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# A rapid and precise assay for peroxide as 'active oxygen' in products, by flow injection analysis in a high pressure system with spectrophotometric detection☆

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**Abstract**

A simple, rapid and automated assay for 'active oxygen' originating from hydrogen peroxide, or other organic peroxides, in products is presented employing flow injection (FI) analysis. The product is dispersed and peroxide dissolved in a solvent of 5% (v/v) acetic acid, which constitutes the carrier stream. Ammonium molybdate can be added to this carrier stream to increase sensitivity as required. The sample solution is injected into the acid carrier stream, which is then merged with iodide ion in situ in a two-stream manifold. The 'active oxygen' in the product oxidises acidified iodide to iodine, which is detected spectrophotometrically at 350 nm. The closed conditions prevent interference from atmospheric oxygen and the short reaction time minimises the potential for interference from side reactions. Standard HPLC equipment is used throughout, employing a back-pressure to improve precision (high pressure flow injection). Conditions have been investigated using screening multivariate experimental design (two-level quarter fractional factorial design incorporating centre points) to identify and optimise the critical variables. The method has been fully validated (with sample solution R.S.D.s typically $< 0.5\%$, LOQs of 0.04 or 0.006 $\mu g\,ml^{-1}$ as 'active oxygen' for acid or acid/molybdate carriers respectively) and is quicker and simpler than the currently employed manual titration approach. It should be applicable to a range of 'active oxygen' products.


*Keywords:* Flow injection; Analysis; Determination; Hydrogen peroxide; Active oxygen; Spectrophotometry; Assay; Experimental design; Fractional factorial; Multivariate optimisation

## 1. Introduction

Hydrogen peroxide ($H_2O_2$) and its derivatives are now used extensively in the chemical industry, finding increasing new uses; not only for traditional bleaching applications such as the chlorine-free bleaching of pulp/paper but also for the manufacture of fine chemicals and pharmaceuticals [1]. The materials are used for chemical purification of organic compounds, including oils, fats, waxes, alcohols/phenols and many other oxygen, sulphur and nitrogen containing organic materials. They are used for surfactant product bleaching to give a material of acceptable odour and colour to the consumer. In consumer healthcare products, peroxides such as benzoyl peroxide have found widespread use in maintaining the cleanliness of skin pores, assisting in the reduction and prevention of spots and 'acne'.

Many new healthcare products are now being developed for the consumer, exploiting the oral health benefits of $H_2O_2$ as 'active oxygen', in a safe, efficient and innovative manner. These typically can use $H_2O_2$ directly, or as derivatives such as the urea hydrogen-bonded complex of $H_2O_2$, known as UHP (urea hydrogen peroxide) or carbamide peroxide.

It is essential to assay for the level of 'active oxygen', the active agent released from peroxide in aqueous solutions in routine use, to both demonstrate product efficacy in development and as a quality control test for product manufacture. $H_2O_2$ and its derivatives are used in many products manufactured by GlaxoSmithkline.

$H_2O_2$ is routinely quantified in our products by an iodimetric titration procedure. 'Active oxygen' liberates iodine from a solution of acidified iodide, which is then determined

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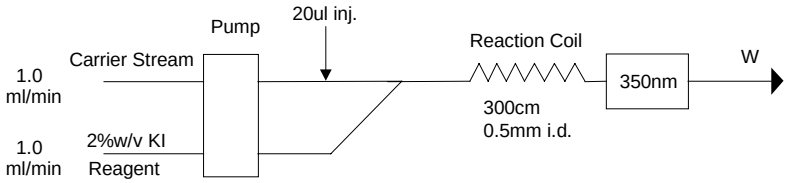


Fig. 1. The 'active oxygen' manifold.

by manual titration with sodium thiosulphate using starch as an indicator. This approach is reported in USP pharmacopoeia [2] and is also routinely used in the fats and oils literature [3]. Other titration approaches have also been reported including the use of cerium(IV), permanganate or dichromate as titrants [1]. Titration procedures are time consuming however and the iodimetric procedure suffers from interference at trace levels due to atmospheric oxygen. For routine analysis of the large number of samples in this laboratory a more rapid and preferably, automated assay was required.

