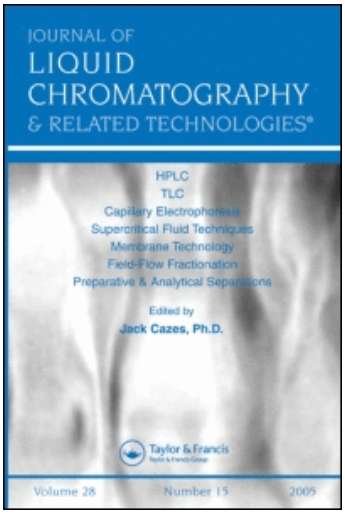

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

The Ion Interaction Reversed Phase High-Performance Liquid Chromatography and the Voltammetry With Microelectrodes In the Analysis of Milk

M. C. Gennaro[a]; P. L. Bertolo[a]; M. A. Baldo[b]; S. Daniele[b]; G. A. Mazzocchin[a]
[a] Dipartimento di Chimica Analitica, Universita' di Torino, Torino, Italy [b] Dipartimento di Chimica Fisica, Universita' di Venezia Calle, Venezia, Italy

To cite this Article Gennaro, M. C. , Bertolo, P. L. , Baldo, M. A. , Daniele, S. and Mazzocchin, G. A.(1991) 'The Ion Interaction Reversed Phase High-Performance Liquid Chromatography and the Voltammetry With Microelectrodes In the Analysis of Milk', Journal of Liquid Chromatography & Related Technologies, 14: 1, 115 — 138
To link to this Article: DOI: 10.1080/01483919108049601
URL: http://dx.doi.org/10.1080/01483919108049601

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(1), 115-138 (1991)

THE ION INTERACTION REVERSED PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND THE VOLTAMMETRY WITH MICROELECTRODES IN THE ANALYSIS OF MILK

M. C. GENNARO[1], P. L. BERTOLO[1],
M. A. BALDO[2], S. DANIELE[2],
AND G. A. MAZZOCCHIN[2]
[1]*Dipartimento di Chimica Analitica*
Universita' di Torino
Via P. Giuria
5 - I- 10125, Torino, Italy
[2]*Dipartimento di Chimica Fisica*
Universita' di Venezia
Calle Larga S. Marta
2137 - I-30123, Venezia, Italy

ABSTRACT

Some anionic species present at the natural pH of milk (6.72), namely hydrogen-orthophosphate, L(+)-ascorbate and hydrogen-citrate were detected in samples of crude bovine-milk both by ion interaction reverse phase HPLC chromatography and by amperometry based on microelectrodes.

The RSD ranged between 2 and 5 % for the former technique and between 3 and 4 % for the latter. The accuracy was evaluated by comparing the data obtained with the two techniques and discrepancies between the methods never exceeded 9%.

INTRODUCTION

Facing with analysis of complex-matrix samples like milk, one would wish to overcome problems connected with the sample pretreatment. The goal would be to carry out measurements directly on the milk sample without any pretreatment. This paper is within this scope. It presents two independent techniques,like reversed phase HPLC based on the use of ion interaction reagents [1-4] and voltammetry employing microelectrodes as working electrodes[5], for determining and monitoring both organic and inorganic species present in milk.

It has been shown [6] that microelectrodes are suitable to this purpose for some electroactive species. On the other hand, to our knowledge, no HPLC method has been previously proposed for analysis of milk without requiring any pretreatment, derivatization or sample preconcentration processes.

It must to be mentioned that the two techniques here employed are becoming increasingly related, since the growing attention towards electrochemical detectors for HPLC and the miniaturization of cells makes microelectrodes, among voltammetric probes, the most attractive [6]. Moreover, the comparison of data relevant to those species (mostly negatively

charged ions at milk pH), which can be determined by the two techniques, gives direct indication of the accuracy of both methods.

As a last remark, it must be emphasized that with the use of these methods, the undeniable advantage of not altering chemical equilibria present in milk is also achieved.

MATERIALS AND METHODS

Apparatus .

Chromatography.

Chromatographic analyses were performed by using a Varian LC 5000 chromatograph,equipped with a Vista 401 Data System and UV-100 spectrophotometric detector.A Wescan 213 A conductometric detector was also employed: 1 V exit interfaced it to the Vista 401 Data System.

