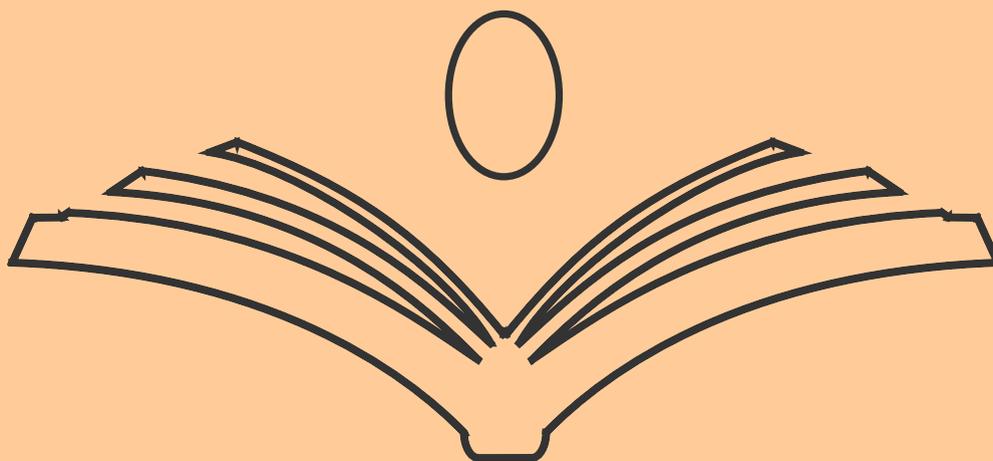


JOURNAL ACADEMICA

VOLUME 1, NO. 1, May 6 2011

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Full Length Research Paper

***Pasteurella multocida* X73 Able to Develop Natural Competence in Presence of Hyaluronidase-Producing *Staphylococcus aureus* Strain**

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Accepted March 7 2011

Bacteria presenting hyaluronic acid-rich capsules were thought to be unable to develop natural competence and to uptake exogenous DNA. In this article, genetic transformation of *Pasteurella multocida* X73 is achieved with the aid of physiological concentrations of calcium ions. The encapsulated *Pasteurella multocida* X73 was cultivated as a mixed culture in proximity to a hyaluronidase-producing strain of *S. aureus*. The *Pasteurella*-*Escherichia coli* shuttle vector pPBA1100 is investigated to test the competence ability of *P. multocida* X73 under natural conditions using bottled mineral water, which contained between 0 and 11 mM Ca²⁺. It was found that transformation frequencies with *P. multocida* X73 were similar to those reported for other gram-negative bacteria known to develop natural competence. This result will contribute to the understanding of how bacterial horizontal gene transfer is happening in natural ecosystems. Other studies should be carried on other Bacteria presenting hyaluronic acid-rich capsules and hyaluronidase-producing strains.

Key words: Natural competence, *Pasteurella multocida* X73, *Staphylococcus aureus*

INTRODUCTION

The gram negative bacterium *P. multocida* is a heterogeneous species, which exhibits a broad host range upon most mammals, birds and even human being. It can cause specific diseases such as hemorrhagic septicemia and fowl cholera. It is also a major cause in contributing in diseases such as the respiratory tract of different animals (Adlam and Rutter, 1989). *P. multocida* can be capsulated or non capsulated.

The capsulated strain can be separated into five serological groups A, B, D, E, or F (Carter, 1967; Rimler and Rhoades, 1987). The X73 strain belongs to the serological group A (A: 1) with a high amount of hyaluronic acid and confers a highly mucoid colony morphology (Pandit and Smith, 1993). A hyaluronic acid consists of a polymer of N-acetylglucosamine and glucuronic acid probably arranged as alternating units in a flexible chain, which can be depolymerized or hydrolyzed in presence of the mucolytic enzyme Hyaluronidase into N-acetylglucosamine. Hyaluronidases are produced by a variety

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of organisms and can be divided into three categories (Kreil, 1995):

- 1- The testicular type hyaluronic acid-4-glycanohydrolases.
- 2- The hyaluronic acid-3-glycanohydrolases produced by leeches and hookworms.
- 3- The hyaluronic acid lyases or bacterial Hyaluronidases.

Hyaluronidase-producing strain of *Staphylococcus aureus* is one of those bacterial organisms implicated in infections (Adlam and Rutter, 1989).

In 1989, Bredy and Botzler reported that Sodium, protein, calcium, and magnesium have been associated with both the survival of *P. multocida* and with avian cholera mortality.

On the other side, it was shown that increased calcium and magnesium levels have been associated with persistence of *P. multocida* and avian cholera mortality (Windingstad et al., 1984; Windingstad et al., 1988; Price et al., 1992). In addition, it was demonstrated that *P. multocida* has been isolated from the water and sediment of wetlands experiencing avian cholera epizootics, and the bacteria can persist in wetland soil and water (Rosen and Bischoff, 1949; Backstrand and Botzler, 1986; Rosen, 1969; Korschgen et al., 1978; Price and Brand, 1984; Ausubel et al., 1987; Samuel et al., 2003).

