

A LABORATORY INVESTIGATION OF ACONITIC ACID ISOMERISATION AND SOME OBSERVATIONS ON ISOMERISATION IN FACTORY PROCESSING

SN WALFORD

Sugar Milling Research Institute, University of Natal, Durban, 4041

Abstract

The kinetics of the geometrical isomerisation of *trans* to *cis* aconitic acid have been studied. Variables included pH (5 to 8), temperature (70 to 97°C), buffer strength (0 to 150 mM) and relevant cations (sodium, potassium, calcium and magnesium). Isomerisation rates increased with increasing temperature and buffer concentration, but decreased with increasing pH. Monovalent cations had no effect whilst the divalent cations marginally inhibited the isomerisation rate. The equilibration ratio of the isomers was most affected by pH. Decarboxylation rates of the *trans* isomer to itaconic acid were influenced by buffer strength and divalent cation concentration. The *cis* to *trans* isomer ratio was found to change in factory streams across factory unit operations in agreement with the trends found in this study. Higher levels of aconitate scale and lower *cis/trans* ratios in the scale were found in inland mills.

Introduction

Aconitic acid is the major non-nitrogenous acid found in extracted cane juice and factory process streams (Walford, 1996). Behr established its natural origin in sugarcane in 1877. Being an unsaturated acid (carbon-carbon double bond) it can exist in two geometrical forms, the *trans* isomer (the predominant form in fresh cane juice) and the *cis* isomer (Figure 1). In solution, the *trans* converts to the *cis* isomer until an equilibrium state is attained. The changing of one geometrical form to another is known as isomerisation. Little information on the fundamentals of the rate of *trans/cis* isomerisation or the possible consequent processing effects are recorded in the literature. Kinetic studies of this isomerisation and possible subsequent aconitate degradation could have benefits in the study and understanding of scale formation in evaporator stations (Walford and Walthew, 1996). A study was undertaken to establish the rate of isomerisation as a function of pH, temperature, buffer strength and selected monovalent and divalent cations present in sugar processing streams.

Experimental

Reaction equipment

All reactions were carried out in a 500 ml Schott bottle which had been modified by the addition of four inlet/outlet screw ports and a modified cap for insertion of a condenser. Separate Pt100 (temperature) and pH probes connected to a Crison micropH 2002 pH meter utilised two of these inlets.

Nitrogen sparging was used to ensure no oxidation occurred and to help mix the solution. The fourth inlet was used for continuous sampling through a Technicon peristaltic pump to an autosampler connected to an High Performance Liquid Chromatography system for *trans* and *cis* analysis. Reaction temperature was maintained by placing the bottle in a constant temperature oilbath. A schematic diagram is shown in Figure 2.

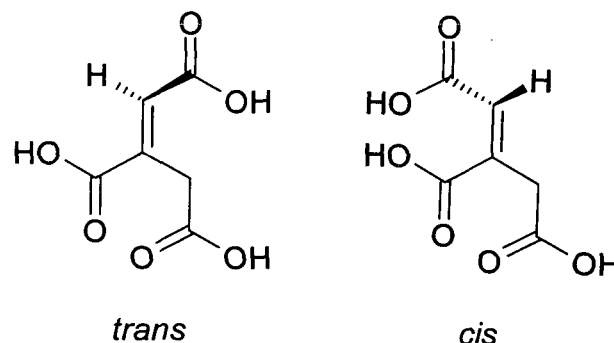


Figure 1. Geometric isomers of aconitic acid.

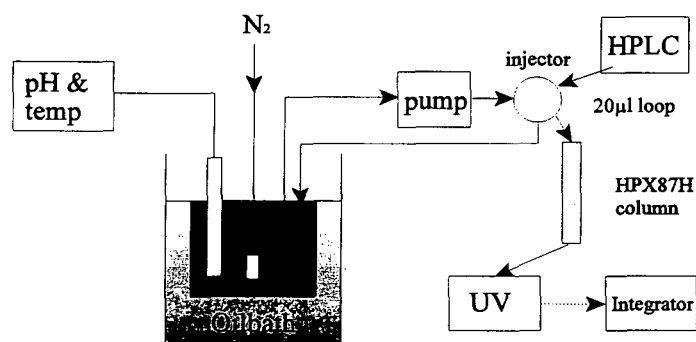


Figure 2. Schematic diagram of reaction vessel and ancillary equipment.

HPLC analysis

Concentrations of the *trans* and *cis* isomers were measured on-line using an HPLC system consisting of an SP IsoChrom pump, an SGE LS3200 Autosampler (20 µl injection volume), a Linear PHD 206 UV detector (210 nm) and a HP3396 integrator. Separation was achieved using a BioRad HPX87H

column held at 65°C. The eluent was 0,01 N H₂SO₄ at a flow rate of 0,5 ml/min. Samples were analysed every 17, 30 or 60 minutes depending on the speed of isomerisation. Manual sampling was used for the reactions at pH 8 (97°C) and at pH 7 (70°C) due to the slowness of isomerisation. Individual *trans* and *cis* standards were run at the beginning of each isomerisation experiment analysis. The organic acid composition of mixed juice, clear juice, syrup, molasses and intermediate products was determined as described previously (Walford, 1996).

