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Comparison of fluorimetric, voltammetric and biosensor methods for the determination of total antioxidant capacity of drug products containing acetylsalicylic acid

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Abstract

Antioxidant capacity of several drug specialities containing as mean component acetylsalicylic acid were experimentally evaluated using an enzymatic electrode, recently developed by the present authors, based on superoxide dismutase (SOD) enzyme. The precision of this method of analysis was found to be good (for drug samples RSD $\leq$ 5%). The results were also compared with those ones by a traditional spectrofluorimetric method and by two other methods, respectively, based on cyclic and pulsed voltammetry, recently trialled by the present authors.

Keywords: Antioxidant capacity; Evaluation; Drugs; Acetylsalicylic acid

1. Introduction

Antioxidants are not a homogeneous category of substances: to it vitamins, inorganic compounds, essential amino acids, polyphenols [1], etc., belong. Their capacity to scavenge free radicals is now universally recognised although the true mechanism by means of which they act, for instance in animal organisms, is still not fully lighted. They usually act by neutralising the highly unstable free radical molecules by supplying them with electrons, thus preventing or at least limiting the chain reactions that cause tissue damage [2]. It is important to stress that the different types of hydrosoluble or liposoluble antioxidants can either act individually or interact at the time they are oxidised.

On the other hand, the pace of life and air pollution, particularly in large urban agglomerations, together with an unhealthy diet, facilitate the development of free radicals in the human body. These radicals are considered to be the main factors causing the so-called "oxidative stress", which may be considered a true disorder as it can facilitate the onset of serious diseases, some of which are very difficult to be treated [3]. All this has aroused an interest in modern medicine in the antioxidant and radical scavenging properties of both foodstuffs, especially of plant origin [4], and of actual drug specialities, the adoption of which is justified by the large number and severity of the disorders that can apparently be prevented at varying levels by the regular ingestion of these products. In response to these advances in medical science, the modern pharmaceuticals industry has marketed both products classified as diet integrators [5], and drug specialities, some of which however were and still are marketed not only, and often not principally, for the antioxidant properties of the active principles they contain. For instance it is well known that even some of the more popular drug specialities based on acetylsalicylic acid, although marketed essentially for their painkilling properties [6], have a specific antioxidant effect. In the present research we therefore considered of great interest to evaluate and compare also the total antioxidant capacity of several of the better known specialities of this type which for some time have been readily available in drugstores and for which it would be interesting to point out any differences in antioxidant capacity for equal amounts of drug ingested.

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Although several different methods have been proposed for the measurement of radical scavenging properties, no truly official method still exists to determine total antioxidant capacity. Consequently, in order to validate the results obtained correctly, in the present work, the determination of the antioxidant capacity of various acetylsalicylic acid based specialities was performed by several different methods simultaneously: a biosensor based method recently developed in our laboratory [7,8], one of cyclic voltammetric type [9] recently used on several occasions in our laboratory [10,11] and a spectrofluorimetric method [12] that is among the better known and most frequently used methods described in the literature. Lastly, we also investigated the possibility of applying a new classic differential voltammetric method, recently reported in literature [13,14], but modified by us using differential pulsed voltammetry (DPV), currently under test in our laboratory. The principal aims of the work were therefore: (1) to compare the total antioxidant capacity of several different commercial drug specialities under conditions of equal weight of the formulation or equal concentration of the active principle after drug solubilisation; (2) to assess the validity of widely differing methods for measuring total antioxidant capacity, then comparing the results obtained when they are simultaneously applied to the analysis of different drug specialities all containing acetylsalicylic acid, but in some cases of widely varying complexity as it regards their pharmaceutical formulation.

2. Experimental

2.1. Chemicals

Xanthine oxidase 0.39 U mg^{-1}, cellulose acetate, κ-carrageenan, sodium perchlorate monohydrate and glycerine were supplied by Fluka AG, Buchs (Switzerland); polyvinylacetate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was supplied by Aldrich (Germany); xanthine (2,6-dihydroxy purine) sodium salt, ethylene diamino tetracetic acid (EDTA), superoxide dismutase 4980 U mg^{-1}, dialysis membrane (D-9777) and β-phycoerithryn were supplied by Sigma (Milan); monobasic potassium phosphate, anhydrous dibasic potassium phosphate RPE, anhydrous sodium acetate, analytical grade and methanol RPE were supplied by Carlo Erba (Milan); potassium chloride, sodium sulfate, acetylsalicylic acid, diethyl ether and glycine were supplied by Merck (Germany); 2,2′-azobis(2-amidinopropan) dihydrochloride (ABAP) was supplied by Waco Chem. (Richmond, VA, USA).

