

hydro-2*H*-pyridazino[4,3-*c*]cinnoline-3,4-dione formed by the aromatic replacement of the *ortho*-fluorine atom in the pentafluorophenyl substituent by the amino group of the arylhydrazone fragment with the elimination of the HF molecule, was observed. This is a characteristic reaction of the test compounds containing arylhydrazone and pentafluorophenyl groups.<sup>3</sup> The other most important peaks can correspond to the products of stepwise degradation of the ion of the resulting tricyclic pyridazocinnoline system.<sup>4</sup>

The cyclocondensation of esters **1a,b** with phenylhydrazine is a regiodirected reaction because it results in the formation of heterocycles from the same regiosomeric series. It is likely that this results from the primary addition of a dinucleophile at the ester group of the parent ester. The regiosomeric structure of pyridazinodiones **3a,b** was demonstrated by the impossibility of their intramolecular cyclisation to pyridazocinnolines **4**. It is evident that, unlike alternative pyridazine-3,4-diones, pyridazine-5,6-diones **3a,b** cannot close to form a cinnoline ring. We failed to perform the cyclisation of compounds **3a,b**. The proposed isomeric structure of pyridazinodiones **3a,b** was additionally supported by the absence of a [M – HF]<sup>+</sup> peak, which was observed in the case of pyridazinodione **2**, from the mass spectra of compounds **3a,b**.

**4-(4-Methylphenylazo)-3-pentafluorophenyl-2-phenyl-1,2-dihydro-5H,6H-pyridazine-5,6-dione 3b.** After recrystallisation from ethanol, 225 mg (46%) of product **3b**, mp 161–162 °C, was obtained. <sup>1</sup>H NMR ( $\text{CDCl}_3$ ) δ: 2.36 (s, 3H, Me), 6.96–7.73 (m, 9H,  $\text{C}_6\text{H}_4$ , Ph), 14.15 (br. s, 1H, NH). <sup>19</sup>F NMR ( $\text{CDCl}_3$ ) δ: 1.21 (m, 2F), 11.64 (m, 1F), 22.09 (m, 2F). <sup>13</sup>C NMR (100 MHz,  $\text{Me}_4\text{Si}$ ,  $\text{CDCl}_3$ ) δ: 21.6 (Me), 113.3, 118.4, 119.4, 123.6, 128.2, 129.4, 130.3, 143.1, 50.7, 152.2, 137.2–145.9 (m,  $\text{C}_6\text{F}_5$ ), 190.1 (C<sup>6</sup>), 205.7 (C<sup>5</sup>). IR (Vaseline oil,  $\nu/\text{cm}^{-1}$ ): 3264, 1595 (NH), 1688, 1650 (C=O), 1614, 1550, 1523 (C=C, C=N, N=N). IR (0.01 mmol  $\text{cm}^{-3}$  in  $\text{CHCl}_3$ ,  $\nu/\text{cm}^{-1}$ ): 3684, 3621, 1597 (NH), 1683, 1662 (C=O), 1613, 1530 (C=N, N=N). MS, *m/z* ( $I_{\text{rel.}}$  %): 472 (100) [M]<sup>+</sup>, 395 (12.11), 248 (17.67), 119 (25.82), 109 (10.11), 91 (15.35), 77 (60.76), 65 (11.59). Found (%): C, 58.32; H, 2.83; F, 20.55; N, 11.62. Calc. for  $\text{C}_{23}\text{H}_{13}\text{F}_5\text{N}_4\text{O}_2$  (%): C, 58.48; H, 2.77; F, 20.11; N, 11.86.

