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Kinetics of Isothermal and Microwave Extraction of Essential Oil Constituents of Peppermint Leaves into Several Solvent Systems

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The rates and extents of extraction have been measured for three major constituents of peppermint oil, namely 1,8-cineole, menthone and menthol, using the leaves of the black mint (*Mentha* × *piperita* L.). The solvents used were hexane, ethanol and mixtures of composition 90 mol% ethanol + 10 mol% hexane and 90 mol% hexane + 10 mol% ethanol. The extractions were carried out isothermally at 25, 35 and 45°C as well as in an electrically and mechanically modified domestic microwave oven where the temperature increase varied from c. 10 to 30°C. The rates of both isothermal and microwave extractions were sensitive to the solvent employed and decreased in the order 90 mol% hexane > 90 mol% ethanol > hexane > ethanol. The rates of microwave extraction were also affected by the microwave power output and the size of the sample load. The activation energies for the extractions were in the range 30–90 kJ mol^{-1}, again dependent on the solvent used. Scanning electron microscopy on the spent leaves provided evidence of a link between the kinetics of extraction and structural changes on the glands.

KEY WORDS Extraction kinetics; microwave extraction; solvent extraction; rate constants; essential oils; peltate glands; peppermint (*Mentha* × *piperita* L.); 1,8-cineole; menthone; menthol; dielectric properties

INTRODUCTION

Peppermint oil is a plant extract of economic importance, produced on a large scale by steam distillation.[1,2] The essential oil is contained only in the peltate glands or trichomes of the leaves.[3–5] It is known that the chemical composition of the oil is greatly affected by factors both extrinsic (light, water, temperature, soil and nutrients)[6–8] and intrinsic (hereditary, i.e., genotype, and stage of development of leaves and glands, i.e., ontogeny).[6–10]

In spite of the simplicity of steam distillation and its low cost, it is possible that modification of the odour of the essential oil could occur through the high temperature and moisture involved.[11,12] The milder process of solvent extraction has never been studied quantitatively. A kinetic investigation of the solvent extraction of peppermint oil was therefore undertaken using a non-polar solvent (hexane), a polar solvent (ethanol) and their mixtures.

Although solvent extraction can be a relatively slow process, it has recently been reported that microwave extraction of the oil from peppermint leaves in hexane accelerated extraction by a factor of about 180, and produced yields similar to those using steam distillation.[13] As in other published work on microwave extraction,[14–17] the enhancements were reported in terms of yield of oil to weight of raw materials, and the composition of the oil. No experiments were carried out on the rate of extraction under microwave conditions and information on this aspect is provided in the present paper. Ethanol and hexane were again chosen as solvents because of their dissimilar dielectric properties.

For experimental reasons, only the three dominant components 1,8-cineole, menthone and menthol have been analysed in the extracts collected from the kinetic studies.

EXPERIMENTAL

Sample Materials and Analysis

Several trial runs were necessary to locate a suitable type of peppermint plant. The black

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peppermint (*Mentha* × *piperita* × *piperata*) obtained from Iden Croft Herbs, Kent, UK was eventually found to be the most appropriate and was propagated in pots in the open air during the spring and summer seasons, and in the greenhouse during the colder seasons.

Because the essential oil composition and yield were highly dependent on the herbage age, care was taken to pick leaves of about the same colour and size. These two parameters were used as guides for foliage maturity. The individual leaves in a sample were therefore gathered from different pots of plants, as it was impossible to obtain the necessary weight from a single plant.

The density ρ of 2 g of leaves cut into 1-cm^2 pieces and soaked in hexane was determined using a 25-ml pycnometer. Details of the experimental procedure have been given elsewhere.[18] The dielectric properties (relative real permittivity, ε' and dielectric loss factor, ε'') of the leaves were measured with a coaxial probe and HP 8720 network analyser, and the specific heat capacity, C_p, with a differential scanning calorimeter (Perkin-Elmer DSC7), as described previously.[19]

Scanning electron micrographs of the air-dried fresh leaves as well as spent leaves collected at the end of the extractions were taken with a Philips 500 SEM. The samples were sputter-coated (Polaron SEM coating unit E5000) in gold prior to examination.

Extraction Procedures

The solvents used were hexane (BDH, GPR) and ethanol (BDH, AnalaR) as well as hexane–ethanol mixtures of composition 10.1 mol% hexane + 89.9 mol% ethanol (referred to as 90 mol% ethanol), and 89.7 mol% hexane + 10.3 mol% ethanol (90 mol% hexane). The weight of leaves in most experiments was 2.0 g and the solvent volume was kept at 50 ml.

The isothermal extractions were carried out in a thermostatted water-bath at temperatures of 25, 35 and 45°C. Extractions were performed in a three-necked flask fitted with a water condenser to reduce solvent evaporation during a run. Nitrogen was bubbled through the solvent via a gas distribution tube to ensure uniformity of composition and of temperature in the extracting solution.[18] At specific intervals, 0.5-ml samples were withdrawn from the extracting solution using 1-ml syringes.

A domestic microwave oven (Hitachi MR-8140) with a maximum power of 800 W was modified electrically to produce stable partial power outputs, and modified mechanically to permit extractions at atmospheric pressure with minimal solvent losses, as described elsewhere.[19,20] Temperature changes during the microwave extraction process were monitored with a gas thermometer whose bulb was immersed in the solutions.[19,20]

Microwave power outputs ranging from 200 to 642 W were used in the extracting experiments, which fell into two main categories. In one, 2.0 g leaves in 50 ml solvent were irradiated with a mean power of 200 W with 12-s doses in hexane, 90 mol% hexane and 90 mol% ethanol, and 10-s doses in ethanol in order to obtain similar temperature increases, ΔT, of 10–12°C. Higher power outputs ranging from 463 to 642 W were used in another group of experiments to study the effect of higher intensities of microwave energy. After each radiation dose, the extraction system was cooled for 5 min so as to return to ambient temperature (20–25°C). Samples were withdrawn at regular intervals at the end of the radiation doses after addition of the necessary small volumes of solvent to compensate for evaporation losses.[19]

The samples were analysed by gas chromatography (GC) after the addition of an internal GC standard (tridecane). The splitless injection technique of sample transfer into the capillary column was used because of the dilute nature of the sample solutions. Further experimental details have been given in the preceding papers.[18–20]

RESULTS AND DISCUSSION

Equilibrium Properties

The pronounced changes in the chemical composition with herbage age were reflected in the equilibrium concentrations, c_∞, of the three major components listed in Table 1. The equilibrium concentrations for 1,8-cineole ranged from 0.4 to 7.5 μmol (g of leaf)$^{-1}$, for menthone from 4.2 to 29.8 μmol (g of leaf)$^{-1}$, and for menthol from 3.6 to 25.6 μmol (g of leaf)$^{-1}$.