2.2. Apparatus

A Model 551 Amel (Milan) potentiostat was used as potentiostatic power supply and also to convert the current signal into a tension signal, which was recorded using an Amel Model 631 differential electrometer (Milan, Italy), coupled to an Amel Model 868 analog recorder; an Amel Model 332/P electrode was used to assemble the biosensor.

The tests were carried out at 25 °C in a 15 ml thermostatted glass cell equipped with a forced water circulation jacket, coupled to a Model VC 20B Julabo (Germany) thermostat. The solutions used in the tests were kept under constant stirring using a microstirrer supplied by Velp Scientifica (Italy).

An Amel analyzer, Model 433, manufactured by Amel (Milan), equipped with a printer and interfaced with a PC, was used for voltammetric measurements.

A Model LS-5 Perkin Elmer spectrofluorimeter, coupled to a Model 561 Perkin Elmer recorder, was used for fluorimetric measurements.

2.3. Samples

Tests were run on six acetylsalicylic acid based drug specialities, all in tablet form and purchased at the drugstore. Table 1 indicates also the composition of these drug specialities as specified on the respective packages. In the same table also the pure active principle, i.e. acetylsalicylic acid, is shown.

3. Methods

3.1. Pre-treatment of drugs

For the analysis of each speciality five tablets were taken, carefully ground up, combined and homogenised. Then an amount sufficient for the preparation of 20 ml of solution, 5×10^{-2} M, of active principle, i.e. acetylsalicylic acid, to perform measurements using the biosensor method or the cyclic voltammetric method, or the fluorimetric method, was weighed out, or else the amount necessary to prepare 100 ml of solution, 1×10^{-3} M, employed to perform the measurements by the differential pulse voltammetric method.

The weighed quantity of each drug speciality was dissolved in the proper buffer, depending on the method used and then homogenised (at 10 000 rpm for 5 min). The homogenate was then analysed directly using both the biosensor and the DPV voltammetric method, or else was centrifuged (at 3000 rpm for 15 min) and the supernatant analysed directly in the case of the fluorimetric and cyclic voltammetric methods.

In the case of measurements carried out using the biosensor based method but on the same quantities by weight of each sample, 150 mg of each drug, carefully ground up and homogenised as described above, were weighed, dissolved in 20 ml of the buffer solution and then analysed directly.

3.2. SOD biosensors

The biosensor was obtained by coupling a transducer (an amperometric electrode for hydrogen peroxide) with

Table 1
Acetylsalicylic acid based drugs tested and their composition

Product and drug form	Composition	Content per tablet (or capsule) (mg)	Content per tablet (or capsule) (%)	Excipients
1: tablets	Acetylsalicylic acid Ascorbic acid	400 240	12.5 7.5	Monosodium citrate Sodium bicarbonate, sodium carbonate, anhydrous citric acid
2: tablets	Acetylsalicylic acid Ascorbic acid	330 200	9.5 5.8	Glycine Anhydrous citric acid, sodium benzoate, sodium hydrogen carbonate
3: tablets	Acetylsalicylic acid	325	48.2	Corn starch, magnesium carbonate, magnesium oxide, calcium carbonate, anhydrous citric acid, magnesium stearate, monobasic sodium phosphate, povidone, sorbitan monolaurate, propylene glycol, mineral oil, polysorbate 20, medical antifoam, carnauba wax, hyroxypropylmethyl cellulose, blue colorant C.I. 42090, white colorant (deionized water, propylene glycol), titanium dioxide
4: tablets	Acetylsalicylic acid	500	23.9	Mannitol, malic acid, polyvinyl pyrrolidone, citric acid, sodium bicarbonate, sodium carbonate, sodium saccharine, lemon aroma, sodium dioctylsulfosuccinate
5: tablets	Acetylsalicylic acid	500	83.3	Corn starch, powdered cellulose
6: tablets	Acetylsalicylic acid	100	83.3	Powdered cellulose, corn starch, sodium saccharine, raspberry aroma
7: standard	Acetylsalicylic acid (pure)		99.9 (% purity)	

the superoxide dismutase enzyme immobilised in a gel-like κ-carrageenan membrane.

The whole assembly of the biosensor, the preparation of the κ-carrageenan membrane and the immobilisation of the SOD enzyme in the gel membrane were described in detail in previous papers [7,8,15].