The stationary phases used and compared were :Merck Hibar Lichrospher RP-18, 250 x 4, 5 μm, Merck Hibar Lichrospher RP-8,250 x 4, 5μm ,Merck Hibar Lichrosorb C-18,250 x 4, 10μm and Waters Bondapak C-18, 150 x 3.9, 10 μm columns,equipped with a guard column Merck Hibar Lichrocart Lichrosorb RP-18, 25

x 4.It is worth to mention that the Waters Bondapack column is endcapped [7] and characterized by a carbon load percentage of 10 %, whilst the Merck Hibar Lichrosorb is characterized by a carbon load percentage equal to 22% [8].

As previously described [9-12], the solutions of the ion interaction reagents were prepared by dissolving the weighed amount of the amine in ultra-pure water and bringing the solution to a pH 6.7 ± 0.2 by additions of salicylic acid. In these pH conditions, taking into account the acidic formation constants values, the amines are present as protonated ammonium forms.Moreover, even if the composition of the eluents so prepared is not exactly stoichiometric, they will be mentioned henceforth,for simplicity, as ammonium salicylates.

In order to condition the chromatographic system, eluent was allowed to flow through the column until a stable baseline was obtained : generally time of about an hour is required. Eluent solution was prepared every third day.

For sequential analyses in the same conditions of eluent preparation and column conditioning , the reproducibility of retention times was always within 2 % . Anyway,for the sake of the most general validity, standard deviation estimates are evaluated for different preparations.

Between uses, columns were regenerated by flowing a water-methanol 1/1 v/v solution. This treatment assures that no particular deterioration of the column lifetime occurs.

Voltammetry

The preparation of the 12.5 μm disc-electrode was described elsewhere [6] . The reference electrode used was always a saturated calomel electrode (SCE). For the experiments with the microelectrode, a two electrode cell configuration was always employed and the cell was maintained in a Faraday cage made of sheets of aluminium. Linear sweep and cyclic voltammetry were generated by a PAR 175 function generator; a Keithley 485 picoammeter served as a current measuring device and data were plotted by an X-Y (Hewlett Packard 7045 B) recorder.
For pH measurements a Metrohm 605 pH-meter was used.

Reagents.

Ultra-pure water from a Millipore Milli-Q was used for the preparation of solutions. Heptylamine , octylamine, decylamine, lithium orotate and caseine from bovine milk were Fluka

analytical grade reagents.Hyppuric acid was a Merck chemical. Salicylic acid and all other reagents were Carlo Erba analytical grade chemicals. Nitrogen 99.99 % from SIAD was used for deoxygenation in the electrochemical experiments.

Preparation of samples of milk.

Most bovine milk samples were kindly supplied by Parmalat s.p.a. , Parma, Italia.

The voltammetric analyses were usually performed on the samples as withdrawn from the pack .About 20 ml of milk were transferred into the cell for the measurement and maintained under nitrogen atmosphere.

For HPLC analyses, samples were filtered through Nucleopore Syrfil 25 mm 0.45 μm filters and ,for comparison, some experiments were also carried out after centrifugation at 4000 rpm : it was so possible to exclude side effects caused by cream.

Dilutions of at least 1/5 v/v were required. The analyses for samples at different dilutions permitted to exclude appreciable dilution effects.

RESULTS AND DISCUSSION

Ion Interaction Reagent Reversed Phase High-Performance Liquid Chromatography.

In previous works [9-12], the use of the ion interaction reagent reversed-phase chromatography has been shown to be very advantageous in the analysis of anions (both organic and inorganic) and of amines. Commercial reversed-phase columns were used as the stationary phases and salicylates of aliphatic amines of different chain length as the interaction reagents.

Since the salicylate anion is characterized by relatively low ionic equivalent conductivity and by relatively high absorptivity in the UV region ($\varepsilon = 308 \pm 2$ 1 $mol^{-1}cm^{-1}$ at λ = 254 nm), its use as the interaction reagent allows conductometric and spectrophotometric ,both direct and indirect, detections.It has also been shown the relevant effect played on retention and resolution by different parameters and chromatographic conditions (such as the length of the alkyl chain of amine , the concentration of the interaction reagent, its flow-rate, the packing of the stationary phase ,as well as the kind of detection)[9].

In order to find out the optimal conditions for the analysis of anionic species present in milk, in this paper the use of salicylates of heptylamine, octylamine and decylamine as the ion interaction reagents (flowing at different flow-rates) was compared .Stationary phases characterized by reverse phase C-18 and C-8 packings, spherical and irregular , with 5 and 10 μm particles and different carbon load percentage and end-capping degree were used.

