JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Impact of pH on the Kinetics of Acrylamide Formation/Elimination Reactions in Model Systems

KRISTEL DE VLEESCHOUWER, IESEL VAN DER PLANCKEN, ANN VAN LOEY, AND MARC E. HENDRICKX*

Laboratory of Food Technology, Center for Microbial and Food Technology, Faculty of Bioscience Engineering, Katholieke Universiteit Leuven, Kasteelpark Arenberg 22, B-3001 Heverlee, Belgium

The effect of pH on acrylamide formation and elimination kinetics was studied in an equimolar (0.1 M) asparagine−glucose model system in phosphate or citrate buffer, heated at temperatures between 120 and 200 °C. To describe the experimental data, a simplified kinetic model was proposed and kinetic parameters were estimated by combined nonlinear regression and numerical integration on the data obtained under nonisothermal conditions. The model was subsequently validated in a more realistic potato-based matrix with varying pH. By increasing acidity, the reaction rate constants at T_{ref} (160 °C) for both acrylamide formation and elimination can significantly be reduced, whereas the temperature dependence of both reaction rate constants increases. The introduction of a lyophilized potato matrix (20%) did not affect the acrylamide formation reaction rate constant at reference temperature (160 °C) as compared to the asparagine−glucose model system; the elimination rate constant at T_{ref}, on the contrary, was almost doubled.

KEYWORDS: Acrylamide; kinetics; pH; buffer type; thermal treatment; food

INTRODUCTION

In April 2002, the Swedish National Food Administration announced the finding of unexpected high levels of acrylamide in different foodstuffs, mainly heat-treated carbohydrate-rich foods, e.g., French fries and potato chips. Because this compound has been classified as a potential carcinogen to humans by the IARC (*1*) and also shows some genotoxic and neurotoxic effects, this announcement has led worldwide to intensive research in several areas. Shortly after, Mottram et al. (*2*) identified a possible formation mechanism, showing that acrylamide is formed through the Maillard reaction between an amino acid, mainly asparagine, and a reducing sugar at elevated temperatures, i.e., higher than 100 °C. Several studies confirmed this pathway as being the most important one (*3*−*5*). Simultaneously, different techniques were developed, which allow an accurate analysis of acrylamide. The most widely used methods are gas chromatography with mass spectrometric detection (GC-MS) and high-performance liquid chromatography (HPLC) with tandem mass spectrometric detection (LC-MS/MS) (*6*). The knowledge on both the formation mechanism and the methods of analysis facilitated studies on the effects of different product and process-related factors (e.g., heating temperature and time, moisture content, pH, and matrix components) on acrylamide formation and elimination reaction(s).

Most of the ongoing research focuses on minimizing acrylamide levels in different foodstuffs, mainly potato and cereal products. Reduction of acrylamide formation can be obtained in different ways: by minimizing frying temperature, by lowering the content of one or more of the required acrylamide precursors, i.e., asparagine and reducing sugars, or by selectively adapting product composition. Lowering temperature has proven to reduce acrylamide formation (*7*−*9*) but also implies reduced browning. In the case of frying, other product characteristics such as flavor and fat content will inevitably also be affected. Several authors have demonstrated that lowering asparagine and/or sugar content results in a considerable decrease in acrylamide formation. On the one hand, this can be accomplished by selecting raw materials with a low content of acrylamide precursors. Additionally, in the case of potatoes, sugar content can be controlled by selectively choosing storage temperature (*10*−*12*). On the other hand, pretreatments that (partly) remove asparagine and/or reducing sugars can be applied. Pretreatments proven to be efficient in the case of potatoes are soaking in hot or cold water (*13*, *14*) and enzymatic treatment with asparaginase to reduce asparagine concentration (*4*). Additionally, acrylamide formation can be reduced significantly by introducing other amino acids, such as cysteine, lysine, or glycine, which would compete with asparagine for the carbonyl compounds in the Maillard reaction and/or enhance acrylamide eliminaton (*15*, *16*). The elimination of acrylamide is ascribed to reaction with other reactive groups present in the food matrix or formed during the heating process (*17*). Control of product conditions such as moisture content, acidity, etc. has been studied in a less extensive way. So far, very little data are available on the effect of moisture content on acrylamide formation; Elmore et al. (*18*)

* To whom correspondence should be addressed. Tel: +32-16-32-15-85. Fax: +32-16-32-19-60. E-mail: marc.hendrickx@biw.kuleuven.be.

10.1021/jf0611264 CCC: $33.50 © 2006 American Chemical Society
Published on Web 09/02/2006

observed an increased acrylamide formation with decreasing moisture content with the formation being most pronounced at moisture contents below 5%. The impact of acidity on acrylamide formation, however, has been demonstrated in model and potato systems by Jung et al. (*19*). According to these authors, a reduction of pH from 7 to 4 results in a 99.1% inhibition of acrylamide formation in asparagine−glucose model systems after thermal treatment during 30 min at 150 °C. This was explained by the increased proton concentration at lower pH, resulting in a higher amount of protonated amino groups of asparagine. Because the nucleophilic unprotonated amino group of asparagine is required in the first step of acrylamide formation through Maillard reaction, the formation reaction will be blocked.

The aim of this paper was to study the effect of pH on the acrylamide formation and elimination reaction on a kinetic basis. Detailed kinetics allows description of changes in a quantitative way and prediction of changes based on temperature−time data and thus of design of processes resulting in low acrylamide levels, either by diminishing the formation and/or by enhancing the elimination. The influence of acidity was first analyzed under idealized conditions in a closed asparagine−glucose model system using different buffer types. Second, the estimated kinetic parameters were validated using a model system with increased complexity in which a lyophilized potato matrix was used as a background.

MATERIALS AND METHODS

Preparation of the Potato Matrix. A batch of potatoes of the variety Bintje (year of harvest, 2004) was purchased from a local farmer. They were cut into slices (with a thickness of 5 mm) and blanched for 10 min at 70 °C to prevent enzymatic browning. The blanched potato slices were mixed and stored in the freezer at −40 °C. Before lyophilization (0.01 mbar vacuum pressure, Alpha 2−4, Christ, Osterode, Germany), the potato mixtures were immersed in liquid nitrogen. The resulting freeze-dried matrix was characterized in terms of amino acid, sugar, and starch contents.