3.3. SOD biosensor method

The antioxidant capacity using the SOD biosensor was checked as follows: the superoxide radical is produced by the oxidation in aqueous solution of the xanthine to uric acid in the presence of enzyme xanthine oxidase:

$$\text{xanthine} + H_2O + O_2 \xrightarrow{\text{xanthine oxidase}} \text{uric acid} + 2H^+ + O_2^{\bullet -}$$

the disproportion reaction of the superoxide radical, catalysed by the superoxide dismutase immobilised on the H_2O_2 electrode, releases oxygen and hydrogen peroxide:

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \xrightarrow{\text{superoxide dismutase}} H_2O_2 + O_2$$

the H_2O_2 is monitored by the amperometric sensor for hydrogen peroxide.

The hydrogen peroxide released is oxidised at the anode, generating an amperometric signal variation (of the order of tens of nA) that is proportional to the concentration of superoxide radical in solution.

The addition of a sample characterised by antioxidant properties results in a decrease of signal strength as the antioxidant species react with the superoxide radical, thus reducing its concentration in solution. There is a consequent decrease in the H_2O_2 released and thus also in the intensity of the amperometric signal and, in the case of several additions, in the value of the slope of the obtained straight line.

For the performance of the measurement the electrode is placed in a glass cell thermostatted at 25 °C containing 15.0 ml of phosphate buffer 5×10^{-2} M at pH 7.5 and allowed to stabilise under constant magnetic stirring. After the addition of a fixed amount of the enzyme xanthine oxidase (1.2 mg), a series of further additions of 200 μl of the xanthine solution 1×10^{-2} M is performed, waiting for the signal to be stabilised after each addition before proceeding to read off the current. The current values (read after signal stabilisation) are then recorded in the graph as a function of increasing xanthine concentration, thus obtaining a straight line calibration curve from which the slope may be measured. The same type of measurement is then repeated, but this time adding also 0.5 ml of the sample solution to be tested, obtained by dissolving and homogenising the weighed sample in the same phosphate buffer solution, to the cell containing the phosphate buffer and then, after stirring, proceeding as described above. If the sample displays antioxidant properties, the observed signal variation will be lower than in the preceding case, in proportion to the concentration decrease of the superoxide radical in solution; the new calibration curve thus obtained will present a lower slope value than in the preceding case. By comparing the values of the two slopes it is possible to determine the total antioxidant capacity [16] of our sample.

The value of the relative antioxidant capacity is expressed by the algorithm:

$$\text{Relative antioxidant capacity (RAC)} = 1 - \frac{m_b}{m_a}$$

where m_a is the slope of the straight line obtained by successive xanthine additions and m_b the slope of the straight line obtained by successive xanthine additions, but in the presence of the sample to be tested for its antioxidant properties.

3.4. Cyclic voltammetric method

The 20 ml of a 0.05 M solution in the active principium is prepared as previously described and analysed. The solvent used to prepare these solutions is a 1 M solution of phosphate buffer at pH 7.5; this solution also acts as supporting electrolyte for the voltammetric measurement. The measures are performed by connecting the voltammetric apparatus to a cell thermostatted at 25 °C where the test sample has been introduced in the presence of a supporting electrolyte. The method entails the use of three electrodes: (a) a glassy carbon electrode as indicator electrode, (b) a calomel electrode as reference electrode, and (c) a platinum electrode as counter electrode.

In order to avoid the diminishing of its sensitivity the indicator electrode is cleaned at the end of each cycle by rubbing the surface on alumina that has been previously moistened with a minimum quantity of distilled water. After further washing, the electrode is then ready for further tests.

The voltammogram is recorded by scanning the potential over the range (−200 to +1300) mV at a scanning rate of 400 mV s^{-1}, with a scale bottom of 40 μA.

For the purpose of the test the area (in cm^2) subtended by the anodic curve of the voltammogram is measured. The method is actually based on the correlation between the anodic area and the antioxidant capacity of the sample.

3.5. ORAC spectrofluorimetric method

In the presence of free radicals or oxidant species the protein β-phycoerithryn (β-PE) loses over 90% of its fluorescence within 30 min [12]. The addition of antioxidant species, which react with the free radicals, inhibits the diminution of the fluorescence of this protein. The inhibition caused by the action of the free radicals is correlated with the sample's antioxidant capacity. The 2,2′-azobis-(2-amidinopropane) dihydro chloride is used to generate peroxide radicals.

