Simultaneous Determination of Ascorbic Acid, Caffeine and Paracetamol in Drug Formulations by Differential-pulse Voltammetry Using a Glassy Carbon Electrode

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A simple, rapid and accurate method for the simultaneous determination of ascorbic acid, caffeine and paracetamol in drug formulations has been developed. Peak currents were measured with a glassy carbon electrode at +0.350, +0.618 and +1.425 V *versus* a saturated calomel electrode for ascorbic acid, paracetamol and caffeine, respectively. Perchloric acid (0.1 M) - methanol (1 + 1) was used both as a solvent and supporting electrolyte. The optimum modulation amplitude, pulse repeat time and scan rate of the polarographic analyser were found to be 50 mV, 0.5 s and 5 mV s^{-1}, respectively and the linear calibration ranges for ascorbic acid, caffeine and paracetamol were 0–35, 0–50, and 0–55 μg ml^{-1}, respectively. The relative standard deviations for 9.30 μg ml^{-1} of ascorbic acid, 8.50 μg ml^{-1} of caffeine and 7.30 μg ml^{-1} of paracetamol were 1.3, 2.5 and 0.7%, respectively. Results are reported for several commercially available drugs.

Keywords: *Ascorbic acid determination; caffeine determination; paracetamol determination; differential-pulse voltammetry; glassy carbon electrode*

Paracetamol (*N*-acetyl-*p*-aminophenol or acetaminophen) is a widely used analgesic anti-pyretic drug having actions similar to those of aspirin, and a suitable alternative for patients who are sensitive to aspirin.[1] Many methods have been described for the determination of paracetamol[2] including titrimetry, chromatography, electrochemistry, spectrophotometry and high-performance liquid chromatography (HPLC).[3] In most of these methods, considerable time is required for the extraction and the reaction steps. Further, ascorbic acid interferes with the titrimetric methods based on the reducing properties of paracetamol. In some spectrophotometric methods,[2,4] ascorbic acid and acetylsalicylic acid interfere and give rise to irreproducible results.

Caffeine (1,3,7-trimethylxanthine) is sometimes included in analgesic preparations because of its diuretic action[5] and it has been determined by titrimetry,[6] spectrophotometry[7] and HPLC.[8] Both titrimetric and spectrophotometric methods involve a time-consuming extraction process prior to the determination.

Large doses of ascorbic acid have been used for prophylaxis of the common cold, although conclusive evidence of its efficacy is still required.[9] The BP method for the determination of ascorbic acid is based on titration with iodine solution.[10] Many other methods have been developed including visible[11] and ultraviolet spectrophotometry,[12] flow injection,[13] differential-pulse polarography,[14] microfluorimetry[15] and HPLC.[16]

However, very little work has been performed on the simultaneous determination of ascorbic acid, caffeine and paracetamol, which often occur together in certain drug formulations. As ascorbic acid and paracetamol are oxidised at very different potentials, electrochemical methods should offer higher selectivity for the simultaneous determination of these two compounds. In contrast, ascorbic acid interferes seriously with some spectrophotometric methods[2,4] for the determination of paracetamol and with titrimetric methods based on the reducing properties of paracetamol. Further, when subjected to HPLC on the common octadecylsilane column, the retention time for ascorbic acid is extremely short and the peak is often obscured by those of additives in the drugs. The purpose of the work described here was to develop a voltammetric method for the simultaneous determination of these three compounds in analgesic drugs using a glassy carbon electrode.

Experimental

Instrumentation

The differential-pulse voltammograms were recorded with a PAR Model 174A polarographic analyser equipped with a Houston Model RE0089 *x* - *y* recorder. A glassy carbon electrode (PAR Model G173; surface area, 7 mm^2), a saturated calomel electrode (SCE) and a platinum wire counter electrode were used. The modulation amplitude, pulse repeat time and scan rate were 50 mV, 0.5 s and 5 mV s^{-1}, respectively. All voltammograms were scanned in the direction from 0 to +1.5 V *versus* the SCE and all experiments were performed at 25 °C.

As the glassy carbon electrode usually became contaminated with the products of oxidation after scanning, the electrode was polished between scans by buffing the electrode surface with a polishing cloth and an abrasive slurry of 0.5 g of alumina in de-ionised or distilled water as in the PAR Polishing Kit Instructions.

Reagents

All reagents used were of analytical-reagent grade.

