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The diffusion of sennoside A through a cellulose membrane

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The diffusion rate of sennoside A through a cellulose membrane into water increased irrespective of the temperature conditions under which diffusion took place, when the membrane was irradiated with ultrasound. The results are consistent with the hypothesis of boundary layer disruption at the phase interface and their significance in the effect of ultrasonic energy on the aqueous extraction of senna pericarps is discussed.

Although ultrasonic energy may increase the extraction rate of plant material, the means whereby that result is achieved remains obscure. Morrison & Woodford (1967) observed that ultrasonic irradiation of an aqueous suspension of senna pericarps resulted in an increase in the extraction rate of both sennosides and free anthraquinones, and further work (Woodford & Morrison, 1969) showed that although insonation produced visible damage to occasional epidermal cell walls, only slight gross physical damage to whole pericarps resulted. Because many pericarp cell walls were visibly undamaged by insonation, experiments were made to see if ultrasonic energy is capable of increasing the diffusion rate of sennoside molecules through an intact cellulose barrier.

EXPERIMENTAL

Construction of diffusion cell

The diffusion cell was constructed in Perspex to the design of Wood, Rising & Hall (1962). An RBT 16 thermoelectric unit* was embodied into the rear chamber of the cell so that the inner face of the unit was continuous with the wall of the diffusion chamber. The unit was connected in series with a 6 V accumulator through an ammeter and rheostat so that a current of 1 to 10 A could be passed through the thermoelectric junctions. By adjustment of the direction and amount of current the unit could be used either to increase or decrease the temperature of the liquid in the cell at a predetermined rate.

Modification of cellulose film permeability to sennoside A

The permeability of additive-free regenerated cellulose film (British Cellophane) to sennoside molecules was increased by a zinc chloride treatment modified from the methods of McBain & Stuewer (1936), Craig & Konigsberg (1961) and Pierce & Free (1961).

Suitably-sized squares of film were immersed in a 64% w/w aqueous solution of zinc chloride at 35° for 20 min. The squares were placed in 0·01N hydrochloric acid for 2 min, the procedure repeated using a similar volume of acid, and the membranes washed with purified water until free from chloride. Squares of both treated and

* Salford Electrical Instruments Limited, Salford, Lancs.

untreated cellulose film were stored in purified water at room temperature for 14 days before being used. The thickness of these samples was measured using a horizontal metroscope (Zeiss). The thickness of the dry film was 21·6 μm, of the untreated film soaked in water 42·8 μm (98% increase over dry film); the thickness of the treated film soaked in water was 136·5 μm (532% increase over dry film). Each value is the mean of 10 measurements. The increase in area of soaked treated film over dry untreated film was 88%.

Aqueous solutions of sennoside A [decomposition range 215–220°; Stoll, Becker & Kussmaul (1949) give 200–240°] were prepared using potassium bicarbonate and their pH adjusted to 5·40, the pH of aqueous extracts from senna pericarps. The diffusion of sennoside A through untreated and treated cellulose film into purified water at 20° was determined using the diffusion cell and the results are shown in Fig. 1. The amount of sennoside diffused was determined from a linear calibration graph of absorbance against concentration at the wavelength of maximum absorption 268 nm (E 1%, 1 cm = 226 in purified water).

Effect of ultrasonic energy on the diffusion of sennoside A through treated cellulose film

The potassium salt of a (28 ml of a 90 mg% w/v) solution of sennoside A, adjusted to pH 5·40, was placed in the donor chamber of the diffusion cell and the same volume of purified water in the receptor compartment. Small glass stirrers were inserted into each chamber and the amount of sennoside diffused after 10 min was determined spectrophotometrically at 268 nm. The cell was emptied and the experiment repeated using diffusion times of 20 and 30 min. Separate experiments showed that the size of the stirrer and the rate of stirring had no effect on the diffusion rate of sennoside A.