The analytes considered were inorganic and organic anions likely present in milk , namely chloride,ortho-phosphate, nitrate, formate,lactate, L(+)-ascorbate, iodide, orotate, citrate, maleate, tartrate, fumarate, hyppurate.

The results confirmed , as previously [9,11] evidenced, that a longer alkyl chain of amine of the interaction reagent causes higher retention of anions. For this reason, for instance, the use of decylammonium salicylate seemed at first the most suitable for the separation of anions characterized by lower retention such as acetate, formate and lactate. But, on the other hand, the sensitivity offered by this eluent resulted to be very lower with respect, for instance, to that achievable with octylammonium salicylate. And this last, in turn ,allowed lower sensitivity

levels when compared with the use of heptylammmonium salicylate. The role played by the alkyl chain length on sensitivity has been already observed [11] but has not yet found explanation.

As it concerns the effect on retention due to stationary phase packings,it has been observed that greater retentions correspond to stationary phases characterized by spherical packings (with respect to irregular ones) and by the highest carbon load percentage. These parameters likely affect the degree of the so called " functionalization" induced onto the column by the flowing interaction reagent.

The most suitable reagent was shown to be octylammonium salicylate . Table 1 lists the retention times obtained (with the use of this interaction reagent and different stationary phases) for the principal inorganic and organic components identified in the bovine-milk samples investigated.

The chromatograms of figures 1-3 present typical examples of the above considerations. Figure 1 shows , for comparison,the chromatograms of a sample of fresh whole milk (figure 1a) and of a UHT-sterilized-skim one (figure 1b) (both diluted 1/10 v/v) recorded under the same chromatographic conditions (i.e. a Lichrospher C-8 5 μm column, octylammonium salicylate 0.005 M as

TABLE 1

Retention Times of some Anions present in Milk, obtained with the use of 0.005 M Octylammonium Salicylate (Flow-rate = 1.0 ml/min) as the Interaction Reagent and different Reversed Phase Stationary Phases.
Standard Deviation Estimates are based on at least Four Replicates.

	Merck Lichrospher C-18 ,5 μm	Merck Lichrospher C-8,5 μm	Merck Lichrosorb C-18,10μm
chloride	5.7 ±0.3	5.9 ±0.3	6.1 ±0.3
L(+)-ascorbate	6.2 ±0.2	6.2 ±0.2	5.6 ±0.3
iodide	6.7 ±0.2	6.7 ±0.2	
orotate	7.9 ±0.4	8.4 ±0.3	6.2 ±0.3
o-phosphate	10.0 ±0.5	9.5 ±0.4	8.0 ±0.3
citrate	13.0 ±0.5	14.2 ±0.5	12.5 ±0.4
maleate		25.2 ±0.5	
fumarate		32. ±1.	
hyppurate		>50.	

eluent, flow 1.0 ml/min and spectrophotometric detection). In both samples chloride, orotate,orthophosphate and citrate are present, whilst L(+) -ascorbate and iodide are present only in the fresh milk (figure 1 a), accordingly to their likely degradation during the sterilization processes.

Due to UV detection (λ=254 nm) , chloride, orthophosphate and citrate, which are characterized at this wavelength by null absorptivity, come out as negative peaks, whilst L(+)-ascorbate,