Horizontal gene transfer's studies between bacteria in natural aquatic systems have shown that the occurrence takes place by conjugation (O'Morchoe et al., 1988), transduction (Saye et al., 1987), transformation (Paul et al., 1991; Stewart, 1992; Stewart and Sinigalliano, 1990) and cell contact-mediated transformation (Price and Brand, 1984).

It was reported that, in gram-positive bacteria, competence is usually induced and controlled by competence factors which are secreted into the medium (Saunders and Saunders, 1988). In such cases, the competence is induced as soon as the competence factor has reached a certain concentration (Stewart and Carlson, 1986). In contrast, in gram-negative bacteria, competence is usually internally regulated (Paget and Simonet, 1994; Stewart and Carlson, 1986). The problem with studies of natural competence development is finding the relevant environmental parameters which trigger this induction. In 1994 Lorenz and Wackernagel have reviewed the known parameters. The bacteria have to be metabolically active, and a shift to unbalanced growth, e.g., by nutrient limitation, can trigger competence development in many gram-negative bacteria. The multiple variations in the required conditions found illustrate the different ecologies of the variety of bacteria chosen. For example, *Azotobacter vinelandii*, a typical soil bacterium, is best transformable after growth in minimal media (Page and Sadoff, 1976) while *Acinetobacter calcoaceticus*, a ubiquitous human pathogen (opportunistic) usually found on the skin develops competence in complex as well as minimal media (Palmen et al., 1993).

It was shown that incubation of *E. coli* cells with small temperature shifts (5 or 10°C) and even in the absence of temperature variations achieved the highest transformation frequencies which could be obtained under environmental conditions (Baur et al., 1996). *E. coli* can develop genetic competence under environmental conditions when in contact with surface water originating from calcareous regions. Calcium concentrations above 1 mM, which are

often found in spring water and river water, are sufficient to make gram-positive and gram-negative bacteria competent, and no additional competence-promoting factors or buffering substances are needed. (Trombe, 1993. Baur et al., 1996).

In 1975 Carter and Rundell developed a simple test in which *P. multocida* X73 (serogroup A) strains are recognized by depolymerization of the capsule after growth in proximity to a hyaluronidase-producing strain of *S. aureus*. On the basis of their findings and the author's one (Abdelhak, 2009), this study investigates for the first time, how the bacterial horizontal gene transfer is happening in natural ecosystems for those presenting hyaluronic acid-rich capsules.

Table 1. Bacterial strains and plasmids used in this study.

Strain or plasmid	Relevant characteristic(s)	Source or reference
Strains		
<i>P. multocida</i> X-73	Serotype A:1 wild-type strain	Institute of Microbiology and Epizootics, FU-Berlin, Germany
<i>S. aureus</i>	Hyaluronidase-positive	Institute of Microbiology and Epizootics, FU-Berlin, Germany
<i>S. aureus</i>	Catalase-negative, Hyaluronidase-negative	Department of infectious diseases, Centre hospitalier universitaire de Batna, Algeria
Plasmids		
pPBA1101	<i>E. coli</i> - <i>P. multocida</i> shuttle vector, Kan ^r	Homchampa et al., 1997

Oligonucleotides

Two antisense (M13 universal primer and M13 universal reverse primer) primers were custom synthesized, they had following sequences: Oligonucleotide pba-1, 5'-ACTGGCCGT CGTTT TAC-3', and pba-2, 5'-GTCATAGCTGTTTCCTG-3'. Both of them are sitting in inverse position on the pPBA1100 and were qualified to amplify the whole pPBA1100.

MATERIALS AND METHODS

Bacteria, plasmid, media and growth conditions

Bacterial strains and plasmids used in this study are shown in Table 1. The encapsulated *P. multocida* X73 and a hyaluronidase-producing strain of *S. aureus* were separately cultivated overnight at 37°C in brain heart infusion (BHI) (Oxoid, Hampshire, England).

The mineral water samples were sterilized by filtration through nitrocellulose membrane filters (0.22-mm pore size). The mineral water samples used and their calcium contents are presented in Table 2.

Natural transformation protocol

P. multocida X73 and *S. aureus* were grown separately in brain heart infusion (BHI)(Oxoid, Hampshire, England) overnight at 37°C. The overnight cultures were used to inoculate fresh culture which was grown at 37°C to an optical density at 600 nm of 0.6. The cells were centrifuged at 10,000 x g for 10 min, washed very gently twice with ice cold

sterile mineral water sampled and finally separately resuspended in 40µl aliquots. 1 µl from each aliquot were then mixed in 38µl sterile water and 2.5 ng of the *Pasteurella-Escherichia coli* shuttle vector pPBA1100 were then added. The samples were then shuttled gently for 10 days at 4°C. Immediately after transformation, 1 ml SOC medium was added to the different samples and incubated at 37°C for two hours. Finally the cultures were plated overnight by 37°C on Columbia agar plates (Oxoid, Hampshire, UK) containing 5% sheep blood supplemented with 50 µg/ml of kanamycine (Kan). Control experiments are presented in table 2.

