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The simultaneous determination of caffeine, aspirin and paracetamol by principal components regression using automatic dilution and calibration

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Abstract

The development of continuous and stepwise automatic dilution and calibration systems that enable analyte dilution and enhancement in multicomponent analyses are described. The continuous system has been used with a robot and the stepwise configuration integrated into a flow-injection analysis system. All analyses are performed using a UV/Visible diode-array detector and data analysis performed using principal components regression. Results of both systems are presented for the simultaneous determination of caffeine, aspirin and paracetamol in pharmaceutical formulations. © 1997 Elsevier Science B.V.

1. Introduction

The automatic preparation of suitable calibration standards for presentation to a measurement system usually mimics human operations. Dilutions and standard additions can be performed batchwise to produce samples containing analyte in the required, often narrow, working range of the analytical technique. The discrete steps involved in conventional, manual calibration methods can be automated by using computer-controlled, piston-driven pipettes, and this is undertaken in many automatic systems. Mechanised operations eliminate the possibility of human error, but at the expense of losing the flexibility and experience of the analyst. For example, the sample response may initially not fall within the working range of either the calibration graph or the instrument itself and feedback from the measuring instrument to the computer-control system is therefore necessary to initiate further dilutions. In cases where the signal response is beyond the linear working range of the instrument, the factor by which it is exceeded cannot be determined. This makes intelligent feedback difficult and may result in the necessity for further dilutions, with the possibility of over-dilution and, consequently, loss of sample.

A better procedure would be to eliminate these discrete steps altogether, thereby removing a potential source of error. To achieve this, a continuous automatic dilution and calibration system has been developed. The system exploits the phenomenon of diluting or enhancing a sample, or its components, in

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an unsegmented, continuously flowing stream. The principles of the flowing system are similar to flow injection analysis (FIA), which embodies the principle of continuous automatic dilution in its use of a "continuum of concentrations" [1], arising from the dispersion of the injected sample in the carrier stream. There are several techniques that make use of this concentration gradient. For example, FIA titration, dating from 1977 [2–4], which utilises the reproducible concentration gradients obtained from the dispersion of the sample plug in the continuously flowing stream of titrant of fixed concentration. The range of these concentration gradients can be changed by varying the diameter and length of the carrier stream tubing.

The concept of continuous dilution has been applied in flame atomic absorption spectrometry and achieved using a concentration gradient chamber [5,6]. The basic apparatus consists of a sealed, continuously stirred fixed-volume unit. This unit, initially filled with water, has a single component standard solution pumped into it. The solution concentration measured is an exponential function of time described by the equation

$$C_t = C_m [1 - \exp(-ut/V)] \quad (1)$$

where C_t is the solution concentration at time t, C_m is the concentration of the standard solution added, u is the flow-rate, and V is the volume of the mixing chamber.

Olsen et al. [7] developed a system in which electronic selection of different segments along the gradient could be used to provide an extended concentration range. Dilution systems based on FIA gradient techniques are still being developed. Garrido et al. [8] have produced a fully automated dilution system in which computer-controlled rotary valves select portions of the gradient at different times for resampling and analysis. The authors claim up to 10,000-fold dilution factors.

In addition to FIA gradient dilution a number of authors have described other methods of sample dilution, in particular for atomic absorption spectrometry (AAS) and flame emission spectrometry (FES) [9–13]. These include FIA systems having on-line, continuous flow and automatic dilution facilities incorporated into the system. For example, in 1993, Vaughan et al. [9] described an automated system in which soil extracts could be diluted using a peristaltic pump, prior to analysis by AAS. The diluent (distilled water) and each of the samples in turn are pumped in parallel and then merged at a T-connection before AAS analysis. The rate of dilution is varied by altering the pump speed and internal diameter of the tubing. Another alternative to gradient dilution is the system developed by Giné et al. [10]. The introduction of the sample and solvent at precise volumes is achieved using software controlled solenoid valves. The system provides reproducible analyses, but requires a dilution factor to be input via the keyboard, and hence prior knowledge of the sample.

As well as automatic dilution there are standard addition methods dating from 1983 [14]. The standard addition procedure is based on merging standard solutions with the sample zone. The resulting sample gradient consists of different sample-to-standard ratios, hence permitting standard addition [15–18]. The most recent of these is by Agudo et al. [18] in which an open/closed flow loop was used.