The technique of flow injection (FI) analysis has been widely used for this purpose [4] and for pharmaceutical and healthcare analysis [5]. A review of the literature reveals several methods for the analysis of $H_2O_2$ employing this approach. For high sensitivity various chemiluminescence [6,7] and fluorescence [8] methods are reported. Also electrochemical [9] and ion selective electrode (ISE) [10,11] methods including ISE with the iodine redox chemistry [12]. Biosensors employing peroxidase have also been employed [13,14] and the determination of peroxides and hydroperoxides in oil [15]. Spectrophotometric methods comprise iron(iii) catalysed colouration [16], titanium(iv)-complexes [17], tetrazolium [18], thio-micheler's ketone [19] and use of the iodide/iodine redox chemistry for $H_2O_2$ [20] and hydroperoxides in foods, lipids and oils [21–23]. The use of spectrophotometry to automate and simplify the iodide/iodine redox chemistry (currently employed for the iodimetric titration) was chosen for this analysis.

Peroxide, as active oxygen, readily oxidises acidified iodide according to the following reaction:

$$ROOH + 2I^- + 2H+ \rightarrow ROH + I_2 + H_2O$$

(with excess iodide ion $I_2 + I^- \rightarrow I_3^-$).

The liberated iodine, which is proportional to the level of peroxide and hence 'active oxygen' is determined on-line spectrophotometrically at 350 nm. The product is dispersed and injected into a carrier stream of acetic acid which is then mixed with the iodide in situ. The closed reaction conditions in a two-stream manifold prevent interference, commonly encountered in manual methods for trace analysis, from excess iodine released by atmospheric oxygen due to the reaction:

$$4I^- + O_2 + 4H+ \rightarrow 2I_2 + H_2O$$

Experimental conditions have been studied using multivariate experimental design. This approach has been used previously in these laboratories for development and optimisation of other FI methods [24–27]. A quarter 'fractional factorial' design was applied to identify and optimise the critical variables.

High, or low, sensitivity method options have been developed, either with or without ammonium molybdate in the carrier stream to catalyse the reaction. The method has been validated and is quicker and simpler than the currently employed titration approach. It should be applicable to a range of 'active oxygen' products.

## 2. Experimental

### 2.1. Apparatus

The flow injection manifold is shown schematically in Fig. 1. All development work was carried out using Agilent 1100 series HPLC equipment. This consisted of a G1365B multiwavelength detector (equipped with a 10 mm path length, 17 μl interval volume, silica flowcell), two G1310A pumps, a G1313A autosampler and a G1316A column-oven (maintained at 25 °C). PTFE (0.5 mm i.d.) reaction tubing (Whatman Ltd.) was used throughout. A back pressure regulator (Thames Chromatography Ltd.), adjusted to 100 psi, was connected after the flow cell. An 'Atlas' (Thermo Lab systems) integration system was employed for peak area and height quantitation in this work.

### 2.2. Reagents

Potassium iodide, acetic acid, ammonium molybdate and hydrogen peroxide (30% (v/v) solution) were obtained from BDH Chemicals (Pool, Dorset, UK). Distilled water was used throughout.

### 2.3. Flow injection conditions

The reagent stream was a solution of 2% (w/v) potassium iodide in water. The carrier stream was either 5% acetic acid, or 5% acetic acid containing 0.001% (w/v) ammonium molybdate, dependent upon the required sensitivity of the method. Generally for a sample solution concentration greater than 16 μg ml$^{-1}$ 'active oxygen', the former was used; for sample solutions less than 16 μg ml$^{-1}$ the molybdate carrier stream was employed. When freshly prepared, both carrier and reagent solutions were sonicated

for approximately 10 min to degas. The carrier and reagent solutions were stable for 2 months at ambient temperature.

Both streams were pumped at a flow rate of 1.0 ml $min^{-1}$ through a 300 cm PTFE reaction coil (0.5 mm i.d.), held at 25 °C. An injection volume of 20 μl was used for the 5% (v/v) acetic acid carrier stream, or 10 μl for the acid carrier stream containing molybdate. A detection wavelength of 350 nm was employed with a reference of 900 nm (both bandwidths set at 50 nm).

#### 2.3.1. Diluent

The chosen carrier stream, either with or without molybdate, was used as diluent for standard and sample solutions (i.e. if a carrier containing molybdate was employed then both sample and standard solutions would be prepared with the same carrier containing molybdate). When freshly prepared, all solutions were sonicated for approximately 10 min to degas.