Plasmid isolation and digestion

The pPBA1100 was prepared and digested with the restriction endonuclease *EcoRI* (Life Technologies, UK) as described previously (Ausubel et al., 1987). Endonuclease-digested DNA was electrophoresed through a 0.7% agarose gel.

Polymerase chain reaction (PCR)

PCR amplification was performed directly from single colonies grown on sheep blood agar plates. A pipette tip was lightly touched onto a colony. This

sample was then resuspended in PCR amplification mixture. DNA amplification was performed with the Expand high-fidelity PCR kit, using the reaction conditions specified by the manufacturer (Roche Molecular Biochemicals) and carried out using a thermal programmer (Gene Amp PCR System 2400; Perkin-Elmer Cetus Inc., Norwalk, Conn.). PCR-amplified DNA was then electrophoresed through a 0.7% agarose gel.

RESULTS

The encapsulated *P. multocida* X73 is truly able to develop natural competence in presence of hyaluronidase-producing *Staphylococcus aureus* strain.

Table 2, gives the number of transformants obtained when *P. multocida* X73 cells were incubated in natural water of known Ca^{2+} concentrations. Bidistilled water alone gave no transformants at all. Incubation of *P. multocida* with pure Hyaluronidase (Roche Molecular Biochemicals) in Bottled mineral water increases the number of transformants in contrast to native hyaluronidase produced by *Staphylococcus aureus*.

Table 2. Ca²⁺ concentrations of water samples and related numbers of transformants.

Sample type or source	Sample no.	[Ca ²⁺] (mg/l)	No. of transformants/ml
Bottled mineral water (Algeria)			
Hayet (Danone) + <i>S. aureus</i> Hyaluronidase-positive	1	120	576
Vie pure (Nestlé) + <i>S. aureus</i> Hyaluronidase-positive	2	57.9	322
Ifri + <i>S. aureus</i> Hyaluronidase-positive	3	81	208
Control solutions			
Bidistilled water	4	0.0	0
Bidistilled water + <i>S. aureus</i> Hyaluronidase-negative	5	0.0	0
Bidistilled water + <i>S. aureus</i> Hyaluronidase-positive	6	0.0	0
Bidistilled water + Pure Hyaluronidase	7	0.0	0
Hayet (Danone) + Pure Hyaluronidase	8	120	2211
Vie pure (Nestlé) + Pure Hyaluronidase	9	57.9	1122
Ifri + Pure Hyaluronidase	10	81	1812
Bottled mineral water Hayet (Danone)	11	120	0
Bottled mineral water Vie pure (Nestlé)	12	57.9	0
Bottled mineral water Ifri	13	81	0

The titration experiments were undertaken using different concentration of the mentioned enzyme and different concentrations of the hyaluronidase-producing *Staphylococcus aureus* strain. The maximal transformation efficiency was obtained at 1.85U/ml for the pure enzyme and 1µl for the native one produced by *Staphylococcus aureus* strain (data no shown).

The 2.5 ng DNA concentration was largely sufficient to obtain recombinants. Recombinants *P. multocida* X73 were checked using *EcoRI* endonuclease (Fig. 1) and PCR (Fig. 2).

We have used a small plasmid to obtain maximal transformation frequencies because it has been demonstrated that the transformation frequencies obtained are inversely proportional to the size of the transforming plasmid (Hanahan, 1983; Inoue et al., 1990).

No recombinants *P. multocida* X73 were detected by the control experiment

involving a non-hyaluronidase-producing strain *S. aureus* substantiating the effect of the staphylococcal enzyme.

DISCUSSION

The hyaluronic acid-rich capsules make affected bacteria relatively retractile to transformation. For the first time, this study demonstrates that encapsulated bacteria are also able to develop natural competence in presence of those producing hyaluronidase.

Natural genetic transformation in this work was only performed on *Pasteurella multocida* X73 using a hyaluronidase-producing *Staphylococcus aureus* strain. Other researchs should be studying other capsulated and hyaluronidase-producing strains like *Streptococcus pyogenes* (Hynes et al., 2000) and *Clostridium perfringens* (Canard et al., 1994).

Low temperature (4°C) of incubation was required to inhibit a rapid bacterial division. The increase of the number of transformants using pure hyaluronidase

was expected because of the direct effect of the enzyme with the substrate.

Natural transformation efficiencies are related to many factors, such as the nature and concentration of plasmids and the state of the plasmid DNA (supercoiled, circular, linear, or multimeric form) (Norgard et al., 1978; Hanahan, 1983; Stewart and Carlson, 1986; Lorenz and Wackernagel, 1994).