Characterization of the Potato Matrix. The analysis of amino acids was performed by an external company, Ansynth Service B.V. (Roosendaal, The Netherlands). An asparagine concentration of 9.39 g/kg lyophilized potato mixture was measured.

Because 90% of all sugars in potatoes consist of glucose, fructose, and sucrose, sugar analysis was performed on these three components. The concentration was determined using the enzymatic test kit from r-Biopharm GmbH (Darmstadt, Germany). Prior to analysis, the samples were deproteinized with Carrez reagents. The lyophilized potato matrix contained 12.7 g/kg glucose, 10.22 g/kg fructose, and 7.11 g/kg sucrose. The total starch content was determined using the enzymatic test kit of Megazyme (Wicklow, Ireland) and amounted to 17% on a fresh weight basis.

Preparation of the Model Systems. The effect of buffer type and concentration was studied in an equimolar model system consisting of 0.1 M L-asparagine (≥99.5%, Sigma-Aldrich) and D-glucose (99.5%, Sigma-Aldrich) dissolved in citrate or phosphate buffer at pH 8 with variable concentration (0.025−0.5 M).

The kinetic experiments were performed using two different types of model systems. The first simplified model system was prepared by dissolving 0.1 M L-asparagine and 0.1 M D-glucose in a 0.05 M citrate or phosphate buffer with an initial pH value of 4, 6, or 8. The pH, after the reactants were dissolved, remained unchanged in all model systems studied, except for the system in citrate buffer at pH 8 where the value dropped to 6.96. The more complex model systems consisted of 20% lyophilized potato matrix, in which the glucose and asparagine concentration was selectively adapted to a final equimolar level of 0.1 M for both components as in the simplified model systems. The remaining 80% consisted of a 0.05 M phosphate buffer with a pH value of 4, 6, or 8. Note that all of the pH values reported in this work refer to room temperature.

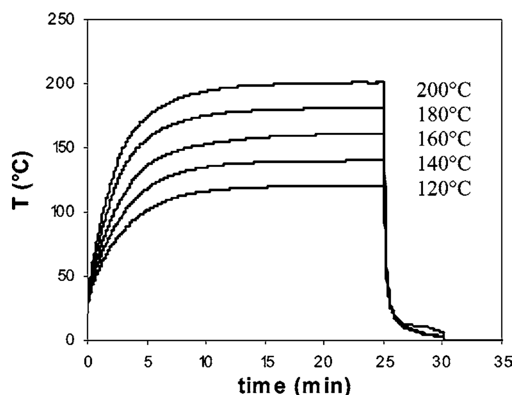


Figure 1. Typical temperature–time profile of samples heated in closed reactors at different temperatures.

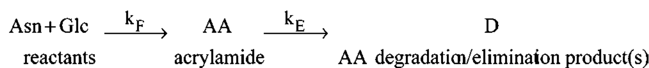
Heat Treatment. Samples were heated in hermetically closed reactor tubes (stainless steel, 8 mm × 100 mm, custom-made) in order to avoid as much as possible side phenomena during heating, such as fluctuations in water activity due to water evaporation and seeping in of oil, which could affect acrylamide formation/elimination. Heat treatment was performed in a thermostated oil bath (UH2D, Grant Instruments Ltd., Cambridge, England) at 120, 140, 160, 180, and 200 °C. For the experimental part concerning the effect of buffer type and concentration, a 20 min heat treatment was applied; for the kinetic experiments, samples were taken at different heating times, depending on the treatment temperature. After thermal treatment, samples were immediately cooled in ice water to stop any further reaction. During the reaction and subsequent cooling phase, the temperature of the samples was registered within the closed reactor tubes at regular time intervals (4 s) using thermocouples (type T, Thermo Electric Benelux, Balen, Belgium) connected to a datalogger (TM 9616, Ellab, Roedovre, Denmark). An example of a typical temperature profile is given in **Figure 1**.

Analysis of Acrylamide. The analysis of acrylamide was performed by GC-MS with chemical ionization based on the method described by Biedermann et al. (*20*), without prior derivatization of acrylamide. After acrylamide extraction and further cleanup, the sample was analyzed using the 5973 inert GC-MS system (Agilent Technologies, Diegem, Belgium). One microliter was injected at low temperature, i.e., 60 °C, on an HP-InnoWax column (30 m × 250 μm i.d., 0.25 μm ft, Agilent Technologies) with a 0.5 m × 530 μm i.d. precolumn of deactivated fused silica. The chromatographic separation was carried out with a constant flow rate of 2 mL/min of helium as the carrier gas using the following temperature program: Initially, the oven temperature was set to 60 °C (0.2 min), from which the temperature was increased to 100 °C at a rate of 35 °C/min and further augmented at a rate of 12 °C/min to 230 °C (3 min). The detection was performed with a quadrupole mass spectrometer operating in positive chemical ionization mode with 20% methane as the ionization gas in selected ion monitoring at m/z 72 (acrylamide), m/z 86 (methacrylamide), and m/z 88 (butyramide). The acrylamide concentration was quantified using methacrylamide (≥99%, Merck) as an internal standard, which was added at the start of the sample preparation step and was used to account for losses during sample preparation and analysis. By comparing this internal standard with a second internal standard, i.e., butyramide (≥98%, Fluka), added to the samples prior to injection, the recovery of the extraction procedure could be determined. If the recovery dropped below 40%, the extraction procedure was repeated (*20*).

Repeatability of the Heat Treatment and the Subsequent Acrylamide Analysis. For every temperature−time combination, two reactor tubes were heated in the oil bath, which were combined after cooling in ice water. From the resulting sample, the acrylamide concentration formed was determined once. In order to assess the standard error on the heat treatment and subsequent analysis of acrylamide, five identical samples were heated independently of each other at each of the five temperatures applied (120, 140, 160, 180, and 200 °C). For each temperature, the resulting standard error on the acrylamide concentration

formed of the five identical samples was calculated and was found to be 10% maximum.

