfully employed such agents for extracting belladonna, hyoscyamus, cinchona, and ipecac. They used several surfactants and extracted the drugs by the official percolation method. Brochmann-Hanssen (10) followed the use of surfactants for the extraction of cinchona and ipecac. These investigations induced the present authors to undertake the inquiry of the effect of two surface-active agents, Tween 20 (polyoxyethylene sorbitan monolaurate) and Tween 80 (polyoxyethylene sorbitan mono-oleate), not so far studied by the previous workers, on the extraction of belladonna herb (*Atropa belladonna*), an important drug of the Indian Pharmacopoeia. The above two surfactants belong to the nonionic group and are practically nontoxic (11, 12).

A surfactant concentration of 0.2%, usually employed in pharmaceutical formulations, was used in 70% alcohol and in water. Extractions were done by percolation and mechanical agitation. Throughout this study we employed 70% alcohol where alcohol was utilized and maintained the concentration of the surfactants at 0.2%.

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## EXPERIMENTAL

A sample of belladonna herb (*A. belladonna*) powdered to No. 40 and assayed by the method of the Indian Pharmacopoeia of 1955 (13), was used all through the present work. The average result of six readings of assay was 0.08782% w/w of alkaloids calculated as hyoscyamine.

**Extraction by Percolation.**—In the first series of experiments 100 Gm. of the drug powder (No. 40) was extracted by the percolation process in the following ways: (*a*) simple percolation using alcohol as menstruum (control for comparison); (*b*) percolation without 24-hour maceration using alcohol containing Tween 20 for moistening only and alcohol for the rest of the process; (*c*) simple precolation using alcohol containing Tween 20 for moistening as well as for maceration and alcohol for the rest of the process; and (*d*) percolation without 24-hour maceration using alcohol containing Tween 20 for the entire process.

In every case the same size percolator was used and the rate of percolation was maintained at 30 drops per minute. A 700-ml. quantity of the percolate was collected in five fractions—three 100 ml. and two 200 ml. Each fraction was assayed for its alkaloidal content and from the data thus obtained the progressive percentage of alkaloids extracted from 100 Gm. of the drug was calculated. Three sets of experiments were done in each case and the average values are recorded in Table I. The total solids (T.S.) extracted from 100 Gm. of drug was determined and the solvent selectivity index (s.s.i. = alkaloids × 100/total solids) was calculated therefrom. These values are also noted in Table I.

Table I.—Results of the Extraction of Belladonna Herb by the Percolation Process

| Fraction No. | Progressive Percentage of Alkaloid Extracted (a) | (b) | (c) | (d) |
|---|---|---|---|---|
| 1 | 55.7 | 47.3 | 51.3 | 53.2 |
| 2 | 83.8 | 74.4 | 80.5 | 81.2 |
| 3 | 93.2 | 84.6 | 89.0 | 92.1 |
| 4 | 97.7 | 89.6 | 94.4 | 98.3 |
| 5 | 100.4 | 91.4 | 96.6 | 100.6 |
| s.s.i. | 0.662 | 0.625 | 0.570 | 0.632 |

It is clear that complete extraction of alkaloids from belladonna herb is effected by percolation without 24-hour maceration when Tween 20 is incorporated in the total volume of alcohol used as menstruum. The efficiency of extraction procedure *d* is similar to simple percolation with 24-hour maceration, and plain alcohol procedure *a*. The solvent selectivity, however, is slightly reduced in this case. The other modifications adopted, *b* and *c*, have not proved successful.

**Extraction by Mechanical Agitation.**—In order to have more controllable conditions and obtain comparable results, extraction by mechanical agitation with or without maceration was used in the following series of experiments. For this purpose 20 Gm. of drug powder (No. 40) was taken with 150 ml. of menstruum in a well-closed 250-ml. bottle and agitated in a mechanical shaker at the rate of 200 strokes per minute. At the end of the specified time the clear extract was decanted off. Duplicate samples were prepared in each case and each sample was assayed twice for alkaloids taking 50 ml. of extract each time; total solids were also determined. Total alkaloids and solids present in the total volume of menstruum added (150 ml.) were calculated. This was done to find out the percentage of the alkaloids in 20 Gm. of drug (calculated value = 0.0176 Gm.) taken up by the total volume of the menstruum at the end of the process. In this way the results obtained were rendered comparable.

The effect of the duration of time of agitation was first determined; 20 Gm. of the drug and 150 ml. of alcohol containing Tween 20 were first macerated for 24 hours and then shaken for 5, 10, 20, and 30 minutes. As a control for comparison, 20 Gm. of the drug was also extracted with plain alcohol and agitated for 30 minutes by this method. The results are recorded in Table II.