Further studies, like it was done in 1996 from Baur et al. should be carried on to understand if there is correlation between Transformation frequency and

1. Ca²⁺ concentration
2. DNA concentration
3. Temperature shifts

Regarding the author's discussion on horizontal gene transfer in natural ecosystems, this also could be happening for other bacteria presenting hyaluronic acid-rich capsules in presence of those producing hyaluronidase. Future studies should be carried out to understand how natural genetic transformation affect's this type of bacteria.

On the basis the author's founding, it seems legitimate to presume that the natural development of genetic competence in *P. multocida* X73 is biologically possible. In this case, the transformation process will be highly dependent on the phenotypical characteristics of each strain and on the type and characteristics of the transforming DNA.

ACKNOWLEDGMENTS

We thank B. Adler (Department of Microbiology, Monash University, Clayton, Victoria, Australia) for providing plasmids pPBA1100, L. H. Wieler (Institute of Microbiology and Epizootics, FU-Berlin, Germany) for providing *P. multocida* X-73, Hyaluronidase-positive *S. aureus*, and

L. A. Kessah (Department of Infectious Diseases, Centre Hospitalier Universitaire de Batna, Algeria) for providing non-hyaluronidase-producing strains of *S. aureus*.

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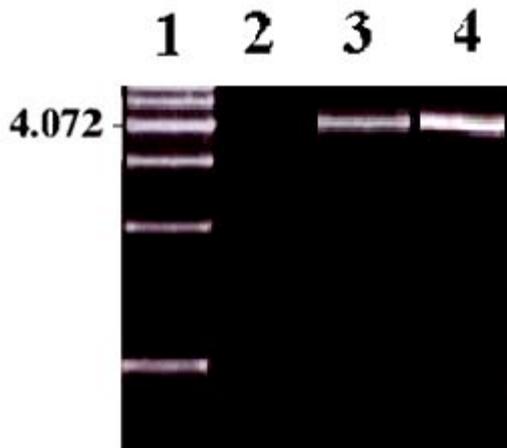


Figure 1. Agarose gel (0.7%) electrophoresis of the digested pPBA1100 with *EcoRI* (life technologies) isolated from *P. multocida* X73. Lane 1 = 1 kb ladder (life technologies); Lane 2 = No recombinants were produced in experiment using non-hyaluronidase-producing strains of *S. aureus*; Lane 3 = pPBA110 as positive control; Lane 4 = pPBA1100 isolated from recombinant *P. multocida* X73.

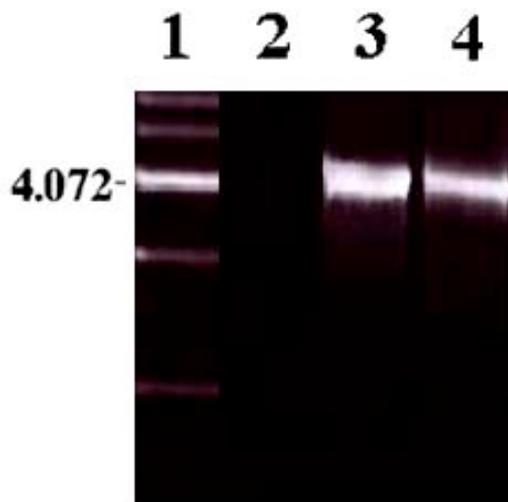


Figure 2. Agarose gel (0.7%) electrophoresis of the PCR-amplified pPBA110 (directly from single colonies grown on sheep blood agar plates from recombinants *P. multocida* X73) using the inverse primers pba-1 and pba-2. Lane 1 = 1 kb ladder (life technologies); Lane 2 = No recombinants were produced in experiment using non-hyaluronidase-producing strains of *S. aureus*; Lane 3 = pPBA110 as positive control. Lane 4 = pPBA110 isolated from recombinant *P. multocida*X73.

Full Length Research Paper

Influence of concentrated supernatants from *Bacillus cereus* var. *Toyoi* strain on the metabolic activity of vero cells

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Accepted March 15 2011

This report summaries results concerning an influence of concentrated bacterial supernatant from *Bacillus cereus* var. *Toyoi* CNCM I-1012/NCIMB 40112 (probiotic-Toyocerin) on Vero cells. To test the influence of the supernatant on the metabolic activity of Vero cells, supernatant was concentrated 5-fold using the “Centriprep Kit concentrator” concentration system with a cut off of 3.000 Da. and were incubated for 2 h on Vero cell monolayers. The bacterial supernatant was tested in triplicate in a dilution of 1:8 (12.5 ml supernatant + 87.5 ml RPMI) on Vero cells (Vero I ATCC CCL 81). As negative controls, wells received 5-fold concentrated supernatant from *Bacillus thuringiensis* T 26001 and BHI + 1% Glucose. *Bacillus cereus* 1230 was used as positive control. The negative metabolic effects/cytotoxic effects was objectified through the use of MTT [3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyl tetrazolium bromid]. This assay is based on the cleavage of the yellow tetrazolium salt MTT to purple formazan crystals by metabolic active cells (Cory et al., 1991; Mosmann, 1983). The solublized formazan product was spectrophotometrically quantified using an ELISA reader.