Ascorbic acid stock solution, 1000 μg ml^{-1}. Prepared fresh as required by dissolving 0.1000 g of the acid in 100 ml of 0.1 M perchloric acid in a calibrated flask.

Caffeine stock solution, 1000 μg ml^{-1}. Prepared by dissolving 0.1000 g of caffeine in methanol and diluting with the same solvent to 100 ml in a calibrated flask.

Paracetamol stock solution, 1000 μg ml^{-1}. Prepared by dissolving 0.1000 g of the compound in methanol, and diluting with the same solvent to 100 ml in a calibrated flask.

Supporting electrolyte. Prepared by mixing equal volumes of methanol and 0.1 M perchloric acid, which was prepared previously in doubly de-ionised water.

Procedure

Determination of paracetamol in tablets and cough syrup

The tablets were weighed accurately and ground into a powder about 0.1 g of which was weighed accurately and dissolved in 100 ml of the supporting electrolyte to give sample solution A. Similarly, 1 ml of cough syrup was dissolved in the supporting

electrolyte to a final volume of 100 ml to produce sample solution B. An aliquot of the sample solution (0.3 ml of sample solution A or 1 ml of sample solution B) was diluted to 25 ml with the supporting electrolyte. The resulting solution (20 ml) was pipetted into the cell, and the voltammogram was recorded by scanning from 0 to +0.8 V *versus* SCE. The peak height of the signal was measured at +0.618 V *versus* SCE, with the base line constructed across the shoulders of the peak. The amount of paracetamol in the samples was determined by a multiple standard additions calibration.

Simultaneous determination of ascorbic acid, caffeine and paracetamol in tablets

Because the content of paracetamol was much higher than that of either ascorbic acid or caffeine, the current range was set to a higher value in the region of the peak potential of paracetamol.

About 0.5 g of the powdered tablets was weighed accurately and then dissolved in 100 ml of the supporting electrolyte; the resulting solution was protected from the light to avoid decomposition of the ascorbic acid. The base line was constructed by using the voltammogram of the supporting electrolyte (20 ml) recorded by scanning from an initial potential of 0.0 V, and with the current range set to 50 μA in the range 0–0.5 V, 100 μA in the range 0.5–0.9 V and 50 μA in the range 0.9–1.5 V. After 0.3 ml of the sample solution was pipetted into the cell containing the supporting electrolyte, the scan was repeated as described above. The peak heights of the signals for ascorbic acid, paracetamol and caffeine were measured at +0.350, +0.618 and +1.425 V *versus* SCE, respectively. The amounts of ascorbic acid, caffeine, and paracetamol in the sample were determined by a multiple standard additions calibration.

Simultaneous determination of ascorbic acid and caffeine

The procedure was similar to that above for the determination of the three components except that 1.0 g of aspirin - phenacetin - caffeine (APC) and 5.36 g of the powdered ascorbic acid - caffeine sample were used instead, and the current range was fixed at 50 μA.

Results and Discussion

In the proposed method, a glassy carbon electrode was employed because this electrode is inert and allows a potential more positive than +0.3 V, which is required for the oxidation of paracetamol and caffeine, to be applied.

Selection of pH for Measurements

The effect of pH on the peak potentials and the sensitivities for the determination of ascorbic acid, caffeine and paracetamol was studied using Britton - Robinson buffer solutions. It was found that no signals were detected for caffeine around pH 8.3 and that ascorbic acid is also unstable in alkaline media. Hence, it can be concluded that the simultaneous determination of these compounds should not be carried out at pH > 8.3.

Further, for paracetamol and ascorbic acid, the signals were found to increase with decreasing pH. Unfortunately, no signals were detected for caffeine at pH < 4.1 and it appeared that Britton - Robinson buffers were not suitable for this work. However, it was clear from these preliminary experiments that the determination should be carried out in acidic medium and attempts were then made to use acids as the supporting electrolytes.

Selection of Supporting Electrolyte

Three acids, namely, sulphuric, phosphoric and perchloric, were assessed for their suitability as supporting electrolyte. Hydrochloric acid was not used as the chloride ion may also be oxidised and interfere with the method, and similarly dilute nitric acid was not employed because of its powers of oxidation.

It was found previously that the use of methanol could improve the precision of the determination of methyl salicylate and thymol by differential-pulse voltammetry using a glassy carbon electrode, and that the best precision was obtained when 1 + 1 water - methanol was used.[17] In view of this observation, 1 + 1 dilute acid (0.1 M) - methanol was employed.