TABLE II.—RESULTS OF EXTRACTION OF BELLADONNA HERB BY MECHANICAL AGITATION AFTER 24-HOUR MACERATION

| Agitation, Minutes | Menstruum Used | Alkaloid Extracted, % | s.s.i. |
|---|---|---|---|
| 5 | Alcohol with Tween 20 | 67.9 | 0.420 |
| 10 | Alcohol with Tween 20 | 71.7 | 0.442 |
| 20 | Alcohol with Tween 20 | 83.2 | 0.510 |
| 30 | Alcohol with Tween 20 | 90.9 | 0.535 |
| 30 | Alcohol (control) | 46.1 | 0.294 |

The results in Table II show evidence of the superiority of the extraction of alkaloids from belladonna herb with alcohol containing Tween 20 over the extraction with plain alcohol; the extraction with agitation for 30 minutes in the former case is nearly double (90.9%) of that in the latter (46.1%). Even agitation for 5 minutes with the potentiated menstruum extracts nearly one and a half times more alkaloids than in the control. It is interesting to note that the solvent selectivity index is also higher in the case of the potentiated menstruum.

In the following experiments the 24-hour maceration before agitation was eliminated and 20 Gm. of the drug was agitated with 150 ml. of solvent. The time of shaking was increased as shown in Table III. Three types of solvents were used: (i) plain alcohol as control for comparison, (ii) alcohol containing Tween 20, and (iii) purified water containing Tween 20. The results obtained are given in Table III.

TABLE III.—RESULTS OF EXTRACTION OF BELLADONNA HERB BY MECHANICAL AGITATION WITHOUT MACERATION

| Agitation, Minutes | Menstruum Used | Alkaloid Extracted, % | s.s.i. |
|---|---|---|---|
| 30 | Alcohol with Tween 20 | 26.4 | 0.296 |
| 60 | Alcohol with Tween 20 | 34.4 | 0.377 |
| 90 | Alcohol with Tween 20 | 73.9 | 0.480 |
| 120 | Alcohol with Tween 20 | 97.6 | 0.595 |
| 60 | Water with Tween 20 | 53.1 | 0.143 |
| 120 | Water with Tween 20 | 66.4 | 0.187 |
| 120 | Alcohol (control) | 42.5 | 0.313 |

It is clear that 2 hours of agitation of the drug with alcohol containing Tween 20, without maceration, extracts it almost completely (97.6%), purified water containing Tween 20 extracts 66.4% of the alkaloids, while plain alcohol under similar conditions extracts only 42.5% of the alkaloids. It is also significant to note that Tween 20 in alcohol gives a much higher value for the solvent selectivity index than the value obtained in the case of water.

Having examined the effect of Tween 20, some experiments were also done with Tween 80, both in alcohol and water. Extraction of 20 Gm. of the drug was done by mechanical agitation without maceration in 150 ml. of the menstruum. Duplicate samples were prepared and each sample was assayed twice. The results are given in Table IV. For the sake of ready comparison, the values of per cent alkaloids extracted by alcohol and water both potentiated separately and severally with Tween 20 and Tween 80 are tabulated in Table V.

TABLE IV.—RESULTS OF EXTRACTION OF BELLADONNA HERB BY MECHANICAL AGITATION WITHOUT MACERATION

| Agitation, Minutes | Menstruum Used | Alkaloid Extracted, % | s.s.i. |
|---|---|---|---|
| 30 | Alcohol with Tween 80 | 72.7 | 0.519 |
| 60 | Alcohol with Tween 80 | 83.87 | 0.555 |
| 90 | Alcohol with Tween 80 | 92.2 | 0.592 |
| 120 | Alcohol with Tween 80 | 100.7 | 0.640 |
| 60 | Water with Tween 80 | 61.5 | 0.159 |
| 120 | Water with Tween 80 | 69.9 | 0.181 |

TABLE V.—RESULTS OF EXTRACTION OF BELLADONNA HERB BY MECHANICAL AGITATION WITHOUT MACERATION

| Agitation, Minutes | Alkaloid Extracted, %: Alcohol, 70% — Tween 20 | Alcohol, 70% — Tween 80 | Water — Tween 20 | Water — Tween 80 |
|---|---|---|---|---|
| 30 | 26.4 | 72.7 | ... | ... |
| 60 | 34.3 | 83.9 | 53.1 | 61.5 |
| 90 | 73.9 | 92.2 | ... | ... |
| 120 | 97.6 | 100.7 | 66.4 | 69.9 |

Tween 80 in the same concentration potentiates both alcohol and water more than Tween 20 does;

and, it also acts better in alcohol than in water. It may be noted that the alcohol incorporating either Tween 20 or Tween 80 extracts the drug almost equally. However, the solvent potentiated with Tween 80 acts more quickly in the beginning as evidenced by the values of per cent alkaloids extracted within short durations. Further, alcohol incorporating Tween 80 acts as a more selective solvent than that containing Tween 20 as indicated by the respective s.s.i. values (0.640 and 0.594).