**6-(4-Methylphenyl)-2-phenyl-7,8,9,10-tetrafluoro-2,3,4,6-tetrahydro-pyridazino[4,3-*c*]cinnoline-3,4-dione 4.** Phenylhydrazine (1.43 ml, 2.9 mmol) was added to a solution of ester **1b** (1.247 g, 2.9 mmol) in ethanol (25 ml). The reaction mixture was boiled for 10 min and then cooled to 20 °C. The resulting precipitate was filtered off and washed with chloroform. 189 mg (54%) of product **4**, mp 244–245 °C, was obtained. <sup>1</sup>H NMR [ $(\text{CD}_3)_2\text{SO}$ ] δ: 2.34 (s, 3H, Me), 7.31–7.73 (m, 9H,  $\text{C}_6\text{H}_4$ , Ph). <sup>19</sup>F NMR [ $(\text{CD}_3)_2\text{SO}$ ] δ: 2.68 (m, 1F), 14.97 (m, 1F), 20.28 (m, 1F), 30.75 (m, 1F). IR (Vaseline oil,  $\nu/\text{cm}^{-1}$ ): 1683, 1665, 1650 (C=O, C=N). Found (%): C, 58.74; H, 2.63; F, 16.01; N, 12.16. Calc. for  $\text{C}_{23}\text{H}_{12}\text{F}_4\text{N}_4\text{O}_2$  (%): C, 58.98; H, 2.58; F, 16.23; N, 11.96.

The presence of two weak-field signals of two carbonyl carbon atoms (C<sup>5</sup>, C<sup>6</sup>) in the <sup>13</sup>C NMR spectrum of compound **3b** indicates that products **3a,b** have pyridazine-5,6-dione structures.

According to IR-spectroscopic data (one high-frequency absorption band of the carbonyl group), pyridazine **2** occurs in an hydrazone-enol form in a  $\text{CHCl}_3$  solution or in a solid state, unlike N-phenyl-substituted pyridazines **3a,b**, which occur as azo-keto tautomers. The low-frequency shift of carbonyl group absorption bands in the IR spectra of heterocycles **2**, **3a,b** is due to their conjugation with other C=C and C=N bonds.

Pyridazino[4,3-*c*]cinnoline **4** was prepared by the reaction of ester **1b** with phenylhydrazine in boiling ethanol. It is likely that, under the specified conditions, ester **1b** initially reacted with a dinucleophile at the  $\gamma$ -carbonyl (this reaction site can be activated under conditions of a proton-donor solvent). Next, the intermediate underwent consecutive cyclisation reactions to form the pyridazino[4,3-*c*]cinnoline system.

Considerable electron density redistribution due to the presence of pentafluorophenyl and arylhydrazone substituents can be responsible for the unusual behaviour of esters **1** in the reactions with hydrazines. Evidently, this redistribution decreases the probability of a nucleophilic attack of the  $\alpha$ -carbonyl carbon atom by the amino groups of hydrazine, as is the case with 2,4-dioxobutanoates and their derivatives.<sup>1,2</sup>

Thus, we found that 3-arylhyclazono-4-pentafluorophenyl-2,4-dioxobutanoates **1** can undergo cyclocondensation with dinucleophilic reagents at the  $\gamma$ -dicarbonyl fragment, unlike their fluoroalkyl and nonfluorinated analogues and unsubstituted 2,4-dioxobutanoates.

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## QSAR of inhibition of classical pathway of complement activation by dicarboxylic acids

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The influence of distance between charges, hydrophobic and electronic parameters of aliphatic and aromatic dicarboxylic acids on the complement-inhibiting activity (CIA) has been ascertained using the QSAR method.

The excessive activation of the complement system, a part of the immune system, is a major cause of tissue injury in many pathological conditions [myocardial infarct, sepsis, asthma, allergic reactions, glomerulonephritis, rheumatoid arthritis, Alzheimer's disease, organ rejection (transplantation), myasthenia and multiple

sclerosis].<sup>1–3</sup> As there are no clinically available drugs that inhibit complement activation, great research efforts are directed towards the discovery of complement inhibitors ranging from low-weight molecules<sup>4–6</sup> to monoclonal antibodies and other proteins.<sup>7</sup> Negatively charged polymers and low-molecular-weight compounds

**Table 1** Properties of dicarboxylic acids: complement-inhibiting activity, distance between carbonyl carbon atoms (*R*), lipophilicity (cLog *P*) and the values of the partial positive charges at carbonyl carbon atoms (*q*).