The purpose of the system developed in this study is to simultaneously dilute and enhance individual components in multicomponent sample solutions. The automatic system was initially designed for dilution of single component solutions [19,20]. As a consequence of the success of the system, and the rarity of single component samples in practice, the functionality was extended to provide for multicomponent analysis by simultaneous dilution and standard addition. Due to problems encountered with the design of the continuous apparatus, the ideas were incorporated into an FIA system. The development of the system is described, along with the equations necessary for modelling up to three components. Results of preliminary investigations in pharmaceutical tablets containing caffeine, aspirin and paracetamol are presented.

2. Background

To implement the continuous automatic dilution technique proposed, a suitable experimental set-up was designed and has been reported [19,20]. The schematic of the automatic dilution system used for

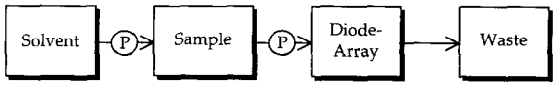

Fig. 1. Schematic of the continuous automatic dilution and calibration system for one/two components.

this analysis is shown in Fig. 1, where the solvent is distilled water and the sample is a single component solution. Sample transport to the spectrophotometer is performed simultaneously with the addition of solvent to the sample vessel, thereby providing continuous dilution of the sample. The input of solvent and output of resultant sample solution are carried out at equal flow-rates, the sample volume therefore remaining constant. The dilution of the sample, and hence the absorbance measurement at the spectrophotometer, can be described by a first-order rate equation of the form

$$\ln A_t = \ln A_0 - kt \qquad (2)$$

where the measured quantity is absorbance A_t, which within useable limits is proportional to concentration, t is the time (min), and k is the dilution rate (min^{-1}). A plot of the natural logarithm of the measured absorbance against time is linear with slope of $-k$ and intercept $\ln A_0$.

Typical plots of absorbance and log(absorbance) against time for the dilution of single component standards with distilled water render exponential and linear plots, respectively. The linear region can be used as a calibration graph, its calculated slope gives the dilution rate, and its extrapolated intercept gives the logarithm of the absorbance value predicted by Eq. (2) which can be used to determine the concentration of the sample or starting solution by comparison to reference standards.

A major advantage of the continuous dilution technique described, other than bringing samples automatically into the normal working range of the instrument, is that it results in several (many) measurements being recorded for each sample, rather than a single measurement which is often the case. The benefit of numerous measurements, and performing a least squares fit through the linear region of data to obtain the intercept, is that it averages random noise in the system, or measuring instrument, giving a more precise result.

2.1. Modelling one and two component analyses

The single component studies described above serve to introduce continuous automatic dilution and enhancement as an analytical technique, providing the motivation for multicomponent applications necessitating the derivation of equations to model these systems. If a sample solution containing two components is diluted (or enhanced) with a standard solution containing a known concentration of one component, then one component will be diluted to a known concentration, i.e. that of the standard, and the other will be diluted by the addition of the standard. The schematic shown in Fig. 1 would be applicable, the sample vessel now containing the two component solution, the solvent being a one component standard. The equation to model this system is

$$y_t = x_0 - (x_0 - y_0)e^{-kt/V} \qquad (3)$$

where k is the flow-rate ($ml \cdot min^{-1}$), x_0 is the concentration in the solvent ($mg \cdot ml^{-1}$), y_0 is the sample starting concentration ($mg \cdot ml^{-1}$), y_t is the sample concentration ($mg \cdot ml^{-1}$) at any time t (min), and V is the sample volume (ml).

The system can be demonstrated using caffeine and paracetamol. Eq. (3) is modelled using Mathcad (Version Plus 6.0, Mathsoft, Massachusetts) and substituting spectra for concentrations. The spectra are shown in Fig. 2. The concentration of caffeine is increased to the caffeine-standard concentration, whilst the paracetamol is effectively being diluted from its starting concentration to approach zero, by the addition of the caffeine solution. Fig. 3(a) illustrates the change in absorbance with time at characteristic wavelengths of the components. Plotting absorbance against $e^{-kt/V}$ gives a straight line (Fig. 3(b)) as predicted by Eq. (3).

Eq. (3) can also be used to model one component systems, where the solvent is distilled water or a different concentration of the analyte, and systems with complex matrices where one of the two components could be the matrix of a sample and the other the analyte. In the latter case, the analysis could be considered a type of standard addition, in which the analyte is enhanced and the matrix diluted by addition of the solvent.