In performing the measurements the wavelengths are set at 540 nm for excitation and 565 nm for emission. Initially 80 μl of the sample in phosphate buffer at pH 7, in the case of the drug specialities, first homogenised and then filtered, are placed in the cuvette, together with 15 μl of phosphate buffer (75 mM, pH 7), and 1.46 ml of β-phycoerythrin (18.3 nm in phosphate buffer), prepared and allowed to stand at 37 °C for 15 min before use. The cuvette is placed in the spectrofluorimeter and the initial fluorescence read off after 30 s. Then a further 20 μl of phosphate buffer are added to the solution in the cuvette together with 20 μl of ABAP (0.32 M in phosphate buffer). After stirring, the fluorescence is read off after 0.5 s and then every 2 min, for a total time of 70 min. A similar procedure is also carried out using 80 μl of a 20 μM solution of Trolox instead of the sample.

All the products were analysed by this way. The final results are expressed in "ORAC units" (micromoles of Trolox equivalent per litre of sample):

$$\text{ORAC value} = 20k\frac{S_{\text{sample}} - S_{\text{blank}}}{S_{\text{Trolox}} - S_{\text{blank}}}$$

where k is the dilution factor for the sample and S the integral of the fluorescence curve of the sample, of the Trolox, or of the "blank".

3.6. Differential pulse voltammetric method

The differential pulse voltammetric method was applied by performing potential scans from +150 to −150 mV versus Ag, $AgCl/Cl^-$ reference electrode with a scale bottom of 24 μA and a scanning rate of 5 mV s^{-1}; a Pt electrode was used as counter electrode. In practice, what was recorded was above all the first oxygen reduction peak when using a mercury suspended drop electrode in 15 ml of an aqueous solution of sodium sulfate 100 mM. The solution in the measuring cell was then replaced with 15 ml of an aqueous solution of sodium sulfate 100 mM containing also the sample to be tested at a concentration of 0.25 mM and the first oxygen reduction peak again recorded. Lastly, a nitrogen current was bubbled through the solution for 5 min so as to completely deoxygenate the solution and the curve obtained by DPV was then recorded under the same conditions. It was thus possible to determine the antioxidant capacity of the test sample using the following expression:

$$\text{Antioxidant capacity (\%)} = \frac{i_{\text{blank}} - i_{\text{sample}}}{i_{\text{blank}}}$$

where i_{blank} and i_{sample} are, respectively, the peak current intensity of oxygen reduction obtained during the first scanning operation (performed on the support electrolyte alone) and during the second operation (performed in the presence of the sample), in both cases after subtracting the current intensity recorded during the third scanning operation (performed in the absence of oxygen).

4. Results and discussion

The research work was above all aimed at making a comparison of the antioxidant capacity, determined using the three main methods described above, of several important commercial drugs all containing acetylsalicylic acid as well as of pure acetylsalicylic acid itself. However, in the present

