

Determination of Osmolarity of Small Latex Samples by Vapour Pressure Osmometer

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An accurate, sensitive and rapid method for measuring osmotic concentrations of small latex samples with the vapour pressure osmometer is described. Presence of the rubber hydrocarbon in the latex did not affect the determinations. It was thus possible to measure the osmotic concentration of small latex samples representative of latex within the tree.

Accurate measurement of osmotic concentrations of small latex samples is essential for the study of water relations and latex flow phenomena of *Hevea brasiliensis*.

The freezing point technique has been used to determine the osmotic concentration of latex taken from different parts of the tree (ARISZ, 1918) and to study changes in tapped latex during flow (RUBBER RESEARCH INSTITUTE OF MALAYA, 1962). The size of sample needed—a minimum of 30 ml—limits its use to studies on tapped latex. Since considerable dilution of the latex occurs as a response to the tapping operation itself, observations on tapped latex do not necessarily reflect conditions within the latex vessels in the tree.

Vapour pressure osmometers now available are capable of measuring osmotic concentrations of small volumes of biological fluids. Changes in the latex within the tree can be studied by measurements on small quantities of latex drawn from the latex vessels at the point of sampling.

Latex is a heterogeneous fluid consisting of a serum phase containing dispersed rubber and non-rubber particles (COCKBAIN AND SOUTHORN, 1962). The object of the study was to investigate whether this heterogeneity affects the measurement of osmotic concentrations by a vapour pressure osmometer, and if the method could be applied to the contents of the latex vessels.

MATERIALS AND METHODS

The experiments were carried out on 14-year-old budded trees of clone Tjir 1, which were

tapped on a half-spiral cut, alternate daily. Latex was collected in glass containers surrounded by crushed ice to maintain a temperature of 5°C until the measurements could be made.

A Mecrolab 301A vapour pressure osmometer was used with an aqueous thermistor probe at an operating temperature of 37°C. The instrument was calibrated with sodium chloride and mannitol solutions of known osmotic concentrations over a range of 50–1000 milliosmoles. The principle of the thermoelectric osmometer has been fully discussed by BRADY *et al.* (1951). Drops of pure solvent and solution, (in this case water and latex respectively), are placed upon balanced thermistors enclosed in a temperature-controlled chamber saturated with solvent vapour. Since the vapour pressure of the solution is lower than that of the pure solvent, solvent vapour condenses on the solution sample causing its temperature to rise because of the heat of condensation. The temperature difference between solvent and solution, which is proportional to the vapour pressure lowering and hence to the solute concentration, is measured as a difference in resistance (ΔR) between the two thermistors.

RESULTS

Time of Equilibration of Latex

The change in ΔR with time for latex and three concentrations of mannitol, is shown in *Figure 1*. The initial rates of change in ΔR are dependent upon the concentration of the

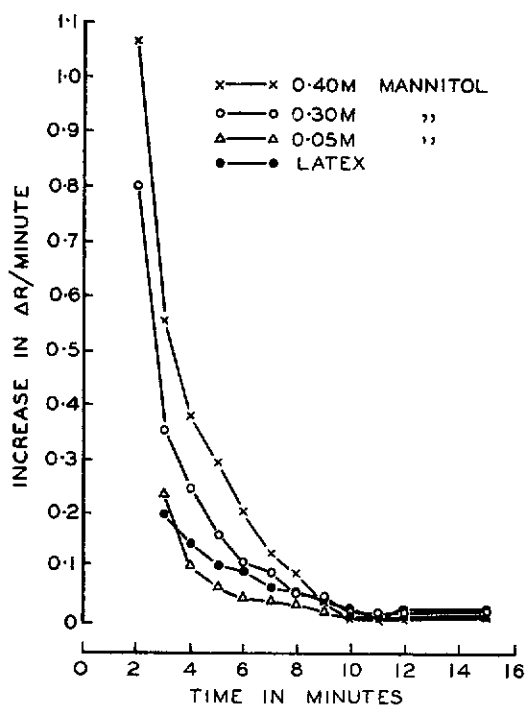


Figure 1. Rate of equilibration for latex and three concentrations of mannitol solutions.

sample, solutions of lower concentration reaching a steady state more rapidly. The three mannitol solutions and latex both reached a steady state within 12 minutes, indicating that the heterogeneity of latex did not affect this. Subsequent determinations of osmotic concentrations of latex were made therefore 12 minutes after the introduction of the sample into the instrument.