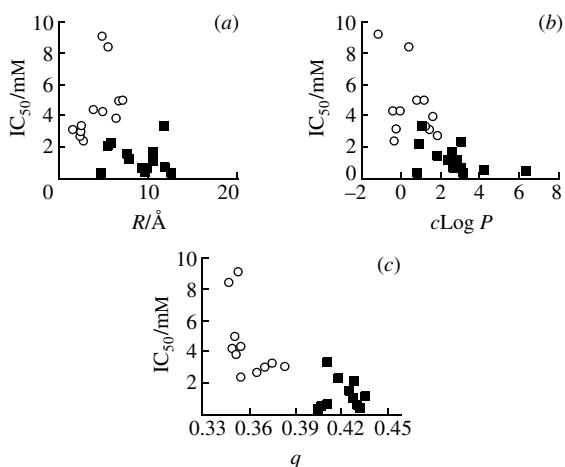
No.	Compound <sup>a</sup>	<i>R</i> /Å <sup>b</sup>	cLog <i>P</i>	<i>q</i>	Log (1/IC <sub>50</sub> )	Pred Log (1/IC <sub>50</sub> ) <sup>c</sup>	Dev	IC <sub>50</sub> <sup>d</sup> , mM
<b>1</b>	Oxalic acid	1.52	-0.24	0.384	-0.49	-0.66	0.17	3.11±0.01
<b>2<sup>e</sup></b>	Malonic acid	2.69	-0.31	0.355	-0.38	-0.80	0.41	2.42±0.16
<b>3</b>	2-Benzylmalonic acid	2.51	1.87	0.365	-0.44	-0.46	0.02	2.76±0.43
<b>4</b>	2-Phenylmalonic acid	2.56	1.47	0.371	-0.49	-0.48	-0.01	3.11±0.52
<b>5</b>	2-Cyclopentylmalonic acid	2.53	1.27	0.375	-0.52	-0.49	-0.03	3.34±0.65
<b>6<sup>e</sup></b>	Cyclopropane-1,1-dicarboxylic acid	2.49	0.42	0.419	-0.63	-0.41	-0.22	4.31±0.17
<b>7</b>	Succinic acid	3.92	-0.38	0.354	-0.63	-0.81	0.18	4.31±0.39
<b>8</b>	Glutaric acid	5.03	0.02	0.350	-0.63	-0.78	0.15	4.24±0.28
<b>9</b>	Adipic acid	5.49	0.42	0.348	-0.92	-0.73	-0.19	8.36±0.50
<b>10</b>	Pimelic acid	6.82	0.81	0.351	-0.70	-0.66	-0.03	4.99±0.70
<b>11</b>	Suberic acid	7.11	1.21	0.351	-0.69	-0.61	-0.09	4.95±1.10
<b>12</b>	Azelaic acid	6.40	1.61	0.352	-0.58	-0.55	-0.03	3.81±0.56
<b>13</b>	Iminodiacetic acid	4.89	-1.12	0.353	-0.96	-0.92	-0.04	9.13±0.72
<b>14<sup>e</sup></b>	<i>N</i> -Benzoyliminodiacetic acid	3.48	0.46	0.371	-0.33	-0.62	0.29	2.13±0.12
<b>15<sup>e</sup></b>	Furan-2,5-dicarboxylic acid	4.86	-1.28	0.453	0.25	-0.49	0.74	0.56±0.48
<b>16</b>	Pyridine-2,5-dicarboxylic acid	5.73	1.00	0.430	-0.34	-0.29	-0.05	2.17±0.13
<b>17<sup>e</sup></b>	Pyridine-2,6-dicarboxylic acid	4.87	0.93	0.431	0.48	-0.29	0.77	0.33±0.02
<b>18</b>	Naphthalene-2,6-dicarboxylic acid	7.96	2.45	0.428	-0.08	0.01	0.23	1.20±0.19
<b>19</b>	Quinoline-2,6-dicarboxylic acid	7.92	1.93	0.425	-0.16	-0.04	-0.31	1.44±0.16
<b>20</b>	Biphenyl-4,4'-dicarboxylic acid	10.04	3.13	0.431	0.24	-0.06	-0.17	0.58±0.06
<b>21</b>	Biphenyl-2,2'-dicarboxylic acid	6.01	3.13	0.419	-0.36	0.00	-0.04	2.27±0.23
<b>22</b>		10.75	2.66	0.429	-0.23	-0.10	0.02	1.71±0.19
<b>23</b>		10.69	2.87	0.436	-0.05	-0.18	0.02	1.11±0.48
<b>24</b>		9.62	4.26	0.430	0.25	0.16	0.09	0.56±0.17
<b>25</b>		9.77	6.36	0.407	0.38	0.34	0.03	0.42±0.08
<b>26</b>		11.94	1.15	0.412	-0.52	-0.34	-0.17	3.30±0.50
<b>27</b>		11.88	2.73	0.412	0.15	-0.13	0.28	0.70±0.12
<b>28<sup>e</sup></b>		12.67	3.22	0.406	0.46	-0.09	0.54	0.35±0.02