Kinetic Data Analysis. Because acrylamide concentrations measured in food are the net result of simultaneous formation and elimination reactions (*21*, *22*), the overall changes in acrylamide content due to different factors, e.g., reaction temperature and time, can be modeled by a simplified reaction schedule of two consecutive reactions as presented in the following scheme (*23*) in which k_F and k_E, respectively, represent the rate constants of acrylamide formation and elimination reaction(s).



It has been shown that the formation of acrylamide can be modeled by a second-order reaction, i.e., first-order in asparagine and first-order in glucose (*24*). For the acrylamide elimination reaction, Biedermann et al. (*25*) proposed a (pseudo)-first-order kinetics based on experiments using D_3-acrylamide. Consequently, the rate for each step proposed in the preceding scheme can be written as:

$$\frac{dC_{Asn}}{dt} = -k_F \times (C_{Asn} \times C_{Glc}) \quad (1)$$

$$\frac{dC_{Glc}}{dt} = -k_F \times (C_{Asn} \times C_{Glc}) \quad (2)$$

$$\frac{dC_{AA}}{dt} = k_F \times (C_{Asn} \times C_{Glc}) - k_E \times C_{AA} \quad (3)$$

$$\frac{dC_D}{dt} = k_E \times C_{AA} \quad (4)$$

with C_{Asn}, C_{Glc}, C_{AA}, and C_D the concentrations of asparagine, glucose, acrylamide, and degradation/elimination products, respectively, and t the reaction time. Initially, at time $t = 0$, the concentrations of both reactants equal 0.1 M and the concentration of acrylamide and degradation/elimination products can be considered zero.

The effect of temperature on the reaction rate constant k can be expressed by the Arrhenius equation, in which the temperature dependence of k is quantified by the activation energy E_a (J/mol) according to

$$k = k_{ref} \exp\left[\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right] \quad (5)$$

with R the universal gas constant (8.314 J/K mol), T the temperature concerned (K), and k_{ref} the reaction rate constant at reference temperature T_{ref} (160 °C). This equation is considered to apply for each rate constant involved in the kinetic model describing acrylamide formation and elimination. However, because the model systems studied were heated under nonisothermal conditions, the integrated effect of temperature on the reaction rate has to be taken into account.

After insertion of eq 5 in eqs 1−4, the resulting differential equations were solved by numerical integration of the registered temperature−time profile of each sample and kinetic parameters describing acrylamide formation and elimination (k_{Fref}, E_{aF} and k_{Eref}, E_{aE}, respectively) could be estimated by nonlinear regression using the statistical software package SAS (v8, Cary, NC).

The precision of model fitting and parameter estimation was evaluated in terms of the output statistics, i.e., the sum of squares (SSQ) and the standard errors (SE) associated with the parameter estimates. Commonly, the quality of fit of a linear model is expressed as the R^2 value. This value cannot be used as such to evaluate nonlinear models. Therefore, a measure closely corresponding to R^2 in the nonlinear case was defined (*26*)

$$\text{pseudo-}R^2 = 1 - \frac{\text{SSQ(residual)}}{\text{SSQ(total}_{\text{corrected}})} \quad (6)$$

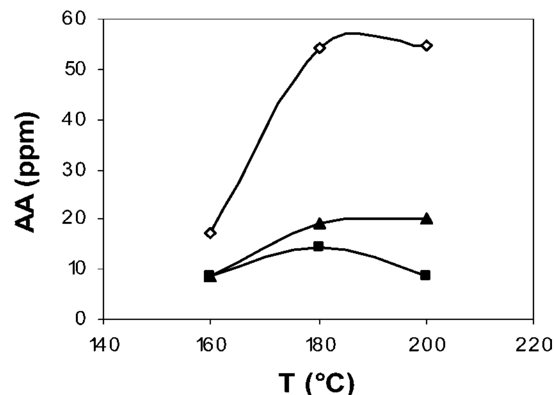


Figure 2. Effect of buffer type (0.5 M) on acrylamide formation as compared to the reaction in water in an equimolar (0.1 M) asparagine–glucose system at pH 4.83 as a function of temperature for a 20 min treatment: water (◇), phosphate buffer (▲), and citrate buffer (■).

In addition, the adequacy of the kinetic model can be evaluated graphically by plotting the QQ plot, showing the relation between experimental and predicted values (*27*).

RESULTS AND DISCUSSION

Influence of Buffer Type and Concentration. To evaluate the possible effect of buffer ions on the formation reaction of acrylamide, the precursors, i.e., asparagine and glucose, were dissolved in both an unbuffered (i.e., demineralized water) and a buffered model system for which phosphate or citrate buffer was used. The pH of the system was chosen at 4.83, since this pH was reached when dissolving the reagents in water. The results are illustrated in **Figure 2**. This figure shows that the net acrylamide formation is much lower in the buffered systems as compared to the unbuffered model system. A possible explanation could be the change in pH in the unbuffered system due to the reaction(s) occurring in that mixture, which would be less pronounced in the case of the buffered systems. Moreover, it has to be kept in mind that the pH could only be measured at room temperature and that the exact pH at temperatures applied is unknown. Because the buffer concentration used was relatively high, especially in relation to the precursor concentration, the much lower acrylamide formation could possibly be attributed to an inhibiting effect of the buffer ions on the acrylamide formation reaction. Therefore, different buffer concentrations were tested for both types of buffer (**Figure 3a**,**b**). A distinct effect of buffer concentration on acrylamide formation can be observed, which is comparable for both buffers tested. For the model systems with a buffer concentration lower than the precursor concentration, i.e., 0.025 and 0.05 M, the concentration of acrylamide formed seems to evolve toward a plateau at higher temperatures for a heating time of 20 min. This is not the case for the model systems with buffer concentrations higher than or equal to the precursor concentration, where the acrylamide concentration decreases again at higher treatment temperatures. This observed reduction is presumably the result of an enhanced elimination reaction, which could be related directly or indirectly to the buffer concentration. Increasing buffer concentrations from 0.1 to 0.5 M also seems to lower the measured concentration of acrylamide, which is the net sum of simultaneous formation and elimination. The lower acrylamide content can be the result of a reduced formation, an enhanced elimination, or a combination of both. This could plausibly be attributed to the sterical hindrance caused by the high amount of buffer ions. Because there was only a limited difference between phosphate and