The sulphuric acid - methanol mixture exhibited a broad peak at about +0.230 V *versus* SCE, which was very close to the ascorbic acid peak at +0.300 V. In addition, the signal due to caffeine was detectable only at the lowest scan rate of 2 mV s^{-1}.

The phosphoric acid - methanol mixture exhibited a broad peak at +0.130 V *versus* SCE, which overlapped with the peak due to ascorbic acid. A scan rate of 2 mV s^{-1} was again required to observe the caffeine signal.

When perchloric acid - methanol was used, the peaks of ascorbic acid, paracetamol and caffeine were all well defined. Further, the caffeine signal could be observed at a higher scan rate. Hence, the 0.1 M perchloric acid - methanol (1 + 1) mixture was the best of the three systems under study, and 0.1 M perchloric acid was chosen as the supporting electrolyte.

Selection of Organic Solvent

Different organic solvents were employed to study the possibility of improving the sensitivities and peak separations further. In addition to methanol three other solvents, namely, acetonitrile, dimethyl sulphoxide (DMSO) and ethanol, were

Table 1. Effect of organic solvent on the sensitivity of measurement in 0.1 M perchloric acid - organic solvent (1 + 1) of ascorbic acid, caffeine and paracetamol. Modulation amplitude, 50 mV; pulse repeat time, 1 s; and scan rate, 5 mV s^{-1}

	Slope of calibration graph/μA μg^{-1} ml			
Ingredient	Acetonitrile	DMSO*	Ethanol	Methanol
Ascorbic acid	0.44	—	0.11	0.24
Caffeine	0.64	—	0.26	0.89
Paracetamol	3.75	2.29	2.17	2.16

* The peak potential of ascorbic acid varied with concentration; the peak for caffeine was obscured by the large residual current.

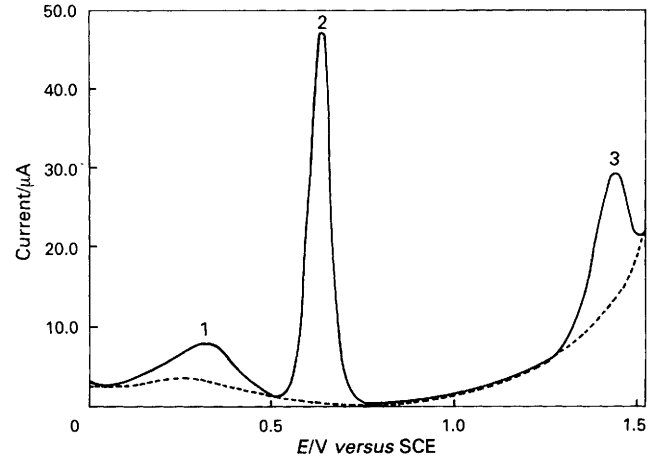

Fig. 1. Typical voltammogram of a mixture of 1, ascorbic acid (20.40 μg ml^{-1}); 2, paracetamol (18.15 μg ml^{-1}); and 3, caffeine (19.62 μg ml^{-1}) in 0.1 M perchloric acid - methanol (1 + 1). The base line was constructed using the voltammogram of the pure supporting electrolyte

Table 2. Concentrations of three dyes that cause the peak currents of ascorbic acid (7.82 μg ml^{-1}), caffeine (6.95 μg ml^{-1}) and paracetamol (7.02 μg ml^{-1}) to vary by 5% from the expected values

	Ascorbic acid		Caffeine		Paracetamol	
Interferent	[Interferent]*/ μg ml^{-1}	Mass ratio†	[Interferent]*/ μg ml^{-1}	Mass ratio†	[Interferent]*/ μg ml^{-1}	Mass ratio†
Amaranth	4.07 (−)	0.52	35.00 (+)	5.03	4.16 (+)	0.59
Ponceau 4R	6.97 (+)	0.89	6.97 (+)	1.00	27.46 (+)	3.91
Tartrazine	1.80 (−)	0.23	1.80 (−)	0.26	3.57 (−)	0.51

* The sign of the deviation is given in parentheses.
† Mass ratio = [interferent]/[analyte].

