Determination of *Escherichia coli* Negative Charge Concentration From XPS Data and Its Variation with pH

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The negative charge concentration, the surface functional groups, the molecular composition, in terms of proteins, polysaccharides and hydrocarbon-like of three *Escherichia coli* strains cultivated in different media as well as the electrophoretic mobility were investigated. These characteristics were generally influenced by the composition of culture medium. The negative charge concentration considered as the sum of [$RCOO^-$] and [$R_2PO_4^-$] at the cell surface was estimated by using the acid-base equilibrium and the concentration of chemical functions. The latter was computed using the surface chemical composition given by X-ray Photoelectron Spectroscopy and the carbon concentration of each constituent (Proteins, Polysaccharides and hydrocarbons-like). A high correlation between negative charge concentration and the electrophoretic mobility was found suggesting that the carboxyl and phosphate groups contribute to the negative charges present at *Escherichia coli* cell surface. Moreover, the concentration of both [$RCOO^-$] and [$R_2PO_4^-$] groups determined at pH 7 and pH 3 showed a clear difference between the two groups. This suggested that the contribution of each group was pH-dependent. A high correlation with 1/1 ratio was obtained at pH 3 between the sum of surface concentration of anions ($RCOO^-$, $R_2PO_4^-$) and the sum of surface concentration of cations (Na^+, K^+, NH_4^+) indicating that these positive charges are the counter-ions of these negative charges. Therefore, this data suggested that at this pH, the carboxylate and the phosphate groups were the sole source of the negative charge on *Escherichia coli* cell surface.

1. INTRODUCTION

The surface charge properties of bacterial cells are known to play an important role in interfacial phenomenon such as microbial adhesion to surface (cell-substratum) and aggregation phenomenon (cell-cell interactions). Several works (Amory et al.[1], Cowan et al.[2], Cuperus et al.[3], Latrache et al.[4], Mozes et al.[5,6], Van der Mei et al.[7,8]), have reported some relations between the surface charge of a number of microbial strains and the overall chemical surface composition of the cells as probed by X-ray Photoelectron Spectroscopy (XPS). Different methods have been proposed to characterize this surface charge including electrostatic interaction chromatography, Pedersen[9], aqueous two-phase partitioning, Liang et al. [10], Magnusson et al.[11], acid-base titration, Van der wal et al. [12], and microelectrophoresis, Harkes et al. [13], Latrache et al.[14], Van der Mei et al.[15]. Previous studies, Van der wal et al. [12], James [16], reported that the surface charge could be originated from acidic and basic groups such as carboxyl, phosphate, and amino groups. Dengis and Rouxhet [17] have proposed that the surface charge concentration may be deduced from acid-base equilibrium using the concentrations of chemical functions. The latter was estimated using the surface chemical composition given by XPS and the carbon concentration of each constituent: protein, polysaccharide and hydrocarbon-like.

In this work, we used the same method reported by Dengis and Rouxhet [17], to estimate the concentration of chemical functions of three strains of *Escherichia coli* (*E. coli*) cultivated in different conditions and to follow the concentration of anionic groups $RCOO^-$ and $R_2PO_4^-$ at different pH. The negative charge concentration is considered as the sum of the concentration of [$RCOO^-$] and [$R_2PO_4^-$]. In this study, we also examined the correlation between the concentration of negative charge ($RCOO^-$ and $R_2PO_4^-$) and the surface charge determined by microelectrophoresis, as well as the correlation between the sum of anionic groups ($RCOO^-$ and $R_2PO_4^-$) and the sum of cationic groups (Na^+, K^+, NH_4^+) at different pH.

2. Materials and Methods

Bacteria

Three *E. coli* strains were used: (i) HB101, a K12 strain, non-pathogenic, (ii) 382 and (iii) AL52, both isolated from patients with urinary tract infections. Each strain was grown either in liquid Luria Bertani medium (LLB) or on solid Luria bertani agar (SLB). For testing the effect of medium composition, the 382 strain was also grown in liquid minimum medium (LMin), composed of 22 mM KH_2PO_4, 48 mM $Na_2HPO_4.2H_2O$, 9 mM NaCl, 19 mM NH_4Cl, 0.5 mM $MgSO_4.7H_2O$, 0.07 mM $CaCl_2.2H_2O$, 0.02 mM $FeSO_4.7H_2O$ and 28 mM D-glucose. Bacteria were cultured at 37°C in two steps: preculture for 18 h and culture for 22 h.

Microelectrophoresis

The electrical properties of the bacteria were characterized by measuring their electrophoretic mobility. The cells were washed twice with 0.9% NaCl solution, and treated with 1% formol solution during 20 min at room temperature to eliminate the bacterial motility. The formol was removed by centrifugation and the bacteria were suspended in distilled water. A portion of this suspension was diluted in 10^{-3} M KNO_3. The pH was adjusted by HNO_3 or KOH and the electrophoretic mobility was determined with a zetameter Zm77 (Zeta-meter Incorporation, New York).

