

# Instantaneous rate constants in physical organic chemistry: application to acyl transfer reactions of *p*-nitrophenyl acetate to hydroxide ion<sup>†</sup>

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**ABSTRACT:** The instantaneous rate constant (IRC)–time profiles for single-step mechanisms are straight lines with zero slope. The IRC–time profiles for reactions taking place in more than a single step depend upon whether decay of reactant or evolution of product concentration is monitored. When reactant decay is monitored, the IRC–time plot extrapolated to zero time is equal to the rate constant for the initial step ( $k_p$ ) in the reaction while the IRC–time profile for product evolution extrapolates to zero at  $t=0$ . The IRC–time profiles for monitoring reactant decay and product evolution converge to a plateau value which can be equated to the rate constant under steady-state conditions ( $k_{s,s}$ ). Two independent procedures for calculating IRC–time profiles were developed and tested for both simple and complex mechanisms. The first method is initiated with a least squares correlation over the first 11 points of a 2000-point  $\ln(1-ER)$ –time profile (ER designates extent of reaction). The IRC at the midpoint (IRC(6)) of the time interval is assigned the value of  $-S$  (where  $S$  is the slope of the correlation). The second correlation is over points 2–12 and provides a value of IRC(7). This procedure is continued until IRC(6) to IRC(1995) have been generated. The second method involves a 5th order polynomial smooth of the  $(1-ER)/\text{time}$  profile before calculating IRC at the midpoints between successive points. The limitations of the two IRC procedures are discussed. The IRC procedures are applied to experimental absorbance–time profiles for the acyl transfer reactions of *p*-nitrophenyl acetate to hydroxide ion in water and in aqueous acetonitrile. In water, no significant deviations from pseudo first-order kinetics were observed. A dramatic change in IRC/time profiles was observed on changing solvents to aqueous acetonitrile. Under the latter conditions the IRC analyses were observed to be consistent with a 2-step mechanism. Copyright © 2006 John Wiley & Sons, Ltd.

**KEYWORDS:** instantaneous rate constants; acyl transfer reactions; *p*-nitrophenyl acetate; hydroxide ion; hydrolysis

## INTRODUCTION

Our recent work<sup>1–11</sup> suggests that some of the fundamental reactions of organic chemistry follow complex mechanisms, which do not quickly reach a steady state. At an early stage in the development of physical organic chemistry, Hammett<sup>12</sup> recognized that the kinetic response of the reversible consecutive second-order mechanism (Scheme 1) differs from that of the simple irreversible second-order mechanism at short times. However, he concluded that the deviations from simple mechanism behavior are small and differentiation of the mechanisms could not be done with the precision of kinetic measurements at that time. More recently, Bunnett<sup>13</sup> expressed the general belief that rate constants of organic reactions are generally obtained with errors of the order of  $\pm 5\%$ .

Digital technology has greatly increased the potential precision of kinetic measurements and attention should be called to the fact that the standard deviation of the mean (equal to

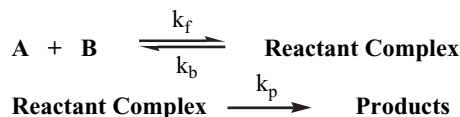
$$X_{\text{best}} = X_{\text{mean}} \pm \sigma/N^{0.5} \quad (1)$$

$\sigma/N^{0.5}$ ) where  $\sigma$  is the standard deviation, varies with the square root of the number of measurements ( $N$ ), is the appropriate parameter to use to estimate the practical level of precision available.<sup>14</sup> The necessity of gathering large quantities of kinetic data in order to take advantage of this fact is readily accommodated when using stopped-flow spectrophotometry as the measurement technique. We find that the precision in rate constant measurements of first-order reactions can be of the order of  $\pm 0.5\%$  using that method.

In the time period before the steady-state is reached during a reaction following a complex mechanism, the decay in reactant concentration leads the evolution of product concentration assuming a 1/1 stoichiometry between reactant and product. This means that the apparent rate constant for the reaction will vary with time during the *pre-steady-state* time period. The latter, along with the availability of kinetic data of greater precision,

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Scheme 1

suggests that if instantaneous rate constants (IRC) were available, IRC–time profiles should provide a very effective method for differentiating between simple and complex mechanisms. In this paper, the methodology for generating IRC–time profiles is developed using both experimental and calculated extent of reaction (1–ER)–time profiles.

## EXPERIMENTAL

### Solvents and reactants

Acetonitrile was of the highest purity level commercially available and used without further purification. *p*-Nitrophenyl acetate was of the highest purity level available and recrystallized before use.

### Kinetic measurements

A Hi-Tech Scientific SF-61 Stopped Flow Spectrophotometer with a Techne Flow Cooler FC-200 thermostat to control the temperature of the cell block within  $\pm 0.2^\circ\text{C}$  in a glove box under a nitrogen atmosphere was used for kinetic measurements. Data, 2000 points over a time range about 10% greater than the first half-life, were collected under pseudo first-order conditions.

### Kinetic procedure

The stopped-flow instrument used a single-beam light path. This necessitated subtraction of background absorbance, which was obtained by having the excess reagent in one syringe in twice the concentration used in kinetic experiments and pure solvent in the other syringe. Three background shots were recorded before each set of kinetic shots and all data points were averaged. A minimum of 20 shots under kinetic conditions were recorded in each set of measurements. Product extinction coefficients were obtained under the reaction conditions by averaging three shots with duration of about 20 half-lives of reaction. The kinetic absorbance–time curves were first averaged, background absorbance was subtracted and the zero-time absorbance ( $\text{Abs}_0$ ) was calculated by a linear least squares procedure. The absorbance–time arrays were converted to (1–ER)/time profiles and either converted to  $\ln(1-\text{ER})$ /time profiles or fit to 5th order polynomial equations. The reactant

extinction coefficient was calculated from  $\text{Abs}_0$  and the known reactant concentration.

Prior to beginning kinetic studies, diode-array UV-VIS spectra were recorded over a period of about 20 half-lives to follow the progress of all reactions. Infinity absorbance values were evaluated by single wavelength measurements over about 20 half-lives.

## Calculations

Fortran programs were compiled using the Microsoft Visual Fortran compiler. All calculations were carried out on desktop or laptop computers with processor speeds of 1.7 GHz or greater.

## RESULTS

### Instantaneous rate constants

The apparent IRC ( $k_{\text{inst}}$ ) for a reaction taking place in a single microscopic step (simple mechanism) is time independent and equal to the microscopic rate constant for that step. However, for a reaction taking place in more than one consecutive step, the opportunity exists to observe time dependent  $k_{\text{inst}}$ . This opportunity can only be taken advantage of in the event that the *pre-steady-state* time period can be accessed. By definition, a  $k_{\text{inst}}$  is evaluated over an infinitesimally short time period. In practice, it is convenient to evaluate  $k_{\text{inst}}$  over a finite time period as the rate constant at the time of the mid-point of the time range. The validity of this procedure was readily established by showing that the resultant value is independent of the magnitude of the time interval when the latter is small.