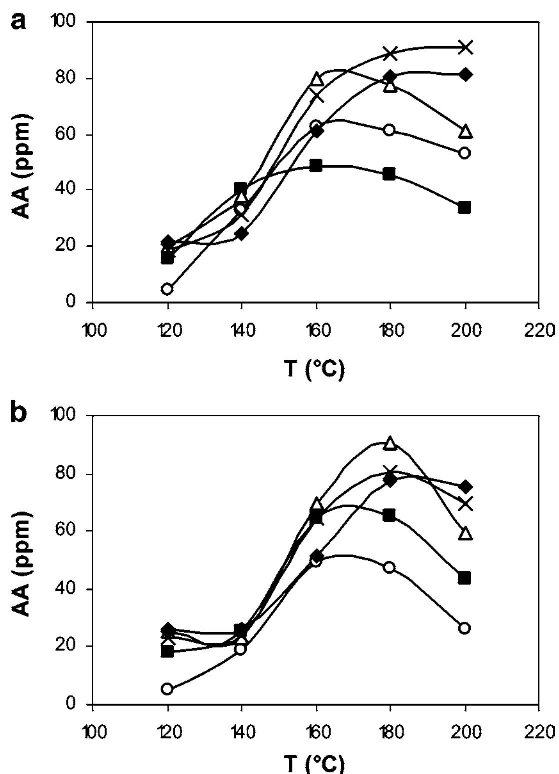


Figure 3. Acrylamide formation as affected by buffer concentration studied in an equimolar (0.1 M) asparagine–glucose model system at pH 8 in phosphate buffer (**a**) and in citrate buffer (**b**) as function of temperature for a 20 min treatment: 0.025 (◆), 0.05 (×), 0.1 (△), 0.25 (■), and 0.5 M (○).

citrate buffer, it can be assumed that the type of the buffer ions is not or only to a limited extent affecting acrylamide formation. Bell (*28*) on the contrary, when studying the impact of buffer type and concentration on the Maillard reaction in the same range of magnitude, observed a difference between phosphate and citrate buffers. The amino acid content and browning was followed as a function of time at 25 °C and pH 7 for different buffer concentrations. He observed an increased reaction rate for both responses measured with increasing buffer concentration in the case of phosphate buffer, whereas no changes were observed in the case of citrate buffer tested under the same conditions. These data suggest that phosphate ions enhance the Maillard reaction in contrast to citrate ions, which do not seem to influence the reaction. The stimulating effect of phosphate ions on the Maillard reaction was explained by the bifunctional catalytic activity of phosphate on the nucleophilic reaction of the amine (i.e., from the amino acid) with the carbonyl (i.e., from the sugar). The larger structure of citrate ions does not allow for bifunctional catalytic activity, so the catalytic effect due to the donation and acceptance of protons from citrate is reduced as compared to that of phosphate buffer salts (*29*). The observations of Bell (*28*) were confirmed by others (*30*, *31*). The discrepancy between our study concerning acrylamide formation and the results of Bell (*28*) and others could possibly be attributed to the fact that most studies concerning the effect of buffer on the Maillard reaction focus on the extent of browning, expressed as the melanoidin concentration. These brown-colored products are the result of a complex series of reactions, whereas acrylamide is formed in the earlier stage of the Maillard reaction. Furthermore, our data are only a momentary representation of the effect of buffer concentration on the net acrylamide formation as only 20 min treatments were studied.

Acrylamide Formation/Elimination Kinetics. The influence of pH on acrylamide formation has already been demonstrated (*19*, *22*). Increasing the acidity has been shown to effectively lower acrylamide formation. This was explained by the fact that lowering the pH converts the nucleophilic free nonprotonated amino groups of asparagine to protonated amino groups. This effectively blocks the formation reaction of acrylamide. However, because studies concerning pH effects have only been conducted in a qualitative way, there is a need to quantify the combined effect of pH, temperature, and time. In order to avoid disturbances by the presence of a food matrix, this study was conducted using an aqueous asparagine−glucose model system. Once a suitable model can be found to describe the experimental data and determine the kinetic parameters, this will be validated in a more realistic potato model system.

Because acrylamide formation occurs through the Maillard reaction, in which H^+ ions are formed, a decrease of the pH of the system can be expected (*32*). Consequently, a buffer is necessary when studying acrylamide formation in model systems. Previous studies concerning the Maillard reaction in general or acrylamide formation in particular were mostly conducted using phosphate buffer (*19*, *33*). However, as mentioned above, this buffer seems to enhance the Maillard reaction in contrast to citrate buffer. For this reason, kinetics of acrylamide formation and elimination were analyzed in both phosphate and citrate buffer for different levels of pH. For all model systems tested, the influence of temperature and time on either reaction was clearly visible (**Figure 4a−f**). The maximum content of acrylamide formed not only increases with increasing temperature but is also attained after shorter heating times. At elevated temperature ($T > 160$ °C), the acrylamide concentration decreased again after prolonged heating. Because the acrylamide content measured is the net result of simultaneous formation and elimination/degradation reaction(s), it can be stated that acrylamide formation dominates during the first stage of heating, whereas elimination is all-important during the second phase with decreasing acrylamide content. This elimination phase is, however, only at higher temperatures (180 and 200 °C) clearly visible. This does not necessarily mean that elimination reaction(s) do not occur at the lower temperatures studied. As, however, it was postulated that elimination most probably occurs through binding of acrylamide with other reactive compounds formed or present within the reaction mixture (*25*), it can be assumed that higher heating temperatures result in a higher amount of reactive compounds, which leads to a more outspoken elimination. The experimental data for each model system studied were modeled by means of a second-order formation reaction, followed by a first-order elimination reaction. The proposed kinetic model describes accurately the acrylamide content in all model systems tested, as can be seen by the curves superimposed on the data points in **Figure 4a−f**, regardless of the type of buffer used or the pH of the system. The goodness of fit of the model on the data is confirmed graphically by the QQ plots, in which a high correlation is found between predicted and experimental values (**Figure 5a−f**). This correlation is confirmed by the pseudo-R^2 value, which corresponds to the correlation coefficient of the QQ plot (**Table 1**). On the basis of the experimentally determined temperature−time profiles and acrylamide contents, the rate constants k_{Fref} and k_{Eref} and the activation energies E_{aF} and E_{aE} could be estimated by nonlinear regression. The kinetic parameters and their SEs are listed in **Table 1**. When comparing the model systems with a different initial pH value, it can be seen that the reaction rate constants k_{Fref} and k_{Eref} for acrylamide formation