XPS analysis

The surface chemical composition was analysed by XPS. Bacteria were harvested after 22 h of growth (late stationary phase). Collected by centrifugation (35000 min), suspended in distilled water and washed twice by successive resuspensions and centrifugations. The pellet of the last centrifugation was transferred to glass vial, frozen in liquid nitrogen and kept at -80°C until freeze drying (Lyovac GT4 Thermovac TM, Leybold Heraeus). The samples were placed on the precooled (-50°C) shelf of the lyophilizer, evacuation was started and when the pressure reached 60 Pa, the temperature was raised to -10°C; lyophilization lasted about 18 h. The powder of freeze-dried cells was mounted in a stainless steel trough and pressed to obtain a macroscopically smooth surface. The analyses were carried out in an SSX-100 spectrometer (model 206) of surface science instruments, interfaced with a Hewlett- Packard 9000/310 computer allowing instrument control, data accumulation and data treatment. The X-rays were generated from a monochromatized aluminum anode, the pressure in the chamber during analysis was about 10^{-6} Pa, the flood gun energy 6 eV, and the pass energy of the analyser 50 eV. Resolution, determined on a gold standard, (FWHM Au $4f_{7/2}$) was 1.0 eV. The size of the analysed area was about 0.5 mm^2 or 1.4 mm^2. The order of peak analysis was C1s, O1s, N1s, P2p, K2p, Na1s, S2p, C1s. The duplication of C1s registration at the end provided an estimate of sample degradation under X-ray irradiation during the period of data accumulation (about 3.5 h). The binding energies were calibrated with respect to the C-(C,H) component of the C1s peak set at 284.8 eV. Atom fractions were calculated from the peak areas normalized after non-linear background subtraction, and with sensitivity factors supplied by the spectrometer manufacturer. Major complex peaks were decomposed using a least-squares best fitting routine with a Gaussian /Lorenzian ratio of 85/15, employing literature information concerning component binding energies and fixing the full width at half maximum height at a constant value for all the components of a given peak. Samples of silica (quartz Sikron SF800, or Silica 27620298 from Prolabo) were prepared in parallel with the bacterial samples (centrifugation, freezing, lyophilization) and analyzed by XPS in order to evaluate the amount of contaminating carbonaceous compounds.

All the XPS analyses were performed in duplicate (two independent cultures for each condition). The atomic concentration ratios were calculated by correcting the intensity ratios with experimental sensitivity factors computed by Wagner et al.[18]. The confidence interval of atomic concentration ratios is typically of the order of 10 to 20% [19]. The reproducibility was satisfactory; variations between duplicates were in the range of 1% for carbon, 5% for oxygen, up to 10% for nitrogen and phosphorus and up to 24%

for the minor elements.

The functional composition was obtained by the decomposition of complex XPS peaks (Fig. 1). The carbon peaks were generally decomposed into three components, attributed to carbon bound only to carbon and hydrogen, C-(C,H), at a binding energy of 284.8 eV; to carbon singly bound to oxygen or nitrogen, C-(O, N), including alcohol or amine, at binding energy of 286.2 eV; and to carbon making two single bonds with oxygen, C=O, including carbonyl, carboxylate, amide etc, at a binding energy of 287.9 eV. The