Most of the reactions of interest can be studied under pseudo first-order conditions with the second reactant (B in Scheme 1) in large excess. Under these conditions the appropriate form of the rate law for the simple mechanism is (2) in which ER represents the extent of reaction. Equation (2) transforms to Equation (3) when the decay of  $[R]$  is monitored and to Equation (4) when evolution of  $[P]$  is followed. The appropriate equations for evaluating instantaneous pseudo first-order rate constants (IRC)<sup>16</sup> over a finite time interval ( $\Delta t$ ) when monitoring reactant decay or product accumulation are Equations (5) and (6), respectively.

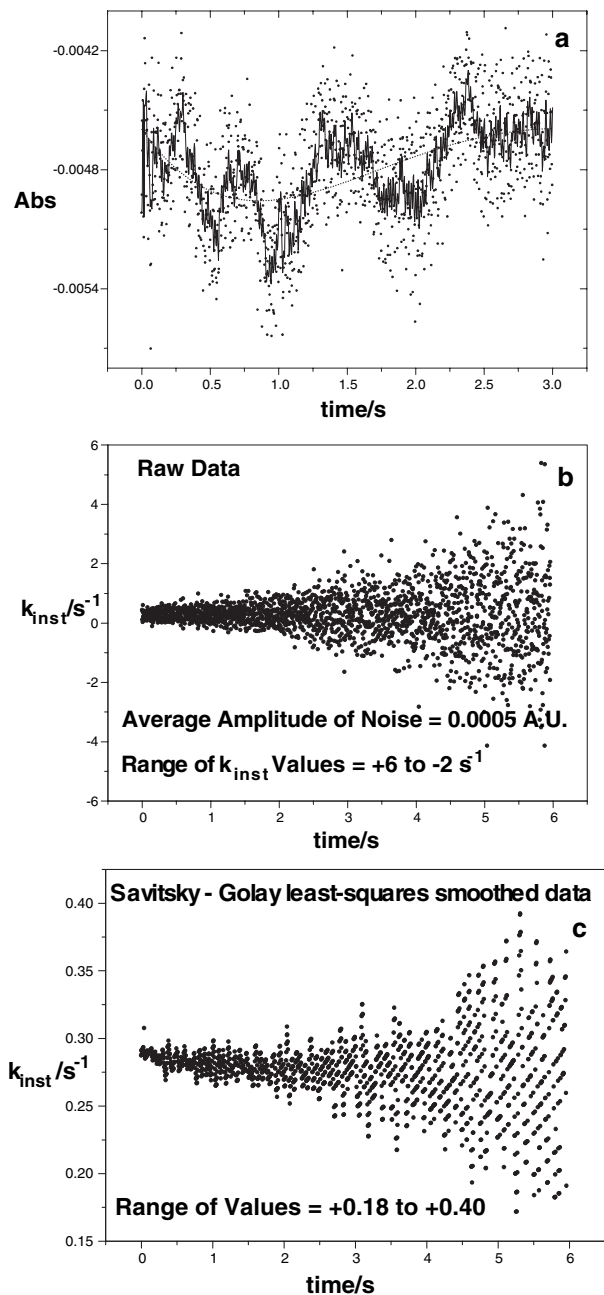
$$-\ln(1 - \text{ER}) = kt \quad (2)$$

$$-\ln([R]/[R]_0) = kt \quad (3)$$

$$-\ln(1 - [P]/[R]_0) = kt \quad (4)$$

$$k_{\text{inst}} = -\Delta \ln([R]/[R]_0)/\Delta t \quad (5)$$

$$k_{\text{inst}} = -\Delta \ln(1 - [P]/[R]_0)/\Delta t \quad (6)$$



**Figure 1.** Spectrometer output noise (a), 2-point IRC analysis of experimental absorbance–time data for a reaction with an apparent rate constant of 0.28/sec, raw data (b), with data smoothed using the Savitsky–Golay procedure (c)

**Spectrometer output noise.** Due to the fact that there is always some scatter and noise in experimental kinetic data it is not practical to evaluate IRC directly from the raw data. Spectrometer output noise under our experimental conditions is illustrated in Fig. 1a. The solid dots represent the absorbance output from the single-beam spectrometer for water in the absence of solutes. The broken line is the data after a Savitsky–Golay smooth procedure<sup>15</sup> was carried out and the solid line is the best fit of the data to a 5th order polynomial equation. That raw data are unsuitable for the IRC procedure is clearly demonstrated by the data in Fig. 1b. The raw data

consisted of a 2000-point absorbance–time profile obtained for product evolution during the hydrolysis of *p*-nitrophenyl acetate (0.00006 M) in water containing hydroxide ion (0.02 M) at 298 K, conditions under which the apparent pseudo first-order rate constant was found to be equal to 0.280 s<sup>-1</sup>. The spectrometer output noise was observed to be equal  $\pm 0.0006$  AU while the average absorbance change due to product formation during a time interval ( $\Delta t$ ) was equal to about 0.0005 AU. The value of  $k_{inst}$  derived from raw data varied from about -2 to +6. Prior smoothing of the raw data using the Savitsky–Golay procedure<sup>15</sup> (Fig. 1c) resulted in some improvement ( $k_{inst}$  values varied from about 0.18 to 0.40) but still did not result in useful data.

**A sliding *n*-point IRC analysis.** Conversion of the raw data to a  $\ln(1-ER)/\text{time}$  profile set the stage for evaluating a sliding *n*-point procedure ( $n = 11, 21, 51$  or 101) for calculating IRC–time arrays. The first step involves a least squares correlation of  $\ln(1-ER)$  versus time over points 1 to *n*. The slope of this line is assigned to  $-k_{inst}(N)$  where *N* is equal to  $(n + 1)/2$ . The value of *N* is then incremented by taking points 2 to  $n + 1$  as the second correlation to obtain  $-k_{inst}(N + 1)$ . The value of  $-k_{inst}(N + 3)$  is obtained using points 3 to  $n + 3$ . This procedure is continued until all points in the  $\ln(1-ER)/\text{time}$  profile have been used. For example when  $n = 11$ , an IRC–time array consisting of  $-k_{inst}(6)$  to  $-k_{inst}(1995)$  results from the process.