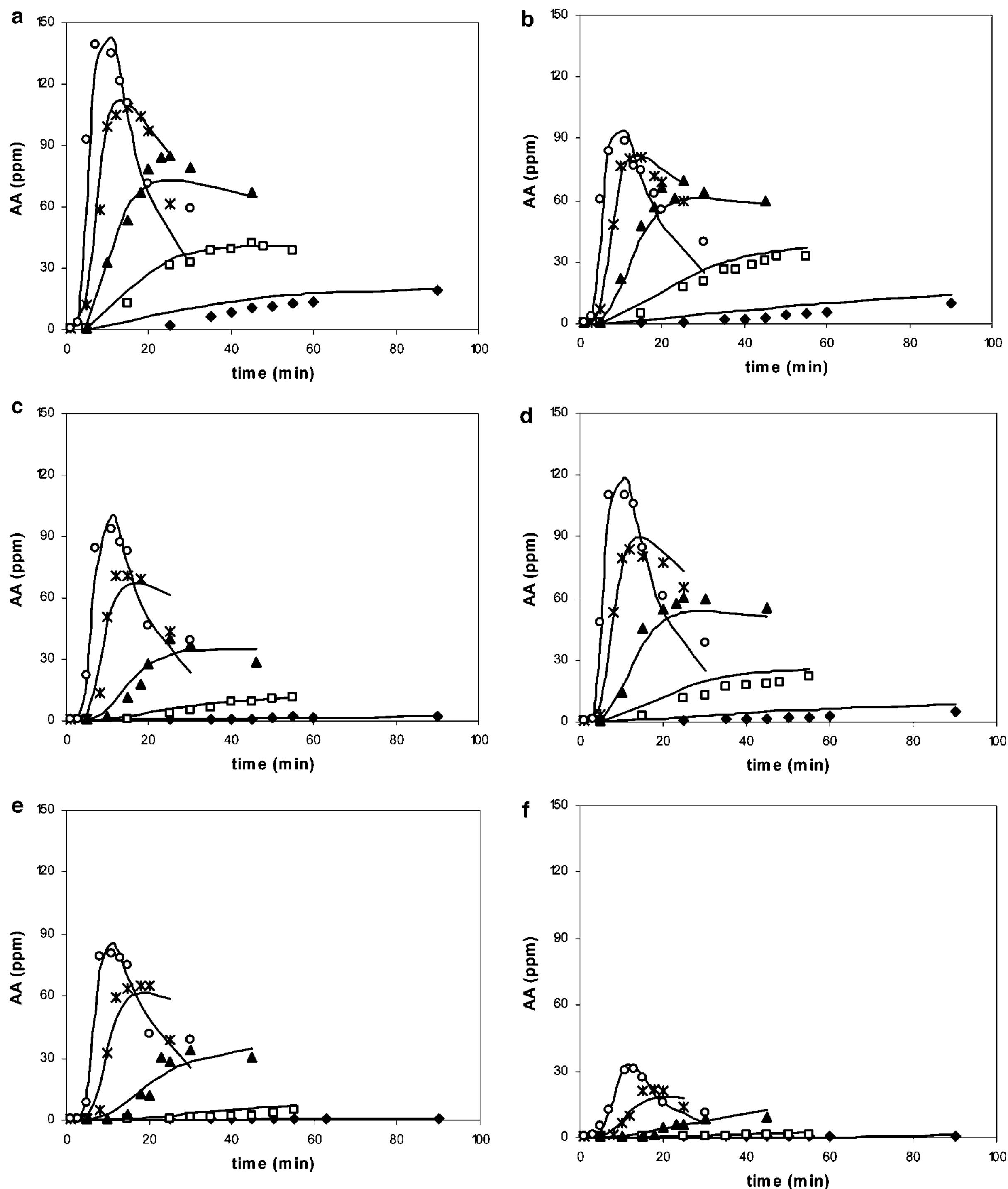


Figure 4. Formation of acrylamide as function of heating time at 120 (◆), 140 (□), 160 (▲), 180 (×), and 200 °C (○) in an equimolar asparagine–glucose (0.1 M) in phosphate buffer (0.05 M) at (**a**) pH 8, (**b**) pH 6, and (**c**) pH 4 or in citrate buffer (0.05 M) at (**d**) pH 8, (**e**) pH 6, and (**f**) pH 4. The full lines represent acrylamide formation/elimination predicted by the kinetic model.

and elimination decrease significantly ($\alpha = 0.05$) with a decreasing pH from 8 to 4. **Figure 6** shows a log−linear relation for both rate constants of acrylamide formation and elimination with pH when studied in phosphate buffer, with correlation coefficients of 0.9986 and 0.9627 for the formation and elimination reactions, respectively. If the slope of the curve is considered to be a measure of the pH dependency of the reaction, this would mean that the formation reaction (slope = 0.5414 ± 0.106) is more sensitive to changes in pH than the elimination reaction (slope = 0.3442 ± 0.013). The log−linear relation between the *k* values and the pH is not valid for the formation and elimination reaction studied in citrate buffer. This is due to the nonsignificant difference between k_{Fref} and k_{Eref} for the model systems in citrate buffer at pH 6 and 8, which can be attributed to the lowered initial pH (=6.96) of the model system prepared in citrate buffer at pH 8, as mentioned above. Nevertheless, although this is still a pH unit higher than the model system at pH 6, the kinetic parameters do not seem to differ significantly, with the exception of the activation energy for acrylamide formation E_{aF}. Thus, by increasing acidity, acrylamide formation is effectively reduced, especially at a pH lower than 6. These findings are in line with earlier qualitative studies concerning the effect of pH on acrylamide formation (*19*, *22*). The temperature dependence of the reaction rate