The effect of ultrasonic energy was determined by subjecting the liquid in the receptor chamber to insonation from a model 60W MSE ultrasonic disintegrator (frequency 20 kHz, probe end diameter $\frac{3}{8}$ inch) for 10 min. The experiment was

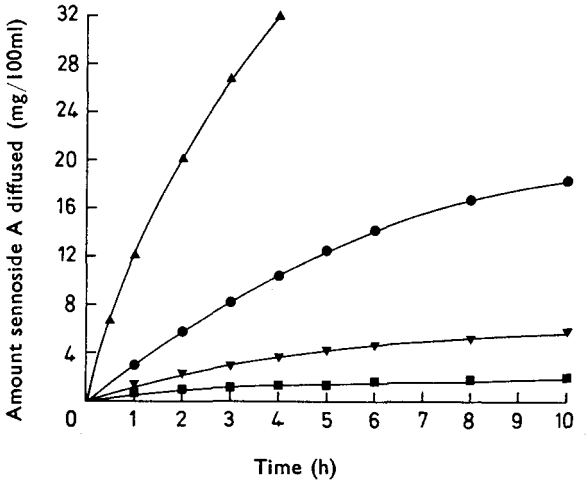

FIG. 1. Diffusion of sennoside A as potassium salt through untreated and zinc chloride-treated cellulose film into purified water at 20°. ■, Initial concentration of sennoside in donor chamber 50 mg/100 ml, untreated membrane; ●, ditto, treated membrane; ▼, initial concentration of sennoside in donor chamber 90 mg/100 ml, untreated membrane; ▲, ditto, treated membrane. Micro-glass stirrers (240 rev/min) were used in donor and recipient chambers.

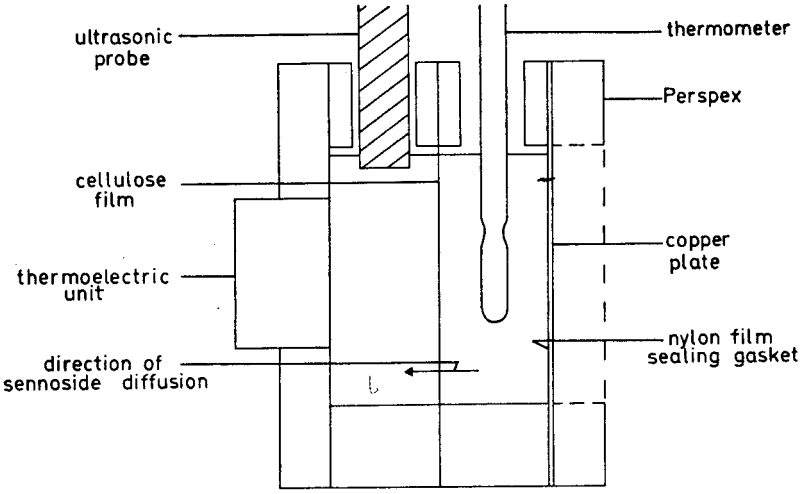

FIG. 2. Diffusion cell employed in the study of the effect of ultrasonic energy on the diffusion of sennoside A through cellulose film.

repeated with fresh sennoside solutions using diffusion times of 20 and 30 min, the absorbance of all solutions being measured at 20°. The rise in temperature of the liquids on either side of the membrane was (time, min/temp.°): 0/20, 5/33·5, 10/43·5, 15/49·5, 20/56, 25/60, 30/63. Preliminary experiments were made to determine the volume of purified water required to be added to each chamber to replace that lost by evaporation.

The contribution of this temperature rise in the diffusion process was determined by passing a current of 7·5 to 8·5 A through the thermoelectric unit in the absence of ultrasonic irradiation. By adjustment of the rheostat the temperatures obtained above could be repeated with an accuracy of $\pm 0{\cdot}3°$ during diffusion periods of 10, 20 and 30 min; in each case fresh sennoside solution was used.

The effect of ultrasonic energy at room temperature was determined in the cell in which the front face was replaced by 0·3 mm thick bright copper plate and the complete assembly was surrounded by solid carbon dioxide (Fig. 2). This removed heat away from the cell at the same rate as it was produced. The plate was held in place by a nylon film sealing gasket impermeable to sennoside A. The carbon dioxide served to augment the cooling action produced by passing a 5 A current through the thermoelectric unit in the reverse direction to that used in assessing the effect of temperature rise on the diffusion process in the absence of ultrasonic irradiation.