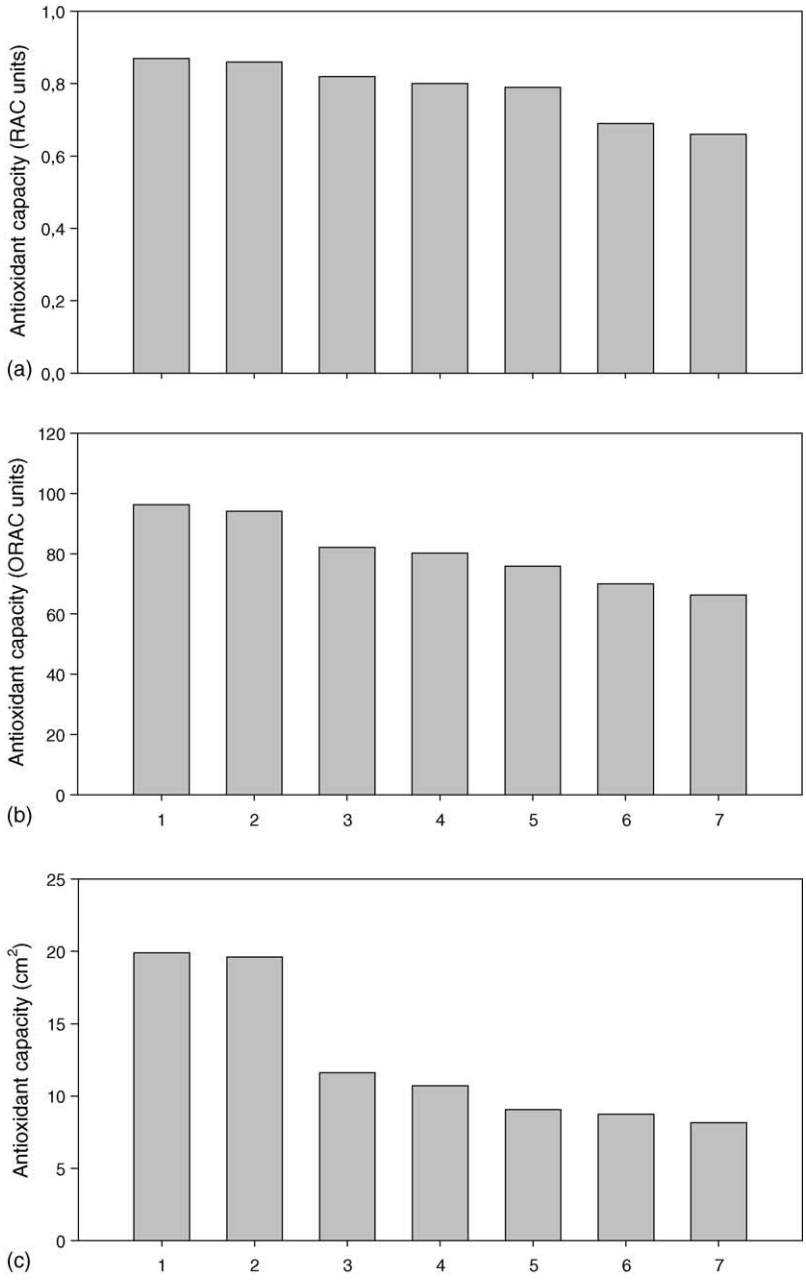

Fig. 1. Comparison of antioxidant capacity trends for the same concentration of acetylsalicylic acid, 5×10^{-2} M, of the six different drug products containing acetylsalicylic acid examined and of pure acetylsalicylic acid, obtained respectively using (a) the biosensor method, (b) the fluorimetric method and (c) the cyclic voltammetric method. The numbers from 1 to 6 refer to the numbering used for these products in Table 1 (7: pure acetylsalicylic acid).

research the antioxidant capacity values obtained for the various products has been determined not only for equal weight of the sample (Table 2) and principally using the biosensor method, as it was done for instance in the case of fresh fruits and beverages, considered in previous researches [11,15,16], but essentially for equal acetylsalicylic acid concentration in the solution obtained and using together all three methods (see Table 3 and histograms shown in Fig. 1). By so doing it was possible to evaluate the effect of the presence of other compounds or excipients contained in the drugs themselves which should thus increase or decrease the antioxidant capacity of these formulations with respect to the antioxidant capacity of the contained principal antioxidant component (in this case the acetylsalicylic acid). It should be noted that these comparisons are possible insofar as these products are true drug specialities, with a perfectly well known and well-defined composition, as declared on the packaging of the manufacturers. For instance, in the case of drugs 1 and 2, the contribution to the total antioxidant capacity made by the ascorbic acid contained in the two drug products is quite clear, as in the case of the contribution due to the small amount of the contained citric acid in the case

Table 2
Values of antioxidant capacity for the same sample weight (150 mg of product) of the six different drug products containing acetylsalicylic acid considered in Table 1, obtained using the SOD biosensor

Sample	Antioxidant capacity (RAC units) ($n \geq 5$)
1	0.12 ± 0.01
2	0.091 ± 0.01
3	0.44 ± 0.02
4	0.21 ± 0.01
5	0.69 ± 0.03
6	0.64 ± 0.03
7	0.73 ± 0.03

The numbers from 1 to 6 refer to the numbering used for these products in Table 1 (7: pure acetylsalicylic acid).

of drugs 3 and 4. In the case of preparations 5 and 6 the antioxidant capacity determined using the fluorimetric and voltammetric methods is nearly similar and lower than that found for the above drugs. In practice it is enough similar to that of pure acetylsalicylic acid. This seems quite logical. Indeed, only excipients are present in these two products in addition to acetylsalicylic acid (see Table 1). However, the albeit slight increase in the RAC of drug 5, which is nevertheless recorded using the biosensor method, with respect to the RAC value of acetylsalicylic acid alone, would thus seem to have been slightly overestimated.

Lastly, it is comforting to observe (Fig. 1) the reasonably good overall agreement found between the trend in antioxidant capacity obtained using the biosensor method or the cyclic voltammetry method and the trends observed using the ORAC method, which was in practice taken as the reference method.

The correlation curves obtained by comparing values for the antioxidant activity of the six examined drugs and for pure acetylsalicylic acid, using three considered methods, respectively, are shown in Fig. 2.