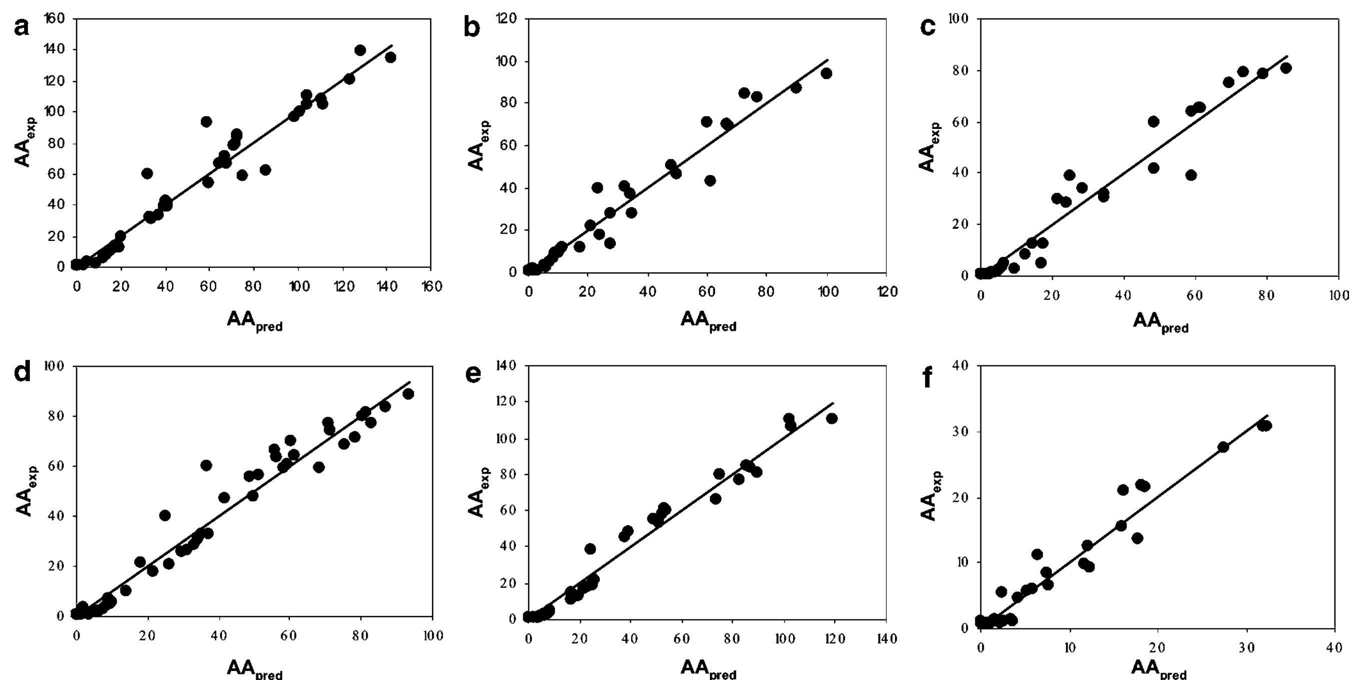


Figure 5. QQ plot of experimentally determined acrylamide values (ppm) and acrylamide values (ppm) predicted by the proposed kinetic model in an equimolar asparagine–glucose (0.1 M) in phosphate buffer (0.05 M) at (**a**) pH 8, (**b**) pH 6, and (**c**) pH 4 or in citrate buffer (0.05 M) at (**d**) pH 8, (**e**) pH 6, and (**f**) pH 4. The full lines have a slope of 1.

Table 1. Effect of pH and Buffer Type on the Kinetic Parameters Describing Acrylamide Formation/Elimination Kinetics in a Buffered (0.05 M) Equimolar Asparagine–Glucose (0.1 M)[a]

T_{ref} = 160 °C	pH	k_{Fref} (×10^{-3} M^{-1} min^{-1})	k_{Eref} (×10^{-3} min^{-1})	E_{aF} (kJ/mol)	E_{aE} (kJ/mol)	pseudo-R^2
phosphate buffer	8	37.5 ± 4.21 a	333.6 ± 41.4 a	130.3 ± 6.12 a	84.19 ± 6.52 a	0.951
	6	8.78 ± 1.34 b	175.3 ± 36.1 b	190.6 ± 8.18 b	128.3 ± 9.54 b	0.961
	4	4.30 ± 0.636 c	84.2 ± 18.8 c	208.1 ± 8.53 c	158.5 ± 10.0 c	0.958
citrate buffer	8	20.1 ± 1.73 d	219.7 ± 25.0 b,d	146.1 ± 4.30 d	115.3 ± 5.23 b,d	0.962
	6	20.8 ± 2.01 d	262.8 ± 31.1 d	159.1 ± 5.15 e	106.3 ± 5.82 d	0.975
	4	0.599 ± 0.134 e	43.1 ± 13.4 e	277.4 ± 13.1 f	221.1 ± 14.2 e	0.946

[a] Values of the same parameter with a different letter are significantly different based on 95% asymptotic confidence intervals.

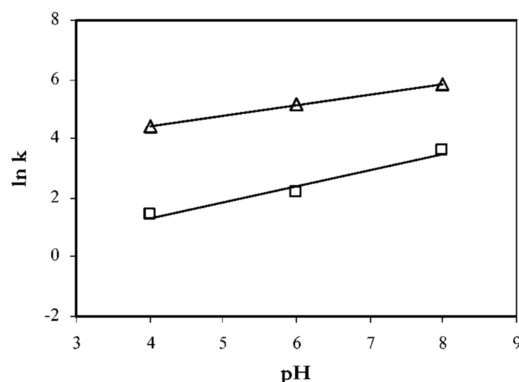


Figure 6. Relation between pH and ln k_{ref} for acrylamide formation (□) and elimination (△) in an equimolar asparagine–glucose (0.1 M) in phosphate buffer.

constants k_{Fref} and k_{Eref}, expressed as the activation energy, increases significantly ($\alpha = 0.05$) with decreasing initial pH. This indicates a higher temperature sensitivity of the rate constants of acrylamide formation and elimination with increasing acidity. This is the opposite of the trend followed by the reaction rate constants themselves.