In order to demonstrate the sliding *n*-point IRC procedure, a  $(1-ER)/\text{time}$  profile for the complex mechanism (Scheme 1;  $k_f = 0.546$  s<sup>-1</sup>,  $k_b = 14.8$  s<sup>-1</sup>, and  $k_p = 12.4$  s<sup>-1</sup>) was simulated. The rate constants were selected to correspond to those published for the Diels–Alder reaction between anthracene and tetracyanoethylene ( $[TCNE] = 0.1$  M).<sup>10</sup> The IRC/time profiles for analyses with *n* equal 11, 21, 51, and 101 are illustrated in Fig. 2 for reactant decay (2a, 2b, 2c, and 2d, respectively) and product evolution (2a', 2b', 2c', and 2d', respectively). There are two trends of interest in the data. As *n* increases from 11 to 101, both (a) the oscillations at the lower end of the  $(1-ER)$  range decrease in amplitude and (b) the range of  $k_{inst}$  values in the steep sections of the plots decrease with increasing *n*. These two effects are in opposite directions with respect to the quality of the IRC analyses.

The effect of changes in the  $k_f/k_p$  ratios on the IRC/time profiles for reactions taking place by the reversible consecutive mechanism (Scheme 1) is illustrated by the 11-point IRC/time profiles in Fig. 3. As the  $k_p/k_f$  changes from 40 (3a) to 2.5 (3e) the  $(1-ER)$  value at which the plateau region is approached changes steadily from  $< 0.95$  (3a) to about 0.55 (3e).

In order to incorporate the advantages of using different *n* values into a single IRC analysis, a procedure was devised to successively increase *n* with increasing time. A superior procedure was observed to involve an initial

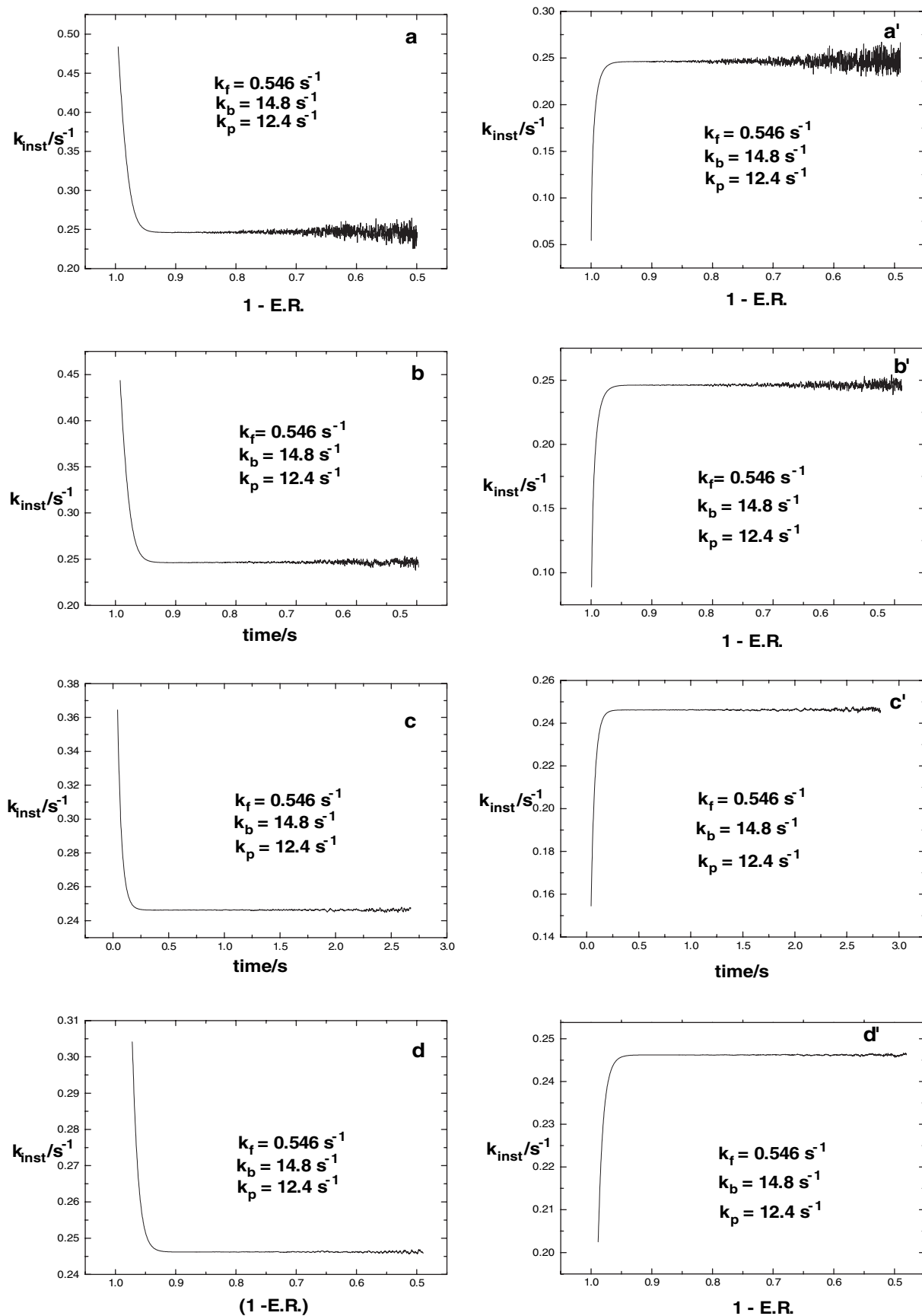
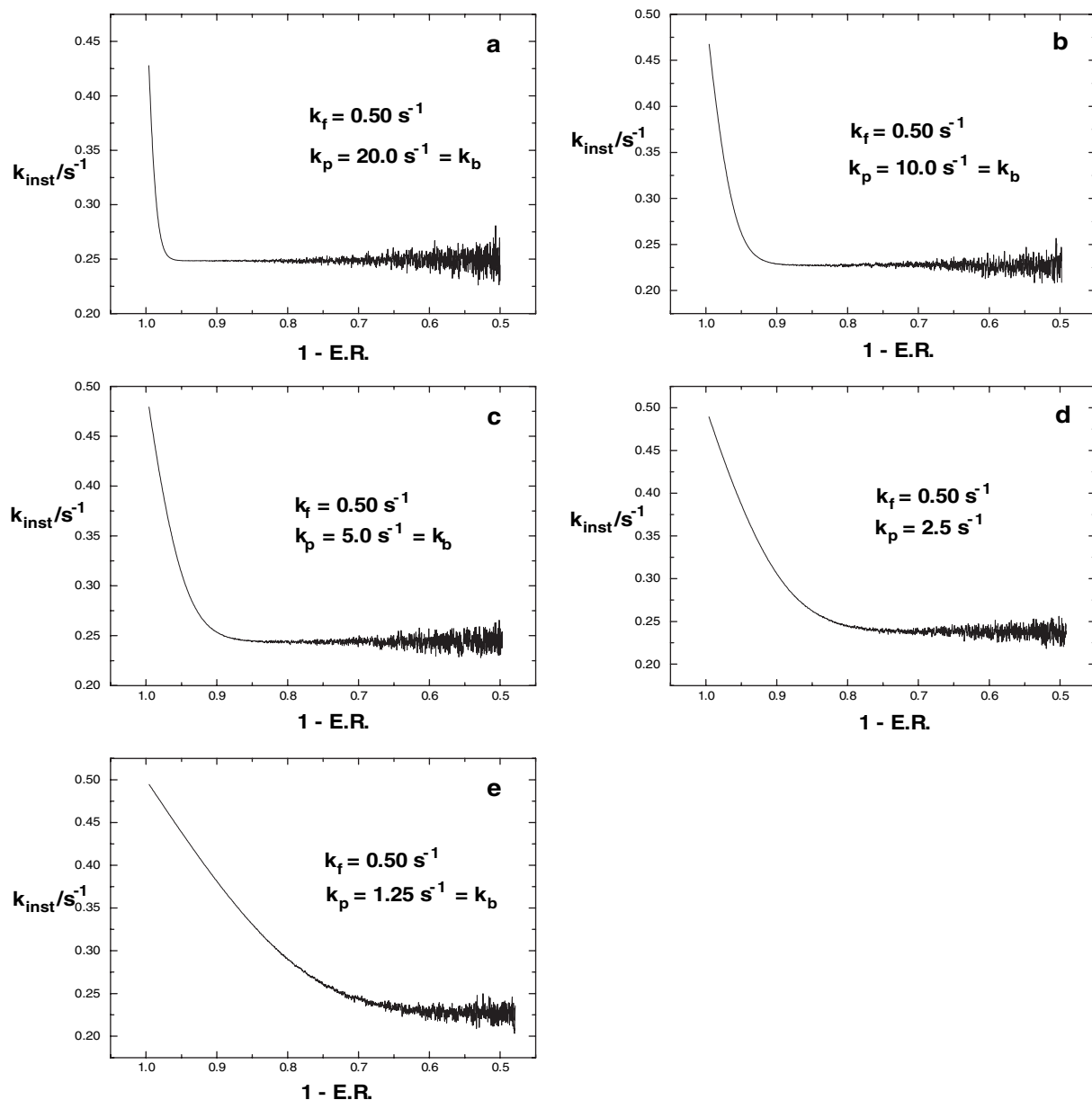