The c_∞ values in each run varied not only with each batch of leaves but also with the solvent used. An example is the exceptionally high concentrations found with 90 mol% hexane. From Table 2, the sum of three successive extraction

Table 1. Values of c_∞, k°_{obs} and $t_{1/2}$ for the isothermal extraction of three major constituents of peppermint oil at various temperatures and in four solvent systems

Compound	Temp. /°C	c_∞/μmol (g of leaf)$^{-1}$				$(k^\circ_{obs}/10^{-2}\ min^{-1})/(t_{1/2}/min)$			
		Hexane	Ethanol	90 mol% ethanol	90 mol% hexane	Hexane	Ethanol	90 mol% ethanol	90 mol% hexane
1,8-Cineole	25	4.5	1.0	1.9	–	1.2 ± 0.1/70	0.7 ± 0.01/111	0.7 ± 0.02/100	–
	25	1.0				1.2 ± 0.1/50	–	–	–
	35	4.0	1.2	1.1	5.7	1.9 ± 0.1/33	1.4 ± 0.1/64	2.6 ± 0.1/41	10.1 ± 0.6/7
	35	2.3		0.4	7.5	2.4 ± 0.2/35	–	2.6 ± 0.2/24	15.5 ± 2.0/5.5
	45	3.4	1.8	2.1		3.0 ± 0.1/24	2.4 ± 0.1/28	5.7 ± 0.3/17	–
Menthone	25	20.6	5.1	7.0	–	0.4 ± 0.03/168	0.6 ± 0.03/118	0.6 ± 0.02/100	–
	25	23.3	–	–	–	0.5 ± 0.03/150	–	–	–
	35	26.6	5.3	4.7	19.7	0.7 ± 0.03/92	1.2 ± 0.1/70	1.7 ± 0.1/45	10.7 ± 0.6/7
	35	–	–	4.2	21.2	–	–	1.8 ± 0.1/36	16.5 ± 1.0/6
	45	29.5	14.2	9.0	–	1.3 ± 0.1/47	1.9 ± 0.1/36	5.8 ± 0.3/17	–
	45	29.8	–	–	–	1.3 ± 0.1/48	–	–	–
Menthol	25	6.7	7.9	6.6	–	0.6 ± 0.1/124	0.9 ± 0.03/90	0.8 ± 0.03/84	–
	25	9.6	–	–		0.6 ± 0.02/138	–	–	
	35	7.2	7.3	4.6	22.9	0.9 ± 0.03/67	1.3 ± 0.1/69	2.2 ± 0.1/37	8.8 ± 0.5/8.5
	35	22.8	–	3.6	25.6	0.8 ± 0.03/84	–	1.8 ± 0.1/38	13.7 ± 2.0/7
	45	4.7	4.1	7.2	–	1.8 ± 0.6/38	1.8 ± 0.1/37	5.1 ± 0.3/18	–
	45	5.2	–	–	–	1.9 ± 0.1/39	–	–	–

The uncertainty limits for c_∞ were ±6% for all three compounds.

runs with fresh hexane gave a total menthol concentration of 24.4 μmol (g of leaf)$^{-1}$, which tallied closely with the c_∞ values obtained in the two experiments using 90 mol% hexane as solvent, i.e. 22.9 and 25.6 μmol (g of leaf)$^{-1}$. Both sets of experiments were carried out in July 1993 with an interval of about two weeks between them. SEM observations reported below show a wide distribution of ruptured glands not observed in the other extraction runs. This suggests that structural changes initiated by 90 mol% hexane have resulted in the high concentrations.

The partition constants, K, for each of the three main components in hexane were determined from the equation:[19]

$$K = \frac{m}{\rho V}\left(\frac{c_\infty(1)}{c_\infty(2)} - 1\right) \quad (1)$$

where $c_\infty(1)$ and $c_\infty(2)$ refer to the equilibrium concentrations after the first and second extractions carried out on the same sample of leaves with fresh volumes V (50 ml) of solvent, while m and ρ (0.68 g cm^{-3}) are the mass and density of the solvent-swollen leaves, respectively. Table 2 lists the equilibrium concentrations obtained from each of three extractions performed on the peppermint leaves. The resulting values of K were fairly similar, being 0.4 for 1,8-cineole, 0.5 for menthone and 0.3 for menthol. The leaves were found to decrease in weight with each extraction, from 2.10 g at the start of the extraction, to 1.60 g for the second extraction and finally to 1.08 g for the third extraction. The partition constants were based on the swollen-weight m as 2.10 g. Because of the change in m, the partition constants serve only to indicate the differences in extraction characteristics of the three components of the essential oil.

From a random light microphotograph showing the distribution of the oil-containing peltate glands on the abaxial side of the peppermint leaf, the number of glands per square centimetre was counted to be c. 7×10^3. A typical peppermint leaf used in the extraction experiments weighed about 96 mg and measured 4.8 cm long by 2.5 cm wide (the broadest width of the leaf). The surface area of the leaf was thus calculated to be c. 10 cm^2 and there were c. 7×10^5 glands (g of leaf tissue)$^{-1}$. This was higher than the literature value of $3–5 \times 10^5$ glands (g of leaf tissue)$^{-1}$.[21] However, the distribution of the glands has been known to differ between the abaxial and adaxial surface of the leaf, as well as between the upper and lower sections of the same side of the leaf. From Table 2, the amount of 1,8-cineole, menthone and menthol per peltate gland is thus approximately 1×10^{-2}, 4×10^{-2} and 3.5×10^{-2} nmol, respectively.

Kinetics of Isothermal Solvent Extractions

Figure 1 shows the concentration–time (c–t) plots for the extraction of menthol into the various solvent systems at 35°C. The c–t plots for 1,8-cineole and menthone were similar to those of menthol. As mentioned earlier, the c_∞ values varied with every kinetic run depending on the batch of leaves and the solvent used. However, it can be seen that with all the solvents the concentration increased rapidly at first, and slowed down on approaching equilibrium.

Only the data for hexane fitted a first-order kinetic equation. All the c–t plots were therefore fitted to third-order polynomials and the rate constants at the initial stage of the extraction, k°_{obs}, obtained by differentiating and applying the equation[19]

$$k^\circ_{obs} = \left(\frac{1}{c_\infty}\right)\left(\frac{dc}{dt}\right)_{t\to 0} \quad (2)$$

Table 1 lists the initial rate constants as well as the half-lives, $t_{1/2}$, obtained for the various solvent systems and temperatures.