#### 2.3.2. Standard solution

An accurately prepared 0.015% (w/v) solution (150 μg $ml^{-1}$ or 150 ppm) of hydrogen peroxide (equivalent to 70.59 ppm 'active oxygen') was employed as the standard solution when using 5% (v/v) acetic acid as carrier stream and diluent. Alternatively an accurately prepared 0.00199% (w/v) solution (19.9 μg $ml^{-1}$ or 19.9 ppm) of hydrogen peroxide (equivalent to 9.365 ppm 'active oxygen') was employed as the standard solution when using 5% (v/v) acetic acid containing 0.001% (w/v) ammonium molybdate as carrier stream and diluent.

For the analysis of products containing carbamide peroxide, solutions were prepared as above except an account was made for the molecular weight of the material compared to hydrogen peroxide (414 or 55 μg $ml^{-1}$ carbamide peroxide for carrier streams without or with molybdate, respectively).

#### 2.3.3. Sample solution

An accurately weighted amount of product, dispersed and diluted to volume in carrier stream, to contain the equivalent of either 70.59 or 9.365 μg $ml^{-1}$ 'active oxygen', as above, in carriers without or with molybdate, respectively. A cosmetic gel product was analysed in this exercise.

### 2.4. Procedure

With both carrier and reagent streams flowing through the manifold at 1.0 ml $min^{-1}$, the system was monitored for a stable baseline followed by satisfactory repeatability of measured peak areas for successive injections of the standard solution. Injections of the sample solutions (typically six samples) were then sequentially injected, concluding the analysis with further injections of the standard solution in a single point calibration exercise. By comparison of peak areas of the sample with the mean of the bracketing standard injections, the 'active oxygen' content of the samples was calculated.

*Carrier stream*. The carrier consisted of either 5% (v/v) acetic acid or 5% (v/v) acetic acid containing 0.001% (w/v) ammonium molybdate, depending upon the required sensitivity.

## 3. Results and discussion

FI can readily be applied on existing HPLC equipment, and this was used for all the work described in this paper. It was found that precision was significantly improved with the use of a back pressure regulator which was set at approximately 100 psi. This can be considered 'high pressure FI'. Temperature control of the reaction coil was also considered desirable to comply with strict internal limits applied to drift in peak areas for both FI and HPLC runs. A temperature of 25 °C was employed.

The iodide/iodine redox chemistry in an FI system has been previously applied in these laboratories for the assay of the drug sodium stiboguconate (as pentavalent antimony) and for the determination of trace levels of hydroperoxide and peroxide in lipid products [23,28]. This chemistry was adapted to assay the comparatively simple species of hydrogen peroxide and carbamide peroxide.

Initially a single-stream approach was investigated at the request of the analysts. This consisted of a single-stream of 2% (w/v) potassium iodide in water pumped at a flow rate of 1 ml $min^{-1}$ through a 100 cm reaction coil. Both samples and standard solutions were made up in 5% acetic acid containing 0.01% (w/v) ammonium molybdate. Acidification of the iodide was hence carried out during injection of the solutions. This approach was successful but a small blank contribution was obtained and in routine use this was found troublesome for analysis of trace peroxide levels.

A two-stream approach was hence introduced; standard and sample solutions were prepared in the carrier stream and subsequently injected into the same media, eliminating any solvent contribution. This allowed the potential for analysis of low levels of peroxide. The effect of method variables upon the sensitivity (in terms of peak height) of the method was investigated. The use of molybdate is well known to catalyse the iodide/iodine redox reaction [29] and the effect of this variable was included in the investigation. This was performed using multivariate analysis in a screening 'fractional factorial' experimental design (the software Design Expert DX6, available from Stat-Ease corporation, USA, was employed). The use of a fractional factorial design allows the screening of a large number of variables to find the significant few and to provide a measure of their effect. It also allows the detection of variable interactions, difficult to detect in univariate analysis. A quarter factorial two level design was employed with four centre points (conditions intermediate between the high and low, two level extremes and a useful measure of experiment to experiment variation) This design allowed the investigation of 6 variables in 20 experiments. The design was resolution IV, in