Deviations from linearity have been observed in

practice during extended analysis times. Investigation of possible causes indicated that inequalities in flow-rate to be a significant factor. For differing input and output rates Eq. (3) becomes

$$y_t = \frac{k_1 x_0 - (k_1 x_0 - k_2 y_0)e^{-k_2 t / V_t}}{k_2} \quad (4)$$

where k_1 and k_2 are the input and output flow-rates ($ml \cdot min^{-1}$), x_0 is the solvent concentration ($mg \cdot ml^{-1}$), y_0 is the starting concentration ($mg \cdot ml^{-1}$), y_t is the sample concentration ($mg \cdot ml^{-1}$) at any time t (min), and V_t is the sample volume (ml) at time t.

Modelling of the system using Eq. (4) demonstrates that a very small difference in the diameter of the tubing, and hence in the input and output flow-rates, can cause a significant error. The dilution apparatus was modified to overcome the problem of two pump tubes with potentially different diameters. The beaker was replaced by a sealed flask housing both input and output tubes, thus enabling the solvent to be drawn into the sample flask from its source by the partial vacuum generated when the sample solution is pumped to the spectrophotometer.

2.2. Modelling three component analyses

To enable two component analysis, one component standards solutions are added to two component sample solutions. However, the continuous addition of a two component mixture to a three component sample does not enable three component analysis since all solutions are linear combinations and treated as a single component, i.e. the model only recognises the mixture spectrum as one combined spectrum and not as the two, individual, spectra of its components. To perform three component analysis, the experimental set-up was modified, as shown in Fig. 4. Solvent A is a single component solution and solvent B a mixture of two components, one of which is the same analyte at the same concentration as solvent A. Hence, the addition of standard A to standard B keeps one component the same whilst the second changes with time. The addition of both 'static' and

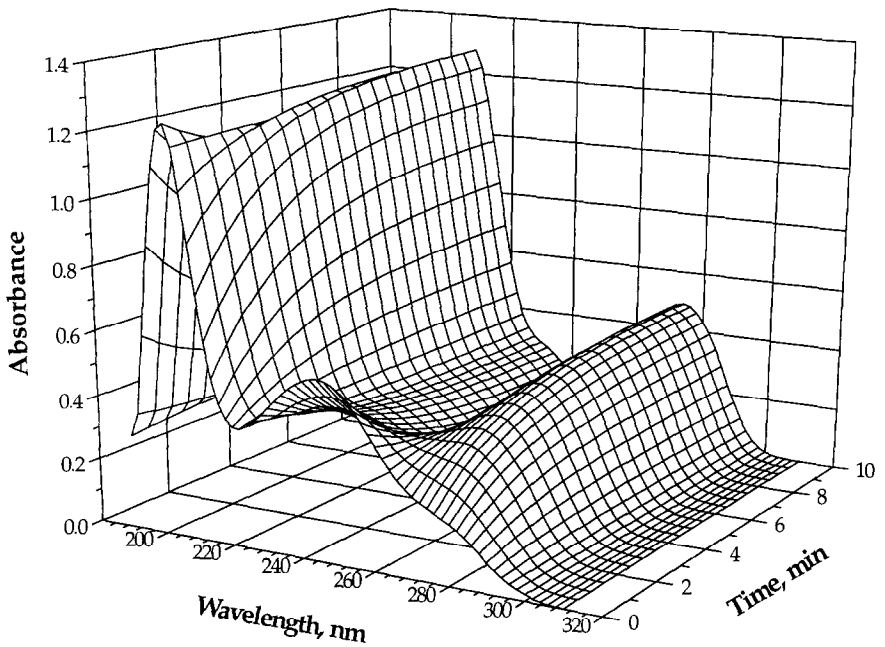

Fig. 2. Continuous analysis of a two component solution, where y_0 is a mixture containing 2 ppm caffeine and 8 ppm paracetamol, x_0 is a solvent solution of 10 ppm caffeine, V is 25 ml and k is 10 $ml \cdot min^{-1}$ (spectra were calculated every 30 s for 10 min).

