

KINETIC STUDIES ON ZINGIBER OFFICINALE

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ABSTRACT

The present investigation deals with the isolation, purification and characterization of gingerol, the major pungent constituent of ginger (*Zingiber officinale*) and its kinetic of extraction using a number of organic solvents. The characterization was carried out through GC and GC-MS. Gingerol has been assayed in the plant material during extraction with various solvents by a HPLC method.

In order to develop a relationship between solvent characteristics such as viscosity and dielectric constant and the rates of extraction, the kinetics of extraction of gingerol has been studied by using twelve different solvents in order to evaluate the solvent efficacy in the extraction processes. It has been observed that both solvent viscosity ($1/\nu$) and dielectric constant (ϵ) show a linear relationship with the rates of extraction (k). An increase in solvent viscosity leads to a decrease in the rates of extraction, similarly an increase in dielectric constant also leads to a decrease in the rates of extraction. This appears to be largely due to an unionizable character of gingerol which does not interact with polar solvents. Thus solvent viscosity and dielectric constant both play an important role in the choice of solvents for the extraction of gingerol. Solvents with relatively low viscosity and dielectric constant are more suitable for the extraction of gingerol from plant material.

INTRODUCTION

Ginger is a rhizome of plant *Zingiber officinale* Roscoe of the family Zingiberaceae. It is chiefly distributed in India, East Asia and Malaysia; widely cultivated in India, Bangladesh, Taiwan, Jamaica and Nigeria (Tyler, 1993 and Mustafa *et al.*, 1993).

In Chinese and Unani-Tibb systems of medicine, ginger is used to treat catarrh, rheumatism, nervous diseases, gingivitis, toothache, painful menstruation, asthma, stroke, constipation, diabetes and migraine (Anonymous, 1991 and Mustafa & Sirivastava, 1990). In Asian medicine, it is used as a carminative (digestive aid), stimulant, diuretic and anti-emetic (Tyler, 1993).

Ginger is valued throughout the world as a spice or flavouring agent (Tyler *et al.*, 1988). The characteristic aroma of ginger is due to a volatile oil that is present in 1-3% quantities. Its pungency is attributed to ginger oleoresin (Tyler, 1993).

The major component of ginger oleoresin is Gingerol that is a mixture of homologues having 10, 20 and 14 carbon atoms in the side chain. They are designated as Gingerols also found in small

quantities are Zingerone and Shogaol (Tyler *et al.*, 1988 and Govindarajan, 1982). A variety of pharmacological activities have been performed on Gingerol, Shogaol and their analogues both separately and in combinations. The results from these experiments suggest that they possess moluscisidal (Adewunmi *et al.*, 1990), antitussive, analgesic, antipyretic, antiemetic and cardiostimulant activities. They also inhibited spontaneous motor activity, prolonged hexobarbital induced sleeping time (Suekawa *et al.*, 1984), inhibited gastric lesions (Yamahara *et al.*, 1985, 1988) and increased bile acid secretions (Adewunmi *et al.*, 1990; Aburada *et al.*, 1982; Suekawa *et al.*, 1984; Yamahara *et al.*, 1985; 1988; 1989 and Kobayashi *et al.*, 1988).

The aim of the present study was to evaluate the efficiency of solvents in the extraction of Gingerol, Shogaol and their analogues. The results thus obtained would lead us to adopt commercially viable method of extraction of these compounds.

In order to rationalize the solvent efficiency, quantitative measurements of the kinetics of the solvent extractions were carried out and a co-relation was developed between kinetic data of solvents and their parameters like dielectric constant and viscosity. The results thus obtained would lead us to adopt an economically viable method for the extraction of Gingerol etc.

EXPERIMENTAL

Plant Material:

The dried rhizome of *Zingiber officinale* Rcoe was purchased from the local market. It was ground into fine powder using a food processor and stored in a closed container until required.

Reagents:

All solvents used during the experiment were of analytical grade and obtained from BDH/Merck. Beside these solvents, freshly prepared distilled water was also used wherever required.