Effect of Latex Constituents upon Determinations of Osmotic Concentration

As solids constitute between 30 and 50% of the latex by weight, their effect on the determination of the osmotic concentration was examined. Moreover, the rubber hydrocarbon forms the greater part of these solids and, although rubber particles would not be expected to contribute to the osmotic concentration owing to their large molecular weight, their effect was tested.

Latex and Salt Solution Mixtures

A pooled sample of latex was collected from several trees and its total solids and dry rubber contents were determined and found to be 42.3% and 39.6% respectively. The ΔR values of a series of aliquots of sodium chloride dissolved in water to give osmotic concentrations of 100, 200, 300, 400, 500, 800 and 1,000 milliosmoles, were compared with a series of similar aliquots of the latex sample, in which the same respective amounts of sodium chloride had been dissolved. The ΔR values for each series are plotted in Figure 2 against the osmotic concentration of sodium chloride solutions. The regression lines for the two series are virtually parallel and the almost constant interval between them indicates the osmolarity of the latex. There is no indication of interference between the salt and latex constituents in the determination of the osmotic concentration.

Re-suspension of Rubber Particles in Buffered Osmoticum

Fifty ml of latex was collected directly into an equal volume of potassium chloride solution buffered by phosphate at pH 7.0 with a measured osmotic concentration of 453 milliosmoles. The mixture was centrifuged at

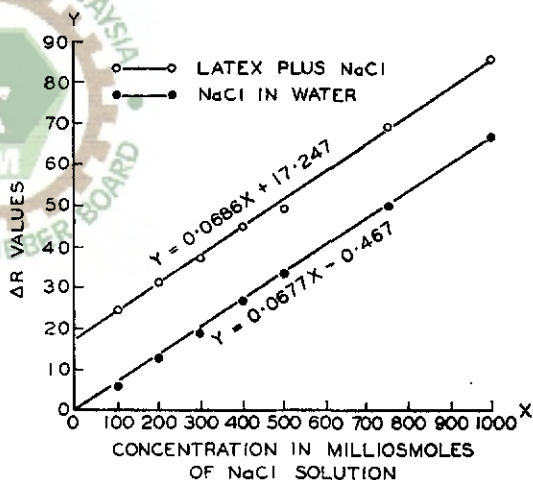


Figure 2. Effect of sodium chloride of known concentrations on the osmotic pressure of latex.

22 500 g max. for one hour. The hydrocarbon layer was washed by re-suspension in a similarly buffered osmoticum and centrifuged. This process was repeated twice and excess solution was removed by blotting lightly with filter paper. A series of suspensions of the hydrocarbon in the buffered osmoticum was prepared covering a range of 5 to 40% hydrocarbon by weight, and the osmotic concentration of each of these suspensions was determined. The results (*Table 1*) confirm that there was no effect of the concentration of suspended rubber particles on the measured osmotic concentrations.

TABLE 1. EFFECT OF HYDROCARBON PARTICLES ON OSMOTIC PRESSURE DETERMINATION

Rubber, %	0	5	10	15	20	30	40	Mean
Osmotic pressure, milliosmoles	453	456	456	452	452	447	456	453

Precision of Osmotic Concentration Determinations

The initial 30 ml of latex from each of four trees was collected separately. Eight determinations were made on each of the four samples to evaluate the accuracy and reproducibility of measurement of osmotic concentrations using the vapour pressure osmometer. Mean osmotic concentrations in milliosmoles for the four latices, given in *Table 2*, show a high degree of precision and reproducibility.