Figure 2. Sliding IRC analysis; 11-point (a, a'), 21-point (b, b'), 51-point (c, c'), 101-point (d, d')



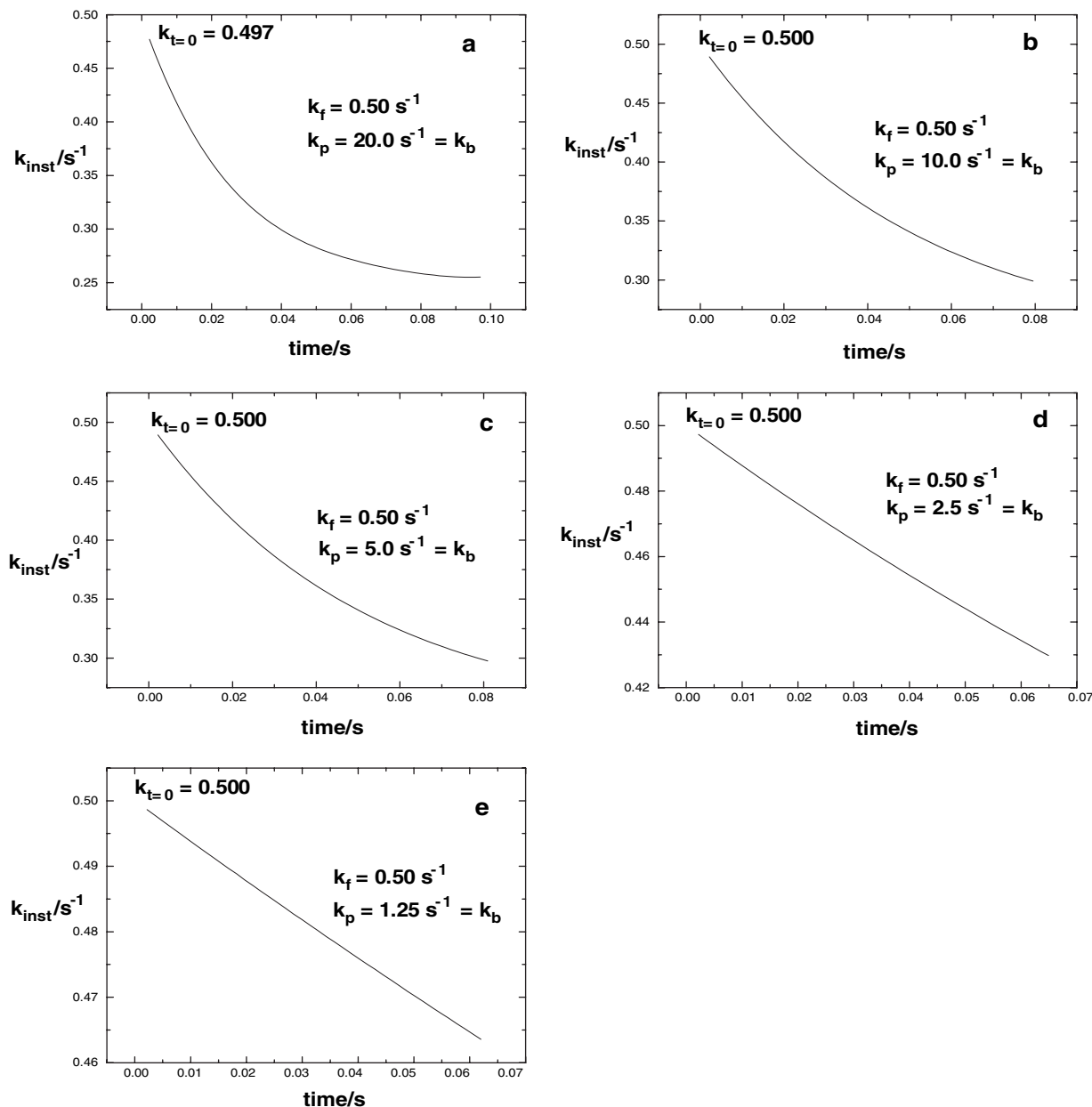
**Figure 3.** Sliding 11-point IRC analysis of complex mechanism data;  $k_p = 20.0 \text{ s}^{-1}$  (a),  $k_p = 10.0 \text{ s}^{-1}$  (b),  $k_p = 5.0 \text{ s}^{-1}$  (c),  $k_p = 2.5 \text{ s}^{-1}$  (d), and  $k_p = 1.25 \text{ s}^{-1}$  (e)

correlation over the first 3 points to give IRC(2), followed by correlations over 5 points (IRC(3)), 7 points (IRC(4)), and 9 points (IRC(5)). The 11-point sliding procedure was then used to give IRC(6) to IRC(50) and this was followed by application of the 101-point sliding procedure to generated IRC(51) to IRC(1950). The IRC analysis was used on the experimental data described later.