Apparatus

HPLC consisted of a model LC-6A Shimadzu pump as a solvent delivery system. Rhodyne sample injector with a 20 μ l loop, precolumn (50 mm x 4.6 i.d.), Main Column (250 mm x 4.6 mm i.d., Whatman) with 5 μ l octadecylsilane (ODS) stationary phase, C-R4A (Shimadzu) Recorder and SPD-6AV (Shimadzu) Detector. The GC-MS was performed on Hewlett Packard GC with 11/73 DEC computer system and 1.2 m x 4 mm packed glass capillary column, coated with gas chrome Q (100-120 mesh, OV 1011%). The column temperature was maintained between 70-250°C with an 8°C/min rate of increase in temperature. The Helium gas was used as carrier gas with a flow rate of 32 ml/min. The injector temperature was maintained at 250°C.

Extraction and Isolation:

Dried ginger (10 kg) was first ground into powder and soaked in acetone for 15 days, then extracted three times. The combined acetone extract was evaporated under reduced pressure on rotary vacuum evaporator to yield 106 gm of oily residue. This oily residue was partitioned between water and n-Hexane. The n-Hexane layer was evaporated under reduced pressure to yield 25 gm of oily extract. It was subjected to column chromatography on silica gel using n-Hexane and diethylether in the ratios of 8:2, 7:3, 6:4 and finally 4:6 (v/v).

The identification of 6-Gingerol was made on the basis of TLC using silica gel fluorescent plates and n-Hexane-diethylether (7:3 v/v) as mobile phase. The spots were detected by spraying

the plate with concentrated sulphuric acid followed by heating at 140°C for 10 minutes. The R_f value of 6-Gingerol was found as 0.29 and is in agreement with that reported (Connell & McLachlan, 1972) value of Gingerol. On the basis of TLC analysis the required fraction was first subjected to GLC and finally assessed on GC-MS. Consequently Gingerols were identified by matching their GC-MS spectra with those reported in literature (Connell & McLachlan, 1972 and Harvey, 1981).

Kinetic Study on Extraction of Gingerol:

Dried ginger powder was sieved by mechanical shaking and stored in an airtight container. The extracts were prepared by using twelve different solvents. This was carried out in previously weighed 100 ml volumetric flasks fitted with stopper and provided with magnetic stirrer. They were filled with 100 ml of each solvent and re-weighed. The solvent was stirred at 450 rpm at 30°C; once the thermal equilibrium was achieved (after about 15-20 min.), accurately weighed ginger powder (3.0 g) was added. The time was noted and stirring continued. At predetermined intervals 2.0 ml of the extract was drawn from each flask using a glass syringe fitted with stainless steel needle. After drawing six samples of each extract, they were filtered through 0.45 µm pore size PTFE filter, collected in sample tube and stored in refrigerator until analyzed on HPLC (After the run, the flask with its stopper and magnetic stirrer was weighed again to determine the total solvent loss). Later on each fraction was subjected to HPLC, using methanol: water (6:4 v/v) as mobile phase flowing at the rate of 2 ml/min. This system gave best resolution for 6-Gingerol peak that was identified by comparing with the peak of isolated Gingerol used as standard. The absorbance of HPLC eluate was measured at 282 nm (SPD-6AV Shimaduz), absorbance position.

RESULT AND DISCUSSION

The data obtained by the analysis of group in various solvents at different time intervals is reported in Table 1. The kinetic treatment of the extraction data was carried out by using the first-order rate equation (Spiro and Page, 1984, Spiro and Selwood 1984 and Spiro *et al.*, 1989).

$$\ln \frac{(C_{\infty})}{C - C_{\infty}} = k_{\text{abs}} t$$

Where C and C_{∞} are the concentration of extracted constituents at time t and infinity, respectively.

For a particular solvent, gingerol concentrations determined at various intervals were plotted as function of time, and a relationship was observed. The apparent first-order rate constants for the process were determined from the slopes of the kinetic curves (Fig. 1). The values of k_{abs} are reported in Table 2 and 3. It is evident from the values of k_{abs} that they differ in the solvent used indicating the influence of the nature of the solvent on the extraction efficacy for gingerol. The rate of extraction may depend upon the affinity of the component with the solvent.