Determination of Osmotic Concentration of Latex in situ

Observations upon latex obtained by conventional tapping methods do not necessarily reflect changes that actually occur within the latex vessels. In an attempt to study changes in the properties of latex that occur within the latex vessels during flow, FERRAND (1941) developed a method of obtaining small samples of latex representative of that in the latex vessels at the point of sampling. He considered that if a small puncture be made into the bark the

TABLE 2. MEAN OSMOTIC CONCENTRATIONS OF INITIAL LATEX FLOW SAMPLES

Tree No.	Mean osmotic concentration in milliosmoles	Standard error of Mean	Coefficient of variation (%)
1	379	±1.8	1.40
2	369	±1.2	0.92
3	365	±1.2	0.96
4	383	±2.7	2.01

first few drops of latex that exude would be squeezed out by contraction of the severed latex vessels and will have undergone little or no dilution. GOODING (1952) could detect little or no change in the percentage total solids in the first six drops of latex exuding from a puncture made by Ferrand's technique.

In a similar experiment, changes in osmotic concentrations in successive drops of latex have been studied. The drops from seven sampling points were pooled to obtain a sufficient volume of latex. Representative results are given in *Figure 3*. There is a progressive fall in the osmotic concentration with drop number, indicating dilution. In this particular tree determinations were made on two successive days at 8 a.m. Extrapolation indicates an osmotic concentration of the latex *in situ* as 451 milliosmoles. The drop in osmotic concentration over the seven drop samples was 73 milliosmoles. Repeated observations demonstrate a similar decline in osmolar concentration of this magnitude.

Ten drops of latex are the minimum volume required for osmotic concentration determinations by this method. If the number of latex drops is limited to two per incision, the sample obtained may be expected to have an osmotic concentration about 20 milliosmoles or 5% less than that in the tree. Restriction to two drops per incision required five incisions for an osmolarity determination.

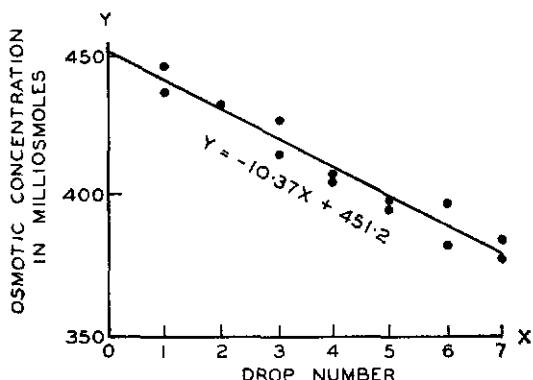


Figure 3. Relationship between the successive latex drop samples from micro-incision and osmotic concentration.

DISCUSSION AND CONCLUSION

Experiments described here indicate that the osmotic concentration of small samples of latex can be rapidly and accurately determined by the vapour pressure osmometer. The rubber particles in latex do not noticeably influence the determinations and this presumably applies to the other substances of high molecular weight or relatively large particles and organelles present. Unlike the freezing point method, which requires large samples which may obscure variation during short intervals, the vapour pressure method is very suitable for determining concentrations in successive small samples during latex flow. However, such observations do not necessarily reflect changes in latex *in situ* as the tapping operation itself causes changes in latex composition, particularly in respect of the degree of dilution.

Using a modified form of Ferrand's method of obtaining latex samples representative of latex within the latex vessels at the point of sampling in conjunction with the vapour pressure osmometer, changes in latex *in situ* can be studied. In contrast with the observations

of GOODING (1952) on changes in total solids of successive samples from a single sampling point, it has been demonstrated that, in terms of osmotic concentration, a degree of dilution occurs. This difference is attributed to the greater sensitivity of the osmolarity determinations compared with those of total solids used by Gooding, the latter displaying greater variation. By restricting the number of drops per sampling point, and increasing the number of sampling points, reasonably reliable estimates of osmotic concentrations within the latex vessels can be obtained.

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