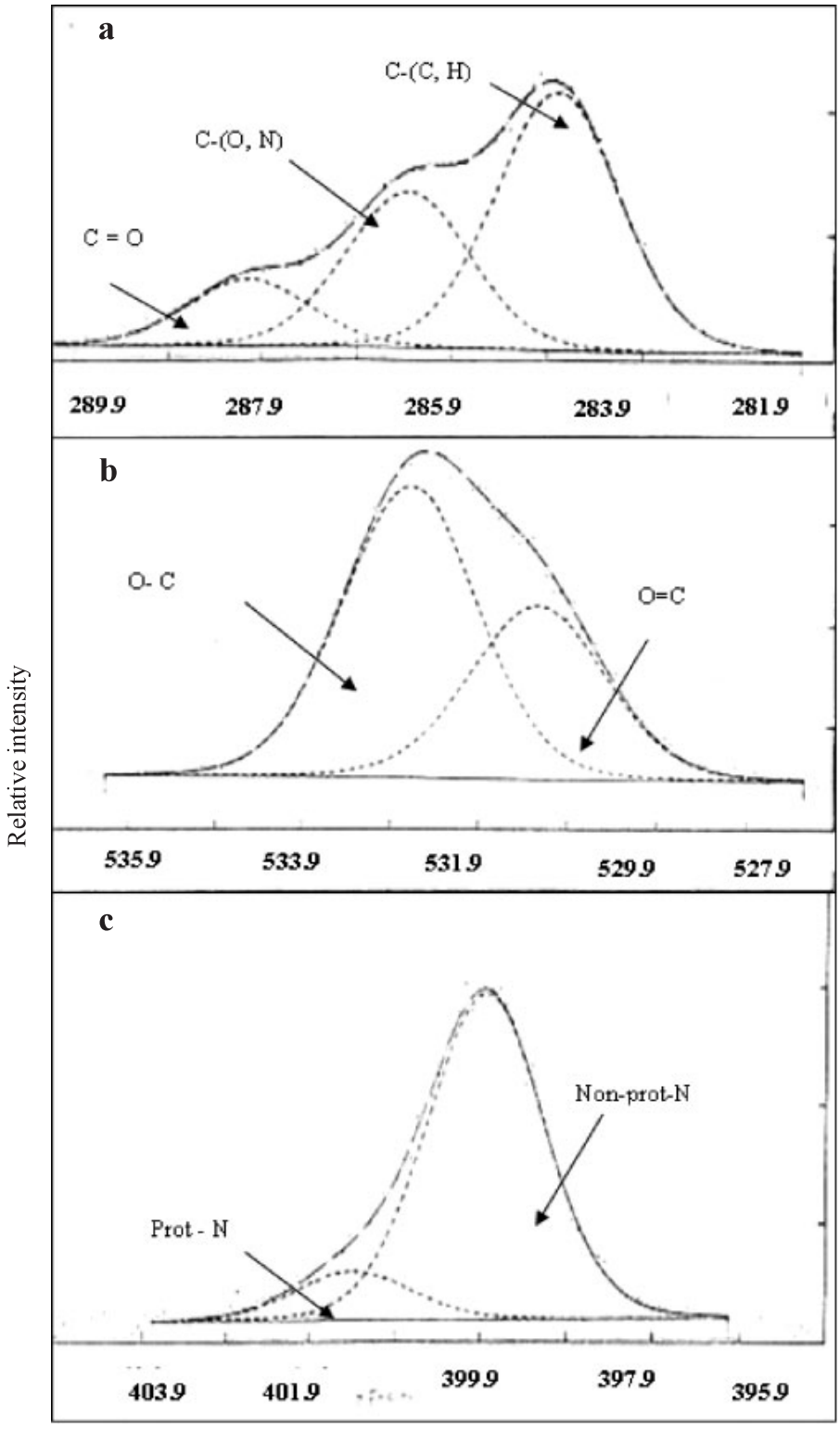

Fig. 1: XPS spectra of HB101 cells: a, C1s; b, O1s; c, N1s

oxygen peak was decomposed into two components. The first one, noted O-C, was attributed to hydroxide, C-(OH), and acetal or hemiacetal, C-O-C functions, at a binding energy of 523.6 eV, and the second one was attributed to oxygen making a double bond with carbon O=C, in carboxylic acid, carboxylate, ester, carbonyl, or amide, at a binding energy around 531.2 eV. The nitrogen peaks were decomposed in two components, attributed to nonprotonated nitrogen (Nnonproton at binding energy of 399.9 eV, involved in amine or amide) and to protonated nitrogen (Nproton at binding energy of 401.6 eV, involved in ammonium ion). The phosphorus peak appearing at 133.4 eV was attributed to phosphate groups.

Surface molecular composition.

Using the (C-(C, H)/C), (C-(O, N)/C) and (C=O/C) ratios given by XPS (Table 2) and the chemical composition of model constituents, Dufrêne et al. [20], (Table 1), the molecular composition of *E. coli* cell surface was modelled in terms of polysaccharide (ps), proteins (pr), and hydrocarbon-like (HC), Dufrêne and Rouxhet [21], Rouxhet et al. [22]. The molecular composition was computed with the following equations based on the three components of the carbon peak, Dufrêne and Rouxhet [21], Rouxhet et al. [22]:

$[(C=O/C)]_{obs} = 0.279(C_{pr}/C) + 0.167\ (C_{ps}/C)$

$[(C-(O,N))/C]_{obs} = 0.293(C_{pr}/C) + 0.833\ (C_{ps}/C)$

$[(C-(C,H)/C]_{obs} = 0.428\ (C_{pr}/C) + 1\ (C_{HC}/C)$

Solving the systems of equations provides the proportion of carbon associated with each molecular constituent: (C_{pr}/C), (C_{ps}/C), and (C_{HC}/C). These proportions can be converted into weight fractions, using the carbon concentration of each model constituent (Table 1).

Estimation of the concentration of RCOOH and of R_2PO_4H.