Table 3. Results of the recovery tests for ascorbic acid, caffeine and paracetamol using the proposed method. Each determination was carried out in triplicate

Sample	Analyte	Analyte added/mg per tablet	Analyte found/mg per tablet	Recovery, %
Cold remedy powder* . .	Ascorbic acid	0	39.12	
		36.54	75.21	98.8
Cortal	Caffeine	0	29.11	
		33.14	61.51	97.8
Tablet	Paracetamol	0	500.9	
		560.0	1051	98.2

* Contents, mg per sachet.

assessed; the results are shown in Table 1. Acetonitrile was unsuitable because the peak separation for ascorbic acid and paracetamol (at +0.460 and +0.625 V, respectively) was not good enough. Dimethyl sulphoxide was also unsuitable, as the peak currents for both ascorbic acid and caffeine could not be measured accurately owing to the variation of the peak potential of ascorbic acid with concentration and to the fact that the caffeine peak was obscured by the very large residual current. Ethanol was less suitable than methanol for this work because the signals for ascorbic acid and caffeine were much weaker in ethanol; hence, methanol was chosen.

It was found previously[17] that the peak currents for methyl salicylate and thymol decreased with increasing concentrations of methanol in water. However, one of the analytes, namely, paracetamol, is very slightly soluble in cold water; hence, higher methanol concentrations are desirable. As a compromise, all subsequent measurements were carried out in 1 + 1 perchloric acid (0.1 M) - methanol, which is also expected to give the best precision in the determination of methyl salicylate and thymol.[17]

Optimisation of the Parameters of the Analyser

In differential-pulse voltammetry, the analyte signals can depend on instrumental parameters such as the scan rate, modulation amplitude and pulse repeat time. The effects of these parameters on the peak potentials and peak currents in the determination of ascorbic acid, paracetamol and caffeine were studied. It was found that these parameters had little effect on the peak potentials of the three compounds. However, the peak currents for caffeine and paracetamol were highest at a scan rate of 5 mV s^{-1} and that for ascorbic acid at 10 mV s^{-1}. Hence a scan rate of 5 mV s^{-1} was chosen.

When the modulation amplitude was varied from 25 to 100 mV, the peak currents of all three compounds increased with increasing modulation amplitude. Unfortunately, the peak for caffeine was obscured by the large residual current at the highest modulation amplitude of 100 mV; hence a value of 50 mV was chosen.

When the pulse repeat time was varied from 0.5 to 2.0 s, the peak currents decreased with increasing pulse repeat time. Hence, the lowest pulse repeat time of 0.5 s was chosen.

A typical voltammogram of a mixture of ascorbic acid, caffeine and paracetamol is shown in Fig. 1, from which it can be seen that the base line was constructed using the voltammogram of the pure supporting electrolyte. However, for the determination of paracetamol alone, the base line could be obtained by drawing a line across the shoulders of the peak. The peaks of ascorbic acid, paracetamol and caffeine occurred at +0.350, +0.618 and +1.425 V *versus* SCE, respectively, under the optimised conditions.

The electrochemical oxidation of ascorbic acid gave a single pH-dependent voltammetric peak, the reaction involving two electrons and two protons and oxidation of the carbon–carbon double bond.[18] As paracetamol is a phenolic compound and the oxidation was carried out in an acidic medium by the proposed method, the electrode reaction for paracetamol should be similar to that for non-ionised phenols[19]; this involves electrophilic attack on the aromatic nucleus and the irreversible removal of two electrons to give a phenoxonium ion. The electrode reaction for caffeine is complicated and was suggested to proceed by two two-electron two-proton oxidations.[20]

Interference Study

In addition to the active ingredients of interest, most pharmaceutical preparations contain other ingredients such as preservatives, dyes and flavours. The effects of a number of substances commonly found in pharmaceuticals were studied and the following compounds were found not to cause any interferences in the proposed method: acetylsalicylic acid, benzoic acid, brompheniramine maleate, chlorpheniramine maleate, doxylamine succinate, 1-ephedrine hydrochloride, fructose, gelatin, metoclopramide, phenacetin, 1-phenylephrine hydrochloride, phenylpropanolamine hydrochloride, starch and sucrose. However, three common dyes were found to interfere in the proposed method to various extents; these were amaranth, ponceau 4R and tartrazine, having peak potentials of +0.830, +0.725 and +1.050 V *versus* SCE, respectively. The concentrations of these interferents required to cause the peak current of each analyte to vary by 5% from the expected value were determined. Fortuitously, the results obtained cover the normal concentration ranges of these dyes (Table 2).