Solvent Effect on Kinetics of Gingerol Extraction:

Solvent Viscosity:

An important parameter in extraction process is the viscosity of solvent. It may influence the infusion process and enhance the rate of extraction (Spiro and Page, 1984, Spiro and Selwood 1984 and Spiro *et al.*, 1989). A consideration of the influence of solvent viscosity on the rate of

extraction may be necessary in the choice of the solvent used for particular extraction process. The values of k_{abs} for the extraction of gingerol were plotted as function of reciprocal of solvent viscosity (cp^{-1}) and are shown in Fig. 2.

It has been observed that there is a linear relation between viscosity (cp^{-1}) and the rates of extraction k_{abs} . As the solvent viscosity decreases the extraction rate increases suggesting that extraction of active ingredients is influenced by the viscosity of the solvent and an increase in viscosity appears to slow down the extraction of gingerol. It may, therefore, be concluded that low viscosity solvents produce better results for the extraction of gingerol, probably due to their greater diffusion in the plant material matrix.

Solvent Dielectric Constant:

The active ingredient is purely a chemical entity in a plant material and may be acidic, basic or neutral in character. The polar or non-polar behaviour of the substances will depend on the chemical structure and ionic character of the molecules. Since the dissolution process largely depends on the solute-solvent interaction (Martin and Swartric, 1983) the maximum interaction could lead to a faster and greater extraction of the solute (i.e. the active ingredient). Normally the polar solvents will have a greater affinity for polar compounds. It could be very helpful if a relation could be developed between the dielectric constant of the solvent (polarity) and the rate of extraction efficacy of the solvent for compounds of different polar and non-polar character.

A plot of k_{abs} for the extraction of gingerol in various solvents (neutral in character) as a function of solvent dielectric constants is shown in Fig. 1. It is interesting to note that a linear relationship does appear to exist between k_{abs} and the dielectric constant (ϵ). Gingerol is a phenolic ketone with a very weak ionic character (phenolic OH) and thus the solvent polarity may have little influence on the compound. It appears that greater the solvent polarity lesser the rate of extraction, suggesting that low polarity solvents are more suitable for extracting gingerol from the plant material. This is because of the fact that non-polar or weakly polar compounds are better extracted with non-polar or weakly polar solvents such as benzene, chloroform and hexane having low dielectric constants. Since the solvent used are almost neutral, those with some polar character or those having a lone pair of electrons (e.g. diethylether, isopropanol etc.) may not be able to interact with gingerol because of its very weak ionic character thus showing low extraction efficacy. On the other hand the extraction in very low polarity solvents is purely a dissolution phenomenon giving a relatively greater extraction efficacy.

Overall Solvent Effects in Extraction Kinetics:

The observations made on the influence of viscosity and dielectric constant on the extraction efficacy of solvent and their correlation with the rates of extraction indicates that the two solvent parameters play an important role in determining the solvent extraction efficacy depending upon chemical nature of the active ingredients to be extracted. Thus it is important to consider these two factors in the choice of a solvent for extraction. Keeping in mind the economic consideration any two solvents may differ in cost but produce almost same results on extraction of active constituent from the plant material. In specific cases mixture of two or more solvents or gradients elution technique may be used to improve the extraction efficacy in single or multi-ingredient extractions. Since there appears to have been done very little work in this field (Spiro and Page, 1984, Spiro and Selwood 1984 and Spiro *et al.*, 1989) and even those authors have not taken into consideration the concept of viscosity and dielectric constant and their influence on the extraction efficacy of the ingredients, it is not possible to point out to any other application of this approach in the development of solvent extraction processes.