Because the maximal yield of acrylamide was only 1−2% of asparagine, depending on treatment temperature and pH, numerous reactions, including alternative pathways of the Maillard reaction, could play an important role in the asparagine−glucose model systems. In this paper, however, a simplified reaction schedule is considered to model acrylamide formation and elimination, without considering the complex chemistry of possible alternative reactions involved. Use of simple reaction order for complex reaction pathways can be useful for modeling chemical changes during processing, when knowledge of pure chemistry or mechanism of the reaction is of less importance. This single-response approach differs from the multiresponse approach used by other authors (*24*, *34*). When multiresponse modeling is applied, various reaction products and pathways are identified and monitored, which can lead to a better understanding of the mechanism of the reaction.

As mentioned above, the pH of the different model systems was measured at room temperature, before subjecting the systems to a thermal treatment. However, it has to be kept in mind that the pH of a system will change when the temperature is increased. In literature, this effect is only described for temperatures lower than 100 °C (*35*). Because thermal treatments were performed at 120 °C or higher, there are no indications about exact pH changes of the model systems during treatment. Besides the pH changes due to increasing temperature, the pH could also be altered as a consequence of chemical reactions. Generally, if a Maillard reaction is assumed to be the most important one, the pH will drop as the reaction

Table 2. Maximal Change in pH Measured at Room Temperature after Thermal Treatment, in an Equimolar Asparagine–Glucose (0.1 M) Model System in Phosphate or Citrate Buffer (0.05 M), As Compared to the pH Value before Thermal Treatment between 120 and 200 °C

	phosphate buffer			citrate buffer		
T (°C)	pH 8	pH 6	pH 4	pH 8	pH 6	pH 4
120	−1.21	−0.39	+0.40	−1.05	−0.17	+0.26
140	−1.71	−0.91	+0.84	−1.40	−0.64	+0.26
160	−2.25	−1.02	+1.01	−1.81	−1.03	+0.53
180	−2.18	−1.32	+1.17	−1.96	−1.00	+0.71
200	−1.95	−1.28	+1.20	−1.85	−0.96	+1.05

proceeds (*32*, *36*, *37*). To evaluate the possible relation between the course of pH and the acrylamide formed, the pH of thermally treated samples was measured at room temperature after thermal treatment. Model systems with an initial pH of 6 or higher showed a decrease of the pH after thermal treatment for different time intervals. This pH drop was more pronounced at higher temperatures and was comparable for both types of buffer tested (**Table 2**). For the model systems originally at pH 4, the pH increased instead of decreasing as a function of time. This is probably the result of other (Maillard) reaction products being formed.

Knowing the kinetic parameters of acrylamide formation and elimination in a simplified asparagine−glucose model system, the applied kinetic model was subsequently validated for more realistic systems. Hereto, a potato-based model system was adapted selectively with asparagine and glucose, until the same concentration (0.1 M) was reached as compared to the simplified model system. The quantitative study of the influence of pH on acrylamide formation and elimination was performed in phosphate buffer only. The combined effect of treatment temperature and time (**Figure 7a−c**) was comparable to the one discussed above for the simplified model systems. For each pH value studied, acrylamide formation was the predominant reaction during the first phase of heating, while after prolonged heating at elevated temperature the elimination of acrylamide was most pronounced. The kinetic model, assuming a second-order formation reaction and a first-order elimination reaction for acrylamide, was successfully applied to model the experimental data accurately, as is presented in **Figure 7a−c** by the full lines. As for the simplified model systems, the goodness of fit can be deduced from the pseudo-R^2 value, listed in **Table 3**. Because these values are close to 1, a very good correlation between predicted and experimental values for acrylamide content in all three systems is obtained. Regardless of the higher degree of complexity achieved by adding a potato matrix, the proposed kinetic model could still be applied to describe acrylamide formation and an elimination reaction. This confirms the generic nature of the proposed kinetic model, even under more realistic conditions. The kinetic parameters, which were estimated by nonlinear regression based on the experimentally determined temperature−time profiles and the acrylamide contents of each sample, are listed in **Table 3**. The effect of pH on the reaction rate constants k_{Fref} and k_{Eref} is comparable to the simplified asparagine−glucose model systems. Model systems with an initial pH of 8 result in the highest formation and elimination rate constant. The difference between the model systems at pH 6 and 4 is less pronounced but is significant (α = 0.05). Moreover, the rate constants for acrylamide formation in the potato-based model systems are not significantly different from the k_{Fref} values estimated for the simplified model systems, with exception of the model system at pH 4. This is not the case for the acrylamide elimination rate constants k_{Eref}, for which