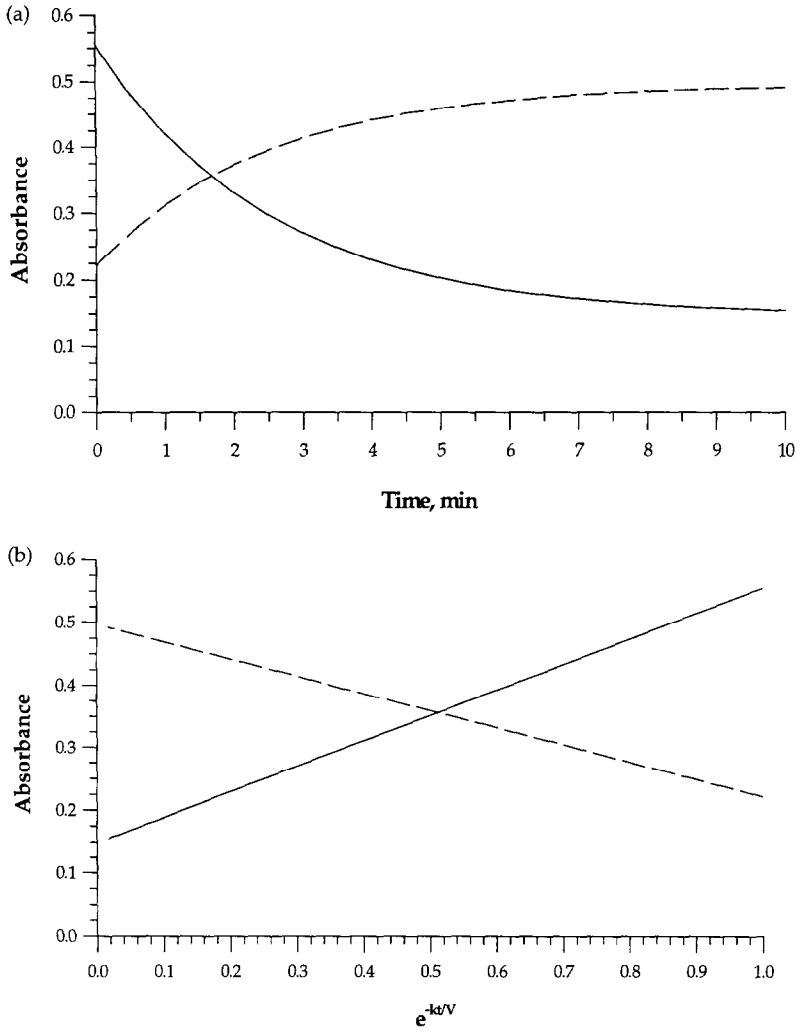

Fig. 3. Plots to illustrate (a) the absorbance versus time and (b) the absorbance versus $e^{-kt/V}$ for the dilution of paracetamol at 244 nm (————) and the enhancement of caffeine at 274 nm (- - -).

'dynamically' changing standards to the sample vessel, containing a mixture of components A, B and C enables three component analysis. It should be pointed out that the first part of this system, i.e. the addition of A to AB can be modelled using Eq. (3). The three component equation to model the system is given by

$$z_t = x_0 - \frac{kt}{V}(x_0 - y_0)e^{-kt/V} + (z_0 - x_0)e^{-kt/V} \quad (5)$$

where k is the flow-rate ($ml \cdot min^{-1}$), x_0 is the analyte concentration in the one component solvent ($mg \cdot ml^{-1}$), y_0 is the analyte concentration of the two component solvent ($mg \cdot ml^{-1}$), z_0 is the analyte concentration in the three component solvent

Fig. 4. Schematic of the automatic dilution and Calibration system for three components.