Table 1
Extraction of gingerol in different organic solvents

Solvents	Concentration	Time					
		0	15	30	60	120	240
Benzene	Conc. $\mu\text{l}/100\text{ ml}$	0	4338	6125	8016	10491	11552
	$\text{Log}(\frac{C_{\infty}}{C_{\infty}-C})$	0	0.2040	0.3281	0.5147	1.0361	1.8600
Hexane	Conc. $\mu\text{l}/100\text{ ml}$	0	3937	5968	7982	9735	10957
	$\text{Log}(\frac{C_{\infty}}{C_{\infty}-C})$	0	0.1932	0.3416	0.5662	0.9526	1.8100
Chloroform	Conc. $\mu\text{l}/100\text{ ml}$	0	6291	7180	8112	9384	10251
	$\text{Log}(\frac{C_{\infty}}{C_{\infty}-C})$	0	0.4131	0.5234	0.6805	1.072	1.7600
Ethanol	Conc. $\mu\text{l}/100\text{ ml}$	0	5249	6289	7438	8783	9921
	$\text{Log}(\frac{C_{\infty}}{C_{\infty}-C})$	0	0.3275	0.4364	0.6015	0.9404	1.6400
Methanol	Conc. $\mu\text{l}/100\text{ ml}$	0	5471	6112	7245	8105	9015
	$\text{Log}(\frac{C_{\infty}}{C_{\infty}-C})$	0	0.4054	0.4921	0.7060	0.9950	1.6500
Acetone	Conc. $\mu\text{l}/100\text{ ml}$	0	3658	4679	5981	7547	8975
	$\text{Log}(\frac{C_{\infty}}{C_{\infty}-C})$	0	0.2273	0.3199	0.4767	0.7983	1.3700
Dichloro-ethane	Conc. $\mu\text{l}/100\text{ ml}$	0	2809	3786	4927	6245	7921
	$\text{Log}(\frac{C_{\infty}}{C_{\infty}-C})$	0	0.1901	0.2823	0.4225	0.6745	1.2800
Acetonitrile	Conc. $\mu\text{l}/100\text{ ml}$	0	3199	3984	4481	5709	7028
	$\text{Log}(\frac{C_{\infty}}{C_{\infty}-C})$	0	0.2637	0.3633	0.4408	0.7265	1.2000
Diethylether	Conc. $\mu\text{l}/100\text{ ml}$	0	630	1343	1983	3079	4987
	$\text{Log}(\frac{C_{\infty}}{C_{\infty}-C})$	0	0.0586	0.1362	0.2201	0.4172	0.8900
Ethyl Acetate	Conc. $\mu\text{l}/100\text{ ml}$	0	1913	2379	2913	3583	4903
	$\text{Log}(\frac{C_{\infty}}{C_{\infty}-C})$	0	0.2147	0.2883	0.3916	0.5698	0.8600
Isopropanol	Conc. $\mu\text{l}/100\text{ ml}$	0	571	960	1785	2698	4815
	$\text{Log}(\frac{C_{\infty}}{C_{\infty}-C})$	0	0.0548	0.0965	0.2011	0.3568	0.7900
Dichloro-methane	Conc. $\mu\text{l}/100\text{ ml}$	0	973	1343	1701	2439	4619
	$\text{Log}(\frac{C_{\infty}}{C_{\infty}-C})$	0	0.1027	0.1496	0.1994	0.3260	0.5800

Table 2
Values of K_{abs}

Solvents	Dielectric constant (ϵ)	Viscosity (cp^{-1})	K_{abs} , min^{-1}
Benzene	2.3	1.773	0.0171
Hexane	1.9	3.401	0.0166
Chloroform	4.8	1.945	0.0141
Ethanol	24.6	0.925	0.0134
Methanol	32.7	1.675	0.0126
Acetone	20.7	0.295	0.0117

Table 3
Values of K_{abs}

Solvents	Dielectric constant (ϵ)	Viscosity (cp^{-1})	K_{abs} , min^{-1}
Dichloroethane	10.6	1.282	0.0109
Acetonitrile	37.5	2.898	9.590×10^{-3}
Diethyl ether	4.3	0.222	7.770×10^{-3}
Ethyl acetate	6.0	2.500	7.676×10^{-3}
Isopropanol	20.1	0.526	7.580×10^{-3}
Dichloromethane	8.9	2.439	4.510×10^{-3}