Calibration Graph and Precision

The calibration graph for ascorbic acid was linear up to 35 μg ml^{-1} having a slope of 0.26 μA μg^{-1} ml and a correlation coefficient of 0.9998. The calibration graph for caffeine passed through the origin and was linear up to 50 μg ml^{-1} with a slope of 0.99 μA μg^{-1} ml and a correlation coefficient of 0.9999. Finally, the calibration graph for paracetamol was linear up to 55 μg ml^{-1} and had a slope of 2.35 μA μg^{-1} ml and a correlation coefficient of 0.9995.

The precision of the procedure was checked by calculating the relative standard deviation of ten replicate determinations of a 9.30 μg ml^{-1} ascorbic acid solution, an 8.50 μg ml^{-1} solution of caffeine and a 7.30 μg ml^{-1} solution of paracetamol; these were found to be 1.3, 2.5 and 0.7%, respectively.

Table 4. Determination of ascorbic acid, caffeine and paracetamol in drugs

Sample	Ingredient	Proposed method*/ mg per tablet	Established method†/ mg per tablet	Label value/ mg per tablet
Paracetamol tablets				
Brand 1	Paracetamol	481.2 475.8 479.9 Mean: 479.0 (0.6)	459.8	500
Brand 2	Paracetamol	528.0 532.8 530.1 Mean: 530.3 (0.5)	516.0	—
Brand 3	Paracetamol	465.0 468.4 465.5 Mean: 466.3 (0.4)	443.0	450
Brand 4	Paracetamol	194.4 207.6 196.8 Mean: 199.6 (3.5)	189.6	200
Panadol syrup‡	Paracetamol	119.0 119.9 123.7 Mean: 120.9 (2.1)	116.0	125
APC tablet	Caffeine	32.0 31.1 31.5 Mean: 31.5 (1.4)	31.0	30
Cortal	Caffeine	29.9 28.7 30.2 Mean: 29.6 (2.7)	31.1	30
Saridon	Caffeine	54.3 53.8 53.0 Mean: 53.7 (1.2)	52.7	50
	Paracetamol	256.0 255.4 250.6 Mean: 254.0 (1.2)	242.0	250
Cold remedy powder§	Ascorbic acid	35.9 36.6 37.3 Mean: 36.6 (1.9)	37.7	40
	Caffeine	45.4 47.7 44.5 Mean: 45.9 (3.6)	43.6	50
Coldrex	Ascorbic acid	29.1 31.2 29.0 Mean: 29.8 (4.2)	30.2	30
	Caffeine	15.5 14.5 16.0 Mean: 15.3 (5.0)	15.3	15
	Paracetamol	310.0 313.8 309.1 Mean: 311.0 (0.8)	299.4	300

* The values in parentheses are the relative standard deviations (%).
† The BP method was employed for ascorbic acid and HPLC for caffeine and paracetamol.
‡ Contents, mg 5 ml^{-1}.
§ Contents, mg per 5.36-g sachet.

Recovery Tests

Recovery tests were performed on three real samples. The mean results of three analyses for added ascorbic acid, caffeine and paracetamol ranged from 97.8 to 98.8% (Table 3), which can be considered to be good recoveries.

Determination of Ascorbic Acid, Caffeine and Paracetamol in Various Drug Formulations

The contents of ascorbic acid, caffeine and paracetamol in several drug formulations were determined in triplicate. The signals of the sample solutions were confirmed as being due to

ascorbic acid, paracetamol and caffeine by enhancement of the peaks at +0.350, +0.618 and +1.425 V *versus* SCE when standard ascorbic acid, paracetamol and caffeine solutions were added to the sample solutions, respectively. The results obtained are summarised in Table 4. The results for caffeine and paracetamol were verified by HPLC[3,8] and those for ascorbic acid were checked by the BP method of iodine titration.[10]

The results given in Table 4 show close agreement between the proposed method and the reference methods and, in addition, the results agreed well with the claimed "label" values. The precision for the determination was also found to be good.

Conclusion

A simple, rapid and accurate voltammetric method has been developed for the simultaneous determination of ascorbic acid, caffeine and paracetamol involving oxidation of these compounds at a glassy carbon electrode. The method is sensitive for caffeine and highly sensitive for paracetamol. The method has been applied successfully and conveniently to the determination of ascorbic acid, caffeine and paracetamol, individually or together, in various drug formulations.

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