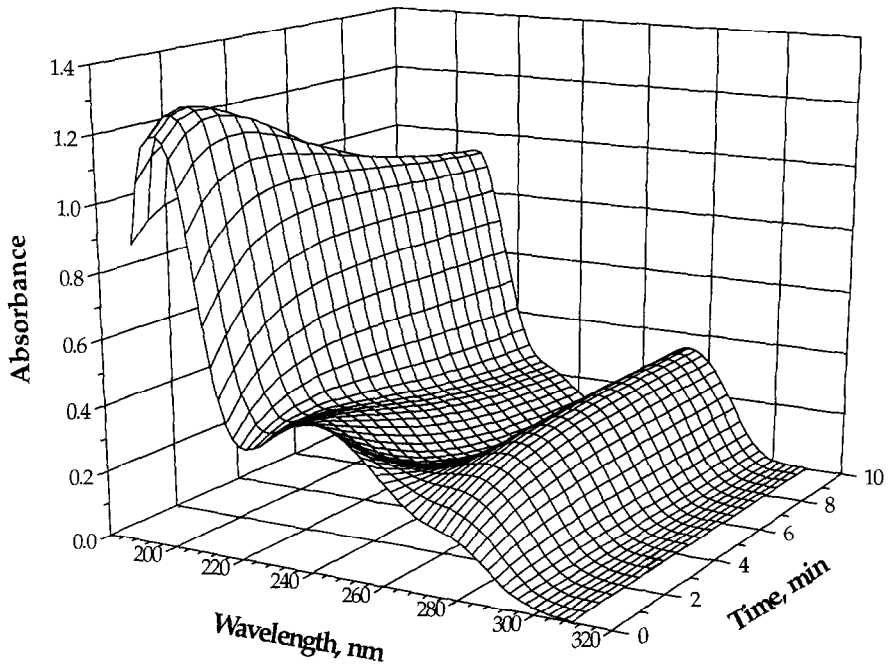

Fig. 5. Continuous analysis of a three component mixture (where the solvent solution x_0 is caffeine (7 ppm), the dynamically changing solvent y_0 is a mixture of caffeine (7 ppm) and aspirin (6 ppm) and the starting solution z_0 contains caffeine (2 ppm), paracetamol (6 ppm) and aspirin (1 ppm), the sample volume is 25 ml and the flow-rate 10 ml · min^{-1}).

(mg · ml^{-1}), z_t is the sample concentration (mg · ml^{-1}) at any time t (min), and V is the sample volume (ml).

Using Eq. (5) and spectra of aspirin, paracetamol and caffeine, which have strongly overlapping peaks, the results are as shown in Fig. 5. Fig. 6 shows the

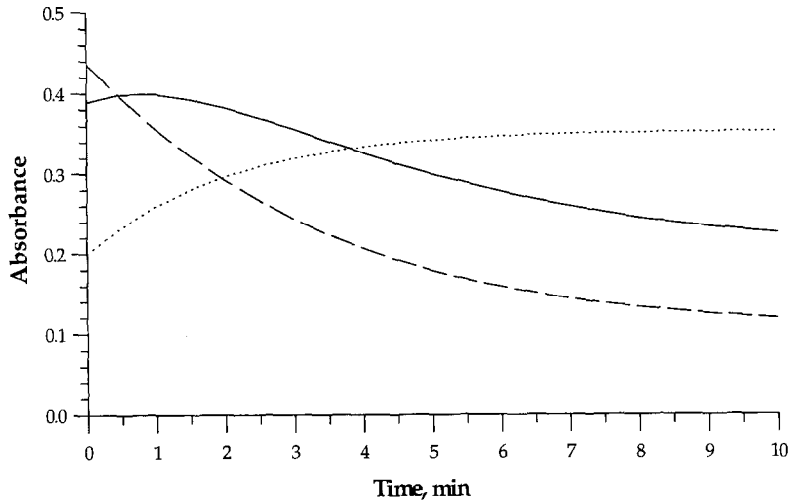

Fig. 6. Illustration of dilution/enhancement at the three characteristic wavelengths of aspirin at 230 nm (· · ·), paracetamol at 244 nm (————) and caffeine at 274 nm (- - -).

change in absorbance with time at the three characteristic wavelengths for the individual components.

3. Continuous automatic dilution and calibration

3.1. Experimental

The continuous automatic system was originally developed for use with a robot system as a way of overcoming the problems posed by having to dilute samples automatically to bring them into the working range of the measurement system [19,20]. The original apparatus was restricted to a design that can be handled by the robot and consisted of a beaker, a lid with a handle that the robot could grip, and two tubes, one for input and one for output of solutions. The input and output rates are controlled using a peristaltic pump (model 312 Minipuls 3, Gilson Medical Electronics, Middleton) fitted with a standard four-channel head. All analyses reported in these studies were performed using a UV/Visible diode-array spectrophotometer (model HP8452A, Hewlett-Packard, Waldbronn) with a 1 s integration time and a 2 nm spectral digitisation resolution.

3.2. Results and discussion

3.2.1. One component analyses

Three types of single component analyses were performed on pharmaceutical tablets each containing approximately 50 mg caffeine (Pro-Plus®, Roche Nicholas Consumer Healthcare). Nine tablet solutions were prepared by dissolving nine tablets in 500 ml of distilled water each. Three tablet solutions were used for each type of analysis. Firstly, a continuous dilution with distilled water was performed on 100 ml of tablet solution. Secondly, a continuous dilution with 5 ppm caffeine standard was performed on 10 ml of tablet solution diluted with 100 ml distilled water, i.e. 110 ml. Finally, a continuous dilution with 10 ppm caffeine standard was performed on 5 ml of tablet solution diluted in 100 ml distilled water, i.e. 105 ml. All analyses were carried out with an input/output flow-rate of 12.75 ml · min^{-1}, a dilution time of 30 min with the absorbance at 274 nm recorded every 30 s. The results of the analyses are shown in Table 1.