In order to estimate the concentration of phosphate groups [R_2PO_4H], and of carboxyl groups [RCOOH] in mol/g, it is necessary to know the P/C and the COOH/C ratios respectively. It is known that the P/C is given directly by XPS data, but no accurate XPS data were available for carboxyl groups. Therefore, in this work we attempted to estimate the percentage of carboxyl groups only from XPS data. It is known that oxygen making a double bond with carbon O=C at a binding energy around 531.2 eV includes carboxylic acid, carboxylate, ester, carbonyl or amide. On the other hand, previous data obtained on phosphate containing compounds such as $ALPO_4$, $CaHPO_4$, KH_2PO_4 and Glucose – 6-phosphate, showed that O_{1s} peak of phosphate has two components appearing in the range of 530.9 to 531.5 and 532.4 to 532.9 eV respectively. The low binding energy peak is attributed to P=O or P-O and the high binding energy peak is attributed to P-OH or P-O-C (for glucose-6-phosphate) [23]. In the case of gram negative bacteria as *Escherichia coli*, the phosphate groups are involved in phospholipids. In this latter, two oxygen atoms of phosphate are singly bound to carbon and are expected to contribute to the component at 532.6 eV. Otherwise, one oxygen is doubly bound to phosphorus, and one is in the form P-O; these are expected to contribute to the component at 531.2 eV, together with O=C. If we neglect the percentage of ester and carbonyl involved in the component O=C we can consider that the subtraction of 2 P and the function O=C include in the amide, from the O=C, could give a relative percentage of carboxylic acid:

$COOH = (O=C) - 2P - (O=C)_{amide}$.

Moreover, the N1s component at 399.9 eV was due to non-protonated nitrogen (Nnp) like amine or amide.

Table 1 Chemical composition of model constituents considered for the deduction of molecular composition of gram negative bacteria

Constituent	C-(C,H)/C	C-(O,N)/C	C=O/C	carbon concentration (mmol/g)
Protein	0.428	0.293	0.279	43.5
Polysaccharide	0.000	0.833	0.167	37
Hydrocarbon	1.000	0.000	0.000	71.4

Table 2 Surface chemical composition from three *E. coli* strains cultivated under different conditions.

E.coli strains	C-(C,H)/C 284.8 eV	C-(O,N)/C 286.2 eV	(C=O)/C 287.9 eV	(O=C)/C 531.2 eV	(-OH, C-O-C)/C 532.6 eV	$N_{nonproton}$/C 399.9 eV	N_{proton}/C 401.6 eV	P/C 133.4 eV	Na/C	K/C
AL52 LLB	0.49	0.37	0.137	0.104	0.318	0.082	0.0079	0.013	0.0041	<dl[a]
AL52 SLB	0.39	0.43	0.176	0.131	0.364	0.076	0.0084	0.011	0.0064	<dl[a]
HB101LLB	0.56	0.31	0.13	0.164	0.251	0.09	0.011	0.023	0.0020	0.0094
HB101SLB	0.58	0.29	0.123	0.143	0.239	0.085	0.0094	0.019	0.0060	0.0012
382 LLB	0.55	0.26	0.143	0.148	0.177	0.123	0.0094	0.014	0.0074	<dl[a]
382 LMin	0.52	0.33	0.142	0.167	0.271	0.093	0.012	0.023	0.0023	0.0077
382 SLB	0.48	0.34	0.170	0.167	0.258	0.130	0.0079	0.015	0.011	<dl[a]

[a]dl, detection limit (0.05%)

Latrache(2001) [24], and Gerin et al., (1993) [25] have reported that the nitrogen is mainly present as amide (O=C-N). Since the function O=C included in the amide function cannot be determined directly by XPS data, we replaced the $O=C_{amide}$ by the Nnp because in the function amide (O=C-N); each O=C corresponds to one N.

Therefore we proposed to estimate the percentage of COOH using this equation: COOH= (O=C) -2P – Nnp.

However, the surface concentration of carboxyl groups expressed in mol/g of material, have been computed by multiplying the estimated ratio of COOH/C by the average concentration of carbon in the material (mol/g). The latter has been calculated from the concentration of model molecular constituents (Table 1), considering the respective carbon concentration of 43.5, 37, and 71.4 mmol/g for proteins, polysaccharide and hydrocarbon-like compounds, respectively, Gerin et al. [25].

The surface concentration of phosphate groups have been computed by multiplying the P/C atom concentration ratio given by XPS (Table 2) by the average concentration of carbon in the material (mol/g).

Evolution of the concentration of $RCOO^-$ and of $R_2PO_4^-$ as a function of pH

The negative charge of microbial cell surface is determined by the deprotonation of phosphate and carboxyl groups; the following acid-base reactions are likely to occur:

$R_2HPO_4 \longleftrightarrow R_2PO_4^- + H^+$ pKa = 2.15

$RCOOH \longleftrightarrow RCOO^- + H^+$ pKa = 3.95

According to Dengis and Rouxhet [17], the concentration of $RCOO^-$ and $R_2PO_4^-$ expressed in mol/g under different pH could be deduced from acid-base equilibrium and from the concentration of chemical functions. Thus this concentration is computed using this relation:

$[B] = [Tc] * 10^{-pKa}/10^{-pH}+10^{-pKa}$

[B]: Concentration in mol/g of $RCOO^-$ or $R_2PO_4^-$

[Tc]: Concentration in mol/g of RCOOH or R_2PO_4H

pKa: acidity constant of RCOOH/$RCOO^-$ or R_2PO_4H/$R_2PO_4^-$

3. RESULTS

Electrophoretic mobility:

The variation of the electrophoretic mobility (EPM) with pH of the three strains grown in LLB is presented in Fig. 2. The EPMs became more negative with an increasing pH. At pH 5-9 the strain HB101 was the most negatively charged, 382 was the intermediate negatively charged and AL52 the least negatively charged strain.