Table 1
Aliases for a quarter fractional factorial resolution IV design for six factors

| Term | Aliases |
|---|---|
| [A] | A + BCE + DEF |
| [B] | B + ACE + CDF |
| [C] | C + ABE + BDF |
| [D] | D + AEF + BCF |
| [E] | E + ABC + ADF |
| [F] | F + ADE + BCD |
| [AB] | AB + CE |
| [AC] | AC + BE |
| [AD] | AD + EF |
| [AE] | AE + BC |
| [AF] | AF + DE |
| [BD] | BD + CF |
| [BF] | BF + CD |
| [ABD] | ABD + ACF + BEF + CDE |
| [ABF] | ABF + ACD + BDE + CEF |

Table 2
Variable levels chosen for the experimental design

| | Min. value | Max. value |
|---|---|---|
| Flow rate (individual, ml $min^{-1}$) | 0.4 | 1.0 |
| Injection volume (μl) | 10 | 24 |
| Ammonium molybdate concentrate (%, w/v) | 0.0 | 0.5 |
| Coil length (cm) | 100 | 300 |
| Acetic acid concentration (%, v/v) | 2 | 5 |
| KI reagent concentration (%, w/v) | 2 | 5 |

which the primary variables are aliased only with three variable interactions (which generally are unlikely to occur) and the two variable interactions are aliased with other two factor interactions. The aliases for this resolution IV design is given in Table 1.

The levels of the variables examined are given in Table 2.

The resulting experiment matrix is shown in Table 3. For ease of quantitation the response of peak height was employed as response. The centre points also provide an ability to predict curvature. All experiments were carried out in run order to eliminate environmental variance.

A statistically significant curved model accounting for 93% of the variance was fitted to the data. A half normal plot showing the normalised cumulative effects of the examined variables is given in Fig. 2a.

The triangles in this plot represent the experiment to experiment variation, calculated from the four centre points ($n-1$ points) and a line is plotted through these, encompassing the variables with equivalent, small effects. Variables lying to the right of the line have the greatest effect and are judged to be the critical variables. The larger is the deflection from the line, the greater is the magnitude of the effect of that variable upon the results. It is clear from the plot that the concentration of molybdate has the greatest effect, followed by the injection volume. Next in rank of importance is a two factor interaction (confounded) effect of both flow rate and acid concentration, followed by the single effect of the flow rate.

A 3D plot of the effects of the two most critical variables, molybdate concentration and injection volume is given in Fig. 2b. This plot demonstrates that an increase in both of these variables leads to an increase in the peak height; molybdate concentration clearly having the greatest effect.

Additionally a 3D plot of the two factor interaction effect of both flow rate and acid concentration (other conditions fixed) is given in Fig. 3a. This same data is also given in a 2D plot in Fig. 3b (this latter plot shows the effect of increasing the flow rate only, at the extremes of both high (5%, v/v) and low (2%, v/v) acid concentration).

Table 3
Experiment matrix and results obtained for the screening experimental design

| Run order | Flow rate (ml $min^{-1}$) | Injection volume (μl) | Ammonium molybdate (%, w/v) | Coil length (cm) | Acetic acid conc. (%, v/v) | KI reagent conc. (%, w/w) | Peak height (units) |
|---|---|---|---|---|---|---|---|
| 1 | 0.6 | 17 | 0.25 | 200 | 3.5 | 3.5 | 4516.0 |
| 2 | 0.8 | 24 | 0.5 | 100 | 5.0 | 2.0 | 5479.9 |
| 3 | 0.8 | 10 | 0.0 | 100 | 5.0 | 2.0 | 40.7 |
| 4 | 0.4 | 24 | 0.5 | 100 | 2.0 | 2.0 | 8431.0 |
| 5 | 0.4 | 10 | 0.0 | 100 | 2.0 | 2.0 | 115.1 |
| 6 | 0.8 | 24 | 0.0 | 300 | 2.0 | 2.0 | 153.9 |
| 7 | 0.4 | 10 | 0.5 | 300 | 5.0 | 2.0 | 2747.7 |
| 8 | 0.4 | 24 | 0.0 | 100 | 5.0 | 5.0 | 502.4 |
| 9 | 0.8 | 24 | 0.0 | 100 | 2.0 | 5.0 | 195.0 |
| 10 | 0.6 | 17 | 0.25 | 200 | 3.5 | 3.5 | 4494.0 |
| 11 | 0.8 | 10 | 0.5 | 300 | 2.0 | 2.0 | 2173.3 |
| 12 | 0.4 | 24 | 0.0 | 300 | 5.0 | 2.0 | 634.9 |
| 13 | 0.6 | 17 | 0.25 | 200 | 3.5 | 3.5 | 4505.2 |
| 14 | 0.8 | 24 | 0.5 | 300 | 5.0 | 5.0 | 5677.0 |
| 15 | 0.4 | 24 | 0.5 | 300 | 2.0 | 5.0 | 6706.0 |
| 16 | 0.8 | 10 | 0.0 | 300 | 5.0 | 5.0 | 181.3 |
| 17 | 0.4 | 10 | 0.0 | 300 | 2.0 | 5.0 | 403.2 |
| 18 | 0.4 | 10 | 0.5 | 100 | 5.0 | 5.0 | 4367.0 |
| 19 | 0.6 | 17 | 0.25 | 200 | 3.5 | 3.5 | 4482.8 |
| 20 | 0.8 | 10 | 0.5 | 100 | 2.0 | 5.0 | 3668.3 |