In practice the method suffers from a delay of 12 s between the solvent and sample vessel and 18 s delay between the sample vessel and the spectrophotometer. These delays must be accounted for in the calculation; the starting volume of the sample has to be corrected for a loss of 12 s worth of sample. In addition, once dilution starts, the solution takes 18 s to reach the detector, and hence the intercept is determined at 30 s and not at 0 s, as was the case with the modelled data. Despite the necessary corrections the results are good.

3.2.2. Two component analyses

For the two component analyses two tablet formulations were used. The first contains 300 mg paracetamol and 200 mg aspirin (declared typical values for Disprin® Extra, Reckitt and Colman Products) and the second 500 mg paracetamol and 65 mg

Table 1
Results of the continuous analysis (standard analysis results in parentheses), where (a) is the dilution with water, (b) is the dilution with 5 ppm caffeine standard, and (c) is the enhancement with 10 ppm caffeine standard

			1	2	3
Proplus	caffeine (mg)	(a)	50.484 (50.622)	49.359 (49.677)	49.526 (50.501)
		(b)	33.944 (32.517)	49.467 (47.727)	49.092 (47.533)
		(c)	49.785 (50.023)	49.867 (50.740)	49.097 (48.393)
Disprin Extra	aspirin (mg)		302.899 (299.912)	307.893 (313.624)	298.991 (312.826)
	paracetamol (mg)		197.307 (192.619)	200.032 (197.315)	195.854 (195.496)
Panadol Extra	paracetamol (mg)		501.286 (500.922)	503.586 (502.468)	493.906 (500.972)
	caffeine (mg)		68.369 (69.103)	67.410 (68.170)	65.177 (71.562)
Anadin Extra	aspirin (mg)		255.535 (295.022)	244.530 (285.469)	255.255 (295.778)
	paracetamol (mg)		192.483 (202.198)	189.370 (196.156)	195.077 (201.802)
	caffeine (mg)		42.567 (43.145)	47.622 (47.843)	45.101 (44.270)

caffeine (declared typical values for Panadol® Extra, Stirling Health). For the analysis of Disprin Extra all solutions were prepared in 0.005 M hydrochloric acid, as aspirin readily hydrolyses to salicylic acid, which has a different ultraviolet absorption spectrum, in the presence of water [21]. Three tablet solutions were used for each analysis by dissolving three tablets in 1 l each. The continuous analysis of Disprin Extra was performed with a starting sample of 2 ml tablet solution diluted with 100 ml HCl and a solvent of 2 ppm paracetamol. For the analysis of Panadol Extra all solutions were prepared in distilled water. The continuous method was performed by diluting a starting sample of 2 ml tablet solution diluted in 100 ml with a 5 ppm caffeine standard. For both sets of samples, the input/output flow-rate was 12.75 ml · min^{-1}, and the spectra were recorded every 30 s for 30 min.

A set of twenty-five calibration solutions were prepared for the two analyses (two components at five concentration levels). Principal component regression was performed in the wavelength range 230 to 300 nm for Disprin Extra and 210 to 300 nm for Panadol Extra. All concentrations and spectra were mean-centred prior to analysis. The number of principal components required for the regression was determined using root mean square error calibration (RMSEC) and was found to be two. The principal components were added into the regression in order of their correlation with the concentration. The concentrations for the two components were determined for each of the 60 solutions in the 30 min dilution, a polynomial fitted to the concentrations and the concentration at 30 s with corrected volume determined. The results, shown in Table 1, are generally satisfactory, but two are in slight error.

3.2.3. Three component analyses

For the three component analyses, tablets containing 300 mg aspirin, 200 mg paracetamol and 45 mg caffeine (Anadin® Extra, Whitehall) according to the manufacturer were used. All solutions were prepared in 0.005 M hydrochloric acid to prevent the hydrolysis of aspirin. Three tablet solutions were prepared by dissolving three tablets in 1 l HCl. The continuous analysis was performed on a starting solution of 2 ml tablet solution diluted with 100 ml HCl. This was continuously diluted with a mixture of 5 ppm caffeine and 5 ppm paracetamol which itself was diluted with a 5 ppm caffeine standard. The input/output flow-rate was 12.75 ml · min^{-1}, and spectra were recorded every 30 s for 30 min.