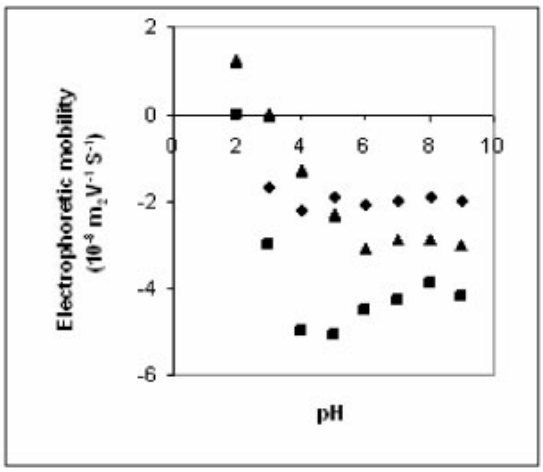

Fig. 2: Effect of pH on electrophoretic mobilty of *E .coli* grown in LLB: ■ HB101; ▲ 382; ◆ AL52

Strains surface molecular composition:

The results from the modelling XPS data in terms of proteins, polysaccharides, and hydrocarbon-like compounds, are presented in Table 3. It was found that the surface proteins content varied from 28% to 45.5%, the surface polysaccharides content varied from 18.5% to 37% and the surface hydrocarbon content varied from 21% to 47%. In addition, it appears that the surface molecular composition of *E. coli* was influenced by the different culture media.

The concentration of chemical functions RCOOH and R_2PO_4H

Table 4 presents the concentration in mol/g of the two chemical functions RCOOH and R_2PO_4H for three strains grown in different media. The concentration of phosphate groups varied weakly for AL52 and HB101 strains and highly for 382 strains when the culture medium changed. In contrast, the concentration of carboxyl groups was found to vary strongly with the different culture media. The concentration of carboxyl groups of 382 cell surface increased from $0.15.10^{-3}$ mol/g (LLB) to $1.4.10^{-3}$ mol/g (LMin). For HB101 strain, the concentration decreased from $1.52.10^{-3}$ mol/g (LLB) to $0.99.10^{-3}$ mol/g (SLB). The most marked changes were found for AL52 where the carboxyl groups concentration was almost twenty fold higher in SLB medium than in LLB.

The variation of the concentration of $RCOO^-$ and of $R_2PO_4^-$ with pH

Using different strains grown in different media, the concentration of $RCOO^-$ and of R_2PO4^- varied with pH (Fig. 3). Except for AL52 grown in SLB media (Fig. 3a) and 382 grown in SLB and LLB media (Fig. 3c), $RCOO^-$ concentration sharply decreased between pH 2 and pH 5 and remained constant above pH 5. All three strains $R_2PO_4^-$ concentration decreased continuously between pH 2 and pH 4 and remained constant above pH 4. Moreover, $RCOO^-$ concentration changes for the three strains differed considerably with the different culture media (Fig. 3). Except for 382 strain (Fig. 3c), the $R_2PO_4^-$ concentration changes of HB101 (Fig. 3b) and AL52 (Fig. 3a) strains were not significantly affected by the composition of the medium. Consequently, the overall negative charges of these strains could be influenced by the culture mode and the medium composition.

Table 3 Surface molecular composition from three *E. coli* strains cultivated under different conditions.

	Proteins	Polysaccharides	Hydrocarbon-like
AL52-LLB	28%	35%	37%
AL52-SLB	41%	37%	21%
HB101-LLB	30%	29%	43%
HB101-SLB	29%	25%	47%
382-LLB	40.5%	18.5%	38.5%
382-LMin	34%	28.7%	37.9%
382- SLB	45.5%	26.3%	28.7%

Table 4 Surface concentration of two functional groups of three *E. coli* strains cultivated under different conditions

E.coli strains	[RCOOH]*10^{-3}mol/g	[R_2PO_4H]* 10^{-3}mol/g
AL52 LLB	0.08	0.7
AL52 SLB	1.48	0.5
HB101 LLB	1.52	1.25
HB101 SLB	0.99	1.105
382 LLB	0.15	0.72
382 LMin	1.4	1.203
382 SLB	0.23	0.75