As the figures in Table 1 show, the fastest extractions by far took place with 90 mol% hexane. Next came 90 mol% ethanol, although at 25°C it was no better than ethanol itself. The pure solvents were least effective: hexane extracted 1,8-cineole faster than ethanol while ethanol extracted menthone and menthol faster than hexane. An attempt to carry out an experiment with 50 mol% hexane at 35°C was thwarted by the initially clear

Table 2. Partition constants and equilibrium concentrations obtained in three successive extractions of the same batch of peppermint leaves into hexane at 35°C

Compound	Equilibrium conc./μmol (g of leaf)$^{-1}$			Partition constant
	$c_\infty(1)$	$c_\infty(2)$	$c_\infty(3)$	
1,8-Cineole	6.3 ± 0.2	0.8 ± 0.05	0.2 ± 0.01	0.4
Menthone	22.5 ± 1.3	2.7 ± 0.2	1.7 ± 0.1	0.5
Menthol	19.3 ± 1.1	3.3 ± 0.2	1.9 ± 0.1	0.3

The error limits for c_∞ values in tables were estimated as ±6%, owing to uncertainties in the GC results and the volume measurements during sample preparation.

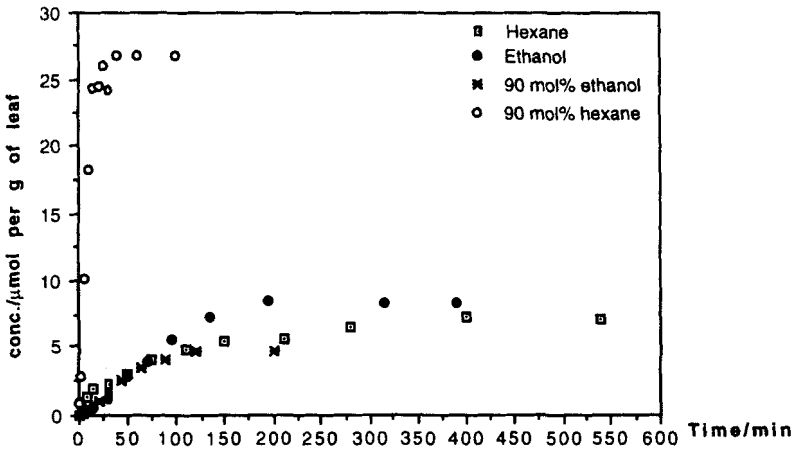

Fig. 1. Solvent extraction of menthol from peppermint leaves into various solvents at 35°C

solvent mixture becoming opaque and separating into two layers as extraction progressed.

The three peppermint oil components were extracted at almost equal rates by ethanol, 90 mol% ethanol and 90 mol% hexane. In the case of pure hexane, however, 1,8-cineole was extracted much more quickly than menthone and menthol. This cannot be attributed to differences in polarity or size of these components: the molecular volumes of 1,8-cineole, menthone and menthol were estimated to be 168, 169 and 178Å^3, respectively.

Inspection of Table 1 shows several instances in which two separate extraction runs in hexane and in 90 mol% ethanol, which gave quite different c_∞ values, yielded fairly similar rate constants and half-lives. For example, c_∞ of 1,8-cineole for two runs in 90 mol% ethanol at 35°C were 0.4 and 1.1 μmol (g of leaf)$^{-1}$, yet the k°_{obs} and $t_{1/2}$ values differed by only 50%. Again, in the case of menthol, two runs in hexane at 35°C yielded c_∞ values of 7.2 and 22.8 μmol (g of leaf)$^{-1}$ but gave the same k°_{obs} of 0.006 min^{-1} and $t_{1/2}$ values of 67 and 84 min, respectively. However, with 90 mol% hexane at 35°C, the c_∞ values were comparable in two separate experiments and so were the half-lives for the extraction of the three components (average value c. 7 min), but the sets of k°_{obs} differed significantly. Moreover, 90 mol% hexane was by far the best solvent. This suggests that a different extraction mechanism operated here.

Table 3. Values of $E^\#$ for the extraction of 1,8-cineole, menthone and menthol in the various solvent systems

Compound	$E^\#$/kJ mol^{-1}		
	Hexane	Ethanol	90 mol% ethanol
1,8-Cineole	35.3	58.0	70.0
Menthone	52.4	43.1	78.8
Menthol	55.2	29.1	71.5

Activation Energies of Extraction

By use of the Arrhenius equation

$$\frac{\mathrm{d} \ln k^\circ_{obs}}{\mathrm{d}(1/T)} = -\frac{E^\#}{R} \qquad (3)$$

the activation energies $E^\#$ for the three extracted compounds were determined from plots of ln (k°_{obs}) against $1/T$, where T is the absolute temperature and R the gas content. Table 3 lists the resulting values.

In hexane extraction, the smallest activation energy belongs to 1,8-cineole which had displayed the highest k°_{obs}, while $E^\#$ was similar for menthol and menthone, both of which showed little difference in their extraction rates. The k°_{obs} values were not very different for the three solutes in ethanol at 35°C although there were significant differences in their activation energies. The much higher $E^\#$ values of about 75 kJ mol^{-1} for 90 mol% ethanol was surprising since the rate constants in this mixed solvent were almost all greater than those in hexane and in ethanol.

The $E^\#$ values with hexane and ethanol were all in the range 30–60 kJ mol^{-1}, similar to those found for the extraction of solubles from some other plant materials such as caffeine from black tea,[22] vitamin C from rose-hip tea[23] (all with water) and [6]-gingerol from ginger rhizome in

acetone.[24] However, much higher activation energies, well over 100 kJ mol^{-1}, had been found for the extraction of several essential oil constituents from rosemary leaves into hexane and ethanol.[18]

Kinetics of Microwave Extractions

With hexane and both solvent mixtures, a 12-s radiation dose at 200 W was sufficient to produce a 10°C increase in temperature over the ambient value (20–25°C), while 10-s radiation doses were adequate for ethanol. Thus similar doses of microwave radiation were required to produce the same temperature rise in the various solvent systems although ethanol is a much better microwave absorber than hexane.[20] This can be attributed to the even spatial distribution of the highly microwave absorbing peppermint leaves throughout the mixture volume. Hence in the leaves and hexane system, the leaves absorbed most of the microwave energy.

Figure 2 shows typical c–t graphs for the four solvent systems. The time scale includes the 5-min cooling interval between each dose of radiation. The c–t plots were fitted to third-order polynomials and k°_{obs} was obtained in the same manner as with the isothermal extractions. Table 4 lists the k°_{obs} values and the half-lives of extraction, $t_{1/2}$. Within any given run, the rate parameters for the three oil components were very similar.