**5th order polynomial fit of (1-ER)/time profiles prior to IRC analysis.** We carried out an extensive comparison of IRC/time profiles generated directly from simulated (1-ER) data to those from fit of the data to 5th order polynomial equations for both simple and complex mechanisms. We found that the simple mechanism (1-ER)/profiles over the first half-life can be fit to 5th

order polynomial equations without distortion and the average deviation in (1-ER)/point between the two profiles is of the order of 0.01%. The 5th order polynomial smoothed data are suitable for the calculation of  $k_{\text{inst}}$  at the midpoint between successive time points.

For complex mechanism (Scheme 1) data, the quality of the fit to 5th order polynomials over the first half-life of reaction was observed to depend upon the relative values of the rate constants. For example, the zero time intercepts of the IRC/time profiles calculated after fit of 5th order polynomial equations to the (1-ER)/time profiles associated with Fig. 3 varied with  $k_p/k_f$  from  $0.257 \text{ s}^{-1}$  ( $k_p/k_f = 40$ ) to  $0.276 \text{ s}^{-1}$  ( $k_p/k_f = 20$ ) to  $0.326 \text{ s}^{-1}$  ( $k_p/k_f = 10$ ) to  $0.406 \text{ s}^{-1}$  ( $k_p/k_f = 5$ ) to  $0.471 \text{ s}^{-1}$  ( $k_p/k_f = 2.5$ ). Since the zero time intercepts in the IRC/time profiles give the



**Figure 4.** Instantaneous rate constant–time profiles over the initial 3% of reaction after 5th order polynomial fit of the  $(1-ER)/$  time profile;  $k_p = 20.0 \text{ s}^{-1}$  (a),  $k_p = 10.0 \text{ s}^{-1}$  (b),  $k_p = 5.0 \text{ s}^{-1}$  (c),  $k_p = 2.5 \text{ s}^{-1}$  (d),  $k_p = 1.25 \text{ s}^{-1}$  (e)

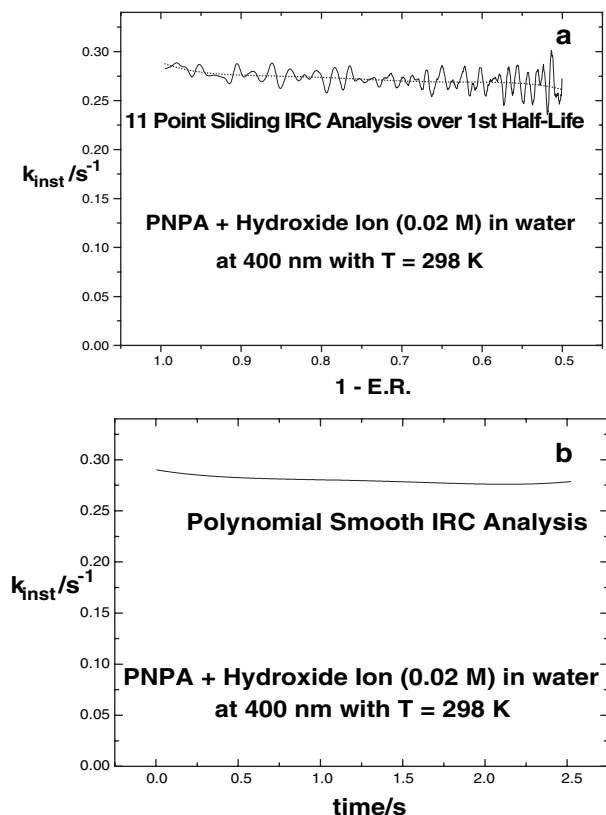
value of the rate constant for the first step in the reaction ( $k_f = 0.500 \text{ s}^{-1}$ ), none of the values are acceptably close.

The apparent reason for the failure to obtain precise fits of 5th order polynomial equations to  $(1-ER)/$  time profiles over the first half-life for reactions following the complex mechanism (Scheme 1) is that there are two time regimes in the data. The two time regimes correspond to the time periods before and after the steady-state has been achieved. The time regime after reaching the steady-state corresponds to that where the reaction follows strictly first-order kinetics and gives excellent fits to 5th order polynomial equations. To test the quality of fit of 5th order polynomial equations to data in the *pre-steady-state* time regime, the fitting procedure described in the previous paragraph was restricted to the

first 3% of the reactions. The IRC/time profiles illustrated in Fig. 4 show the results obtained for the restricted  $(1-ER)/$  time profiles. When  $k_p/k_f$  was equal to 40 (4a) the zero time intercept was  $0.497 \text{ s}^{-1}$  and for all others (Fig. 4b–e) was equal to  $0.500 \text{ s}^{-1}$ . For the case where  $k_p/k_f$  was equal to 40 (Fig. 4a), restricting the 5th order polynomial fit to the first 2% of reaction resulted in  $k_{t=0}$  equal to  $0.500 \text{ s}^{-1}$ , which is equal to  $k_f$  for the simulated data set.

**A comparison of IRC procedures applied to experimental data.** The experimental  $(1-ER)/$  time profile from which the IRC/time profile illustrated in Fig. 1b was obtained was subjected to analysis using the sliding  $n$ -point procedure and to 5th order polynomial smooth followed by the successive 2-point IRC





**Figure 5.** A comparison of the 11-point sliding IRC procedure (a) with the 5th order polynomial smooth IRC analysis (b) on the same (1–ER)/time profile. Data at 400 nm for the reaction of *p*-nitrophenyl acetate (0.00006 M) with hydroxide ion (0.02 M) in water at 298 K

analysis. The results of the IRC analyses are illustrated in Fig. 5. The IRC/time profile (solid line in Fig. 5a) derived from the 11-point sliding procedure shows some oscillations and was smoothed (dashed line in Fig. 5a) for sake of comparison. The corresponding profile obtained after 5th order polynomial smooth of the raw data is shown in Fig. 5b. The average values of  $k_{\text{inst}}$  derived from the two IRC/time profiles are 0.272 (Fig. 5a) and 0.279 (Fig. 5b). This difference arises from the fact that the smoothed line in Fig. 5a bends downward at the extreme right in the plot while that in Fig. 5b has a slight upward bend.

### Application of IRC analysis to acyl transfer reactions between *p*-nitrophenyl acetate and hydroxide ion

The kinetics of the reaction between *p*-nitrophenyl acetate (PNPA) and hydroxide ion were studied in both water and in aqueous acetonitrile (AN/H<sub>2</sub>O (20%)) using stopped-flow spectrometry at 5 nm intervals over the entire accessible uv/vis spectral region. Reactions carried out at 440 nm were selected to demonstrate the response to product evolution and at 270 nm to show that for reactant decay.