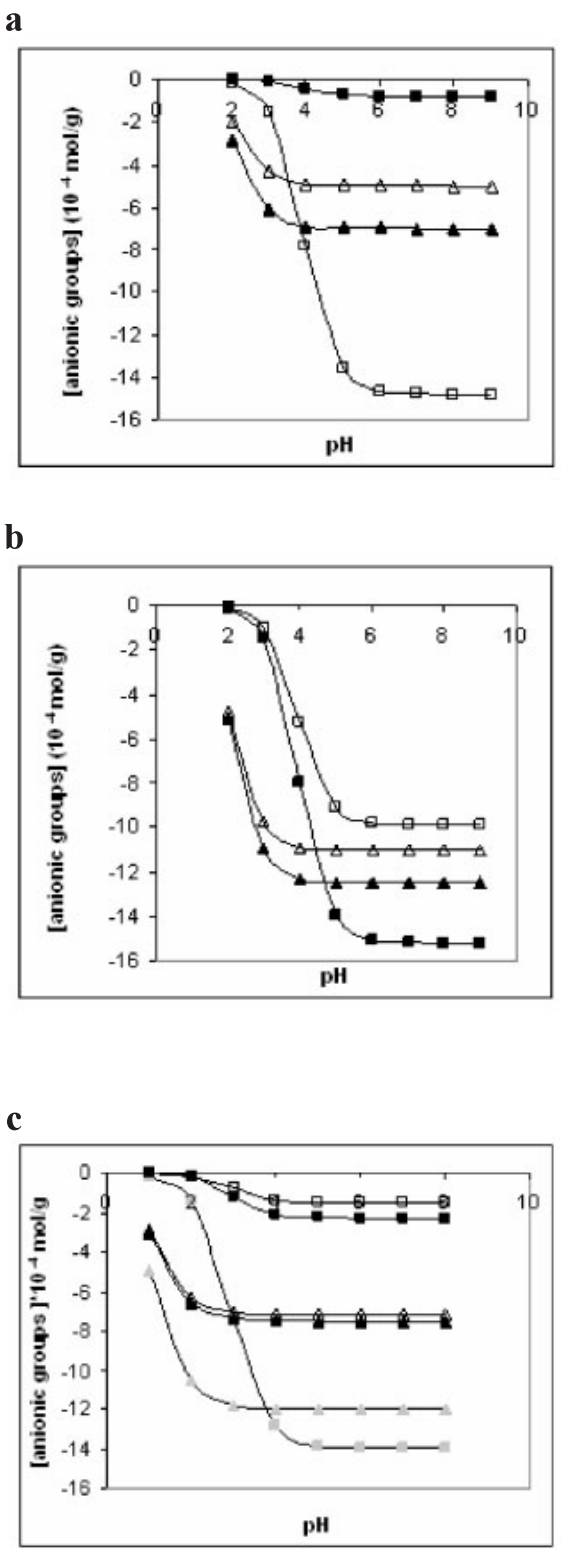

Fig. 3 Effect of pH on carboxylated and deprotonated phosphate concentration of three *E. coli* strains cultivated in Liquid LB, Solid LB and Liquid minimum medium. (a) AL52; (b) HB101; (c) 382: □ [$RCOO^-$] (black: SLB; white: LLB; grey : L Min);△[$R_2PO_4^-$] (black: SLB; white: LLB; grey : L Min).

4. DISCUSSION

Relationship between the negative charge concentration and the electrophoretic mobility.

Several works, Amory et al.[1], Cowan et al.[2], Cuperus et al.[3], Latrache et al.[4], Mozes et al.[5,6], Van der Mei et al.[7,8], have investigated the relationships between the surface charge of cells (yeast, bacteria) and the chemical composition of their surface, in order to investigate the groups involved in determining the negative charge. It is generally accepted that carboxyl and phosphate groups play a role in determining the negative charge on the cell surface. The concentration of negative charge considered as the sum of [$RCOO^-$] and [$R_2PO_4^-$] has been computed at different pH for three strains cultivated in LLB and plotted in Fig. 4.

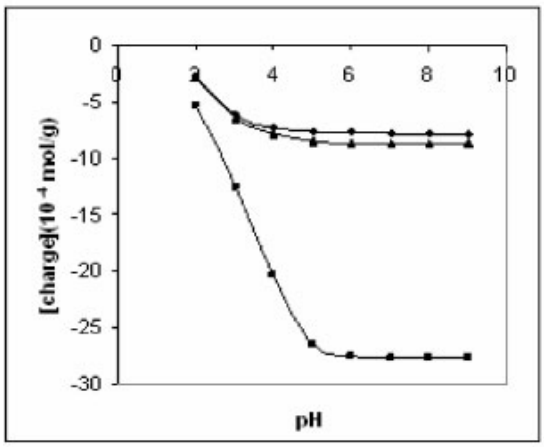

Fig. 4 Effect of pH on negative charge concentration of three *E. coli* strains cultivated in LLB. ■ HB101; ▲ 382; ◆ AL52.