<sup>a</sup>Compounds **1**, **2**, **6–17** are commercially available (Aldrich). Compounds **3–5** were obtained as described below.<sup>†</sup> Compounds **18–28** were received as a gift from Dr. V. A. Vasnev (A. N. Nesmeyanov Institute of Organoelement Compounds, Moscow). <sup>b</sup>Spatial structures were optimised using the PM3 method in MOPAC 7.0; lipophilicity was calculated using the CLOGP program. <sup>c</sup>Values were calculated from equation (4) (Table 2). <sup>d</sup>Data are expressed as means ±SD of IC<sub>50</sub> (*N* = 4–6). <sup>e</sup>Data points not used in deriving equation (4) (Table 2): compounds **6**, **14**, **15** form the test set, compounds **2**, **17**, **28** are outliers.

carrying carboxylic, sulfate, and phosphate groups inhibit the classical pathway of complement activation.<sup>8–11</sup> In this study, we attempted to establish a relationship between the complement-inhibiting activity (CIA) of aromatic and aliphatic dicarboxylic acids and their physico-chemical and structural properties using the QSAR method.

Dicarboxylic acids **1–28** (Table 1) were bioassayed for their ability to inhibit the *in vitro* classical pathway of complement activation in the hemolytic system following the protocol de-

scribed earlier.<sup>11</sup> It was determined that CIA of compound **25** is due to the blocking of the activity of complement components C1r/C1s. According to ELISA, the complex IgG3-C1q obtained on plate was incubated in the presence of the inhibitor **25** with guinea pig serum at different dilutions (as a C1r/C1s source) and an excess of reagent R1 (human serum depleted of C1) as a source of other human complement proteins. Under such conditions, components C1r/C1s were in deficit and we could estimate their activity separately from the activity of other



**Figure 1** Relationship between complement-inhibiting activity of dicarboxylic acids and (a) distance between charges ( $R$ ); (b)  $c\log P$  and (c) partial positive charges at carbonyl carbon atoms ( $q$ ). Circles and squares indicate aliphatic and aromatic acids, respectively.

components. Functional capacities of proteins C1r/C1s were calculated by determination of the quantity of formed C3b using antibodies directed against human C3.<sup>11</sup>

We tested the array of aliphatic acids carrying from two to nine carbon atoms as a model to establish a relationship between CIA and distance between charges ( $R$ ), which was accepted and measured as a distance between carbonyl carbon atoms. However, no satisfactory dependence was obtained [Figure 1(a)]. Glutaric (8) and iminodiacetic (13) acids have close values of  $R$ , but their activity differs more than two times. Figure 1(a) indicates that aromatic acids are more active than aliphatic acids but the most active compounds have quite different  $R$  [9.77 Å (25), 12.67 Å (28)]. Apparently, distances between charges does not have key importance for CIA [equation (1); Table 2] and the effect of aromatic acids is a consequence of hydrophobic and electronic properties. Note that the calculated values of  $R$  for flexible compounds may considerably vary for configurations with close energy values. Such a property of this descriptor limits its application.