All experiments were in triplicate. The diffusion of sennoside A at room temperature through cellulose film previously subjected to ultrasonic waves for 30 min was measured at the completion of the investigations to see if insonation affected the permeability of the membrane. The results are in Fig. 3.

DISCUSSION

The increase in permeability of regenerated cellulose film produced by zinc chloride depended on the concentration of the zinc chloride solution, the temperature, and the time of immersion of the film in the zinc chloride solution. Modified membranes produced as described were of reproducible permeability to sennoside A; film samples

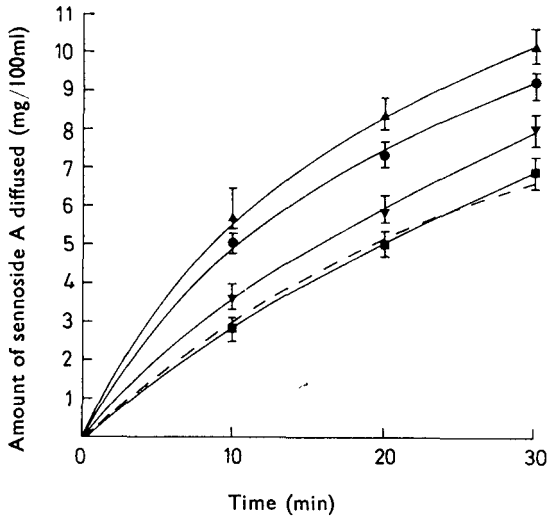

FIG. 3. Effect of ultrasonic energy, controlled heating, and ultrasonic energy at room temperature on the diffusion of sennoside A through cellulose film. ■, Room temperature; ▼, ultrasonic energy at room temperature; ●, controlled heating; ▲, ultrasonic energy. Symbols are the means of 3 experiments, the vertical lines show the range. — — — — Diffusion of sennoside A at room temperature through cellulose film previously subjected to ultrasonic energy. Initial concentration of sennoside in donor chamber 90 mg/100 ml, volume in each chamber 28 ml, surface area of membrane 15·9 cm^2. Micro-glass stirrers (240 rev/min) were in the chambers not containing the ultrasonic probe.

stored in purified water for 14 days did not yield substances absorbing in the ultraviolet region.

Spectrophotometric examination of sennoside A solutions before and after ultrasonic irradiation for 90 min showed that no chemical degradation resulted from insonation. This was confirmed using paper and thin-layer chromatographic techniques.

The use of the diffusion cell enabled the overall heating effect of insonation to be separated from the other effects of ultrasound. The results show that ultrasonic energy produced an increase in the amount of sennoside A passing through the cellulose membrane during a 30 min period, irrespective of the temperature conditions under which diffusion took place. The effect of ultrasonic energy and controlled heating on the diffusion rate of sennoside A through a cellulose membrane was similar to that observed in the extraction of active and inactive constituents from senna pericarps (Woodford & Morrison, 1969). In both cases diffusion of molecules occurred through a cellulose barrier into water which was irradiated with ultrasound.

Ginzburg & Katchalsky (1963) assumed the existence of an unstirred layer adjacent to the cellulose membrane while Nozdreva (1964) stated that the accelerating effect of ultrasound on diffusion rates is due to its disruptive effect on the boundary layer at the phase interface. The present work shows that ultrasonic energy was superior to the stirring procedure previously described in accelerating the passage of sennoside A through cellulose film and that insonation did not produce a permanent alteration in membrane permeability to that glycoside. During the ultrasonic diffusion process marked turbulence together with occasional bubble formation occurred at the membrane surface. These results are consistent with the hypothesis of boundary

layer disruption at the phase interface and indicate that this phenomenon may be an important factor in the non-thermal effect of ultrasonic energy on the extraction of plant material.

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