Reagents

Acetate and phosphate buffers were made using Analar reagents (sodium and potassium acetate, glacial acetic acid, sodium dihydrogen phosphate, and dipotassium hydrogen phosphate). Analar calcium and magnesium acetate salts were used to make up appropriate divalent cation solutions. Chromatographically pure *trans* aconitic acid (Riedal de Haan) and *cis* aconitic acid (96%, Sigma) were used for HPLC calibration. Standard stock solutions of the *cis* and *trans* isomers (0,5 mg/ml in water) were made and kept frozen until required. These were diluted 1:10 with the buffer solution for HPLC calibration.

Procedure

Acetate buffer (380 ml) was placed in the Schott bottle with the pH and temperature probes, sparger and inlet and outlet tubes and heated to the desired temperature. The peristaltic sampling pump was started. A weighed sample of *trans* aconitic acid (20 mg) was dissolved in acetate buffer (20 ml) and immediately added to the solution. HPLC analysis commenced five minutes later to allow mixing.

Treatment of data

Details of calculation methods of rate constants, Arrhenius constants and thermodynamic values from the experimental data are described in Appendix 1.

Results and discussion

Repeatability of the method

In order to compare isomerisation rates determined using this apparatus with others reported in the literature, a measure of the repeatability of the entire method was made. Three replicate isomerisations were carried out at 90°C in 50 mM acetate buffer (pH 5). The results are summarised in Table 1. The precision values include all aspects of experimental error, *viz.* weighing, buffer solution preparation, temperature and chromatographic variability (standard preparation, injection and integration). The standard error for both forward and reverse reactions are similar showing that the same confidence can be placed in both calculations. The replicate values (relative error of less than 5%) obtained were considered acceptable for kinetic investigations.

Effect of ionic strength

The role of buffer concentration was studied on the rate of isomerisation due to the need to use buffered solutions to ensure that the pH remained constant during the course of any

particular experiment. However rates of some reactions can be influenced by the buffer being used due to involvement in the reaction and therefore a check for possible involvement must be made. A typical profile of a run is shown in Figure 3 and the resultant first order plot is illustrated in Figure 4. Table 2 summarises the calculated rates (k_f and k_r) for pH 5 to pH 8 and up to 150 mM buffer. These show that the rate of aconitic acid isomerisation is a function of the ionic strength of the solution in which it is measured and a check for direct buffer involvement is necessary.

Table 1. Repeatability of calculated isomerisation rates at 90°C in 50 mM acetate buffer (pH 5).

Replicate	Forward rate k_f ($s^{-1} \times 10^{-5}$)	Reverse rate k_r ($s^{-1} \times 10^{-5}$)	Equilibrium constant K
1	3,53	8,75	0,40
2	3,98	9,04	0,44
3	3,64	8,55	0,43
Mean	3,72	8,78	0,42
Standard deviation	0,24	0,24	0,02

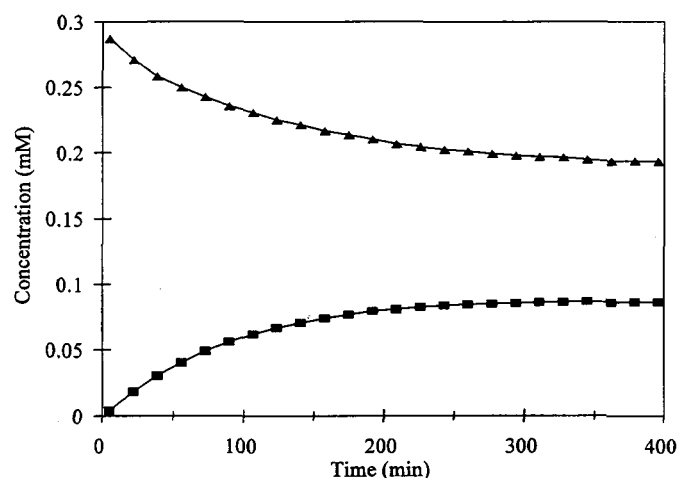


Figure 3. Typical concentration profile of a *trans/cis* isomerisation (\blacktriangle = *trans*, \blacksquare = *cis*).

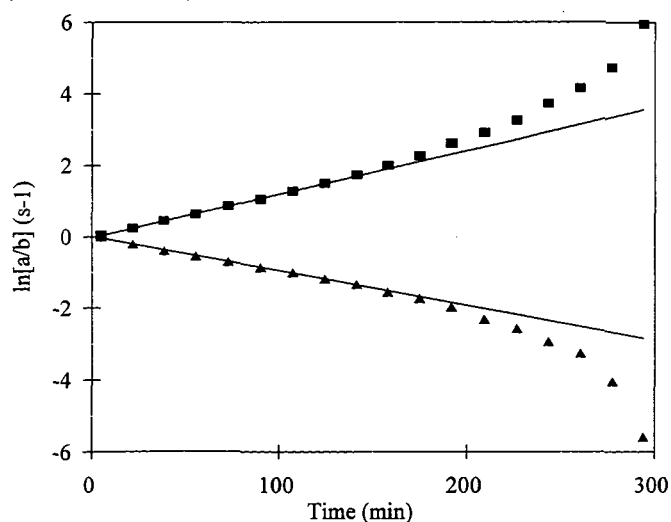


Figure 4. Typical first order plot obtained from a *trans/cis* isomerisation. Slopes equal specific rate ($k_f + k_r$) (\blacktriangle = *trans*, \blacksquare = *cis*).