Inspection of the results shows that for a given solvent system and sample size, higher microwave power outputs generated shorter $t_{1/2}$ and larger k°_{obs} values. This is illustrated in Figure 3 for hexane extraction. With ethanol, $t_{1/2}$ also decreased by a factor of c. 3 when the power was raised from 200 to 555 W but a further rise to 642 W produced another tenfold fall in $t_{1/2}$ although ΔT remained almost the same.

Under the same low microwave heating conditions of 200 W, k°_{obs} as well as $t_{1/2}$ were solvent-dependent. With 2.0 g leaves, all three compounds were extracted with the shortest half-lives of between 8.5 and 12.5 min in 90 mol% hexane, followed by hexane and 90 mol% ethanol in the range 47 to 52 min and finally by ethanol with 73.5–75 min. This trend in the solvents was the same as that exhibited in the isothermal extractions.

When the weight of leaves in 50 ml hexane was doubled from 2.0 to 4.0 g at 200 W power, ΔT was only 1.5°C higher and there was no definite trend in the half-lives of the three components. This indicated that the microwave energy available during the 12-s radiation doses had been almost totally absorbed by 2.0 g of leaves in the extracting mixture. The effect of doubling the mass of leaves was, however, more pronounced at 555 W. Here ΔT increased by 6°C, from 13 to 19°C, and $t_{1/2}$ decreased by about 22%.

Simulated Microwave Extractions

The extraction rates determined for microwave

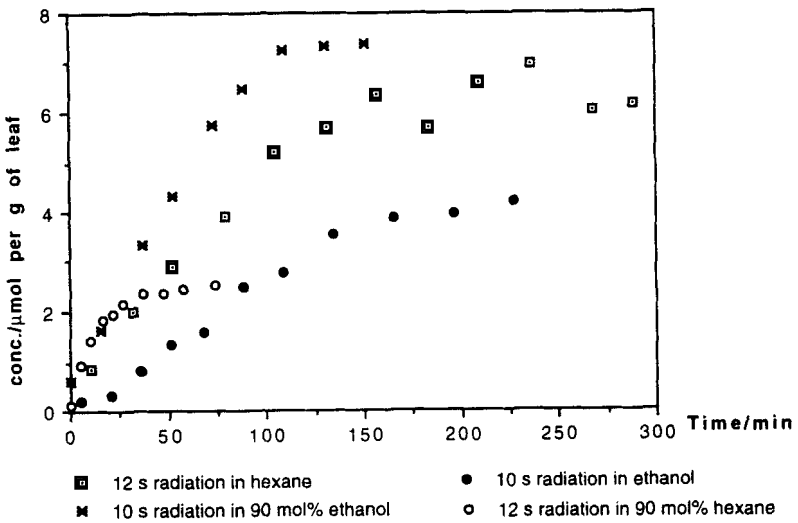

Fig. 2. Microwave extraction of menthol from peppermint leaves into various solvents with temperature increases of c. 10°C after each dose of 200 W radiation

Table 4. Rates of microwave extraction of essential oil components of peppermint leaves into various solvent systems

Compound	Power/dose[a]/ΔT W/s/°C	Solvent	Leaf weight /g	c_∞ /μmol (g of leaf)$^{-1}$	k°_{obs} /10^{-2} min^{-1}	$t_{1/2}$ /min
1,8-Cineole	200/12/9.5	hexane	2.0	3.0 ± 0.2	1.2 ± 0.2	47.0
	200/10/10	ethanol	2.0	1.1 ± 0.0_7	0.7 ± 0.2	73.5
	200/12/12.5	90 mol% ethanol	2.0	1.8 ± 0.1	1.1 ± 0.1	46.5
	200/12/10.6	90 mol% hexane	2.0	2.0 ± 0.1	4.4 ± 0.3	12.5
	200/12/11	hexane	4.0	0.8 ± 0.0_5	1.6 ± 0.1	37.0
	463/12/15	hexane	4.0	0.8 ± 0.0_5	4.2 ± 0.4	17.4
	555/12/13	hexane	2.0	0.3 ± 0.0_2	2.0 ± 0.7	16.0
	555/12/19	hexane	4.0	0.9 ± 0.0_5	5.0 ± 0.3	12.3
	642/12/21	hexane	4.0	0.9 ± 0.0_5	4.1 ± 0.4	11.3
	555/10/30	ethanol	2.0	0.7 ± 0.0_4	2.7 ± 0.4	25.5
	555/10/29	ethanol	2.1	0.8 ± 0.0_5	2.2 ± 0.1	27.0
	642/10/29.5	ethanol	2.0	1.0 ± 0.0_6	–	2.8
Menthone	200/12/9.5	hexane	2.0	15.5 ± 0.9	1.0 ± 0.1	50.0
	200/10/10	ethanol	2.0	9.2 ± 0.6	0.5 ± 0.2	75.0
	200/12/12.5	90 mol% ethanol	2.0	5.7 ± 0.3	1.0 ± 0.1	47.0
	200/12/10.6	90 mol% hexane	2.0	32.2 ± 1.9	4.4 ± 0.2	10.7
	200/12/11	hexane	4.0	6.1 ± 0.4	0.8 ± 0.1	58.0
	463/12/15	hexane	4.0	12.8 ± 1.1	2.0 ± 0.3	17.0
	555/12/13	hexane	2.0	5.5 ± 0.3	1.9 ± 0.3	17.0
	555/12/19	hexane	4.0	20.1 ± 1.2	2.9 ± 0.2	13.5
	642/12/21	hexane	4.0	4.9 ± 1.3	3.7 ± 0.4	10.7
	555/10/30	ethanol	2.0	6.9 ± 0.4	2.3 ± 0.3	31.0
	555/10/29	ethanol	2.1	7.4 ± 0.4	1.9 ± 0.4	30.0
	642/10/29.5	ethanol	2.0	14.6 ± 0.9	–	2.4
Menthol	200/12/9.5	hexane	2.0	6.5 ± 0.4	1.0 ± 0.2	52.0
	200/10/10	ethanol	2.0	4.1 ± 0.2	0.7 ± 0.1	73.5
	200/12/12.5	90 mol% ethanol	2.0	7.3 ± 0.4	1.0 ± 0.1	41.5
	200/12/10.6	90 mol% hexane	2.0	2.5 ± 0.2	5.6 ± 0.3	8.5
	200/12/11	hexane	4.0	2.2 ± 0.1	0.9 ± 0.1	52.0
	463/12/15	hexane	4.0	3.0 ± 0.2	2.0 ± 0.3	19.0
	555/12/19	hexane	4.0	3.1 ± 0.2	4.1 ± 0.4	10.3
	555/10/30	ethanol	2.0	12.3 ± 0.7	1.8 ± 0.5	31.0
	555/10/29	ethanol	2.1	15.1 ± 0.9	1.7 ± 0.3	26.0
	642/10/29.5	ethanol	2.0	6.7 ± 0.4	–	2.4

[a]Dose refers to duration of microwave radiation for each pulse.