The assays revealed that concentrated supernatants of the probiotic strain *B. Cereus* var. *Toyoi* reduced the metabolic activity of Vero cells.

Key words: *Bacillus cereus* var. *Toyoi*, Vero cells, bacterial supernatant, metabolic activity

INTRODUCTION

Bacillus cereus var. *toyoi* is not a member of the normal animals gut flora, but is a common soil inhabitant. A number of studies have assigned probiotic characteristics to this particular Bacillus strain (*Bacillus cereus* var. *Toyoi* CNCM I-1012/NCIMB 40112; ToyoCerin1), and it has been authorized

in the EU for use as a probiotic feed additive for sows and piglets and several other farm animal species (SCAN, 2000). In animal studies with various strains of *B. cereus*, positive effects such as increased weight gain, improved feed conversion ratios and lower mortality rates of piglets have been reported (Kirchgessner et al., 1993; Alexopoulos et al., 2001), and *B. cereus* var. *toyoi* was correlated with a reduced incidence of post-weaning diarrhea (Taras et al., 2005; Scharek et al., 2007).

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In this communication we assessed the effects of concentrated bacterial supernatant from *Bacillus cereus* var. *Toyo* CNCM I-1012/NCIMB 40112 (probiotic-Toyocerin) on Vero cells.

MATERIALS AND METHODS

Bacterial strains tested

The cytotoxicity assay was carried out with the following bacterial strains:

- 1- *Bacillus cereus* 1230 (**positive** enterotoxic strain provided by P.E. Granum Oslo)
- 2- *Bacillus cereus* var. *toyo* CNCM I-1012/NCIMB 40112 (probiotic-Toyocerin)
- 3- *Bacillus thuringiensis* T 26001 (**negative** enterotoxic strain provided by P.E. Granum Oslo)

Cell lines, media and buffer solutions

BHIG: Brain Heart Infusion + 1% glucose (Oxoid, Basingstoke, England)

Vero cells I (vero I): ATCC CRL 1587 (provided from the Institut für Hygiene und Infektionskrankheiten der Tiere, Giessen, Germany)

Cell culture medium: see A4.

Stop solution: 10% SDS in ddH₂O (Sigma Chemical co., St. Louis, U.S.A.)

Phosphate buffer: Formula (g/l)
(10 x PBS pH 7.4) NaCl 80.0 (Carl Roth GmbH + co, Karlsruhe, Germany)
KCl 2.0 (FERAK, Berlin, Germany)

Na₂HPO₄ x 2H₂O 7.6 (E Merck, Darmstadt, Germany) KH₂PO₄ 2.0 (E Merck, Darmstadt, Germany)

MTT: 5mg/ml in 1x PBS (Sigma Chemical co., St. Louis, U.S.A.) (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromid)

Growth conditions and toxin production

The growth conditions of all strains were chosen like described in the SCAN report (5). A single colony of each bacterium

(*Bacillus*) was inoculated into 50 ml of Brain Heart Infusion broth with 1% glucose (BHIG) and then cultured overnight at 32°C with shaking (100 rpm). After overnight culture, 1 ml of each cultivated broth was transferred into 50 ml of BHIG and incubated at 32°C for 6 h with shaking (100 rpm).

The OD_{600nm} was measured using the spectrophotometer "Ultrospec 3000" (Amersham Pharmacia Biotech, Freiburg, Germany), the cells were separated by centrifugation (5000g at 4°C for 20 min) and the supernatant was filtered through 0.22 µm pore-size filters (Millipore co, U.S.A). Proteins of the culture supernatant were concentrated 5-fold using the Centriprep Kit YM-3 MWCO: 3.000 (Millipore Co, U.S.A). Immediately after concentration of the supernatants they were used for the Vero cell cytotoxicity test.

Cell lines and preparation of medium

Vero cells (African green monkey kidney; Vero I, see A2) were cultivated in tissue culture flasks with RPMI 1640 medium (Gibco, NY, U.S.A), supplemented with 10% foetal calf serum (Gibco, NY, U.S.A), 0.75 mM L-glutamine (Gibco, NY, U.S.A.), 40 µg/ml penicillin/streptomycin (Gibco, NY, U.S.A), and 1 µg/ml amphotericin B (Gibco, NY, U.S.A).