A set of one hundred and twenty five (three components at five concentration levels) calibration solutions were prepared. The results were calculated using principal components regression on the spectral region 220 to 300 nm as described for the two component analyses. The results were corrected for the observed time delays; between the two component solvent and the sample vessel and the delay between the sample vessel and the spectrophotometer. The results are shown in Table 1. It is obvious in this particular example that the aspirin results are extremely low, the caffeine results are good and the paracetamol results are slightly low. It is interesting to note that the best results are for the components added to the solution and the worse results are for the component diluted by the addition of the other components.

It is apparent that the problems with the system are increasing with the number of components being analysed. These problems include the length of time taken to perform each analysis and the considerable amount of waste generated by the continuous dilution of the samples, for example, for a 10 min analysis with a flow-rate of 12.75 ml · min^{-1} almost 130 ml of waste is generated for each sample. These problems could be overcome by reducing the starting volume, i.e. miniaturising the system. Another problem has been with the detection system. In some one component analyses the plot of log(absorbance) exhibits deviations from the expected line. Also, for two component analyses the model shows that a plot of absorbance against $e^{-kt/V}$ is linear; unfortunately this was not usually the case as there was slight deviation with time. This may be due to inefficient stirring, although drift in the baseline of the spectrophotometer is a more likely cause and again is due to the length of time required to complete each analysis. In addition to the problems highlighted above, one obvious disadvantage lies in the basic design of the apparatus in that it requires an additional standard, and therefore an extra vessel, each time the system is modified to analyse for another component, making it increasingly complicated and introducing additional sources of error. Other problems

that were recognised include that of the dead-space between beakers, which result in time delays that need to be corrected for.

4. Stepwise automatic dilution and calibration

4.1. Experimental

In an attempt to improve the existing continuous automatic dilution and calibration system, a stepwise system has been developed. This follows the same principles as the continuous system, the main difference being that where the dilution and/or enhancement of the solution's component(s) was achieved through the continuous, simultaneous addition and removal of the sample solution, these are now performed batchwise. Changes in the solution's composition are made by the removal of a portion of sample, followed by the addition of a portion of standard.

Additional instrumentation includes a Gilson autosampler (model 222 Sample Changer, Gilson Medical Electronics, Middleton) that contains a test tube rack that holds twelve 25 ml glass test tubes used for storing water for washing and calibration standards, and a 6-port pneumatic rotary injection valve (Advanced Medical Supplies, Alton) switched using compressed air from a vacuum pump. A diagram of the arrangement is shown in Fig. 7.

4.2. Results and discussion

4.2.1. One component analyses

The three single component analyses performed using the continuous method were repeated for the stepwise system. All tablets were dissolved in 500 ml distilled water. Starting with a 10 ml tablet solution each time, three had five additions of distilled water made, three had five additions of a 5 ppm caffeine standard made (dilution with a standard) and three had five additions of a 10 ppm caffeine standard made (enhancement with a standard).

The results of each analysis were obtained by fitting a line to the six absorbances readings, finding the intercept and determining the concentration by comparison with reference standards. The results of the analyses are shown in Table 2 and appear marginally better than those obtained by the continuous method.

4.2.2. Two component analyses

Three Panadol Extra tablets were dissolved in 1 l distilled water. Stepwise analysis was performed on 10 ml of tablet solution, using an injection loop of 0.5 ml, by making five additions of 10 ppm caffeine followed by five additions of 10 ppm paracetamol. Hence, while caffeine is enhanced the paracetamol is diluted and vice versa. During the addition of caffeine, the dilution of paracetamol is performed by

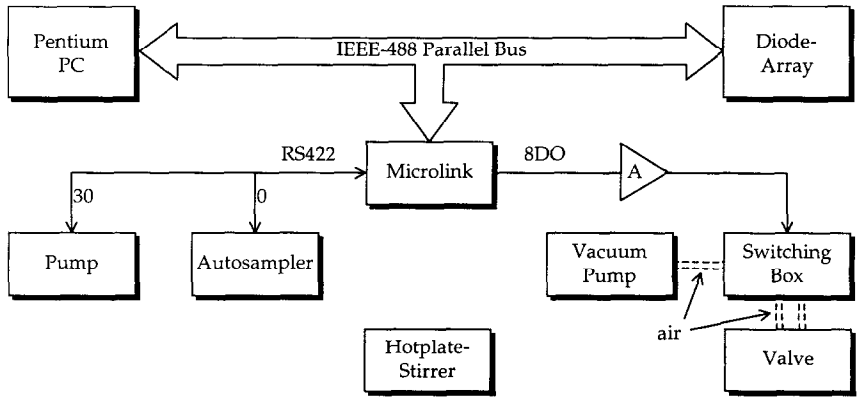

Fig. 7. Interfacing arrangement (A is a buffer amplifier).