It was established that hydrophobic substituents in charged compounds enhance CIA.<sup>10,12</sup> Our results show that hydrophobic properties have critical importance for complement-blocking potency: the most active inhibitors have the highest lipophilicity (24, 25, 28) [Figure 1(b)]. This dependence is distinctly traced on the activity of compounds 26–28. Moreover, the correlation coefficient ( $r^2$ ) of equation (2) (Table 2) expressing the relationship between activity and  $c\log P$ , is sufficiently large to consider the descriptor  $c\log P$  necessary for the QSAR study.

In order to characterise the electronic properties of dicarboxylic acids, we considered ionization constants ( $pK_a$ ), polarizability, hydration energy ( $E_h$ ), energies of lowest unoccupied and highest occupied molecular orbitals ( $E_{LUMO}$ ,  $E_{HOMO}$ ) and partial charges at different atoms of the molecules. We found

<sup>†</sup> Malonic acid derivatives (3–5) were obtained by alkaline hydrolysis of correspondent diethyl esters. For the obtaining 2-benzylmalonic ester benzaldehyde was condensed with malonic ester according to the Knövenagel method followed by hydrogenation on palladium.<sup>17</sup> 2-Phenylmalonic ester was obtained by Claisen condensation from ethyl phenylacetate and diethyl oxalate followed by decarbonylation.<sup>18</sup> Alkylation of malonic ester by cyclopentyl bromide in the presence of sodium hydride led to 2-cyclopentylmalonic ester.<sup>19</sup>

**3:** mp 120–122 °C.  $^1\text{H}$  NMR [Bruker MSL-300, 300 MHz,  $[^2\text{H}_6]\text{DMSO}-\text{CDCl}_3$  (1:1)]  $\delta$ : 7.1 (m, 5H, ArH), 12.5 (s, 2H, COOH), 3.5 (s, 1H, CH), 3.1 (d, 2H,  $\text{CH}_2$ ).

**4:** mp 152–153 °C.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$ : 7.3 (m, 5H, ArH), 11.5 (s, 2H, COOH), 4.5 (s, 1H, CH).

**5:** mp 169–170 °C.  $^1\text{H}$  NMR [ $\text{CDCl}_3-[^2\text{H}_6]\text{DMSO}$  (2:1)]  $\delta$ : 8.0 (s, 2H, COOH), 2.90 (d, 1H,  $\text{CH}(\text{COOH})$ ), 2.3 (m, 1H,  $\text{CH}_2\text{CHCH}_2$ ), 1.6, 1.35, 1.0 (m, 8H,  $\text{CH}_2\text{CH}_2$ ).