Table 2. Kinetic data ($s^{-1} \times 10^{-5}$) for *trans/cis* isomerisation of aconitic acid at increasing temperature and acetate (pH 5) and phosphate (pH 6 to 8) buffer concentration.

Temp (°C)	Buffer conc (mM)	pH 5		pH 6		pH 7		pH 8	
		k_f	k_r	k_f	k_r	k_f	k_r	k_f	k_r
70	0	<i>0,256</i>	<i>0,703</i>	<i>0,006</i>	<i>0,159</i>	<i>0,001</i>	<i>0,058</i>		
	25	0,548	1,405	0,134	0,465	0,019	0,148		
	50	0,703	1,990	0,292	1,121	0,032	0,286		
	75	0,918	2,530	0,405	1,678	0,048	0,388		
	150	1,548	4,045	0,657	3,128	0,094	0,708		
80	0	<i>0,425</i>	<i>1,272</i>	<i>0,116</i>	<i>0,378</i>	<i>0,002</i>	<i>0,157</i>		
	25	0,915	2,948	0,325	1,191	0,049	0,395		
	50	1,657	3,817	0,544	2,168	0,072	0,537		
	75	2,315	5,570	0,793	3,320	0,114	0,868		
	150	3,493	8,402	1,563	6,982	0,202	1,456		
90	0	<i>1,486</i>	<i>3,022</i>	<i>0,324</i>	<i>0,917</i>	<i>0,051</i>	<i>0,334</i>		
	25	2,650	5,283	0,703	2,347	0,117	0,790		
	50	3,717	8,782	1,265	4,488	0,183	1,237		
	75	4,373	10,727	1,551	5,869	0,286	1,943		
	150	6,602	16,850	2,648	11,180	0,436	3,028		
97	0	<i>1,620</i>	<i>4,247</i>	<i>0,486</i>	<i>1,354</i>	<i>0,090</i>	<i>0,471</i>	<i>0,033</i>	<i>0,266</i>
	25	3,780	9,670	1,265	4,247	0,208	1,237	0,035	0,420
	50	5,520	12,745	2,519	8,500	0,374	2,643	0,067	0,527
	75	7,742	18,183	3,317	12,240	0,480	3,448	0,076	0,570
	150	13,322	30,700	6,331	27,010	0,841	6,603	0,091	0,831

Values in italics (0 mM) are extrapolated using equation 6.

The observed data produced a good fit to the reversible first order relationship

$$\ln\left(\frac{a}{a-x}\right) = k_f t$$

(see Appendix 1) but showed obvious systematic departure from the reversible second order rate equation

$$\left(\frac{x}{a-x}\right) = a k_2 t$$

showing that the buffer played no direct role in the isomerisation and the rate increase was due solely to the ionic strength of the solutions used. The increased reaction rate with increasing buffer strength is due to the primary salt effect (Glasstone, 1960). This refers to the influence of the electrolyte concentration on the activity coefficient of the aconitic acid and is a well known physical phenomenon (Connors, 1990; Perlmutter-Hayman, 1971). In order to define the effect of pH, independent of buffer concentration, equation 6 in Appendix 1 was used to calculate the rate at zero buffer strength (shown in Table 2 in italics). No attempt was made to adjust the pH at the reaction temperature (Connors, 1990). The implication of increasing isomerisation with increasing ionic strength in the mill environment is that towards the back-end of the evaporators and raw house the rate of isomerisation will increase producing more of the *cis* isomer. Chemically *cis* isomers are generally regarded as being less stable and hence more labile and reactive (Finar, 1973).

Effect of pH

Isomerisation of *trans* to *cis* aconitic acid has been found to be markedly dependent upon pH. Data reported here show that increasing pH can slow the rate of isomerisation by a

factor of up to 100 times (pH 5 to pH 8 for k_f at 97°C, 75 mM buffer). The unbuffered isomerisation rate as a function of pH can be plotted using the extrapolated rate values. Figure 5 shows this function at 97°C where the log of the forward (k_f) and reverse (k_r) rates in an unbuffered solution are plotted against pH. It can be seen that decreasing pH increases the rate over the range studied (pH 5 to 8), the forward rate increases 50 times whilst the reverse rate increases 16 times. These graphs show that the longer a solution of *trans* aconitic acid is maintained at a lower pH the greater the quantity of *cis* will be formed (up to the equilibrium value for that pH, temperature and ionic strength). Deteriorated cane would exhibit increased levels of *cis* aconitic acid due to this effect. Some processing problems experienced with this type of material could be related to this effect. It has also been found experimentally that as the pH increases, the equilibrium constant (K) decreases (Table 3). It appears that at the higher temperatures (80 to 97°C), K is dependent on pH but independent of temperature.