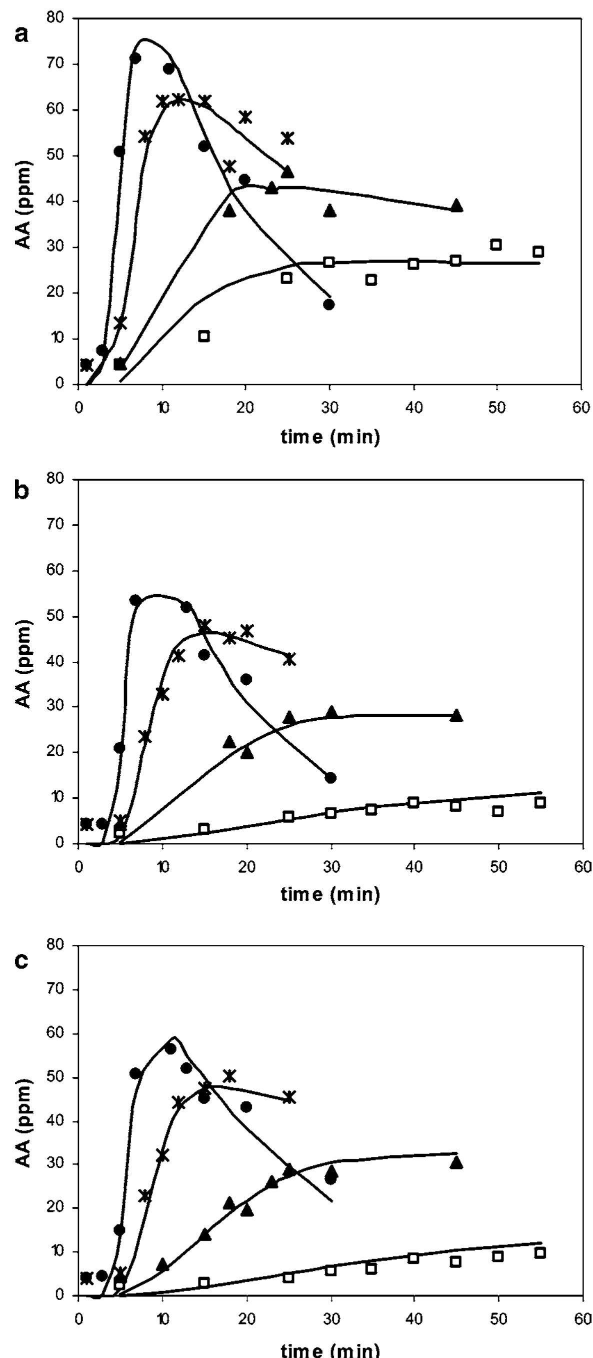


Figure 7. Formation of acrylamide as function of heating time at 140 (□), 160 (▲), 180 (×), and 200 °C (●) in an equimolar potato-based asparagine–glucose (0.1 M) model system in phosphate buffer (0.05 M) at (**a**) pH 8, (**b**) pH 6, and (**c**) pH 4. The full lines represent acrylamide formation/elimination predicted by the kinetic model.

the absolute values are almost twice as high in the potato-based model systems as compared to the simplified asparagine−glucose model system. The higher elimination rate constants combined with the unaltered formation rate constants are also apparent in the lower maximal net acrylamide concentrations formed in the more complex model systems (**Figure 7a−c**). As for the simplified model systems, a log−linear relation could be found between both the rate constants and the pH with a high correlation, i.e., 0.8674 and 0.9557 for the formation and elimination reactions, respectively (figure not shown). The corresponding slopes, which express the pH dependency of the reaction, are 0.4312 ± 0.168 for the formation reaction and 0.3397 ± 0.073 for the elimination reaction. These values are

Table 3. Effect of pH on the Kinetic Parameters Describing Acrylamide Formation/Elimination Kinetics in an Equimolar Potato-Based Asparagine–Glucose (0.1 M) Model System in Phosphate Buffer (0.05 M), Heated between 140 and 200 °C[a]

T_{ref} = 160 °C	pH	k_{Fref} ($\times 10^{-3}$ M^{-1} min^{-1})	k_{Eref} ($\times 10^{-3}$ min^{-1})	E_{aF} (kJ/mol)	E_{aE} (kJ/mol)	pseudo-R^2
	8	40.9 ± 4.61 a	600.9 ± 66.90 a	122.7 ± 6.81 a	83.9 ± 6.60 a	0.961
	6	9.63 ± 0.965 b	236.4 ± 31.7 b	189.1 ± 5.21 b	139.9 ± 6.41 b	0.977
	4	7.29 ± 0.607 c	154.4 ± 19.6 c	178.4 ± 4.91 c	142.0 ± 5.88 c	0.975

[a] Values of the same parameter with a different letter are significantly different based on 95% asymptotic confidence intervals.

not significantly different from the corresponding values obtained in the simplified model systems. The potato matrix, containing about 17% starch on a fresh weight basis, proves to accelerate the acrylamide elimination reaction(s). However, the exact natures of the compounds of this matrix participating in the elimination reaction(s) are not known. Biedermann et al. (*21*) studied the influence of starch on acrylamide formation and elimination. They concluded that starch was not involved in the acrylamide formation reaction, but it seemed to decrease the rate of elimination.

The temperature dependence of the rate constants k_{Fref} and k_{Eref} for the potato-based model systems, expressed as the activation energy, was calculated by the Arrhenius model (eq 5). As in the case of the simplified asparagine−glucose model systems, the temperature dependence increases with increasing acidity, but the difference between the system with an initial pH of 6 and 4 is not significant ($\alpha = 0.05$). Neither E_{aF} nor E_{aE} of the potato-based model systems differs significantly from the corresponding value estimated for the simplified model systems, with the exception of the systems at pH 4.

The aim of this work was 2-fold, namely, to evaluate the effect of pH on the kinetics of acrylamide formation and elimination and to validate the proposed kinetic model in a more realistic, potato-based model system. Despite the seeming simplicity of the proposed kinetic model, it has proven to be applicable to both simplified and more complex asparagine−glucose model systems with varying acidities and buffer types. This kinetic model allows the design of processes resulting in lower acrylamide levels, either by reduction of the formation and/or by enhancement of the elimination. It was demonstrated that by lowering the pH from 8 to 4, a 10-fold reduction of the reaction rate at T_{ref} (160 °C) of acrylamide formation can maximally be obtained in both simplified and potato-based model systems. In the case of actual potatoes, this lower pH can be obtained by pretreatment of real potato pieces in diluted acid solution (e.g., citric acid) (*19*). This pretreatment in combination with lower treating temperatures and longer treating times could result in considerable lower acrylamide contents. However, because changes in pH, temperature, and time of the heat treatment will inevitably induce changes in the types of Maillard reactions, this will result in changes in color and aroma formation. Therefore, it is important for process design that kinetic data on not only acrylamide formation but also color and aroma formation are taken into account. Furthermore, it was proven that adding 20% of potato matrix to the system resulted in an almost doubled rate constant at T_{ref} (160 °C) for acrylamide elimination, depending on the pH of the system. However, information about possible elimination reaction mechanisms is scarce. Further research in this domain can be useful to identify compounds in potatoes that increase the elimination of acrylamide.

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Received for review April 21, 2006. Revised manuscript received August 4, 2006. Accepted August 5, 2006. This research was funded by a Ph.D. grant of the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen) and by the Fund for Scientific Research Flanders (FWO).

JF0611264