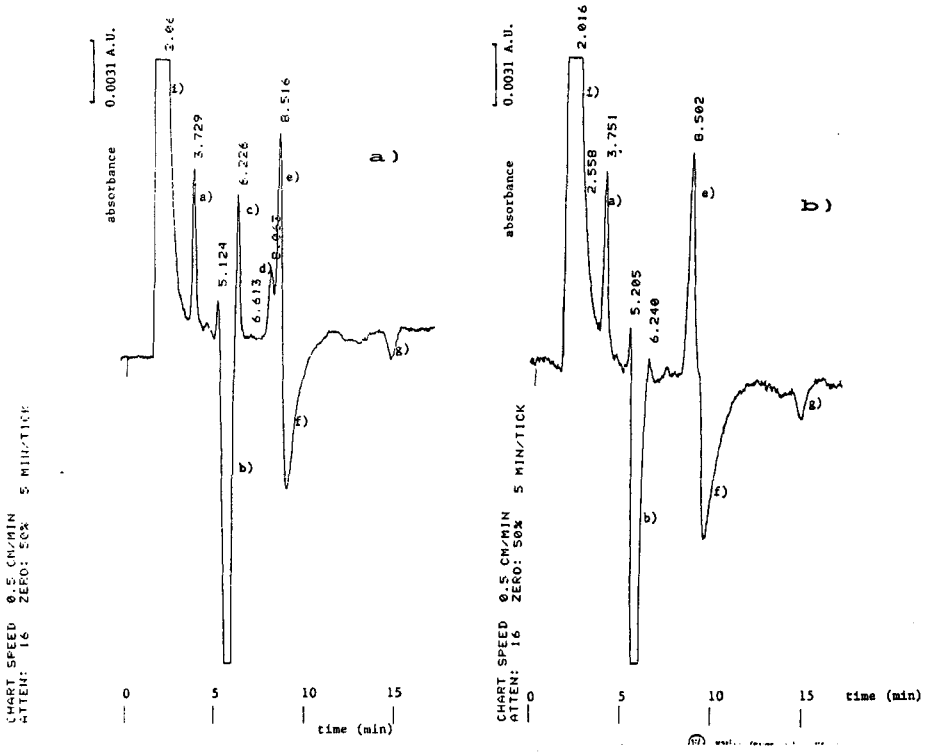

FIGURE 1. Comparison between chromatograms recorded for different samples of milk, diluted 1/10 v/v, under the same chromatographic conditions.
a) fresh whole milk b) UHT-sterilized skim milk
Stationary phase: Merck Hibar Lichrospher RP-8 , 5 μm
Ion Interaction Reagent : Octylammonium salicylate 0.0050 M
Flow-rate : 1.0 ml/min. Injection : 100 μl. Spectrophotometric detection : λ =254 nm.
Peaks :i)injection peak, a)unidentified ,b)chloride, c)ascorbate, d)iodide, e)orotate, f)orthophosphate, g) citrates.

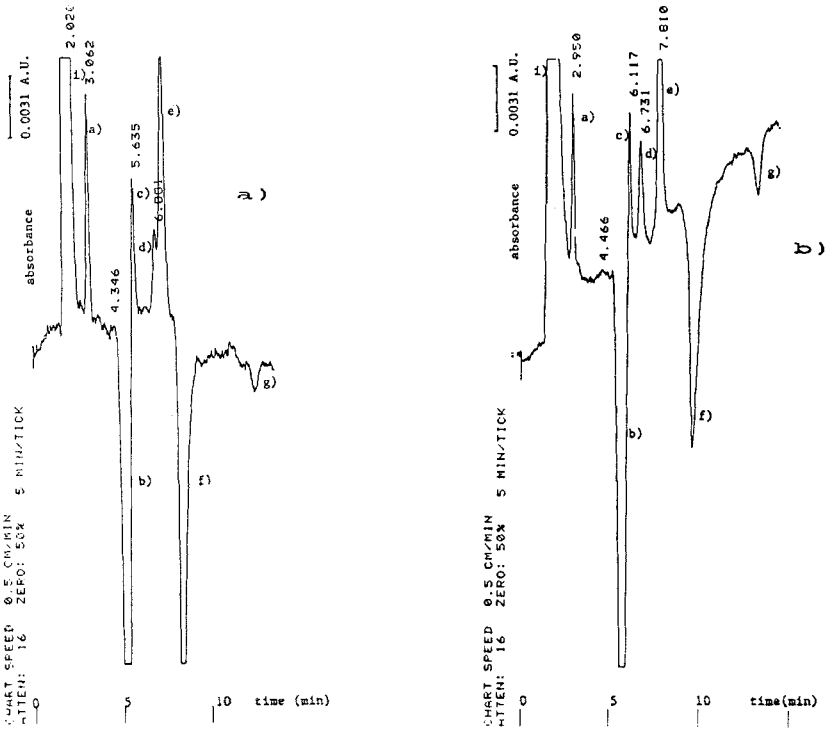

FIGURE 2. Comparison between typical chromatograms recorded for the same sample of fresh whole milk (diluted 1/10 v/v) with different stationary phases.
a) Stationary phase: Merck Hibar Lichrosorb RP-18 , 10 µm
b) Stationary phase: Merck Hibar Lichrospher RP-18 , 5 µ m
Ion Interaction Reagent : Octylammonium salicylate 0.0050 M
Flow-rate : 1.0 ml/min. Injection : 100 µ l. Spectrophotometric detection : λ=254 nm.
Peaks : i)injection peak, a)unidentified ,b)chloride, c)ascorbate, d)iodide, e)orotate, f)orthophosphate, g) citrates.