Table 3. Effect of pH on the average equilibrium constant K.

Temp (°C)	pH 4	pH 5	pH 6	pH 7	pH 8
70		0,372	0,235	0,129	
80		0,410	0,260	0,133	
90	0,591	0,413	0,271	0,147	
97		0,421	0,275	0,144	0,125

Effect of temperature

The rate of the majority of reactions will increase with increasing temperature. From the data presented in Table 2, the isomerisation of aconitic acid follows this trend. The

temperature dependence of the reaction rate can be expressed by the Arrhenius equation (equation 7, Appendix 1). Substituting the data from Table 2 into equation 7 allows calculation of the values of the activation parameters (energy of activation, entropy of activation, etc.) (Table 4). These calculations assume that both ΔH^\ddagger and E_a are independent of temperature (normally valid over a 30-50°C temperature range (Espenson, 1995)).

Enthalpy of activation is a measure of the heat content of the activated complex (the intermediate between the *trans* and *cis* isomers). Entropy of activation is a measure of the amount of energy taken up by the complex whilst the free energy is the amount of energy that would be absorbed or liberated by the activated complex during the isomerisation. The fact that the free energy is positive implies that the isomerisation will only occur with energy or heat being supplied to the isomer. The activation energy and enthalpy are both relatively large indicating a reasonably slow reaction. In most of the data presented, the activation energy increases with an increase in pH at any particular ionic strength. This increasing activation energy shows that the energy barrier to be overcome in order to undergo isomerisation becomes greater with an increase in pH. The differences in free energy between the *trans* and *cis*

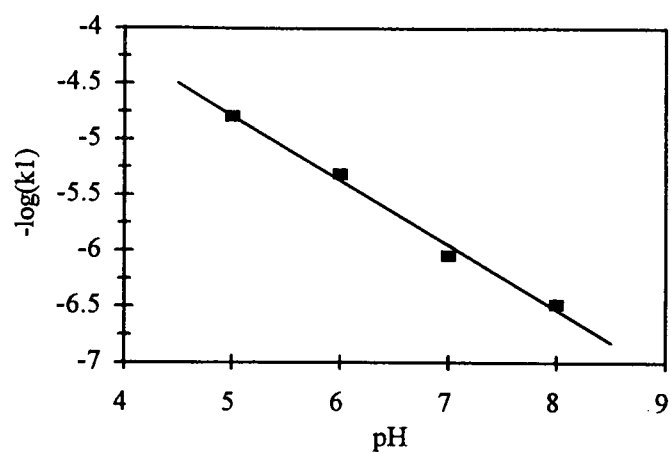
isomers ($\Delta G^0 = \Delta G^\ddagger_{trans} - \Delta G^\ddagger_{cis}$) will give an indication of the favourability of the *trans* to *cis* isomerisation (Table 5). The difference increases with pH, indicating that the isomerisation will become more difficult. The values recorded for the activation energies fall in the same range as reported previously for similar double bond compounds (Davis and Evans, 1955; Tamamushi and Akiyama, 1937).

Effect of different cations

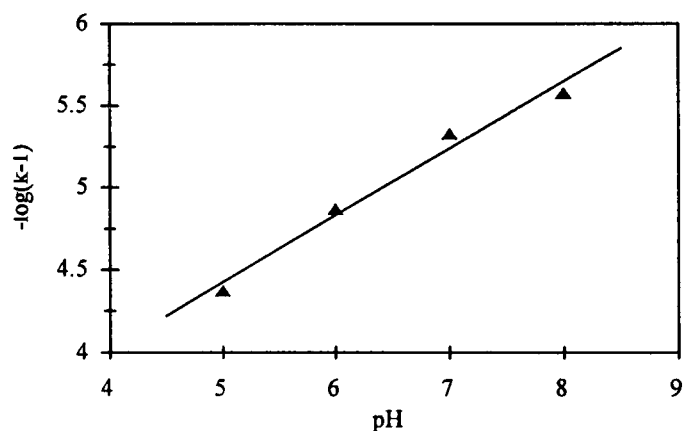
Most of the initial investigations were done using sodium as the cation. Sugar process solutions contain other cations in major concentrations, *viz.* potassium, calcium and magnesium which could affect the rate of isomerisation. Potassium as a counter ion was studied in comparison to 50 mM sodium acetate buffer at pH 5 by substituting with potassium acetate. No difference in the isomerisation rate between the monovalent cations could be found (Table 6). Calcium and magnesium were added to sodium acetate buffer as the acetate salts at 0,3 mM, 3 mM and 9,0 mM concentrations. This represents a 1:1, 1:10 and 1:30 (aconitate:cation) concentration ratio and is typical of normal sugar process solutions (between 1:1 and 1:10 across the factory). Calculated rate and equilibria data are shown in Table 6.