The HB101 strain negative charge concentration was found to be more affected by pH compared to AL52 and 382 strains. Moreover, comparing the negative charge concentration (Fig. 4) to the electrophoric mobility (Fig. 2), it appears that the curves presenting the evolution of the concentration of the negative charge for the three strains were similar in trend with curves obtained by microelectroporesis. Thus we investigated the relationship between the sum of carboxylate and deportonated phosphate groups concentration and the electrophoretic mobility at pH 7. As the sum of deprotonated phosphate and carboxylate concentration increased, the bacteria cell surface became more negatively charged (Fig. 5). These results suggested that both phosphate and carboxyl groups could participate in the determi-

nation of *E. coli* negative charge. These finding could be explained by the fact that at pH 7, the cell surface charge was conferred by these two groups.

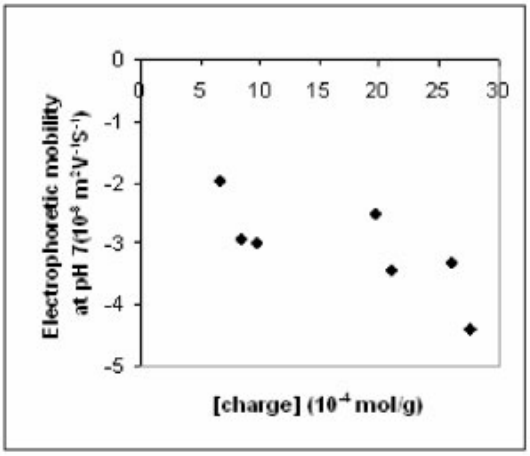

Fig. 5 Correlation between the electrophoretic mobility and the sum of $RCOO^-$ and $R_2PO_4^-$ concentration at pH 7. Coefficient of linear correlation is 0.73.

At pH 7, the carboxylate and deprotonated phosphate groups were found to play a major role in the determination of *E. coli* negative charge (Fig. 6a). This is in agreement with the results reported that the electrokinetic properties of top-fermenting yeasts, Dengis and Rouxhet [17], and of *L.helveticus,* Boonaert and Rouxhet [23], were controlled by carboxylate groups. In contrast, a relation between the electrophoric mobility and the chemical composition of *E. coli* was previously reported by Latrache et al (1994) and suggested that the phosphate groups could be the sole source of the negative charge at the bacteria surface. This difference could be explained by the fact that in the previous study the carboxyl groups concentration was not computed and the phosphate groups concentration was only presented in term of percentage.

Previous works, Amory et al. [1], Latrache et al. [4], Van der Mei et al.[7], Dengis and Rouxhet [17], Mozes et al. [26], have examined the relationship between the chemical composition on the cell surface and the electrophoretic mobility at pH 4. It was reported that the deprotonated phosphate was the only group that played a predominant role in the development of negative charge and the carboxylate

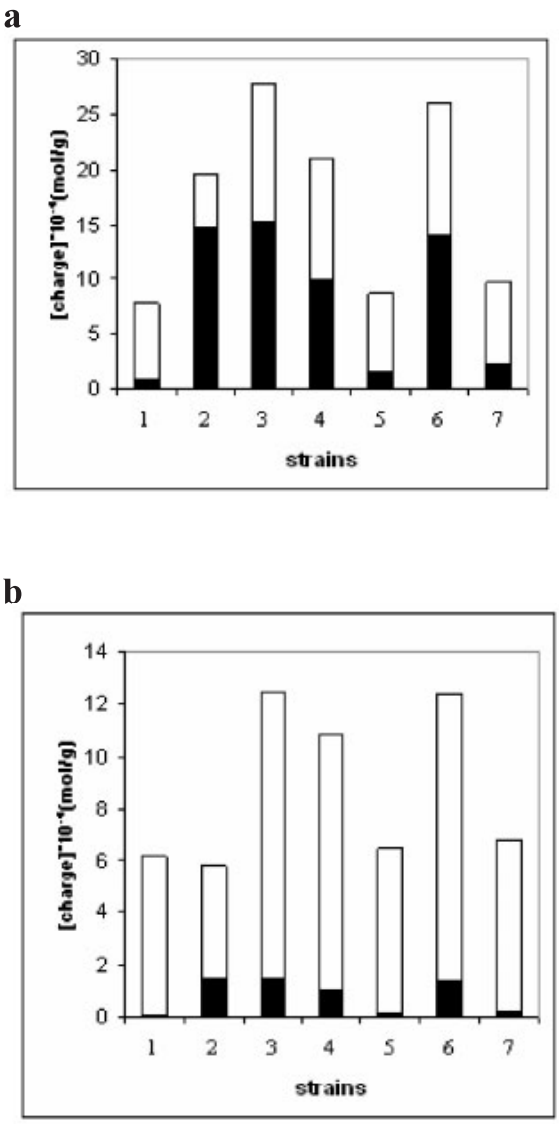

Fig. 6 Contribution of carboxyl and phosphate groups on *E. coli* negative charges concentration at two pHs: (a) pH 7, (b) pH 3. ■ [RCOO-]; □[$R_2PO_4^-$]. 1: AL52 (LLB); 2: AL52 (SLB); 3: HB101 (LLB); 4: HB101 (SLB); 5: 382 (LLB); 6: 382 (LMin); 7: 382 (SLB).