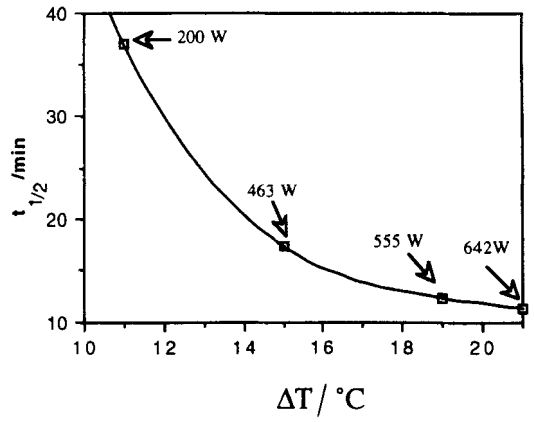

Fig. 3. Variation of half-lives with ΔT in the extraction of 1,8-cineole from 4.0 g leaves into 50 ml hexane at different power levels

extractions cannot be directly compared with those of the thermostatted extractions. This is because the essential oil components were extracted more slowly during the 5-min cooling interval than during the shorter high-temperature microwaving period. Simulated extraction plots as described in a previous paper[19] thus involved alternate combinations of the thermostatted extraction plots at two temperatures (35 and 25°C, or 45 and 25°C). However, in order to allow for the wide variations in the equilibrium concentrations in these runs, the c–t graphs for all three temperatures were first scaled so as to normalize them to the same c_∞ as in the microwave extraction. The resulting simulated microwave extraction plots of menthol into hexane are compared with the actual microwave extraction at 200 W in Figure 4. It is apparent that the latter

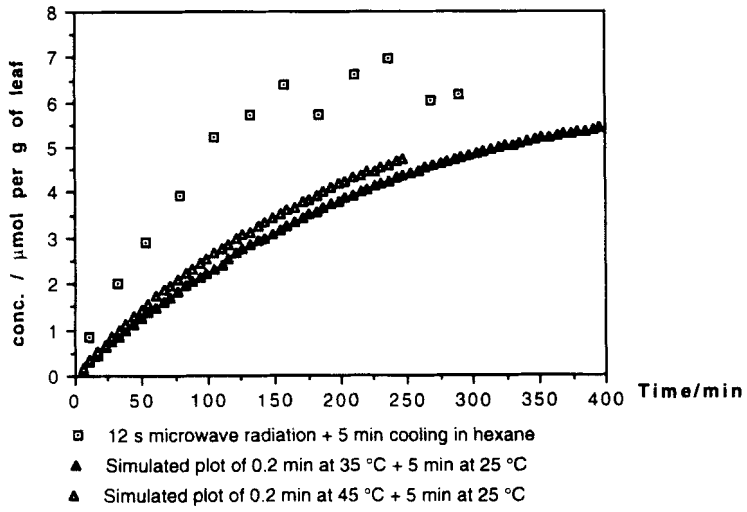

Fig. 4. Simulated microwave extraction plots compared with the actual microwave extraction of menthol into hexane at 200 W, with ΔT at 9.5°C

has proceeded much more rapidly than either of the simulated plots. Thus the $t_{1/2}$ values for the extraction of menthol into hexane were 164, 130 and 52 min for the simulated plots of 35/25°C, 45/25°C and the actual microwave extraction, respectively. The reason for the faster than expected microwave extraction may be attributed partly to the assumption that the entire cooling period was at 25°C as well as to the enhanced deformations of the glands generated by stresses induced by the rapid temperature fluctuations during pulses of irradiations.[19]

Structural Changes during Extraction

The various solvents produced quite different physical changes in the peppermint leaves. After both isothermal and microwave extractions in hexane, the leaves were limp and darkish green, while with ethanol they were discoloured and brittle. The effect of 90 mol% ethanol was the same as that of pure ethanol, while 90 mol% hexane resulted in leaves which were not as brittle or easily breakable as those in ethanol. The 90 mol% hexane also extracted some chlorophyll but less than the mainly ethanol-based solvents.

As regards the detailed structure of the leaf, the peltate trichomes of *Mentha* × *piperita* are ten-celled glandular structures with eight secretory cells.[25,26] As in all species of the Labiate family, the walls of the glands are built of an inner pectic layer, followed by the cuticle proper and finally the outermost cutinized layer.[27] The essential oils accumulate in the subcuticular space between the gland wall and the innermost secretory cells.[25] Brun *et al.*[3] reported on the complete absence of any form of rupturing of the cuticular layer during steam distillation and hexane extraction.

To see what damage had been done to the oil-containing peltate glands during the present experiments, the peppermint leaves were examined under the scanning electron microscope. Some glands had been unaffected, others were deformed but unbroken, some were sunken to varying degrees, and some glands had ruptured. Figure 5 is a micrograph of the untreated leaf which can be compared with the structures of the treated leaves in Figures 6–9. The changes observed for isothermal extractions were similar to those after microwave extractions with ΔT in the region of 10°C, hence comparisons can be made essentially in terms of the solvent effects. However, a significant difference was noted with 90 mol% hexane. Isothermal extractions showed massive bursting of glands (Figure 9) but this was not conspicuous in the microwave extractions with the same solvent. The extracting environment for the two differed in that the former was continually kept at 35°C, while the latter varied between c. 35 and 25°C. The changes which occurred at high microwave power in the pure solvents were not markedly different from those at low power except for the severity of damage in the glands. Ethanol extractions at 642 W did show numerous glands rupturing.