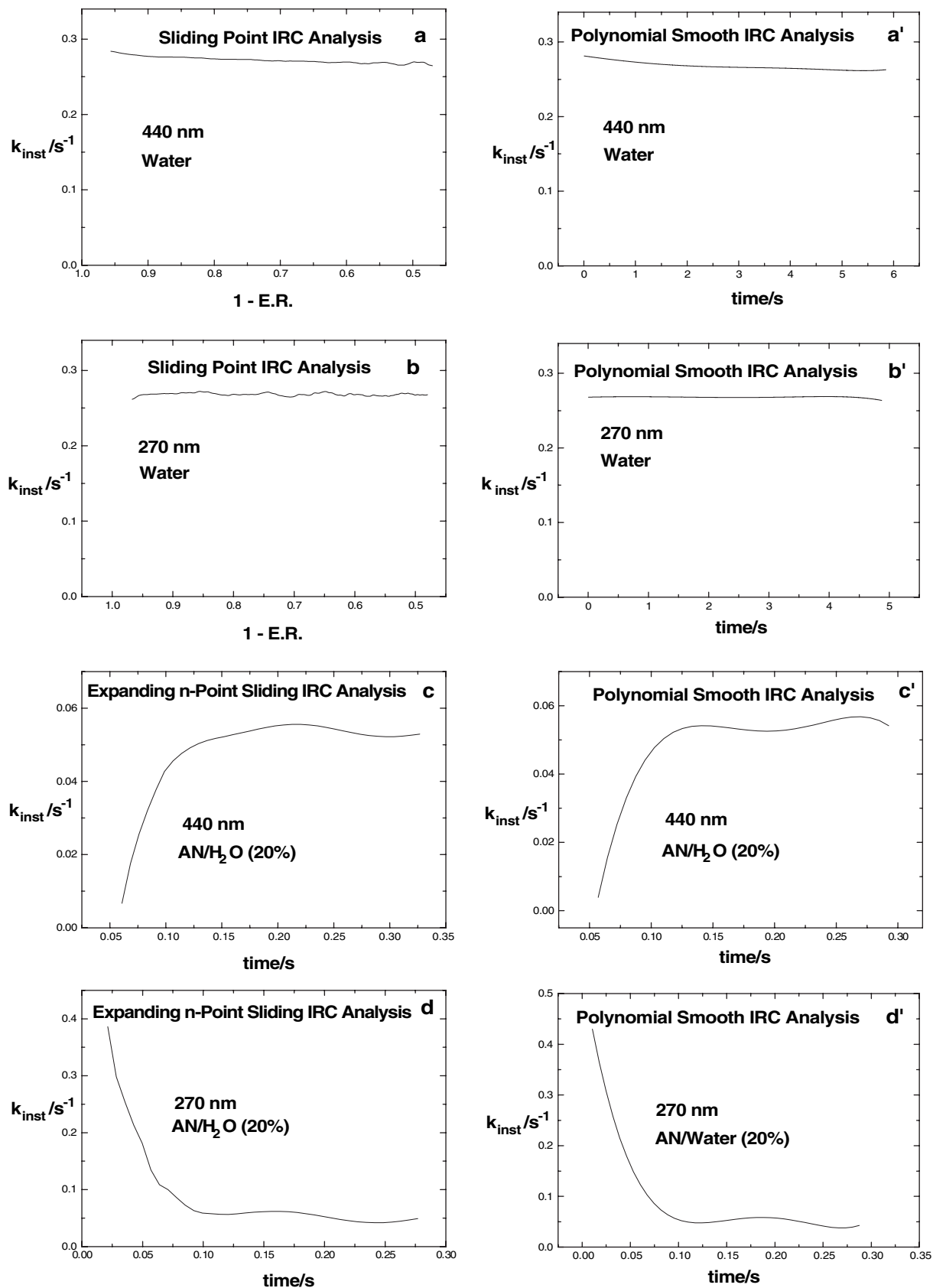
The IRC/time profiles for product evolution at 440 nm for the reaction between PNPA (0.00024 M) and hydroxide (0.02 M) in water are illustrated in Fig. 6a and 6a'. Both the expanding *n*-point sliding IRC (6a) and the polynomial smooth IRC (6a') profiles are nearly flat lines with a slight downward slope. The same reaction monitored at 270 nm resulted in the IRC/time profiles shown in Fig. 6b and 6b'. The response for both IRC procedures are essentially flat and indicate no significant deviations from that expected for pseudo first-order kinetics.

The IRC/time profiles for reactions carried out in AN/H<sub>2</sub>O (20%) and monitored at 440 nm (Fig. 6c and 6c') for product evolution and at 270 nm (Fig. 6d and 6d') for reactant decay show significant deviations from simple mechanism response. During product evolution (440 nm) the IRC/time profiles appear to rise from zero at zero time to the plateau in about 10 msec. The IRC/time profiles for reactant decay (270 nm) decrease rapidly from zero time to the plateau value. That obtained by the 5th order polynomial smooth IRC procedure intersects the zero time axis at  $0.546 \text{ s}^{-1}$ . All of the IRC/time profiles obtained in AN/Water (20%) are characteristic of those expected for complex mechanism behavior.

## DISCUSSION

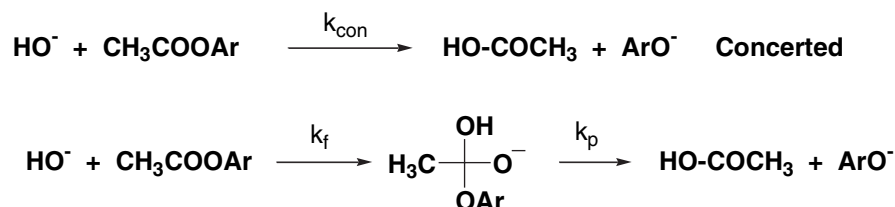
The irreversible consecutive mechanism (Scheme 1) or any other complex mechanism can only be differentiated, by kinetic measurements, from the single-step simple mechanism during the time period before the steady-state is reached. Once steady-state is achieved, the kinetic responses from the two mechanisms are identical. During the *pre-steady-state* time period under pseudo first-order conditions for a reaction following the complex mechanism (Scheme 1), the apparent pseudo first-order rate constant varies with time and depends upon whether reactant decay or product evolution is monitored. Since the steady state is generally<sup>1–10</sup> reached early in the reaction, the  $k_{\text{inst}}/\text{time}$  profile from zero time over the first half-life of a reaction will clearly differentiate between simple and complex mechanism behavior.