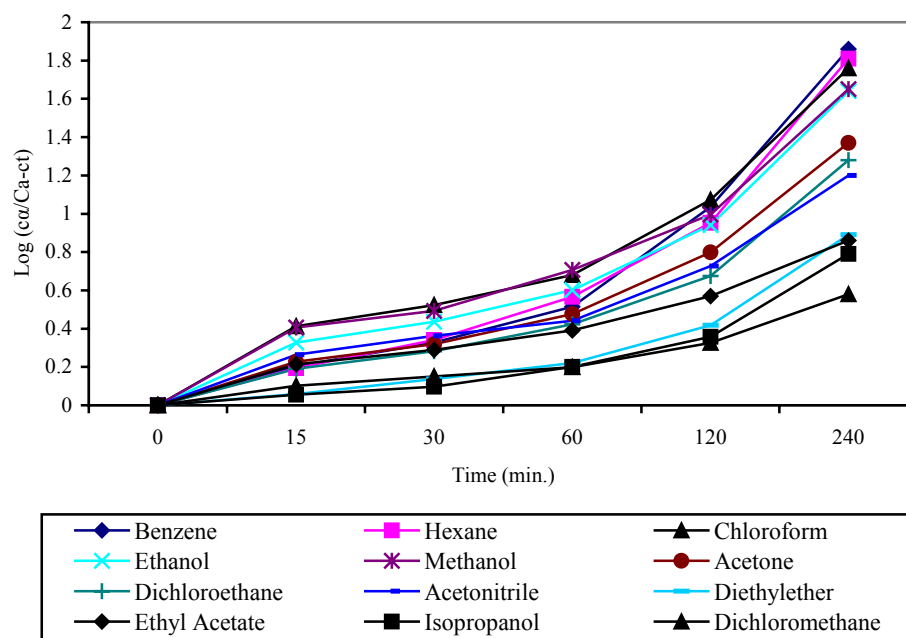


Fig. 1: First-order plot for the extraction of gingerol by different solvents.

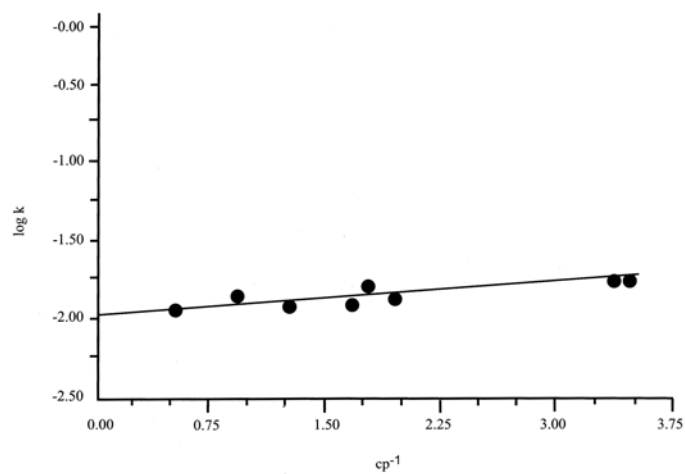


Fig. 2: Dependence of rates of extraction of gingerol on viscosity (cp^{-1}) of the solvents.

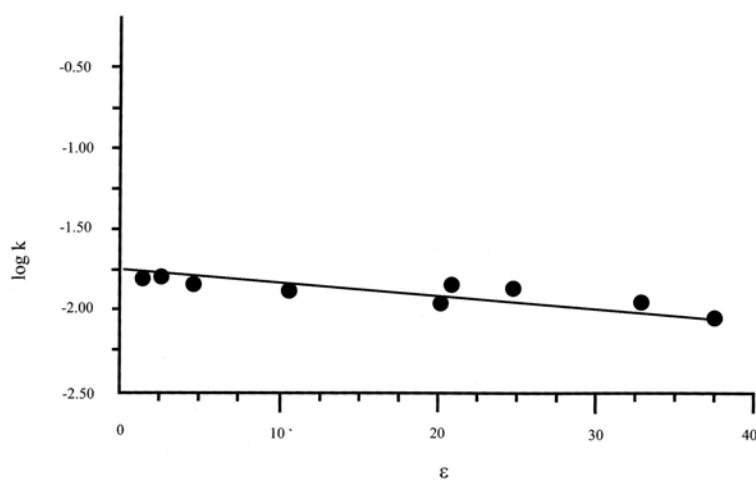


Fig. 3: Dependence of rates of extraction of gingerol on dielectric constant (ϵ) of the solvent.

ACKNOWLEDGEMENT

One of the author (Khan Usmanghani) is thankful to the Ministry of Food, Agriculture and Livestock (MINFAL) for the financial support.

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