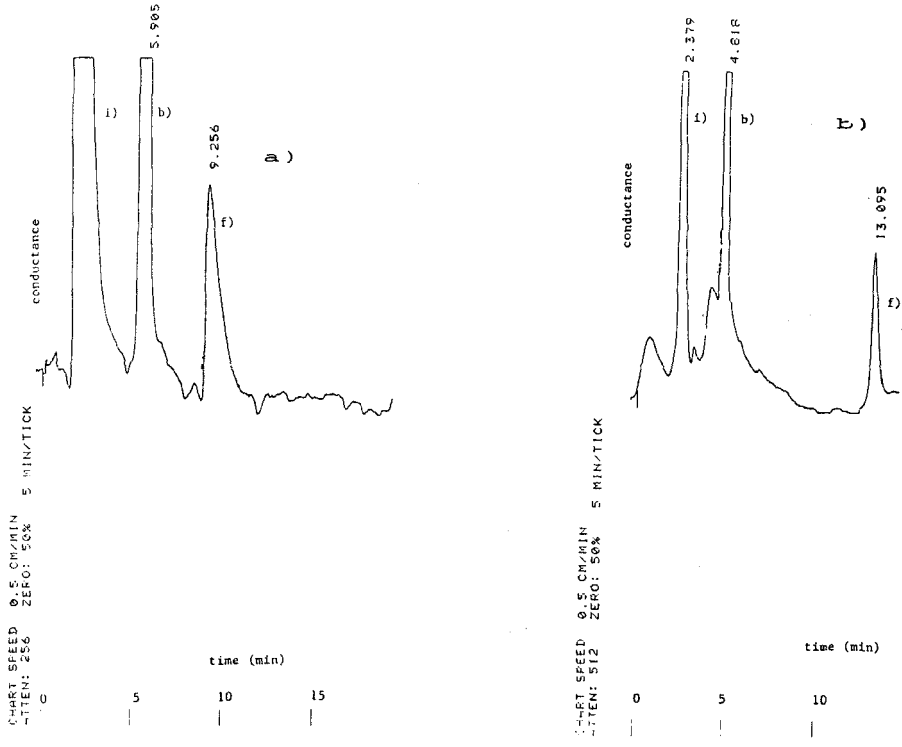

FIGURE 3. Comparison between typical chromatograms recorded for the same sample of UHT-sterilized skim milk,(diluted 1/10 v/v), under different chromatographic conditions and conductometric detection. Injection : 100 µl.
Peaks :j) injection peak, b)chloride,f) orthophosphate
FIGURE 3a)Stationary phase: Merck Hibar Lichrospher RP-8 , 5 µm
Ion Interaction Reagent : Octylammonium salicylate 0.0050 M
Flow-rate : 1.0 ml/min. Dilution of the milk sample:1/10 v/v.
FIGURE 3 b) Stationary phase : Merck Hibar Lichrospher RP-18,5 µm
Ion Interaction Reagent :Heptylammonium salicylate 0.0050 M
Flow-rate : 0.8 ml/min. Dilution of the sample milk :1/50 v/v.

iodide and orotate (whose absorptivity values, evaluated for λ =254 nm ,are respectively $\varepsilon_{ascorbate}$ = $(8.72 \pm 0.04)\ 10^3$, ε_{iodide}= $(2.21 \pm 0.02)\ 10^2$ and $\varepsilon_{orotate}$ =$(3.09 \pm 0.02).10^3$ 1 $cm^{-1}mol^{-1}$) give rise to positive peaks.

Figure 2 shows ,for a comparison between the use of different stationary phases, the chromatograms recorded on the same sample of fresh whole milk ,by using the same interaction reagent (octylammonium salicylate 0.005 M at flow-rate = 1.0 ml/min) and columns with different packings , namely a C-18 Lichrosorb 10 μ m (figure 2a) and a C-18 Lichrospher 5 μ m (figure 2 b).As expected, the column characterized by spherical packing allows higher retentions and a better separation between iodides and orotates.