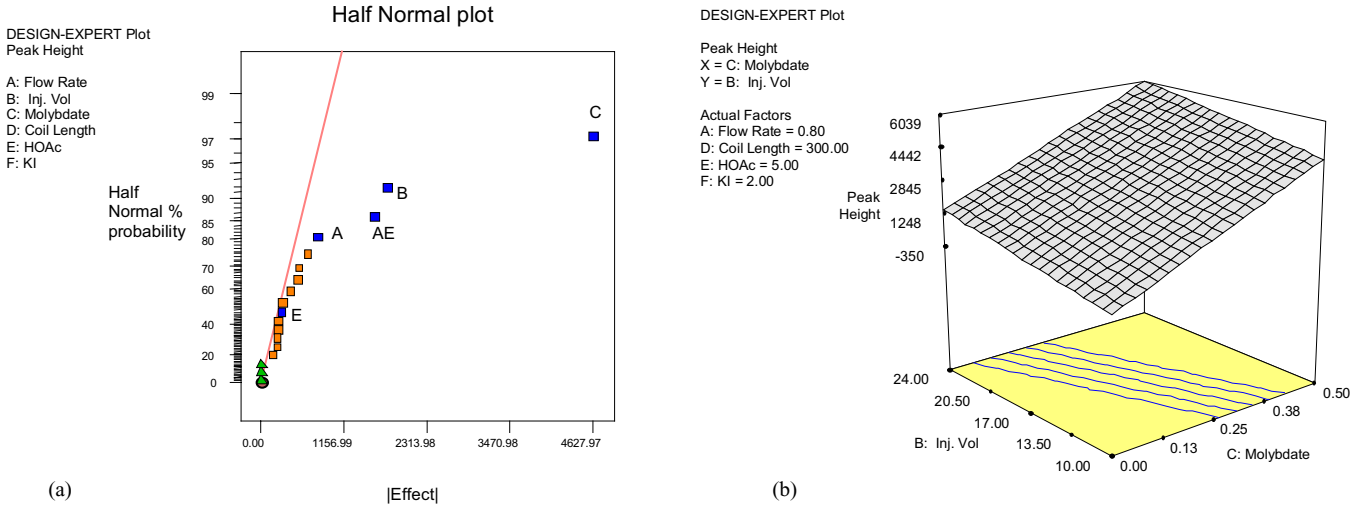


Fig. 2. Plots identifying the most significant variables (a) and the largest effects of molybdate and injection volume (b).

It is clear from Fig. 3a and b that the effect (both magnitude and direction) of an increase in flow rate is dependent upon the acid concentration (different gradients obtained at both high and at low acid concentration). The same is true for the effect of acid concentration at a pre-determined flow rate (see Fig. 3a). This is a good example of a confounded interaction, although for this chemistry the magnitude of these variables is not substantial.

In the final method a flow rate of 1.0 ml $min^{-1}$ for both carrier and reagent streams and a coil length of 300 cm was chosen as the flow and coil length at which acceptable peak shapes, sensitivity and reaction time was obtained. An iodide concentration at the minimum of the examined range, of 2% (w/v), was selected as this concentration is not a critical variable and it is desirable to keep reagent concentration to a minimum to minimise the potential for background absorbance upon storage. An acid concentration of 5% (v/v) was chosen. The molybdate concentration was found to be the most critical factor and for subsequent work, two types of carrier stream were employed, with or without molybdate. These were termed “low sensitivity option” (5% (v/v) acetic acid) or “high sensitivity option” (carrier containing 0.001% (w/v) ammonium molybdate in 5% (v/v) acetic acid) and the decision on which carrier stream to employ was made dependent upon the level of ‘active oxygen’ in the product(s) to be assayed.