Cytotoxicity assay

The cytotoxicity assay was performed as described by Konowalchuk et al. (3) and Dalrymple and Gentry (1). Confluent monolayers were removed with trypsin-EDTA (Gibco, NY, U.S.A), resuspended to approximately 4 x 10⁵ cells/ml in RPMI and 0.1-ml samples were pipetted into each well of a 96-well microtiter plate. After incubation at 37°C in 5% CO₂ for 72 h, the medium was replaced with 1:8 (12.5 µl supernatant + 87.5 µl RPMI; 100 µl end volume) of the

concentrated bacterial supernatants in RPMI and was added to each well. As negative controls, some wells received only RPMI or PBS. 1% SDS (in PBS) was used as positive control. Each sample was tested in triplicate. Cytotoxic effects on Vero cells were detected by monitoring the metabolic activity of the cells through the use of MTT (3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyl tetrazolium bromid).

Before the MTT test started the media containing the bacterial culture filtrates were removed and each well was washed for 2-3 times with 0.89% NaCl. The medium was replaced with 150 μ l 1 x PBS + 25 μ l MTT (5mg/ml in PBS). Then the Vero cells were incubated with the yellow MTT solution for 4 h (e.g. 37°C, 5.0% CO₂, shacked). After this incubation period, purple formazan salt crystals are formed. These salt crystals are insoluble in aqueous solution, but will be solubilized by adding 100 μ l SDS stop-solutions (10% SDS in ddH₂O) and incubating the plates overnight in a humidified atmosphere (e.g. 37°C, 5.0% CO₂, shacked). The solubilized formazan product was spectrophotometrically quantified using an ELISA reader (Dynatech MR5000; Dynex Technologies GmbH).

An increase in number or activity of living cells results in an increase in the total metabolic activity in the sample. The wavelength to measure the optical density (OD) of the Formazan product was 550 nm. Thus the higher the OD, the higher is the metabolic activity of the respective Vero cells.

A negative metabolic effect/cytotoxic effect of supernatants from the 3 test strains was calculated as recommended by the report from the SCAN (2000).

After subtraction of the value for background OD_{550-690nm} the metabolic activity was calculated: (OD_{550-690nm} for Vero cells without "toxin" - OD_{550-690nm}

test sample) x 100/OD_{550-690nm} for Vero cells without "toxin" added.

If the OD_{550-690nm} of the test sample was less than 80% of that of the Vero cells without "toxin", the test sample was considered as "toxin" positive.

RESULTS

Growth of the bacterial strains:

The following tables list the optical densities of the bacterial cultures, immediately before they were used for preparation of the respective supernatants.

Strain	OD _{600nm}
1. <i>B. cereus</i> 1230	2.066
2. <i>B. cereus</i> var toyoi	1.968
3. <i>B. thuringiensis</i> T26001	2.005

Table 1. Results of the OD measurements (600 nm) of the different *Bacillus* strains grown 6 h at 32°C and 100 rpm in BHI + 1% glucose (BHIG) (as suggested in the "SCAN" report)

Results of the cytotoxicity assays

Vero cells were incubated with supernatants from the 3 strains for 2 h. As 12.5 μ l of a 1:5 concentrated supernatant was tested, the final concentration is nearly identical to the one recommended by the SCAN (2000). A metabolic activity of Vero cells incubated with supernatants from the test strains less than 80% of the negative control cells (Vero cells incubated with RPMI or PBS, respectively) was regarded as yielding cytotoxic effects like described in the SCAN report (2000).

Table 2. Optical Density (OD_{550-690nm}) measured (in triplicate) after cytotoxicity test on Vero cells I with the appropriate supernatants dilutions of 3 strains of *Bacillus* (*B. cereus* 1230, *B. thuringiensis* and *B. cereus* toyoi)

	OD_{550-690nm} (Average) 1:8 (12.5µl+87.5µl)
<i>B. cereus</i> 1230 (+)	0.005
<i>B. thuringiensis</i> (-)	0.396
BHI-G	0.377
<i>B. cereus toyoi</i>	0.190

Control + (1% SDS) $0.004 \pm 1.0 \times 10^{-4}$

Control - (1 x PBS) $0.435 \pm 25 \times 10^{-3}$

80 % metabolic activity = OD_{550-690nm} 0.348; values < 0.348 are considered to be toxic

Framed OD: metabolic activity less than 80 % (<OD_{550-690nm} 0.348) of the negative control cells

(+) positive control strain (*B. cereus* 1230); (-) negative control strain (*B. thuringiensis* T 26001) and BHI-G

Note: All S.D. (standard deviation) ranged from 10^{-3} to 10^{-4}

CONCLUSION

A negative metabolic effect/cytotoxic effect of supernatants from the 3 test strains (*Bacillus cereus* var. *toyoi*, *Bacillus cereus* 1230 and *Bacillus thuringiensis* T 26001) was calculated as recommended by the report from the SCAN (2000).