**Table 2** QSAR equations.

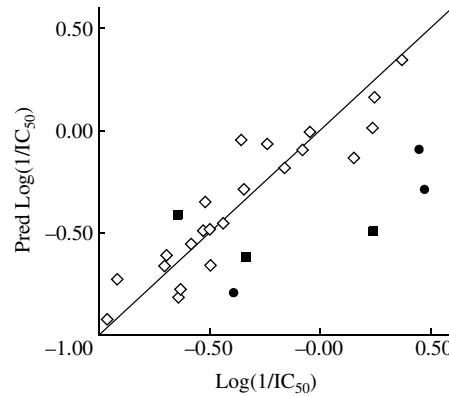
No.	$\log(1/\text{IC}_{50}) = aR + bc\log P + cq + d$							
	$a$	$b$	$c$	$d$	$n^a$	$r^2$	$s$	$F$
1	0.07 ( $\pm 0.02$ )			-0.76 ( $\pm 0.17$ )	25	0.299	0.358	9.81
2		0.18 ( $\pm 0.04$ )		-0.60 ( $\pm 0.09$ )	25	0.528	0.294	25.79
3			9.30 ( $\pm 1.66$ )	-3.94 ( $\pm 0.66$ )	25	0.576	0.278	31.20
4		0.14 ( $\pm 0.03$ )	4.46 ( $\pm 1.23$ )	-2.34 ( $\pm 0.46$ )	22	0.853	0.151	55.35

<sup>a</sup> $n$  is the number of data points used to derive the equation,  $r^2$  is the correlation coefficient,  $s$  is the standard deviation from the regression, and  $F$  is the  $F$  statistics value.

the best correlation between CIA and the simple average of the values of the partial positive charges at carbonyl carbon atoms ( $q$ ) [equation (3); Table 2]. On the whole,  $q$  values of aromatic acids are higher because of donor-acceptor properties of a carbonic framework between charges. The electron density of carboxylic group is delocalised in the aromatic system and solvation spheres around ionised charged groups are less. The decrease in solvation spheres causes the increase in the force of electrostatic interaction of the charged groups of an inhibitor with a molecular target.

Considered features we tried to take into account at QSAR derivation. The formulation of a number of equations and the comparison of their statistic parameters allowed us to derive equation (4) (Table 2) based on  $c\log P$  and  $q$ . For the statistical analysis of the derived QSAR model, two regression coefficients were calculated: a conventional squared regression coefficient ( $r^2$ ) and a cross-validation (CV) coefficient for prediction ( $q^2$ ). The coefficient  $q^2 = 0.744$  for equation (4) allows us to accept this model as a statistically reasonable because it is necessary that  $q^2$  will be greater than 0.6.<sup>13</sup> Three points (compounds 2, 17, 28) having the highest deviation between predicted and experimental activity values were truncated. Statistical coefficients taking these compounds into account reflect the deterioration of model quality in their presence [malonic acid (2,  $r^2 = 0.802$ ,  $q^2 = 0.669$ ), pyridine-2,6-dicarboxylic acid (17,  $r^2 = 0.746$ ,  $q^2 = 0.596$ ), compound 28 ( $r^2 = 0.802$ ,  $q^2 = 0.671$ )]. Dipicolinic acid 17 is an outlier because its high activity is due to strong  $\text{Ca}^{2+}$ -chelating properties,<sup>14</sup> which allow this acid to block  $\text{Ca}^{2+}$ -depending functions of complement system serine proteases nonspecifically. At present, there is no good explanation for other outliers and compound 15 from the test set (see below). Complement is a complex system of proteins and considerable excess of experimental activity over predictive values could be explained by the interaction of these compounds with more than one target.

For the validation of the model quality compounds 6, 14, 15 were studied as a test set. Their activity was predicted using equation (4) and compared with experimental values. Satisfactory



**Figure 2** Plot of predictive vs. experimental complement-inhibiting activities of dicarboxylic acids. Open and solid squares represent compounds of the training set and the test set (6, 14, 15), respectively. Circles symbolise the outliers (2, 17, 28).

results for compounds **6** and **14** allowed us to consider the derived model to be adequate.

It is known that the coefficient at  $c\log P$  ( $b$ ) in QSAR equations can clarify the mechanism of action of compounds.<sup>15</sup> It has been argued that  $b$  values of about unity imply complete desolvation and binding deep into a lipophilic pocket, whereas  $b$  values of about 0.5 reflect only partial desolvation and binding along the surface of a protein. Small regression coefficient  $b$  obtained in equation (4) indicates the binding of an only partly desolvated compound.