The use of both peak area and peak heights was investigated and it was found that superior injection reproducibility (and hence precision) was obtained with the use of peak areas, these were subsequently used for all of the final experiments and validation work.

The effect of molybdate concentration (in 5% (v/v) acetic acid) upon peak area response was examined in detail in a univariate investigation; other conditions were fixed as

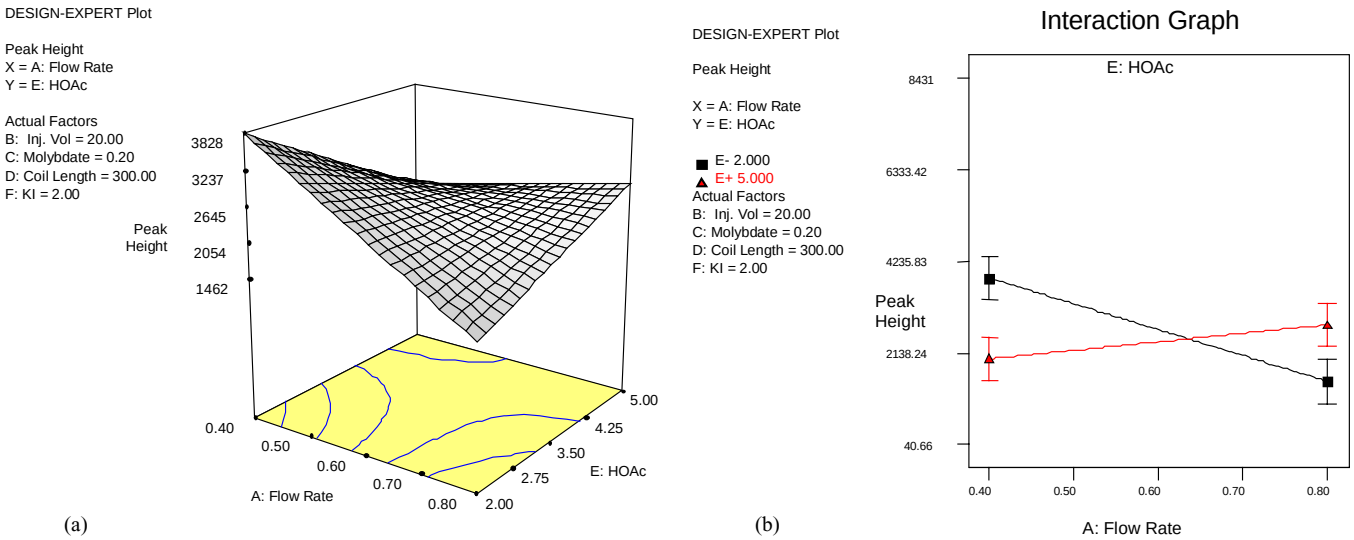


Fig. 3. Plots (3D and 2D) of the two-factor interaction of flow rate and acid concentration.

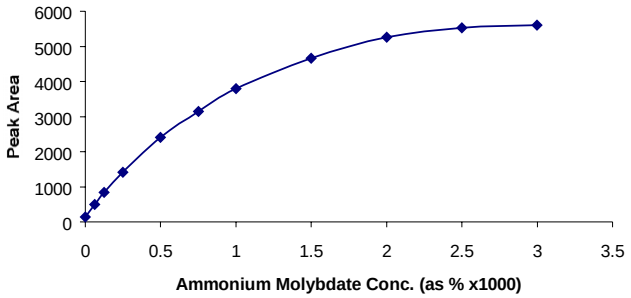


Fig. 4. Univariate plot showing effect of molybdate concentration.

above. The results are given in Fig. 4. This clearly shows that at higher molybdate concentrations the reaction has gone to completion.

The resulting FI conditions are shown in Fig. 1.

## 4. Validation

Validation was carried out predominantly using the carrier stream of 0.001% (w/v) ammonium molybdate in 5% (v/v) acetic acid (higher sensitivity option) as this was the first application to products in this company. This is considered to be a worst-case situation involving the presence of an additional reagent. Confirmatory validation was also carried out on the 5% (v/v) acetic acid carrier stream (lower sensitivity option) which was subsequently employed for all future product analysis in GSK, due to the simplicity in use. All solutions (sample and standard) were prepared in the appropriate carrier stream.