groups were weakly involved. These apparent results differences could be explained by the fact that our results were determined at pH 7 and in term of concentration (Fig. 6a) and while the previous studies were done at pH 4 and in term of percentage. Indeed, comparable results to previously reported works, Amory et al.[1], Latrache et al. [4], Mozes et al.[5,6], Van der Mei et al. [7], Dengis and Rouxhet [17], Mozes et al. [24], were found when we investigated the effect of an acidic pH on the groups involved in the negative charge (Fig. 6b). At pH 3, which is close to iso-

electric point (pH where the electrophoretic mobility was null), the concentration of carboxylate groups involved in the *E. coli* negative charge was almost negligible compared to the concentration of deprotonated phosphate groups (Fig. 6b). These results suggested that this pH was near to the pKa (3 <pKa> 5) of carboxyl/carboxylate groups indicating a beginning of carboxyl groups deprotonation. Our data clearly suggested that carboxyl and phosphate groups contribution to bacterial negative charge determination was pH-dependent.

Correlation between the concentration of positive (Na^+, K^+, NH_4^+) and negative ($RCOO^-$, $R_2PO_4^-$) charges on bacteria cell surface

At pH 7 (Fig. 7a) and at pH 3 (Fig. 7b), a high correlation (coefficient of linear correlation 0.64 and 0.82 respectively) was found between the concentration of the sum of cations (Na^+, K^+, NH_4^+) and the sum of anions ($RCOO^-$, $R_2PO_4^-$). This strong correlation at both pHs, suggested that these cations could represent the counter-ions to $RCOO^-$ and $R_2PO_4^-$ Moreover, a 1:1 ratio between the sum of cations and the sum of anions appeared at pH 3 (Fig. 7b). This suggests that at pH 3 all anions were neutralised by cations. It is known that at pH 7 compared to pH 3, the negative charge is more important; therefore, the reduction of the total negative charge concentration observed from pH 7 to pH 3 could explain the appearance of the ratio 1/1 at pH 3.

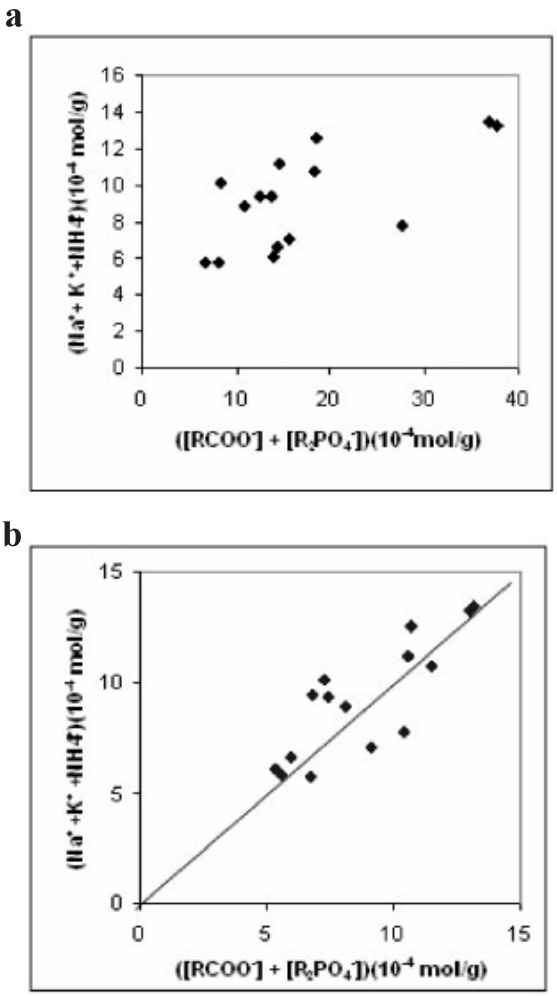

Fig. 7 Correlation between the surface concentration of cations (K^+, Na^+, NH_4^+) and the anions ($RCOO^-$ and $R_2PO_4^-$) at two pHs. (a): pH 7, coefficient of linear correlation is 0.64 (b): pH 3, coefficient of linear correlation is 0.82

4. Conclusion

Our work presents the first attempt to compute the concentration of three *E. coli* strains negative charges and determines the *E. coli* carboxylate and deprotonated phosphate concentration changes with pH. The results obtained herein showed that the profile of the concentration of negative charge was similar to the profile of electrophoretic mobility. Moreover, our results suggested that carboxyl and phosphate groups play the major role in the determination of *E. coli* negative charge at pH 7 determined by electrophoretic mobility. The contribution of each group in the negative charge concentration is pH-dependent.

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