Table 4. Activation parameters for the isomerisation of aconitic acid.

pH	Concentration (mM)	A (s ⁻¹) * 10 ⁶	E _a (kJ mol ⁻¹)	ΔH [‡] (kJ mol ⁻¹)	ΔS [‡] (J K ⁻¹ mol ⁻¹)	ΔG [‡] (kJ mol ⁻¹)	
pH 5	Forward	0	0,54	72,99	70,06	-145,0	122,24
		25	4,23	77,54	74,60	-127,8	120,60
		50	15,37	80,24	77,31	-117,1	119,45
		75	19,96	80,20	77,26	-114,9	118,62
		150	33,53	80,34	77,40	-110,6	117,21
	Reverse	0	0,06	63,85	60,92	-163,1	119,64
		25	1,76	72,25	69,32	-135,1	117,95
		50	3,70	73,42	70,48	-128,9	116,88
		75	8,17	74,89	71,96	-122,3	115,98
		150	26,54	76,99	74,06	-112,5	114,56
pH 6	Forward	0	7,96	85,70	75,53	-143,4	127,16
		25	18,81	85,56	75,70	-135,4	124,44
		50	12,85	82,55	73,42	-136,5	122,55
		75	4,55	78,63	69,97	-143,8	121,72
		150	30,65	82,51	73,62	-126,6	119,91
	Reverse	0	13,04	84,00	73,82	-139,3	123,98
		25	31,43	83,43	73,72	-130,7	120,76
		50	71,67	77,00	68,21	-140,3	118,73
		75	3,23	73,58	65,30	-145,5	117,68
		150	28,84	77,96	69,54	-127,7	115,52
pH 7	Forward	0	177,6	100,35	97,41	-96,7	132,23
		25	40,40	93,32	88,47	-114,3	129,62
		50	82,26	93,95	90,19	-105,4	128,13
		75	26,20	89,44	86,50	-112,6	127,05
		150	56,31	83,28	80,34	-125,4	125,49
	Reverse	0	1,73	80,97	78,04	-135,2	126,70
		25	5,20	81,50	78,57	-126,1	123,95
		50	25,35	84,56	80,18	-116,9	122,26
		75	28,50	83,80	80,86	-111,9	121,15
		150	49,82	83,81	80,88	-107,3	119,50



(a)



(b)

Figure 5. Effect of pH on the isomerisation of aconitic acid, (a) *trans* to *cis* and (b) *cis* to *trans*.

Table 5. Free energy of activation for the isomerisation of aconitic acid at 90°C.

Buffer concentration (mM)	pH 5	pH 6	pH 7
0	2,60	3,18	5,53
25	2,65	3,68	5,67
50	2,57	3,82	5,87
75	2,64	4,04	5,90
150	2,65	4,39	5,99

Two effects are noticeable from this data. Firstly, both calcium and magnesium slow the rates of isomerisation. Calcium slows the rate more with increasing concentration, whereas magnesium appears to affect the rate only at a constant value. This is explained by assuming that the cations are chelating with the *trans* isomer, thereby inhibiting isomerisation. Secondly, calcium has a more inhibitory affect than magnesium, as seen in both calculated rate data and final equilibrium values indicating that the calcium ion, forms a more stable complex than the magnesium ion thereby inhibiting isomerisation. Ambler *et al.* (1945) describe the formation of insoluble calcium and magnesium aconitate salts in the *trans* form at neutral pH. Similar compounds have been found in some evaporator scales from the South African sugar industry (Walthew and Turner, 1995). It is possible that high levels of calcium either in the cane or introduced in the front end of the raw house may inhibit *trans* isomerisation, leading to conditions where the *trans* to calcium ion ratio is sufficient to cause precipitation of insoluble calcium aconitate in evaporators. Direct evidence of this effect was seen in the analysis of melt samples from an ozonation trail done at the Malalene factory (Davis *et al.*, 1998). In 10 separate trials the quantity of *trans* aconitic acid decreased an average of 88,4% between melt and clear liquor with the addition of relatively large quantities of calcium (303 to 35 ppm/Bx). A similar decrease was found to occur in a Japanese refinery (Otake *et al.*, 1961).

Table 6. Effect of selected cations on the isomerisation rate and equilibrium constant of aconitic acid at 90°C in 50 mM acetate buffer.

Cation		Rate ($s^{-1} \times 10^{-5}$)		Equilibrium constant K
Element	Concentration (mM)	k_1	k_{-1}	
Sodium	50	3,717	8,782	0,44
Potassium	50	3,657	8,538	0,43
Calcium	0,3	3,186	7,049	0,45
	3,0	2,639	5,911	0,47
	9,0	1,815	5,277	0,34
Magnesium	0,3	2,829	6,092	0,46
	3,0	2,808	6,091	0,46
	9,0	2,462	6,307	0,39

Decarboxylation of aconitic acid

During this investigation, it was found that breakdown of *trans* aconitic acid occurred in solution. If the isomerisation reaction was left for an extended period of time, the concentration of the *cis* isomer remained constant whilst the *trans* decreased with the appearance of a series of unknown peaks in the chromatogram (Figure 6). The ratio of these peaks changed with buffer concentration and presence of divalent cations. The major unknown compound in an unbuffered solution was identified as itaconic acid, a decarboxylation product from *trans* aconitic acid (Chu and Clydesdale, 1976). The rate at which it forms at pH 5 has been followed and it was found that calcium, magnesium and buffer retard the decarboxylation reaction (Table 7). The divalent ions appear to inhibit the decarboxylation rate in a similar manner to the isomerisation, implying that a common structure is involved in both isomerisation and decarboxylation. Levels of calcium and magnesium are relatively high in process streams, explaining the normally low concentrations of itaconic acid to be found in them.