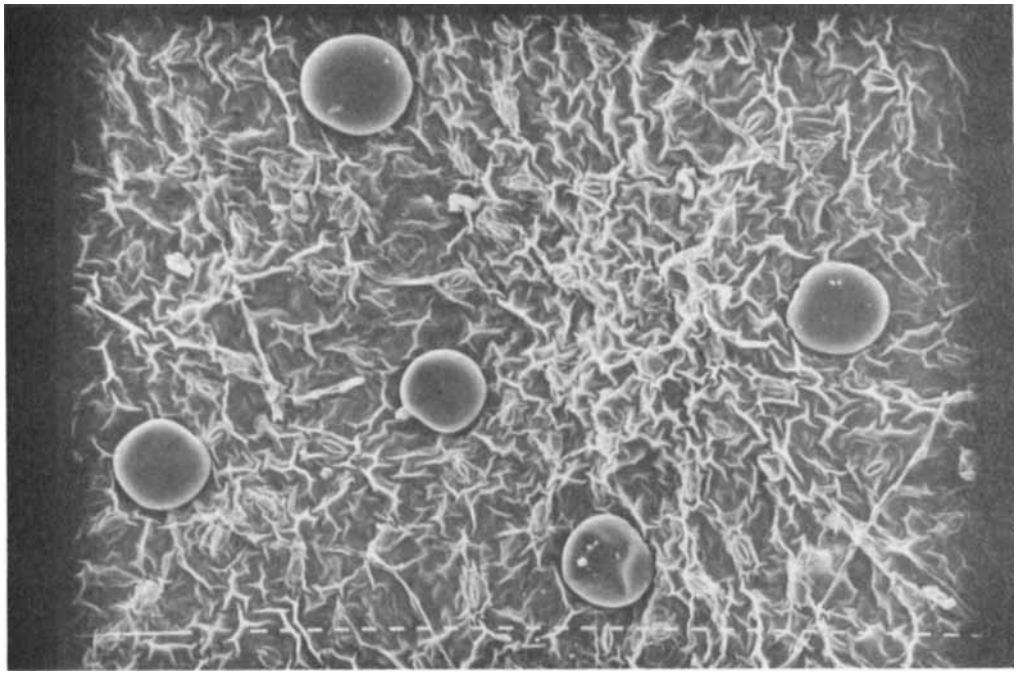

Fig. 5. Untreated leaf showing globular whole glands (10 μm bar, 200× magnification)

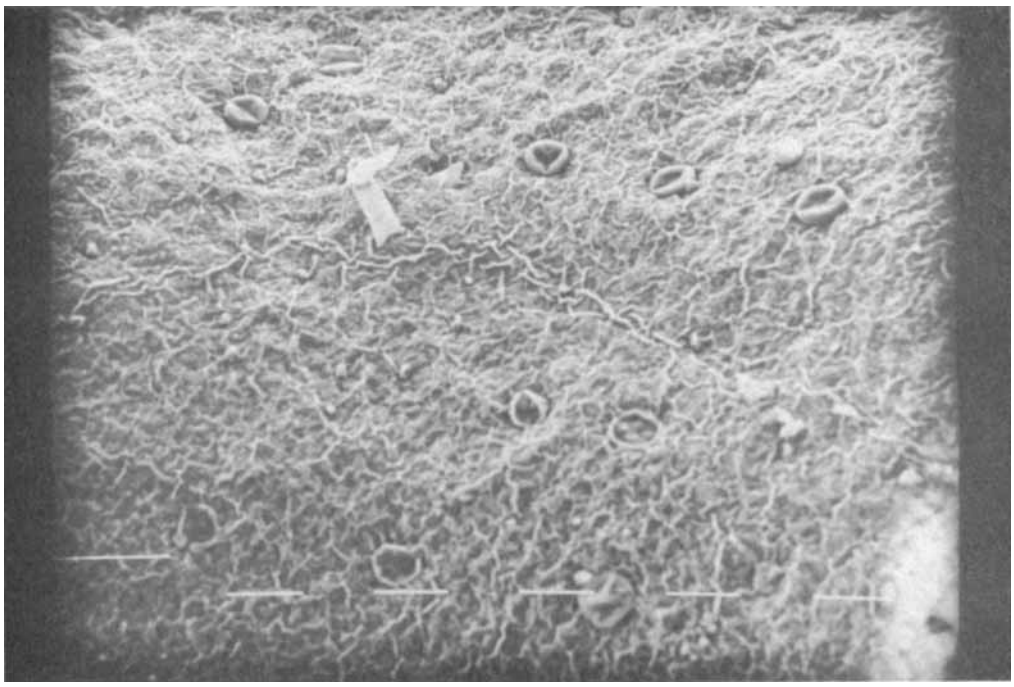

Fig. 6. Glands collapsed to varying degrees in leaves extracted with hexane at 200 W, ΔT c. 10°C (100 μm bar, 100× magnification)

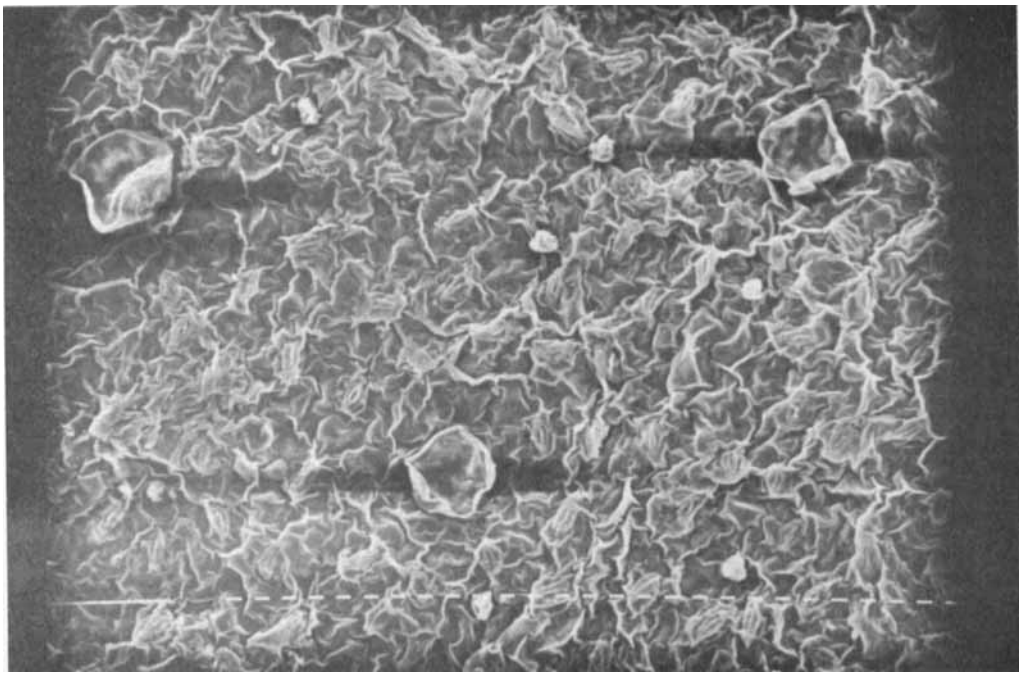

Fig. 7. Shrivelled collapsed glands in extractions carried out using ethanol at a constant temperature of 35°C (10 μm bar, 200× magnification)