Interesting comparisons are offered furthermore by the chromatograms of figure 3, recorded by using conductometric detection : chloride and orthophosphate can be easily detected and separated from the other anionic species characterized by low conductivity. The use of 0.0050 M octylammonium salicylate (figure 3a) and heptylammonium salicylate (figure 3 b) as the interaction reagents was compared. The greater sensitivity offered by heptylammonium salicylate can be observed : notwithstanding figures 3a and 3b are comparable in shapes, the

TABLE 2

Concentrations of some Anionic Species determined on Bovine Milk samples by using the Ion Interaction Reagent Chromatography.

species	concentrations (mM) fresh whole milk		UHT skim milk	
L(+)-ascorbate	0.08	(5.5)	-	
orotate	0.12	(2.7)	0.13	(3.1)
ortho-phosphate	13.50	(3.7)	11.40	(4.2)
citrate	14.30	(2.9)	23.25	(2.1)
chloride	7.70	(3.8)	7.43	(4.0)

( ) RSD % from almost three replicate determinations.

sample of milk was diluted 1/50 v/v when using heptylammonium salicylate(figure 3b) and only 1/10 when using octylammonium salicylate (figure 3a).

Due to the good linear trend of peaks area as a function of concentration, the quantitation of anionic species in milk was carried out. The results obtained with conductometric detection were used for the evaluation of chloride, orthophosphate and citrate and spectrophotometric detection at 254 nm was prefered for the quantitation of L(+)ascorbate and orotate. Some measurements performed on milk with known added amounts of the investigated anions permitted to exclude appreciable matrix effect on the determination of these species. Table 2 lists

the results obtained in samples of whole fresh milk and of skim milk. The estimates of standard deviation are calculated from at least three independent replicates.

Amperometric Determination by using a Platinum Microelectrode.

In a previous paper it has been shown that with a microelectrode useful information have been gained about the electrochemical processes of anionic electroactive species present in milk (6). In particular, it has been demonstrated that the small anodic wave reported in figure 4 ,recorded with a platinum microelectrode on untreated milk samples, is due to the oxidation of ascorbate ion and the catodic peak, made up of two overlapping waves, is due to the reduction of protons delivered by acidic species, mainly $H_2PO_4^-$, at more negative potentials and acids of water-soluble casein at less negative potentials.

To quantify the total amount of the species giving rise to the process (I) , amperometric titrations were done by using NaOH and HCl.

In the milk alkalimetric titrations, the decrease of the peak-current height of the whole peak (I) was recoreded as a

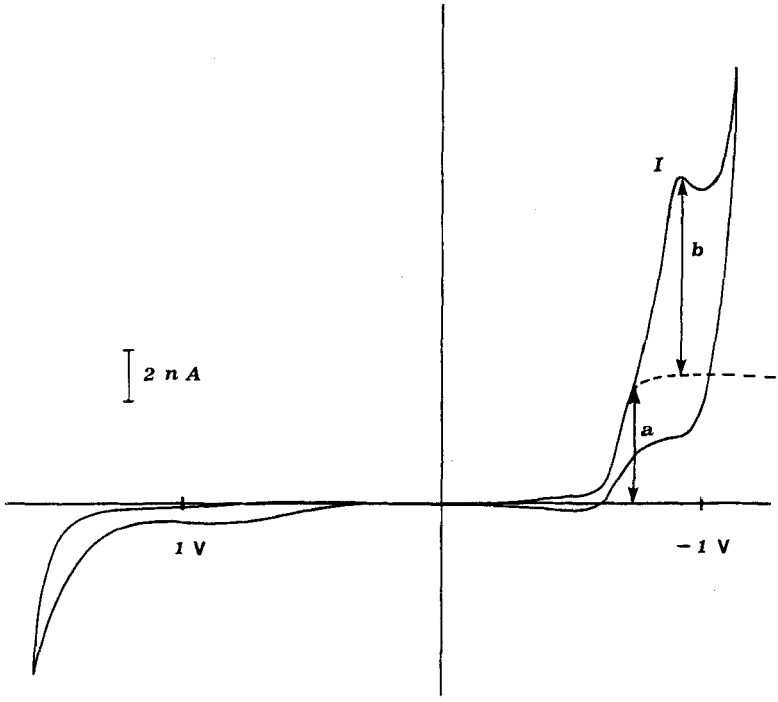

Figure 4. Cyclic voltammogram recorded at platinum microelectrode, 25 μm diameter, on an untreated fresh whole bovine milk sample. Scan rate 5 mV s^{-1}.