One interesting aspect of the present research is that we did not simply make a qualitative comparison of the trends in the values of antioxidant capacity of drugs containing

Table 3
Values of antioxidant capacity of the six drug compounds containing acetylsalicylic acid (considered in Table 1) (compared for equal acetylsalicylic acid concentration, i.e. 5×10^{-2} M) obtained using (a) the biosensor method, (b) cyclic voltammetric method and (c) the fluorimetric method

Sample number	(a) Biosensor method (RAC units) ($n \geq 5$)	(b) Cyclic voltammetry (cm^2) ($n \geq 5$)	(c) Fluorimetric method (ORAC units) ($n \geq 5$)
1	0.87 ± 0.03	19.9 ± 0.26	96.3 ± 8.2
2	0.86 ± 0.03	19.6 ± 0.26	94.1 ± 5.3
3	0.82 ± 0.03	11.6 ± 0.26	82.1 ± 4.1
4	0.80 ± 0.03	10.7 ± 0.26	80.2 ± 7.2
5	0.79 ± 0.03	9.0 ± 0.26	75.9 ± 5.4
6	0.69 ± 0.03	8.7 ± 0.26	70.0 ± 4.2
7	0.66 ± 0.03	8.2 ± 0.26	66.3 ± 3.2

The numbers from 1 to 6 refer to the numbering used for these products in Table 1 (7: pure acetylsalicylic acid).

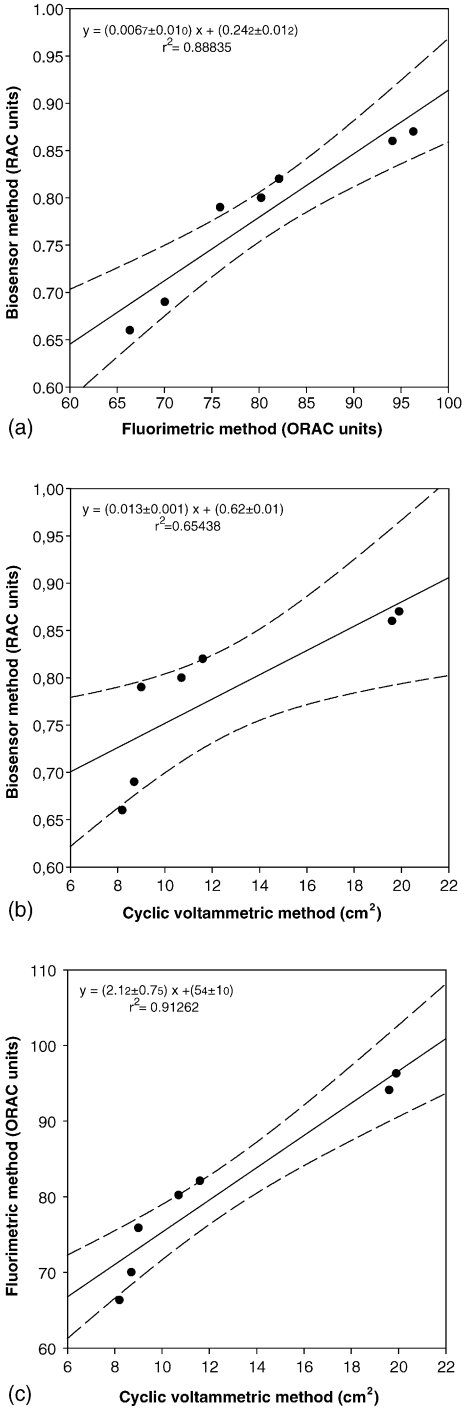

Fig. 2. Correlation curves of antioxidant capacity values obtained, for drugs containing acetylsalicylic acid and pure acetylsalicylic acid, using the SOD biosensor and those found using (a) the fluorimetric and (b) the cyclic voltammetric methods, respectively; (c) correlation curve using fluorimetric method and voltammetric method.

acetylsalicylic acid obtained using the three different methods, but that, by using the correlation curves in Fig. 2, we were able also to make homogeneous quantitative comparisons of the values of antioxidant capacity found by means of the biosensor, cyclic voltammetric and fluorimetric methods

Table 4
Comparison of antioxidant capacity values (all expressed in ORAC units) of the six drug compounds (considered in Table 1) containing acetylsalicylic acid, obtained using (a) the biosensor method and by means of correlation curve equation in Fig. 2(a), (b) the cyclic voltammetry method and by means of correlation curve equation in Fig. 2(c) and (c) the fluorimetric method

Sample number	(a) Biosensor method (ORAC units) (calculated)	(b) Cyclic voltammetry (ORAC units) (calculated)	(c) Fluorimetric method (ORAC units) (measured)	Δ (%) $(c-a)/c$	Δ (%) $(c-b)/c$
1	93.7	96.3	96.3	+3	0
2	92.2	95.7	94.1	+2	+4
3	86.3	78.7	82.1	−5	−10
4	83.3	76.8	80.2	−4	−8
5	81.8	74.2	75.9	−7	−10
6	66.9	72.5	70.0	+4	+8
7	62.4	71.5	66.3	+6	+13

The numbers from 1 to 6 refer to the numbering used for these products in Table 1 (7: pure acetylsalicylic acid).

considered and all expressed in the same (ORAC) units of measure (see Table 4).