If the OD_{550-690nm} of the test sample was less than 80% of that of the Vero cells without "toxin", the test sample was considered as "toxin" positive.

The assays revealed that concentrated supernatants of the probiotic strain *B. cereus* var. *toyoi* reduced the metabolic activity of Vero cells.

The ammonium sulphate precipitation method suggested by SCAN (2000) was established in our laboratory, but unfortunately the protein concentrations varied substantially from batch to batch (data not shown). The ammonium sulphate concentration at which a certain protein will precipitate is dependant from the number and position of the polar

groups, the molecular weight of the protein, the pH value of the solution, and the temperature at which the precipitation is carried out (Bell et al., 1983). Furthermore, removal of the remaining ammonium sulphate by dialysis and the consequent renaturation of the proteins to their native conformation form are not always obvious. To overcome this problem we used another concentration system where proteins in general retain their native form. We utilized a concentration system with a cut off of 3.000 Da., causing substances yielding smaller molecular weights to vanish from the toxin preparation. While the loss of smaller substances clearly is a disadvantage, by this method not only proteins are concentrated, but also fatty acids and sugars will be maintained. These substances may also influence the test result, as toxins of non-protein nature have also been described, i.e. the emetic toxin of *Bacillus cereus*. However, the enterotoxins mentioned in the SCAN-report (2000) for *Bacillus* are larger than 3 kDa in molecular weight, thus they should be detected by this method.

ACKNOWLEDGMENTS

We thank L. A. Kessah (Department of bacteriology, *Centre Hospitalier Universitaire de Batna*, Algeria) for His help and support. This work has been funded by the University of Khenchela.

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Full Length Research Paper

IMMOBILISATION OF CALIX[4]ARENE ONTO MODIFIED SELF AUTOASSEMBLED MONOLAYER GOLD SURFACE FOR ALKALI IONS DETECTION IN WATER

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Accepted May 4 2011

Calixarenes are currently the subjects of study as chemical sensors, biosensors and selective receptors due to their important functionalization and complexations possibilities. A chemical sensor for the detection of alkali ions based on surface-plasmon resonance (SPR) phenomenon was elaborated by using a gold thin film, of which surface was modified with a Calix[4]arene-self-assembled monolayer (SAM). The immobilisation of calixarene onto modified SAM gold surface was confirmed by impedance spectroscopy. The SPR sensor signal based on resonance angle shift is dependent on the concentration of ion in the range from 0 to 1 μM . Three alkaline ions were used K^+ , Na^+ and Ca^{+2} and the influence of pH on ions detection was studied and optimized for each ion. The sensor shows a high sensitivity and a low limit of detection for different alkali ions. The sensitivities obtained are 0.992, 0.742 and 0,122 μM^{-1} for Na^+ , K^+ and Ca^{+2} , respectively. Regeneration of the sensor chip surface was achieved by a pulse injection of 10 mM EDTA.

Key words: *calixarene*, SAM, SPR, alkali ions, sensor, gold surface

1. INTRODUCTION

The determination of alkaline ions is important for routine quality control of beverage products and in medicine and environment fields (Wicker et al., 2002; Wicker et al., 2003; Malon and Maj-

Zurawska, 2005; Ammann, 1986; Qin et al., 2000). A macrocyclic complexes of alkali metal cations have been extensively investigated in aqueous solutions and in a wide variety of neat non-aqueous solvents (Popov and Lehn, 1985; Izatt et al., 1991; Mosier-Boss and Popov, 1985). The complexation reactions of these complexes in mixed solvent systems have been investigated only to a very limited extent (Izan et al., 1985;

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David, 1989; Antesberger et al., 2005). Calixarenes are currently the subjects of study as chemical sensors and selective receptors due to their important functionalization and complexations possibilities. Among this sensors, different calixarene derivative may be found in ions selective electrodes and in chromogenic sensors (McMahon et al., 2003; Nachtigall et al., 2002; Atwood et al., 2002; Thallapally et al., 2005; Purse et al., 2005; Kumar et al., 2006; Jain et al., 2005; Lu et al., 2004). Many work was carried out based on calixarene molecule using polymer support and different measurement techniques to detect traces of ions were reported (Duncan and Cockayne, 2001; Pérez-Jiménez et al., 1998; Chen et al., 2000; Bouazizi et al., 1999; Lu et al., 2002; H. Barhoumi et al., 2005; N. Levit et al., 2002; Love, et al., 2005). The use of polymer as support for calixarene decrease the sensitivity of the sensor (T. Baumgart et al., 2003; Park et al., 1999). It is shown that surface adsorption and bulk adsorption of polymers affect the sensitivity and the response time of sensor and exhibiting low vapor permeability.