The difficulties encountered in attempting to evaluate IRC/time profiles over short time intervals are illustrated in Fig. 1. The root of the difficulty is the fact that the absorbance–time output of a spectrometer varies with time due to noise. Although this time variance is small,  $\pm 0.0005$  AU from the stopped-flow spectrometer used in this study (Fig. 1a) this gives rise to very large variations of  $k_{\text{inst}}$  values ( $-2$  to  $+6 \text{ s}^{-1}$  for a process with  $k_{\text{app}}$  equal to  $0.28 \text{ s}^{-1}$ ) calculated between successive data points. Prior smoothing of the data using the Savitsky–Golay procedure<sup>15</sup> is accompanied by improvement ( $k_{\text{inst}} = 0.18\text{--}0.40 \text{ s}^{-1}$ ) but the result is still not acceptable. The difficulty is readily understood if we consider the fact that the average absorbance change due to the process



**Figure 6.** Sliding point IRC analyses (a, b, c, d) compared polynomial smooth IRC analyses (a', b', c', d') for reaction of *p*-nitrophenyl acetate (0.0006 M) with hydroxide ion (0.02 M) at 298 K





**Scheme 2.** Mechanism of hydrolysis of *p*-nitrophenyl acetate

being monitored is of the order of 0.0005 AU/point (Fig. 1b,c).

Two approaches that result in acceptable IRC/time profiles have been described in the results section. The first is the *n*-point sliding IRC procedure illustrated in Figs. 2 and 3 on simulated (1-ER)/time profiles for both reactant decay and product evolution. An advantage of this approach is that there is no distortion of the data over the entire (1-ER) range. Another important advantage is that raw data are used in the analysis and the numerical treatment of the data is very simple. A disadvantage of the method is that  $k_{\text{inst}}$  values for the first  $(n-1)/2$  points cannot be calculated. This is especially significant when dealing with complex reactions, which reach the steady state at very low degree of conversion. This disadvantage is insignificant when using an improved IRC procedure, which incorporates expanding *n* values; 3 for IRC(2), 5 for IRC(3), 7 for IRC(4), 9 for IRC(5), 11 for IRC(6) to IRC(50), and 101 for IRC(51) to IRC(1950).

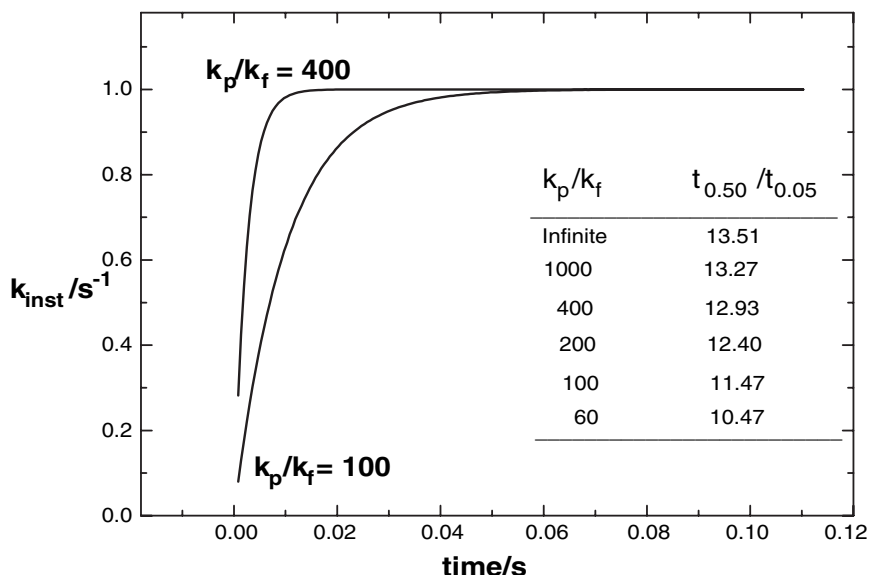
The second approach involves fitting the experimental (1-ER)/time profile to a 5th order polynomial equation before calculating  $k_{\text{inst}}$  as the mid-point between successive time points. The main advantage of this method is that  $k_{\text{inst}}$  are calculated between all successive points in the (1-ER)/time array and the resultant IRC/time profile is smooth. Data from processes, which obey first-order kinetics over the entire range of interest can be fit to 5th order polynomial equations without any distortion. However, data derived from complex mechanisms are in two distinct time regimes corresponding to the *pre-steady-state* and steady-state time periods and (1-ER)/time profiles encompassing both time regimes show significant distortion when fit to 5th order polynomial equations. However, if data are restricted to one or the other of the time regimes the 5th order polynomial smooth procedure does not result in significant distortion of the data. Therefore, limiting the analysis to data, which is clearly in the *pre-steady-state* time regime is the recommended use of this procedure. The 5th order polynomial smooth IRC analyses on simulated (1-ER)/time profiles are illustrated in Fig. 4. The only case where the zero time intercept gives an error (0.6%) is shown in Fig. 4a. This small error can be eliminated by decreasing the time range from 100 to 80 ms. In general, the best procedure to determine the range of data suitable for the analysis is to vary the time range to find the range where the intercept is independent of the latter.

### Application of IRC analysis to acyl transfer reactions between *p*-nitrophenyl acetate and water

Acyl transfer reactions have been the subject of a large number of investigations over the past 40 years and only those particularly pertinent to this work are cited.<sup>17-19</sup> It is generally accepted that acyl transfer reactions take place by one of two mechanisms depending upon a number of factors. The mechanisms include the single step concerted displacement reaction or a 2-step process involving the formation of a tetrahedral intermediate (Scheme 2). When *p*-nitrophenoxide ion is the leaving group the concerted mechanism is favored. A recent theoretical Study<sup>20</sup> resulted in the conclusion that the concerted pathway is energetically favored over the 2-step mechanism for the hydrolysis of PNPA.

The 2-step mechanism in Scheme 2 differs from that in Scheme 1 in that the first step is written as irreversible. This is to take into account the enormous difference in leaving group abilities between *p*-nitrophenoxide and hydroxide ions. An important factor in this difference is the relative  $pK_a$  values of *p*-nitrophenol (7.14 in water<sup>21</sup>) and water (15.7 in water). The assumption for irreversibility of the first step in Scheme 2 likely holds in AN/Water (20%) as well but is not as firmly grounded since the relative  $pK_a$  values are unknown in the mixed solvent. The latter also has consequences for the detection of deviations from pseudo first-order kinetics. The simulated data in Fig. 7 illustrate the effect of the  $k_p/k_f$  ratio for the irreversible consecutive mechanism (Scheme 2) on the kinetic response for product evolution. As  $k_p/k_f$  increases, the IRC/time response in the *pre-steady-state* time regime becomes steeper reaching the plateau value at lower conversions. This trend is also manifested in the data tabulated in the figure. As  $k_p/k_f$  decreases to lower values, the time ratio  $(t_{0.50}/t_{0.05})^4$  decreases significantly from the infinity value (13.51 for first-order kinetics) to lower values. The data in Fig. 7 illustrate the fact that for the irreversible consecutive mechanism, the deviation from first-order kinetics can be detected as long as the  $k_p/k_f$  ratio is of the order of  $10^3$  or less.