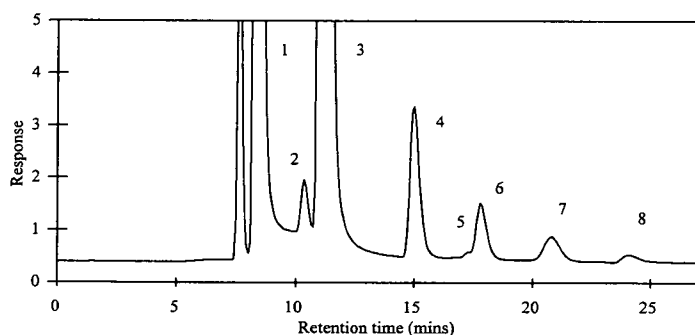


Figure 6. Ion exclusion chromatogram showing *cis* and *trans* isomers and breakdown products of aconitic acid (1=*cis* aconitic, 3=*trans* aconitic, 4=itaconic, 2, 5, 6, 7, 8=unknown).

Table 7. Itaconic acid formation rates as a function of buffer and cation concentration.

Buffer	Buffer cation	Divalent cation	Concentration (mM)	Formation rate ($s^{-1} \times 10^{-7}$)
None		none		9,89
50 mM Acetate	Potassium	none		2,75
		none		2,72
	Sodium	calcium	0,3	2,52
			3,0	1,95
		magnesium	0,3	1,33
			3,0	2,73
		9,0	2,42	
		9,0	1,74	

Application to factory conditions

Analysis of over 250 samples (fresh cane, mixed juice, clear juice, syrup, molasses, melts and sugars) has shown that the ratio of *cis* to *trans* isomer increases from the front to the back end of the factory (Table 8). Growing cane has little of the *cis* isomer present as it is synthesised and utilised in the

Krebs cycle and not stored in the plant. Some *cis* will isomerise to *trans* and, as it is not utilised in the Krebs cycle, the *trans* form is stored in the cell. Any *cis* isomer that may be isomerised from this *trans* will immediately be incorporated back into the cycle, thus ensuring that the plant will only contain the *trans* form. On harvesting, this *trans* isomer will isomerise at a rate dependent upon the temperature of storage and the degree of deterioration (decrease in pH). Some isomerisation may take place in the diffuser or mill, depending on temperatures and lime addition. Heating in juice heaters for clarification will accelerate isomerisation as seen in the small ratio rise across the clarifier. Further isomerisation will occur across the evaporators due to the combined pH drop, heating at the front end and increasing ionic concentration that occurs through the evaporators. The rate of this isomerisation will depend on temperatures, pH drop, total ionic and divalent ion concentration. Increases in the *cis/trans* ratio across the pans can be attributed to the increased ionic concentration as sucrose is removed combined with the decrease in pH between syrup and molasses. The ratio of *cis* to *trans* found in raw sugar is similar to that found in syrup. It is assumed that the transfer rates of the isomers into the crystal are similar and that the values recorded are the same as those found in A massecuite.

Table 8. Average ratio of *cis* to *trans* aconitic acid in the sugar factory.

	Fresh cane	Mixed juice	Clear juice	Syrup	Molasses	Raw sugar (or melt)
Ratio	0,03	0,05	0,07	0,15	0,32	0,18
Range	0,01-0,04	0,03-0,08	0,05-0,11	0,08-0,21	0,25-0,45	0,15-0,21

Studies of the aconitate ratios in sugar streams across individual effects in the evaporator train show that the greatest increase occurs in the first and second effects where the temperatures are highest (Figure 7). Analysis of scale from individual effects shows that the isomer ratio in the scale is similar to that found in the juice. One possible explanation is that both isomers precipitate as the calcium/magnesium salt at the same rate. However studies have shown that the salts of the two isomers do not precipitate at the same rates from the same solution (Ambler and Roberts, 1948), discounting this explanation. A second explanation is that the majority of the aconitate in the scale comes from inclusion of juice as the scale forms.

Isolated incidents of large quantities of precipitated aconitate scale have been recorded from NB and UC (up to 33% of the scale). These mills show consistently higher average concentrations of aconitate in their scale and lower average *cis/trans* ratios than the other mills in the industry (Figure 8). Magnesium from the soil has been proposed as a contributing factor (personal communication).