### 4.1. Linearity of the method

#### 4.1.1. Carrier stream containing molybdate

The linearity of the method over the range 2.12–15.9 $\mu g\,ml^{-1}$ 'active oxygen' in a carrier stream of 5% (v/v) acetic acid containing 0.001% (w/v) ammonium molybdate, was confirmed by injecting a series of standard solutions at levels of 2.1, 5.3, 8.5, 10.7, 12.7 and 15.9 $\mu g\,ml^{-1}$. A linear response of peak area (mv) versus concentration ($\mu g\,ml^{-1}$) was obtained with the equation $y = 165.6x + 30.2$ (correlation coefficient, 0.99985; residual sum of squares 1050; intercept as % nominal conc. 0.0018; % R.S.D. of predicted assay at nominal conc. 1.05). A further experiment to a higher level, 27.8 $\mu g\,ml^{-1}$ (with different reagents) employed standard solutions at levels of 6.9, 9.9, 12.8, 15.8, 18.8, 21.8, 24.8 and 27.8 $\mu g\,ml^{-1}$ with the equation $y = 198.4x - 30.2$ (correlation coefficient 0.99941; residual sum of squares 17330; intercept as % nominal conc. 0.015; % R.S.D. of predicted assay at nominal conc. 1.68).

#### 4.1.2. Carrier stream without molybdate

The linearity of the method over the range 17.7–141 $\mu g\,ml^{-1}$ 'active oxygen' in 5% (v/v) acetic acid was confirmed by injecting a series of standard solutions at levels of 17.7, 35.4, 70.8, 106.2 and 141 $\mu g\,ml^{-1}$. A linear response of peak area (mv) versus concentration ($\mu g\,ml^{-1}$) was obtained with the equation $y = 12.69x - 2.06$ (correlation coefficient 0.99994; residual sum of squares 301; intercept as % nominal conc. 0.002; % R.S.D. of predicted assay at nominal conc. 1.11). A further experiment to a higher level (247.9 $\mu g\,ml^{-1}$) employed standard solutions at levels of 35.4, 70.8, 106.2, 177.0, 212.4 and 247.9 $\mu g\,ml^{-1}$ with the equation $y = 12.128x + 16.7$ (correlation coefficient 0.99990; residual sum of squares 1505; intercept as % nominal conc. 0.019; % R.S.D. of predicted assay at nominal conc. 1.19).

### 4.2. Specificity

A placebo (cosmetic gel) formulation inert in hydrogen peroxide (active oxygen) was passed through the method. A response of 0.023 $\mu g\,ml^{-1}$ (0.25% relative to the response obtained from a simulated active sample) was obtained. This was judged to be satisfactory and the method is considered to be specific in the presence of other components in the formulation.

### 4.3. Limit of quantitation (LOQ)

The limit of quantitation (LOD), for both acid and acid/molybdate carrier streams, was calculated from a range of low level standards, as the standard concentration where the area was 10 times greater than the peak to valley noise level from injections of carrier blank (taken as the analytical background response). Standards corresponding to 0.04 and 0.006 $\mu g\,ml^{-1}$ 'active oxygen' for each carrier stream, respectively, satisfied this requirement (both approximately 0.06% of the appropriate standard solution concentration). The LOD was hence judged to be 0.04 or 0.006 $\mu g\,ml^{-1}$ 'active oxygen' for the acid and acid/molybdate carrier streams, respectively.

### 4.4. Precision

#### 4.4.1. Repeatability

The repeatability of the 9.2 $\mu g\,ml^{-1}$ standard solution for the low sensitivity carrier stream and the 70.6 $\mu g\,ml^{-1}$ (both quoted as 'active oxygen') standard solution for the high sensitivity carrier stream were assessed by carrying out six injections on a single occasion. Relative standard deviation (R.S.D.) values of 0.36 and 0.39 were obtained. These are considered to be satisfactory.