Perhaps even more interesting (see Table 5) was the possibility of first expressing and then comparing values of antioxidant capacity found by the three main methods used, all expressed as Trolox equivalent mM concentration, obtained for the six drugs considered, either directly, using the fluorimetric method, or indirectly (that is, using the correlation curve in Fig. 2(a)), with the biosensor method and lastly in a direct fashion using the voltammetric method (that is, comparing the areas of the cyclic voltammograms obtained for Trolox with those recorded for the respective pharmaceutical formulations, or with that of pure acetylsalicylic acid itself), even though the agreement of the values referring to the three considered methods thus determined, is slightly less good than that observed using values obtained by means of the respective correlation curves shown in Fig. 2.

Recently, another differential voltammetric method was proposed [13,14], based on the following: as the mechanism of electrochemical reduction of oxygen dissolved in aqueous solution at a mercury or carbon electrode taking place at the cathode proceeds in several steps with the formation as intermediate of active superoxide radicals, any antioxidant compound present in solution will react with the superoxide, decreasing its concentration at the electrode. The electrochemical reduction current of oxygen decreases and therefore this decrease can be used as a comparative value of the antioxidant capacity of the antioxidant compound being analysed. Although interesting, in the proposed operating

Table 5
Comparison of antioxidant capacity values (all expressed in mM of Trolox equivalents) of the six drugs compounds containing acetylsalicylic acid, obtained using (a) the biosensor method and by means of correlation curve equation in Fig. 2(a), (b) the cyclic voltammetric method and (c) the fluorimetric method

Sample number	(a) Biosensor method (calculated)	(b) Cyclic voltammetry (measured)	(c) ORAC method (measured)
1	1.87	3.85	1.93
2	1.84	3.79	1.88
3	1.73	2.24	1.64
4	1.67	2.07	1.60
5	1.64	1.75	1.52
6	1.34	1.69	1.40
7	1.25	1.58	1.33

The numbers from 1 to 6 refer to the numbering used for these products in Table 1 (7: pure acetylsalicylic acid).

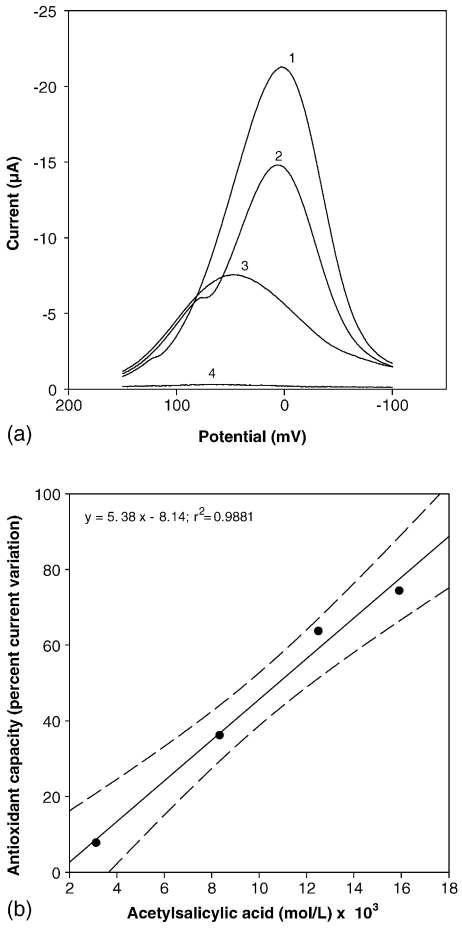

Fig. 3. (a) Differential pulse voltammetric curves, recorded under explained conditions (see Section 3), for increasing (from 1 to 3) acetylsalicylic acid concentration in solution in the presence of dissolved oxygen: curve 4 is recorded after solution deoxygenation by N_2 stream and (b) calibration curve for acetylsalicylic acid by differential pulse voltammetry.