¹DC Walthew, SMRI, University of Natal, Durban, 4041

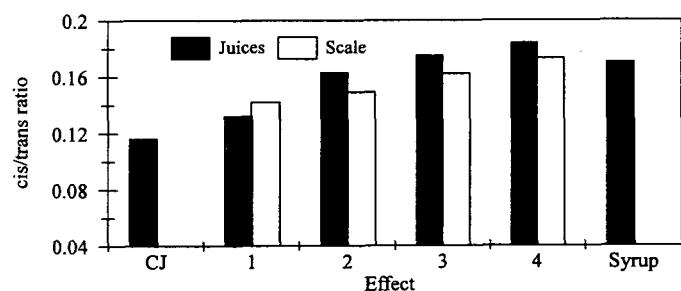


Figure 7. Typical *cis/trans* aconitate ratio for juices and scale measured across the evaporator station.

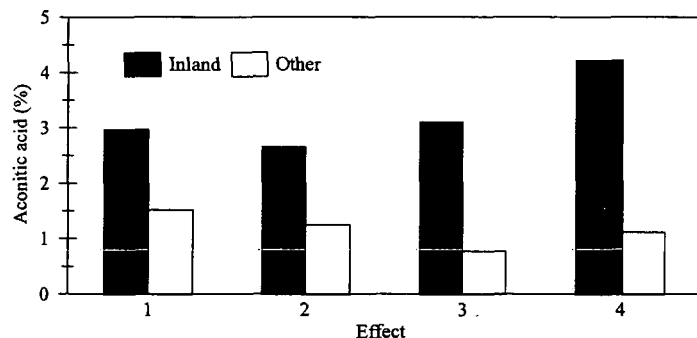


Figure 8. Average total aconitic acid measured in scale from inland mills compared with the remainder of the industry.

Conclusions

Isomerisation of aconitic acid is influenced by temperature, pH, ionic strength and ionic composition. Increasing temperature and ionic strength enhance isomerisation rates whilst increasing pH and divalent cation concentration slow the rates down. The greatest influence would appear to be pH which can account for the enhanced levels of *cis* aconitic acid to be found in back end streams due to pH drop through the factory. Some decarboxylation of aconitic acid to itaconic acid occurs in solution, but the relative speed of this reaction is influenced by ionic strength and divalent cations.

REFERENCES

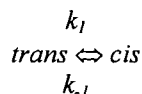
- Ambler, JA and Roberts, EJ (1948). The effect of pH on the stability of *cis*-aconitic acid in dilute solution. *J Org Chem* 13: 399-402.
- Ambler, JA, Turer, J and Keenan, GL (1945). Some salts of aconitic acid *J Am Chem Soc* 67: 1-4.
- Behr, A (1877). *Ber Deut Chem Gessel.* 10: 351.
- Chu, NT and Clydesdale, FM (1976). Decomposition of organic acids during processing and storage. *J Milk Food Technology* 39: 477-480.
- Connors, KA (1990). *Chemical Kinetics. The study of reaction rates in solution.* VCH Publishers Inc, pp 410-411.
- Davis, M and Evans, FP (1955). The kinetics of some *cis-trans* isomerisation reactions in solution. *Trans Faraday Soc* 51: 1506-1517.
- Davis, SB, Moodley, M, Singh, I and Adendorf, M. (1998). The use of ozone for colour removal at the Malene refinery. *Proc S Afr Sug Technol Ass* 72 (in press).
- Espenson, JH (1995). *Chemical Kinetics and Reaction Mechanisms.* 2nd edition. McGraw-Hill Inc, 281 pp.
- Finar, IL (1973). *Organic Chemistry.* Volume 1. Longman, pp 496-498.
- Glasstone, S (1960). *Textbook of Physical Chemistry.* Macmillan and Co, pp 1114-1118.
- Otake, T, Seya, H and Saito, M (1961). Studies on the alternation of organic anions in the sugar refining process and related phenomena. *Proc Res Soc Japan Sugar Refineries' Technol* 10: 39-54.
- Perlmutter-Hayman, B (1971). *Progress in Reaction Kinetics.* Volume 6, Part 5. The primary kinetic salt-effect in aqueous solution. Pergamon Press, pp 240-267.
- Tamamushi, B and Akiyama, H (1937). The paramagnetic isomerisation of maleic acid into fumaric acid in solution. *Bull Chem Soc Japan* 12: 382-389.
- Walford, SN (1996). Composition of cane juice. *Proc S Afr Sug Technol Ass* 70: 265-266.
- Walford, SN and Walthew, DC (1996). Preliminary model for oxalate formation in evaporator scale. *Proc S Afr Sug Technol Ass* 70: 231-235.
- Walthew, DC and Turner, LM (1995). Analysis of scale from some South African sugar mills. *Proc S Afr Sug Technol Ass* 69: 138-143.

APPENDIX 1

Treatment of experimental data.

Calculation of rate constants

The isomerisation of *trans* to *cis* aconitic acid can be described by:



k_1 = forward rate constant k_{-1} = reverse rate constant.