Table 2
Results of the stepwise analysis (standard analysis results in parentheses), where (a) is the dilution with water, (b) is the dilution with 5 ppm caffeine standard, and (c) is the enhancement with 10 ppm caffeine standard

			1	2	3
Proplus	caffeine (mg)	(a)	48.898 (49.180)	49.371 (49.610)	51.571 (51.511)
		(b)	48.768 (48.617)	51.130 (51.196)	50.385 (50.453)
		(c)	51.644 (51.588)	50.197 (50.275)	48.370 (48.439)
Panadol Extra	paracetamol (mg)		502.868 (501.498)	503.931 (503.503)	499.853 (498.501)
	caffeine (mg)		66.698 (66.434)	64.625 (64.364)	64.668 (64.436)
Anadin Extra	aspirin (mg)		296.815 (297.280)	300.853 (301.272)	295.488 (296.380)
	paracetamol (mg)		202.144 (202.832)	203.034 (203.821)	196.449 (196.875)
	caffeine (mg)		48.728 (46.669)	44.859 (42.670)	46.705 (44.716)

removing 0.5 ml of the sample solution and replacing it with 0.5 ml of solvent. Therefore, the starting solution (0) contains 100% paracetamol concentration, after the first removal and addition (1) the solution contain 95% of the initial concentrations, after the second removal and addition the solution contains 90.25%, i.e. 95% of 95%, of the initial concentration, and so on. So, the fifth solution contains 77.38% of the initial concentration of paracetamol.

The results of each analysis were determined by PCR using the calibration set from the continuous analysis. For caffeine this involved fitting a line through the six concentrations (solution 0 to 5), and finding the intercept to give the initial concentration. For paracetamol this process was repeated for solutions 0 to 5 and 5 to 10. In the latter case the concentration had to be scaled up to allow for the initial dilution. The results are shown in Table 2. In the case of paracetamol, the results calculated for the addition of paracetamol are marginally better than for the dilution with caffeine, but not to the extent of justifying the additional stages. In all instances, the results appear better than those obtained by the continuous method.

4.2.3. Three component analyses

As in continuous analysis, all solutions were prepared in 0.005 M to prevent hydrolysis of aspirin. Three Anadin Extra tablets were dissolved in 1 l HCl. Stepwise analysis was performed on 10 ml of tablet solution, using an injection loop of 0.5 ml, by making five additions of 10 ppm caffeine followed by five additions of 10 ppm paracetamol. Hence, while caffeine is enhanced the paracetamol and aspirin are diluted, and during the addition of paracetamol, caffeine and aspirin are diluted.

The results of each analysis were determined as above using PCR and the calibration set from the continuous analysis. The results of the analyses are shown in Table 2 and are significantly better than those obtained by the continuous method.

The stepwise system exhibits a number of potential advantages compared with the continuous system. Stepwise analysis requires considerably less time for each analysis, typically ~ 15 min for the addition of ten standards. The times for the stepwise analysis can be reduced further by increasing the flow-rate, which is currently half that used in the continuous system. In addition, the stepwise system uses much smaller volumes of sample and this coupled with shorter analysis times means that much less waste is generated and the apparatus does not need to be altered with the addition of extra analytes. Sources of error in the stepwise system may include any dead volume in the rotary injection valve, which would mean that the volume of the loop was not actually what was injected or removed. Another source could be the drift on the spectrophotometer, although this is not as significant, owing to shorter analysis times.

5. Conclusion

The mathematical models for continuous dilution/enhancement for one, two and three component automated analyses have been presented and applied to the determination of caffeine, paracetamol and aspirin in commercial pharmaceutical tablet formula-

tions. The results obtained confirm the applicability of the models and demonstrate the use of the technique for multicomponent analysis.

The results obtained from an automated stepwise standard addition/dilution procedure demonstrate the advantages of this technique in terms of greater accuracy and the use of less sample or standard solutions.

The results presented have been obtained from UV/Visible absorption spectrophotometry, but the application of the techniques is quite general and relevant wherever automated generation of multicomponent standards is desirable.

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