function of the amount of the NaOH added. Straight lines with an average coefficient of correlation of 0.997 were obtained for concentrations of NaOH added ranging between 0.5 and 4.0 mM. The end points were then found by the extrapolation to the zero-current ascissa of these straight lines obtained in the titrations. From the titration curves of a series of pasteurized

whole milk samples , the amount of NaOH drawn out from the end points ranged between about 0.25 and 0.30 mmoles per 20 ml of milk corresponding to about 13 and 15 mol of NaOH per 1 of milk. The so obtained values were compared with those determined by using the classical titration method with phenolphtalein as the indicator. With this last method the titrable acidity resulted higher of about 25% than the amperometric one.These discrepancies are probably due to the low precision and accuracy in getting the end point, owing to the difficulty to gather the colour change of the phenolphtalein in a matrix like milk.Conversely, the amperometric titration provides a precision fairly good as shown by the relative standard deviation of 1.5%.

The addition in milk of an external acid characterized by a suitable value of the dissociation constant [6] allows the increase of peak (I) to be observed. This is because acid-base equilibria take place so generating the species reducible at peak (I) [6].

In order to get linear trends ,the concentration of the spiking acid was kept between 0.5 and 3.0 mM. Higher concentrations of acid would have led to a deviation from linearity. A typical equation for the plot current vs.

concentration of HCl is : $i(nA) = 12.9 + 0.996\ C$ ($r=0.997$). The value of acidity thus obtained, compared with that found by amperometric titration with NaOH , gave differences not greater than 4 %.

The contribution of each of the main species (di-hydrogen-phosphate and casein) to the catodic peak (I) of figure 4 was evaluated from the current heights (a) and (b), on the basis of the additivity of responses obtained under steady-state conditions , as are those achievable with microelectrodes by linear sweep voltammetry at low scan rates[5].

Moreover the determination of casein was done by making the approximation that the current height (a) of the shoulder corresponds to the limiting current value of its relevant process, while the concentration of hydrogen-phosphate was evaluated by subtracting the current-height (a) from the overall current-height of peak (I).

Moreover, if we assume that the acid-base equilibria occurring in milk can be treated as in simple aqueous solutions, it is possible to gather further quantitative information on speciation. In particular, the relative amounts of phosphate

TABLE 3

Concentrations of some Anionic Species and Titrable Acidity determined on a Fresh Whole Bovine Milk sample by using both Interaction Reagent Reversed Phase Chromatography and Microvoltammetric Electrodes.

species	concentration (mM)	
	HPLC chromatography	microvoltammetric electrode
L(+)-ascorbate	0.13 (4.7)[a]	0.14 (3.1)[b]
ortho-phosphate	15.5 (3.4)[a]	16.7 (3.7)[b]
citrate	18.8 (2.5)[a]	-
titrable acidity	-	14.5 (1.5)[b]

( ) : RSD% : [a] from three replicate determinations, [b] from five replicate determinations respectively.

species can be evaluated by exploiting the dependence of the distribution of the phosphoric acid species as a function of pH. Thus at pH around 6.7 the following equations hold :

$$C = [\bar{H}_2PO_4^-] + [\bar{H}PO_4^{2-}] \quad (1)$$

$$\alpha_1 = [\bar{H}_2PO_4^-] / C \quad (2)$$

$$\alpha_2 = [\bar{H}PO_4^{2-}] / C \quad (3)$$

At pH 6.72 : $\alpha_1 = 0.602$ and $\alpha_2 = 0.398$

Since the concentration of $H_2PO_4^-$ can be experimentally determined, HPO_4^{2-} is straightforwardly evaluated.

Comparison between Voltammetric and HPLC Results.

The validity of the mentioned methodologies was tested by comparing the data obtained with the two techniques, on the same fresh whole milk at its natural pH of 6.72.

Table 3 lists the results obtained.Agreement within 9% and 8% can be observed for the L(+)-ascorbate and orthophosphate,

On the basis of the pH value and by considering the species distribution coefficients corrected for the average ionic strength of milk (79 mM) , the concentrations of protonated phosphates and citrates can be drawn. Thus the contributions to total phosphate amount from HPLC technique are : $[\bar{H}_2PO_4^-]$ = 9.3 mM, $[\bar{H}PO_4^{2-}]$ =6.2 mM and for citrate : $[\bar{H}Cit^{2-}]$ = 0.3 mM; from voltammetry $[\bar{H}_2PO_4^-]$ = 10.1 mM and $[\bar{H}PO_4^{2-}]$ = 6.65 mM.