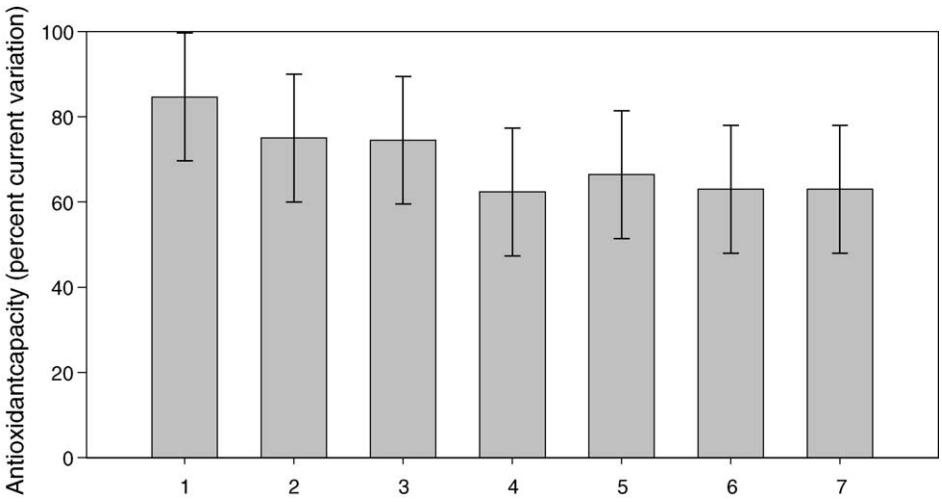

Fig. 4. Comparison of antioxidant capacity trends for the same concentration of acetylsalicylic acid, 1.1×10^{-3} M, of the six different drug products containing acetylsalicylic acid examined and of pure acetylsalicylic acid, obtained using the differential pulse voltammetric method. The numbers from 1 to 6 refer to the numbering used for these products in Table 1 (7: pure acetylsalicylic acid).

conditions [13,14], the method takes a long time to perform (several hours). We are currently evaluating this method after having modified the operating procedure in order to shorten the analysis time (reduced to only 10 min). The method was then applied using differential pulse voltammetry instead of classic slow differential voltammetry. It was thus decided to see whether it was possible to apply the method to determining the antioxidant capacity of the six containing drugs considered as well as that of pure acetylsalicylic acid. To this end the linearity of the method's response to pure acetylsalicylic acid was verified. Fig. 3 shows typical oxygen reduction DPV curves recorded in the absence and in the presence of increasing concentrations of pure acetylsalicylic acid; the same figure also shows the corresponding calibration straight line thus obtained. Lastly, the histogram in Fig. 4 shows the trend in antioxidant capacity obtained using this method for the six drugs considered. Also in this case the trend can be seen to be quite similar to those shown in Fig. 2, which were obtained using the three previously considered methods except for the inversion recorded in the case of products 4 and 5.

5. Conclusions

As far as it concerns the validation of found antioxidant values data, the results obtained in the present work indicate a particularly good correlation between biosensor method and the ORAC spectrofluorimetric method, but also a comparatively good correlation with the cyclic voltammetric method. Overall, as it was found also in previous investigations involving the antioxidant capacity of plant foodstuffs [15,16], beverages [17] and diet integrators [11], the biosensor based method proved to be a reliable method also in the case of the antioxidant capacity of true drug specialities. As well as correlating satisfactorily with the fluorimetric method, which may be considered to some extent as the reference method but which also has the drawback of being extremely expensive, it again proved to be sufficiently precise (RSD = 5% in all RAC measures on drug samples). Lastly, as previously reported [11,16], the method was also found to be sufficiently "rugged" and "robust".

Of course, the trends in the antioxidant capacities of the drugs obtained using these three methods are actually in agreement, even though the differences in antioxidant capacity values found for the same drugs are sometimes found to differ even quite appreciably, in particular when the biosensor and fluorimetric methods are compared to the cyclic voltammetric method. This seems to be due to the fact that, as found on previous occasions [11], the sensitivity of this cyclic voltammetric method can vary to a considerable extent depending on the antioxidant compound analysed. This can perhaps be accounted for by the greater difference found in antioxidant capacity using the cyclic voltammetric method than biosensor method when also ascorbic acid or citric acid are present in pharmaceutical formulations as well as the acetylsalicylic acid. Lastly, the new DPV based method generally seems to be sufficiently in line with the results obtained using the first two methods. However, its precision is not particularly high (RSD > 10%) which probably accounts for the trend inversion found in the case of drugs 4 and 5 and the probable underestimation of the antioxidant activity obtained in the case of drug 2 compared with that of drug 1.

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