**Table 3** Complement-inhibiting activity of aspartic acid, glutamic acid and glutathione.

Compound	$q$	$c\log P$	Experimental activity/mM <sup>16</sup>	Activity predicted by equation (4)/mM
Aspartic acid	0.381	-1.15	2-5	6.3
Glutamic acid	0.374	-0.90	2-5	6.2
Glutathione	0.371	-2.72	10-40	11.4

Takada *et al.*<sup>16</sup> studied the CIA of different amino acids and their derivatives carrying two carboxylic groups. We used equation (4) for calculating the activity of aspartic acid, glutamic acid and glutathione and obtained satisfactory results (Table 3).

In conclusion, the key roles of hydrophobic and electrostatic inhibitor-target interactions at the blocking of the classical pathway of complement activation by dicarboxylic acids have been demonstrated. The QSAR equation taking these parameters into account was derived, and it can be used for predictions in aliphatic, aromatic and amino-dicarboxilic acids.

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## Determination of ampicillin using the Belousov–Zhabotinskii oscillating system

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A new analytical method was developed for the determination of ampicillin by the perturbation of ampicillin to a Belousov–Zhabotinskii oscillating chemical system, which involves the Ce<sup>IV</sup>-catalysed reaction between potassium bromate and malonic acid.

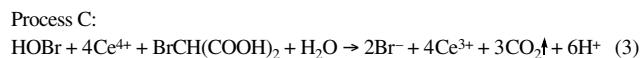
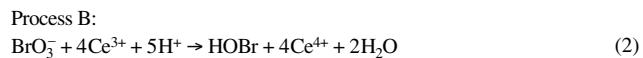
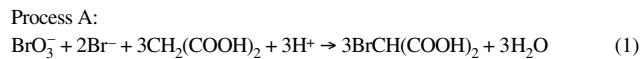
A study in nonlinear dynamics has been well developed. One of the important applications is an oscillating chemical system.<sup>1-3</sup> The kinetics of this oscillating chemical system is of both theoretical and experimental interest,<sup>4-6</sup> since the concentrations of reaction intermediates vary sensitively with time.

A typical oscillating chemical reaction is the Belousov–Zhabotinskii (BZ) reaction.<sup>6</sup> This reaction involves the oxidation of an organic species such as malonic acid by an acidified bromate solution in the presence of a metal ion catalyst. The Ce<sup>IV</sup>–Ce<sup>III</sup> and [Fe<sup>III</sup>(phen)]<sup>3+</sup>–[Fe<sup>II</sup>(phen)]<sup>2+</sup> couples are the two most widely used catalysts. In a closed system, the BZ reaction exhibits a short induction period followed by an oscillating phase. The colour of the solution alternates between yellow and colourless for the Ce<sup>IV</sup>–Ce<sup>III</sup> couple. The oscillations may last over 2 h.

Ultimately, with decreasing concentrations of the major reactants such as potassium bromate and malonic acid, the system proceeds in the direction of decreasing Gibbs energy. Consequently, the oscillation dies out and the system drifts slowly towards its chemical equilibrium.

The mechanism of the BZ reaction has been investigated extensively. The famous FKN mechanism<sup>7</sup> is generally accepted.

Two major processes (A and B) control the BZ reaction alternately and result in concentration oscillations of the intermediate species. A third process (C) is a link between processes A and B. The overall reactions are as follows:



The mechanism is autocatalytic. The concentration of Br<sup>-</sup> in the BZ system plays an important role.<sup>8</sup> It determines whether it is process A or B controlling at a particular time. The function of process A, however, is to remove Br<sup>-</sup> from the system, while process B, also called autocatalytic oxidation, begins when process A has driven [Br<sup>-</sup>] to a sufficiently low value. The onset of process B is accompanied by a rapid production of Ce<sup>IV</sup>, which becomes a reactant in process C to regenerate Br<sup>-</sup>. In addition to supplying Br<sup>-</sup> to suppress process B, process C resets