These findings support the hypothesis advanced in the previous sections about the method to quantify hydrogen-phosphates by voltammetry.

No similar comparison can be made for citrate, because the $HCit^{2-}$ species, which is present at pH 6.72 (α = 0.016), in voltammetry gives the reduction process over the same potential range of acids from casein.

However ,in view of the good agreement found for the other species, the chromatographic data can be employed to calculate the contribution of citric acid to the first process of the cathodic peak reported in Figure 4 . By subtracting from total acidity the contributions due to phosphoric and citric acids, that coming from casein is 4.1 mM.

These data are in good agreement with average values reported in literature (13).

It must be noted that here only the major components have been considered. Other acids which could be electroactive over the potential range investigated and present in milk are at concentration levels which do not contribute significantly to the total acidity.

CONCLUSIONS

The combination of microvoltammetric electrode with interaction reagent chromatographic technique provides good results in the determination of organic and inorganic anionic species in milk. The measurements can be made directly on the crude sample at its natural pH. Information on speciation can be obtained from the evaluation of the total acidity through proper comparison of the data gained by means of both techniques.

This approach can open new prospects for achieving insights on very complex matrices as milk and performing methodologies to be applied in quality control processes.

ACKNOWLEDGMENTS

This work has been supported by a financial aid from "Progetto Strategico Caratterizzazione e Controllo degli Alimenti",Consiglio Nazionale delle Ricerche (Rome, Italy).

REFERENCES

1. Barber W.E., Carr P.W., UV visualization of inorganic anions by reversed-phase ion-interaction chromatography :factors that control sensitivity and detection, J.Chromatogr.,316 , 211 , 1984

2. Dreux M., Lafosse M., Agbo-Hazoume P., Chaabane-Doumandji B., Gilbert M. , Levi Y., Determination of inorganic anions by ion-pair chromatography.Hetaeron adsorption characteristics on some alkyl-bonded silica stationary phases, J.Chromatogr.,354 , 119, 1986

3. Bidlingmeyer B.A., Santasania C.T., Warren Jr.F.V. ,Ion-pair Chromatographic determination of anions using an ultraviolet-absorbing co-ion in the mobile phase, Anal.Chem.59, 1843 (1987)

4. Lookabaugh M., Krull I.S., LaCourse W.R.,Determination of iodide and thiosulphate by paired-ion, reversed-phase high-performance liquid chromatography with ultraviolet absorbance, electrochemical and conductimetric detection, J.Chromatogr.,387,301 (1987)

5. Fleischmann M., Pons S., Rolison D.R., Schmidt P.P., Ultramicroelectrodes, Datatec System, Morganton N.C.,1987

6. Daniele S., Baldo M.A., Ugo P., Mazzocchin G.A., Voltammetric probe of milk samples by using a platinum microelectrode, Analytica Chim.Acta, in press

7. Lullmann C., Genieser H.G., Jastorff B.,Structural investigations on reversed-phase silicas.II.Evidence for endcapping trimethylsilyl groups , J.Chromatogr.,354, 434, 1986

8. Majors R.E., Recent advances in HPLC packings and columns, J.Chromatogr.Sci., 18, 488 (1980)

9. Gennaro M.C. , Role of the alkyl chain length of the ion interaction reagent,flow-rate, column packing and detection in ion interaction reversed-phase high-performance liquid chromatography in separations of anions using amine salicylates, J.Chromatogr. , ,449 ,103 (1988)

10. Gennaro M.C., Bertolo P.L.,Separation of N-containing species by means of a new versatile ion-interaction RP-HPLC methodology, J. Liq.Chromatogr. ,in press

11. Gennaro M.C., Bertolo P.L., Determination of the principal components in wines and soft drinks, by ion interaction reversed-phase high-performance liquid chromatography, J.Chromatogr.472, 433 , 1989

12. Gennaro M.C., Bertolo P.L., L-ascorbic acid determination in fruits and medical formulations by ion interaction reagent reverse phase HPLC technique, J.Liq. Chromatogr. 13,1419 (1990)

13. Walstra P., Jenness R., Dairy Chemistry Physics, Wiley, New York ,1984