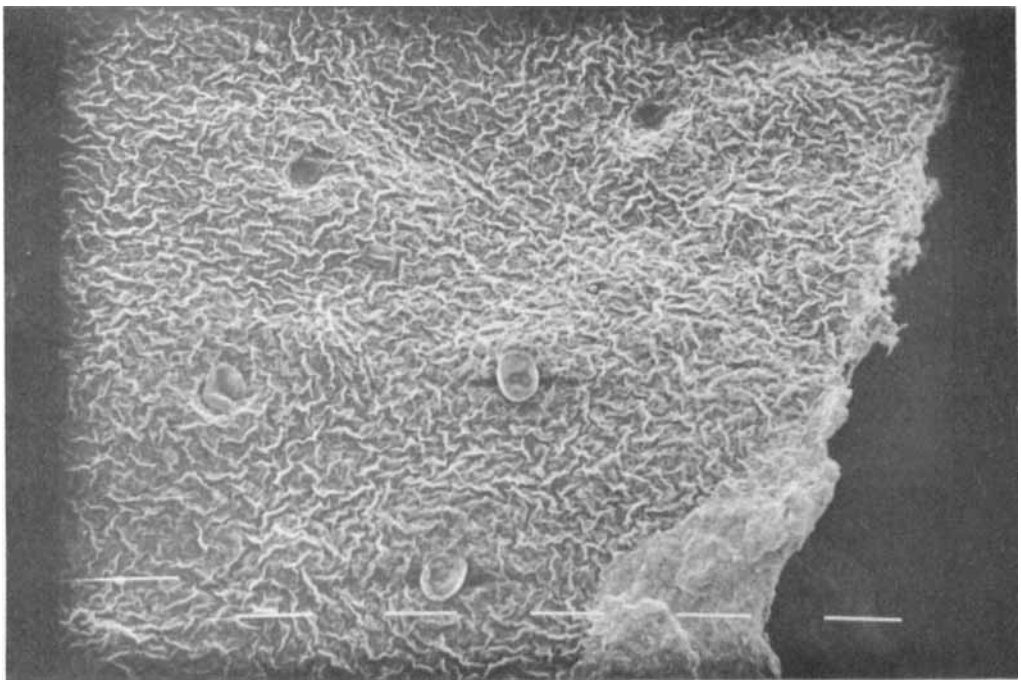

Fig. 8. Glands transformed into deeply sunken cavities after extraction in 90 mol% ethanol at 35°C (100 μm bar, 100× magnification)

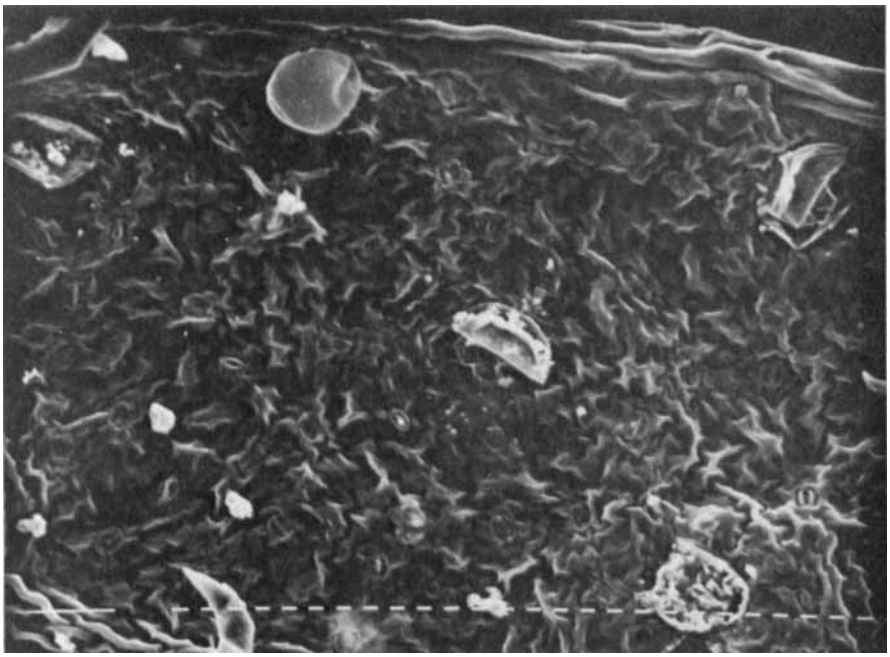

Fig. 9. Glands that have ruptured completely in isothermal extractions carried out with 90 mol% hexane at 35°C. Note the presence of an unaffected gland in the midst of the broken glands. The varied effect of the solvent on the glands could be attributed to their different stages of maturity (10 μm bar, 200× magnification)

Table 5. Effect of various solvent and heating systems on the peltate glands during extraction of peppermint oil

Extraction conditions	Number of glands				
	Globular whole glands	Slightly deformed and sunken glands	Deeply sunken glands	Broken glands	Percentage of damaged glands[a]
Untreated leaf	77	77			50
100% hexane at 35°C	3	359			99
100% ethanol at 35°C		64		7	100
90 mol% ethanol at 35°C		18	132		100
90 mol% hexane at 35°C	13		100	133	95
Microwave/200 W/hexane	26	142		4	85
Microwave/200 W/ethanol	1	221[b]		7	99

[a] Percentage of damaged glands = $\frac{\text{Sum of all damaged glands irrespective of degree of damage}}{\text{Total number of glands}} \times 100$

[b] 15% of the glands looked shrivelled/shrunken.

Table 5 summarizes the effects on the glands produced by the different extractions. Collapsed cuticles were seen to be present even in the untreated leaves although the indentations were generally not severe. After treatment with the three solvent systems of hexane, ethanol and 90 mol% ethanol, the leaves contained predominantly glands with collapsed cuticles. This agrees with the observation of Brun *et al.*[3] Where the glandular wall had collapsed, the degrees of deformity varied from slight indentations to ones so deeply sunken that they appear as cavities as in Figure 10. Deeply sunken indentations were clearly prominent with 90 mol% ethanol, but here there were no broken glands. Ruptured glands were only seen after isothermal 90 mol% hexane extraction.

The micrographs in Figures 10 and 11 provide evidence supporting the absence of burst glands.