Let $[\text{trans}]$ equal the concentration of the *trans* isomer and $[\text{cis}]$ equal the concentration of the *cis* isomer at any time t . At the commencement of the experiment, there is no *cis* present; after time t the concentration of the *trans* and *cis* will be $([\text{trans}] - [\text{cis}])$ and $[\text{cis}]$, respectively. The net rate of reaction at any instant will be given by:

$$\frac{d[\text{trans}]}{dt} = k_1[\text{trans}] - k_{-1}[\text{cis}] \quad (1)$$

At time t_0 $[\text{trans}] = [\text{trans}^0]$ and $[\text{cis}] = 0$ and mass balance requires

$$[\text{trans}^0] = [\text{trans}] + [\text{cis}] \quad (2)$$

Substitute (2) into (1) to give

$$\frac{d[\text{trans}]}{dt} = (k_1 + k_{-1})[\text{trans}] - k_{-1}[\text{trans}^0] \quad (3)$$

At equilibrium $\frac{d[\text{trans}]}{dt} = 0$ and substitution into (3) gives

$$(k_1 + k_{-1})[\text{trans}^{eq}] = k_{-1}[\text{trans}^0] \quad (4)$$

where $[\text{trans}^{eq}]$ is the equilibrium concentration of the *trans* isomer. Substitution of (4) into (3) gives

$$-\frac{d[\text{trans}]}{dt} = (k_1 + k_{-1})([\text{trans}] - [\text{trans}^{eq}]) \quad (5)$$

Integration of (5) gives

$$\ln \left[\frac{[\text{trans}] - [\text{trans}^{eq}]}{[\text{trans}^0] - [\text{trans}^{eq}]} \right] = -(k_1 + k_{-1})t$$

A plot of $\ln[\text{trans}]$ against time will yield the *specific rate* $(k_1 + k_{-1})$. A similar equation can be derived for the *cis* isomer:

$$\ln \left[\frac{[\text{cis}^{eq}]}{[\text{cis}^{eq}] - [\text{cis}]} \right] = (k_1 + k_{-1})t$$

From equation (1) at equilibrium $k_1[\text{trans}^{eq}] = k_{-1}[\text{cis}^{eq}]$ or

$$\frac{k_1}{k_{-1}} = \frac{[\text{cis}^{eq}]}{[\text{trans}^{eq}]} = K$$

where K is the equilibrium constant for the isomerisation. From the measurement of the equilibrium constant and the averaged specific rate constant, the quantities k_1 and k_{-1} can be calculated. Averaging of the rate constant is only valid provided independent measurements of the *trans* and *cis* isomer concentrations are made. In the chromatographic method used both isomer concentrations are measured independent of the quantity of *trans* aconitic acid added to the reaction

vessel. Furthermore, the derivation of the rate constants assumes that no other reactions occur to either of the isomers.

Calculation of isomerisation rates at zero ionic strength

It can be shown (Connors, 1990) that combining the transition state equation relating the rate constant and activity coefficient with the Debye-Hückel equation yields an equation relating the rate constant to the ionic strength:

$$\log k = \log k_0 + \frac{2Z_A Z_B A \sqrt{\mu}}{1 + aB \sqrt{\mu}}$$

k = rate constant Z = charge on the ion μ = ionic strength
 a = ave distance of approach of two oppositely charged ions
 A and B = constants.

For low ionic strength solutions this simplifies to

$$\log k = \log k_0 + 2Z_A Z_B A \sqrt{\mu} \quad (6)$$

A plot of $\log k$ against $\sqrt{\mu}$ will yield $\log k_0$ (the rate constant at zero ionic strength).

Calculation of Arrhenius equation parameters

The Arrhenius equation can be used to express the rate constant as a function of temperature:

$$k = A \exp\left(-\frac{E_a}{RT}\right)$$

E_a = activation energy A = pre-exponential factor
 R = gas constant T = temperature ($^{\circ}\text{K}$).

Taking the logarithmic form of this equation gives:

$$\ln k = \ln A - \frac{E_a}{RT} \quad (7)$$

A plot of $\ln k$ will vary linearly with $1/T$, yielding A from the intercept and E_a from the slope.

Calculation of enthalpy and entropy

The temperature dependence of a rate constant can also be expressed in an equation derived from Transition State Theory (Epsonson, 1995) which yields the standard enthalpy of activation (ΔH^{\ddagger}) and the standard entropy of activation (ΔS^{\ddagger})

$$k = \kappa \frac{k_B T}{h} \exp\left(\frac{\Delta S^{\ddagger}}{R}\right) \exp\left(\frac{\Delta H^{\ddagger}}{RT}\right)$$

k_B = Boltzmann's constant h = Planck's constant
 κ = transmission factor (normally 1).

After taking logarithms and assuming the transmission factor is 1 this equation becomes:

$$\ln\left(\frac{k}{T}\right) = \ln\left(\frac{k_B}{h}\right) + \frac{\Delta S^{\ddagger}}{R} + \frac{\Delta H^{\ddagger}}{RT}$$

A plot of $\ln(k/T)$ will be a linear function of $(1/T)$ with the slope yielding ΔH^{\ddagger} and the intercept ΔS^{\ddagger} .