## DISCUSSION

The effect of two surfactants, Tween 20 and Tween 80, on the extraction of belladonna herb has been studied first by the percolation process (Tween 20 only) and then by the mechanical agitation process. It has been found, in both the processes, the solvent (alcohol) is potentiated by adding Tween 20 and permits the preliminary maceration for 24 hours to be eliminated. The purpose of maceration is to allow the drug to imbibe the solvent to facilitate the subsequent extraction. The most apparent effect of the use of the two Tweens in the solvent is that imbibition takes place so quickly that day-long maceration becomes unnecessary and the whole process is expedited.

Though both the surfactants were shown to be effective in potentiating the solvents, Tween 80 was superior to Tween 20 in the mechanical agitation process. This, probably, is due to the presence of the oleate radical, an 18-carbon chain, in the molecule of Tween 80. The laurate radical present in Tween 20 is only a 10-carbon group.

In the percolation process the potentiation of alcohol by Tween 20 has been found not to improve its solvent selectivity, but in the mechanical agitation process there is a marked enhancement. Both Tween 20 and Tween 80 have definitely higher s.s.i. values. The higher the value of this index, the higher will be the selectivity of the solvent, meaning that it extracts the active principles more than it does the other inert constituents.

## CONCLUSIONS

Polyoxyethylene sorbitan monolaurate (Tween 20) and polyoxyethylene sorbitan mono-oleate (Tween 80) have been found to potentiate 70% alcohol and water as solvents in the extraction of belladonna herb (*A. belladonna*) by percolation and mechanical agitation processes. Tween 80 acts better than Tween 20 in the latter process. Both these surfactants act better in 70% alcohol than they do in water. In the percolation process the selectivity of the solvent (70% alcohol) is not improved but in the mechanical agitation process it is appreciably enhanced.

## REFERENCES

(1) Bay, G., and Gisvold, O., THIS JOURNAL, **37**, 314 (1948).
(2) Greco, S. J., and Dumez, A. G., *ibid.*, **39**, 560(1950).
(3) Carkhuff, E. D., and Gramling, L. G., *ibid.*, **41**, 660(1952).
(4) Campo, J. M., and Gramling, L. G., *ibid.*, **42**, 747 (1953).
(5) *Ibid.*, **45**, 242(1956).
(6) Dean, S. J., Brodie, D. C., Brochmann-Hanssen, E., and Riegleman, S., *ibid.*, **42**, 88(1953).
(7) Head, W. F., Jr., Beal, H. M., and Lauter, W. M., *ibid.*, **45**, 239(1956).
(8) Bose, P. C., Sen, T. K., and Ray, G. K., *Indian J. Pharm.*, **23**, 222(1961).
(9) Butler, W. J., and Wiese, G. A., Jr., THIS JOURNAL, **42**, 382(1953).
(10) Brochmann-Hanssen, E., *ibid.*, **43**, 27(1954).
(11) Sollmann, T., "A Manual of Pharmacology and Its Application to Therapeutics and Toxicology," 8th ed., W. B. Saunders Co., Philadelphia, Pa., 1957, p. 9.
(12) Drill, V. A., "Pharmacology in Medicine," 2nd ed., McGraw-Hill Book Co., New York, N.Y., 1958, p. 212.
(13) "Pharmacopoeia of India," Government of India, Ministry of Health, New Delhi, 1955, pp. 80, 679.

# Absorption and Excretion of Sulfadiazine After Subcutaneous Implantation of Disks in Rats

By BERTON E. BALLARD and EINO NELSON†

**Thin, cylindrical disk-shaped pellets of sulfadiazine were subcutaneously implanted in rats and free and total urinary sulfadiazine excretion followed. It was possible to relate the mean pellet absorption rate per unit area to the mean excretion rate per unit area and to the fraction of a dose of the drug eventually excreted in the urine.**

ABSORPTION of drugs from implants may be studied by following urinary excretion of the unchanged drug, its metabolite(s), or both, by determination of drug blood level, by following excretion of substances such as electrolytes that reflect the drug's action in the body, by studying drug action on a target organ, or by following the decrease in weight of the implants themselves. In previous work it has been shown that the rate-limiting step in absorption after subcutaneous implantation of a number of drugs was the dissolution process at the absorption site (1). The work reported now gives the results of experiments which were conducted to determine the relationship between cumulative urinary excretion of sulfadiazine and acetylated sulfadiazine and change in surface area of thin, cylindrical disks of this material after subcutaneous implantation in rats. A study of this type has not been previously conducted using cylindrical implants; sulfadiazine serves as a readily available and assayable model compound.

## PROCEDURE

Thin, cylindrical disks of compressed drug grade sulfadiazine were prepared and subcutaneously implanted in a manner and site described before (1). Four weighed disks were implanted into each of three Sprague-Dawley rats of both sexes designated as A, B, and C, having weights of 210, 335, and 410 Gm., respectively. They were placed in separate glass metabolism cages from which the urine was collected twice daily for 5 days. The urine was assayed for free and total sulfadiazine

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