The qualitative expectation for the irreversible consecutive mechanism (Scheme 2) in the *pre-steady-state* time regime for product evolution is the same as that for the reversible consecutive mechanism (Scheme 1). When monitoring the evolution of product the IRC/time response is expected to increase from 0 at zero time to

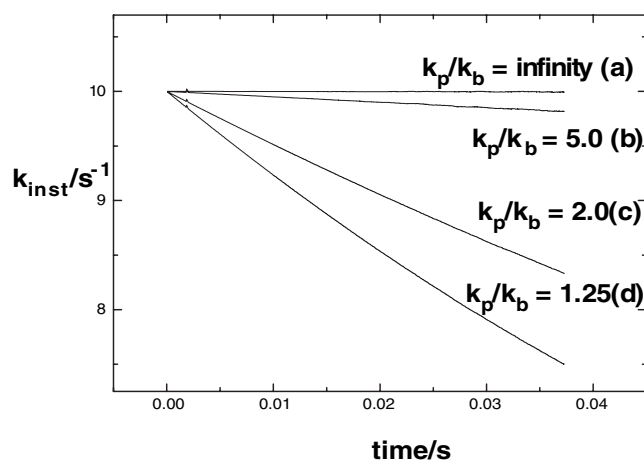


**Figure 7.** Simulated IRC/time profiles for the irreversible consecutive mechanism illustrated in Scheme 2

a plateau value when steady state is achieved. However, the kinetics of reactant decay are only dependent on the first step in the irreversible consecutive mechanism. The IRC/time profile for reactant decay is expected to equal  $k_f$  (the rate constant for the first step in the reaction) at all times. Thus, the reversible and irreversible consecutive mechanisms are readily distinguished by the IRC/time profile for reactant decay. The transition from the reversible consecutive (Scheme 1) to the irreversible consecutive mechanism (Scheme 2) as the partition ratio  $k_p/k_b$  increases is illustrated in Fig. 8.

The IRC/time profiles illustrated in Fig. 6 are representative for monitoring reactant decay at 270 nm and product evolution at 440 nm. Those derived from experimental (1-ER)/time profiles for the reaction in water as solvent (Fig. 6a,a',b,b') do not exhibit any significant deviations from simple mechanism behavior. We are unable to explain the slight downward slope to the curves for product evolution (Fig. 6a,a') but do not regard the deviations to be great enough to question whether or not the reactions follow first-order kinetics over the first half-life of the reaction.

On the other hand, the IRC/time profiles for the reactions carried out in AN/Water (20%) clearly indicate substantial deviations from pseudo first-order kinetics. The forms of the IRC/time profiles for product evolution (Fig. 6c,c') are consistent with either the reversible (Scheme 1) or the irreversible consecutive mechanism (Scheme 2). On the other hand, those for reactant decay (Fig. 6d,d') are clearly inconsistent with the irreversible consecutive mechanism and suggest the reversible consecutive mechanism (Scheme 1). It should also be pointed out that under the same conditions the steady-state rate constant for reactant decay decreased by about a factor of 5 in going from water



**Figure 8.** Transition of the response to the reversible consecutive mechanism (Scheme 1) to that of the irreversible consecutive mechanism (Scheme 2) as  $k_p/k_b$  increases while monitoring the decrease in reactant concentration. In all cases,  $k_f = 10 \text{ s}^{-1}$ ,  $k_p = 10 \text{ s}^{-1}$ , and  $k_b$  was varied in the range from 0 (a), 2.0 (b), 5.0 (c), and  $8.0 \text{ s}^{-1}$  (d)

( $0.28 \text{ s}^{-1}$ ) to 20% water in AN ( $0.05 \text{ s}^{-1}$ ) as solvent. In the mixed solvent, the IRC zero time intercept was  $0.546 \text{ s}^{-1}$ .

The form of the IRC-time profiles in the mixed solvent are consistent with the absorbance at 270 and 440 nm being predominately due to reactant and product, respectively. The IRC-time profiles are generated assuming that the absorbance is due to only reactant and product. At some wavelengths between 270 and 440 nm the IRC-time profiles are more complex suggesting that the intermediate also absorbs in those regions. We have previously suggested that the spectral

properties of the intermediate can be deconvoluted once the kinetics have been resolved.<sup>11</sup>

Does the dramatic change in the IRC/time profiles in going from pure water to aqueous acetonitrile mean that a change in mechanism takes place? It is possible that the mechanism differs in the two solvents but the data do not warrant this conclusion. If the 2-step mechanism is operative in both solvents while the IRC analysis only shows deviation from pseudo first-order kinetics in aqueous acetonitrile the valid conclusion that can be drawn is that the steady state is achieved so rapidly in water as solvent that the *pre-steady-state* time regime has not been accessed during our stopped-flow studies.

A detailed discussion of the mechanism of the acyl transfer reaction from PNPA to hydroxide ion is beyond the scope of this paper. Our data, as well as the previous kinetic isotope effect study<sup>19</sup> are inconsistent with a 2-step mechanism involving a tetrahedral intermediate. All of the data are consistent with the reversible consecutive 2-step mechanism (Scheme 1) in which the intermediate is a non-bonded reactant complex, which is irreversibly transformed to products.

## CONCLUSIONS

The development of an IRC analysis represents the addition of a very effective kinetic tool to the arsenal available for mechanism analysis in *Physical Organic Chemistry*. The analysis unambiguously distinguishes between simple and complex mechanisms as long as the *pre-steady-state* time regime of the complex mechanism can be accessed by the kinetic method employed. Two complimentary and independent methods for obtaining IRC/time profiles are described which, when used to analyze the same (1-ER)/time profiles, provide accurate  $k_{\text{inst}}$ /time profiles which clearly detect any deviation from simple mechanism behavior.

No significant deviations from pseudo first-order kinetics were observed during the study of the acyl transfer reaction between PNPA and hydroxide ion in water when monitoring both reactant decay and product evolution. This kinetic behavior is consistent with either mechanism in Scheme 2 as long as the  $k_p/k_f$  ratio for the 2-step mechanism is about 1000 or greater.

Changing the solvent from water to aqueous acetonitrile brings about a dramatic change in the kinetic response. The IRC/time profiles for the acyl transfer reaction between PNPA and hydroxide ion show significant deviations from pseudo first-order behavior and suggest the reversible consecutive mechanism (Scheme 1). The irreversible consecutive mechanism (Scheme 2) was ruled out on the basis of the IRC analysis for reactant decay. This raises the question as to whether or not a mechanism change accompanies the change in

solvents or if the same mechanism takes place in both solvents and the deviations from first-order kinetics are masked in water due to rapid attainment of the steady state.

## Note added in proof

After submission of this manuscript it was discovered that the IRC analyses for the acyl transfer reaction between *p*-nitrophenyl acetate and hydroxide ion in water show significant deviation from pseudo first-order behavior when the hydroxide ion concentration was increased to values greater than 0.05 M. This observation suggests that the same mechanism takes place in water as in aqueous acetonitrile. In both solvents the data are consistent with the reversible consecutive mechanism (Scheme 1).

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