The method repeatability was also assessed on replicate sample preparations for both carrier streams. Six sample preparations from one batch of product (low sensitivity carrier stream) and a second six sample preparations from a different batch of product (high sensitivity carrier stream), were analysed by a single operator and the 'active oxygen' concentrations measured. Results of 2.73, 2.73, 2,74, 2.77, 2.70, 2.70 $\mu g\,ml^{-1}$ (mean 2.73 $\mu g\,ml^{-1}$, R.S.D. 0.90) and 2.77,

Table 4
Results of the accuracy experiment

| Level 'active oxygen' added ($\mu g\,ml^{-1}$) | Result ($\mu g\,ml^{-1}$) | % 'Active oxygen' recovered |
|---|---|---|
| 4.7 | 4.845, 4.826, 4.812 | 103.3, 102.9, 102.6 |
| 7.3 | 7.381, 7.410, 7.403 | 101.6, 102.0, 101.9 |
| 9.1 | 9.153, 9.135, 9.117 | 100.8, 100.6, 100.4 |
| 10.9 | 11.136, 11.136, 11.125 | 102.2, 102.2, 102.1 |

2.88, 2.84, 2.91, 2.91, 2.91 $\mu g\,ml^{-1}$ (mean 2.87 $\mu g\,ml^{-1}$, R.S.D. 1.96), as 'active oxygen' were obtained, respectively.

On a separate occasion (3-week interval), a second operator prepared six independent sample preparations on the same batch of product with the low sensitivity carrier stream ('Intermediate Precision' experiment). Results of 2.62, 2.59, 2,60, 2.59, 2.63, 2.58 $\mu g\,ml^{-1}$ (mean 2.60 $\mu g\,ml^{-1}$, R.S.D. 0.80) were obtained. The R.S.D. of both data sets was 2.6.

All data was considered satisfactory.

### 4.5. Accuracy

The accuracy was estimated by recovery experiments using the high sensitivity carrier stream. Known amounts of 'active oxygen' (as hydrogen peroxide) were added to an inert product formulation, in triplicate, at levels of 4.7, 7.3, 9.1, and 10.9.5, 2.0 and 2.5 $\mu g\,ml^{-1}$ (equivalent to 50, 80, 100 and 120% of the nominal level in the product) and the mixtures passed through the procedure. The results obtained are detailed in Table 4.

Satisfactory recovery was obtained.

### 4.6. Comparison of results with iodimetric titration

Three different formulations of a cosmetic gel product, stored at both ambient and elevated temperature (2 months at 40 °C) to examine for degradation, were assayed for 'active oxygen' by the FI procedure (low sensitivity option) and by manual iodimetric titration. The results are given in Table 5.

A satisfactory comparison was obtained.

Table 5
Comparison of FI with manual iodimetric titration

| Formulation reference and storage temperature | FI result ($\mu g\,ml^{-1}$ 'active oxygen') | Titration result ($\mu g\,ml^{-1}$ 'active oxygen') |
|---|---|---|
| 1 ambient | 2.84 | 2.86 |
| 1 elevated | 2.73 | 2.73 |
| 2 ambient | 2.85 | 2.84 |
| 2 elevated | 2.84 | 2.85 |
| 3 ambient | 2.81 | 2.77 |
| 3 elevated | 2.76 | 2.82 |
| Mean | 2.81 | 2.81 |
| R.S.D. | 1.8 | 1.8 |

### 4.7. Solution stability

The stability of the hydrogen peroxide standard and sample preparations in 5% (v/v) acetic acid solution containing 0.001% (w/v) ammonium molybdate was demonstrated by analysis of solutions stored at ambient temperature, away from direct sunlight, over a period of 13 and 29 days. The standard and sample solutions were analysed initially, and then after storage, against freshly prepared standards. The comparative results of 'active oxygen' in the standard solution was 101.5 and 102.0% after 13 days and 100.0 and 100.7% after 29 days storage. The comparative results of 'active oxygen' in the sample solution was 101.2 and 102.1% after 13 days and 98.0 and 98.2% after 29 days storage. The solutions are considered stable for storage of up to 29 days hours at ambient temperature.

## 5. Conclusion

An FI procedure employing HPLC with back-pressure to improve precision (high pressure FI) been developed to provide a rapid and precise automated assay for 'active oxygen' from products formulated to contain hydrogen peroxide. The sensitivity of the procedure can be modified by the addition of ammonium molybdate to provide both low and high sensitivity options. Conditions have been investigated using screening multivariate experimental design to identify and optimise the critical variables. The method has been validated and is quicker and simpler than the currently employed titration approach. It should be applicable to a range of 'active oxygen' products.

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