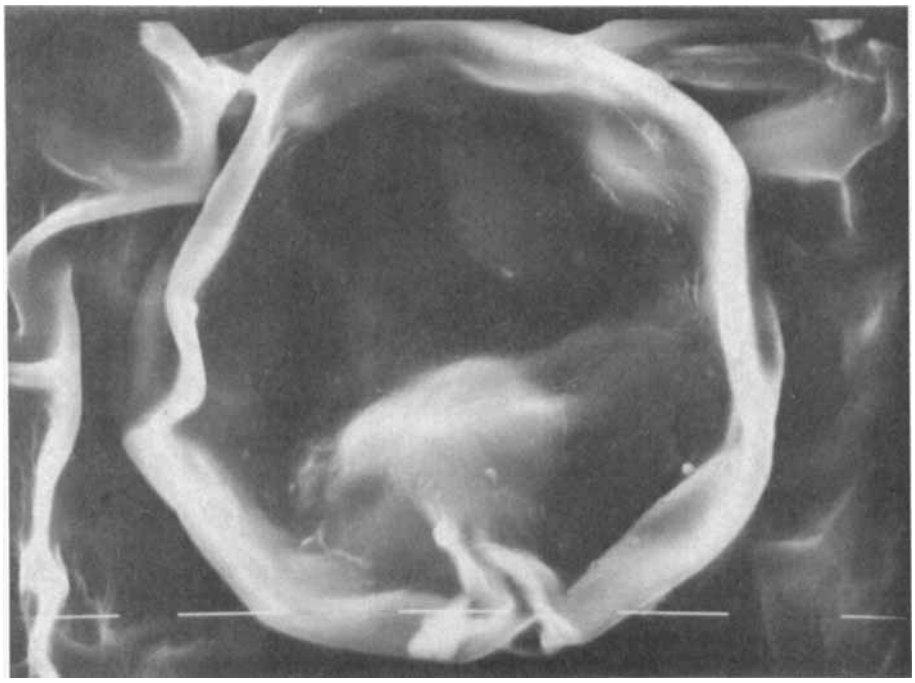

Fig. 10. A badly collapsed gland observed in the microwave extraction with hexane at 463 W and ΔT c. 15°C, showing the initial signs of recovery from its deflated condition (10 μm bar, 1600× magnification)

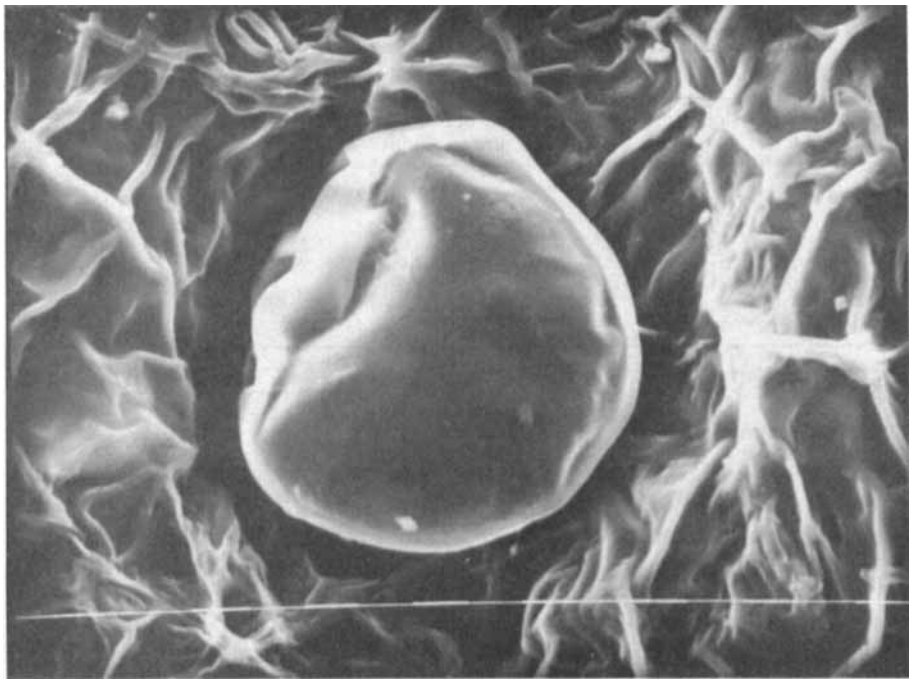

Fig. 11. The inflated gland, due to high magnification focusing, is not restored to its original globular shape but is nevertheless not in a collapsed condition (100 μm bar, 800× magnification)

At high magnification focusing, a number of collapsed glands were observed to bloat up from their flattened topography. This could have been caused by the dissipation of the electron beam energy over a smaller area as magnification increased. The diameter of the electron probe was 320 Å and carried along with it a current of 10^{-10} to 10^{-12} A. Hence an effect was generated causing the gland wall to expand again. This phenomenon could not have occurred had the gland walls burst. Beam damage such as melting of wax deposits or blistering on leaf surfaces are known events in SEM analysis[28] and have been attributed to the electron bombardment on the surface of the specimen. Excessive damage such as this did not occur as the current density would have been reduced considerably at the gland (the gland diameter was c. 60 μm as against the spot size of 32 nm).

Mechanism of Extraction

Two distinct extraction mechanisms become obvious from the micrographs, one involving diffusion of the essential oil across the glandular wall and the other, rapid exudation of the oil into the surrounding solution following rupturing of the glandular walls. Comparison with Table 1 clearly points to gland rupturing as the cause of the much faster extraction rates in 90 mol% hexane. In most of the present work with peppermint leaves, there was no evidence of the explosion said by Pare *et al.* [13,14] to occur at the cell level as a consequence of the sudden temperature rise generated by microwaves. However, Pare *et al.*[13,14] did not introduce a cooling interval in between each dose of radiation as in the present kinetic studies. Hence, their temperature increases will have been higher. Moreover, the leaves in Pare *et al.*'s patents were chopped into pieces prior to the extraction process. This would have brought about additional damage to the glands even before the microwaving stage.

The extraction of essential oil constituents from the peppermint leaves, both isothermally and microwave-assisted, into hexane, ethanol and 90 mol% ethanol seemed to involve mass transfers of the constituents across an unbroken wall. The rate of this transfer increased with temperature. The effects of the different solvents on the glandular walls were difficult to distinguish, although the extent of indentation of the walls increased from hexane to ethanol to the solvent mixtures. As mentioned previously,[18] glands are broken in the presence of a mixed solvent such as 90 mol% hexane through a combination of the dehydrating capability of ethanol, which produces a change in the surface tension of the glandular wall, and the dissolution of the cuticular layers in hexane. The bursting of glands at 642 W during ethanol extraction can be attributed to the more effective dehydrating process with a higher heating rate at the leaves.

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