A self-assembled monolayer (SAM) is a flexible and simple system that permits the interfacial properties of a metal substrate to be tailored for a well-designed functional surface (Ulman, 1991; Gao and Siow, 1996; Sato and al, 1996). The advantages of SAMs include simplicity of preparation, versatility, stability, reproducibility and the possibility of introducing different chemical functionalities with high level of order on a molecular dimension (Freire and Kubota, 2004; Steinberg and Rubinstein, 1992; Mandler and Turyan, 1996; Flink et al., 1998). One of the most widely used systems in the molecular self-assembled method is the

chemisorption of sulfur derivatives (i.e. thiols, disulfides) on gold surfaces (Boussaad et al., 2000; Zacher and Wischerhoff, 2002; Georgiadis et al., 2000).

A large number of physico-chemical techniques such as NMR spectrometry, calorimetry, polarography, spectrophotometry, conductometry and potentiometry have been used to study complexation of alkali metal cations in solutions (Haymore et al., 1982; Sinta et al., 1983; Ungaro et al., 1976; Takeda et al., 1985; Fenton et al., 1981; Lim and Jeong, 2006; Kruppa et al., 2006; Kudo et al., 2006; Bendikov and Harmon, 2006; Katsuta et al., 2005). Surface plasmon resonance (SPR) spectroscopy is a powerful tool for the in situ real-time characterization of a solid/liquid interface and to study interactions between larger molecules (Chah et al., 2004; Boussaad et al., 2000; Zacher and Wischerhoff, 2002; Georgiadis et al., 2000; Sarkar and Somasundaran, 2003; Chah et al., 2002; Chah et al., 2002)..

In this work, an SPR sensor based on gold surfaces modified by self-assembled monolayer of cysteamine was developed and the response of the SPR sensor to alkali ions was investigated. To functionalize SPR sensor, pyridine was made to protect oxygen provider group and catalyze the reaction between the SAM and the alcohol group calixarene.

2. EXPERIMENTAL

2.1. SPR material and Principle:

Surface Plasmon Resonance Spectrometer BIO-SUPLAR 2 (Analytical μ -Systems, Germany) produced by Biacore company was used. It is based on the Kretschmann type

prism and GaAs solid-state laser ($\lambda = 670$ nm).

The principle of detection is that SPR is detected by measurement of the intensity of the reflected light. At the SPR angle a sharp decrease or 'dip' of intensity is measured. The position of the SPR angle depends on the refractive index in the substance with a low-refractive index close to the sensing surface. The refractive index near the sensor surface changes because of binding of ions to the surface. As a result, the SPR angle will change according to the amount of bound ions.

During a binding analysis SPR changes occur as a solution is passed over the surface of a sensor chip. To perform an analysis, one interactant is captured on a sensor surface. The sensor surface forms one wall of a flow cell. Sample containing the other interactant(s) is injected over this surface in a precisely controlled flow. Fixed wavelength light, in a fan-shaped form, is directed at the sensor surface and molecular binding events are detected as changes in the particular angle where SPR creates extinction of light. This change is measured continuously to form a sensorgram (Figure 1), which provides a complete record of the progress of association or dissociation of the interactants.

2.2. Synthesis of the chromogenic amide derivative Calix[4]arene:

The synthesis of tetra-O-substituted Calix[4]arene derivative was performed by the reaction sequence depicted in Figure. The treatment of p-tetrakis(phenylazo)Calix[4]arene with tertiary acetamide (α -chloro-N,N-diethylacetamide) in the presence of

CaH₂ as base gave p-tetrakisphenylazoCalix[4]arene tetra-amide derivative in cone conformation figure 2 (Sarkar and Somasundaran, 2003).

2.3. Reagents and solvents

Several samples necessary to elaborate sensor were prepared. First, 10⁻² M of cysteamine solution was prepared in which sensor was immersed. Calix[4]arene solution was prepared by dissolved 5 mg of Calix[4]arene powder in 2 ml of chloroform. Calix[4]arene was deposited on the sensor by dip-coating technique. This technique makes it possible to have homogeneous layers. PCC or pyridinium chlorochromate, known as Corey's reagent (Chah et al., 2002), was prepared. To 22 ml of 6 M HCl was added 12 g of CrO₃. After ultrasonic mixing during 10 min, 9,5 g of pyridine was added to the homogeneous solution. Ultrasonic mixing over 5 minutes and cooling to 0^o. A dilute sulfochromic oxidant solution and aqueous solutions containing 0 μ M to 1 μ M of the ions K⁺, Na⁺ and Ca⁺² were prepared. Phosphate buffer solution PBS was prepared at pH equal 8.

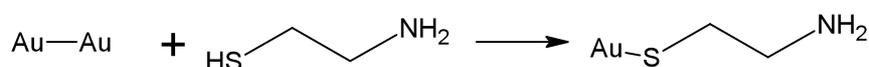
2.4. Immobilisation of calixarene onto modified SAM gold surface

To functionalize Calix[4]arene-SAM onto gold surface in order to make an SPR sensor for ions detection, four stages are necessary:

1. In SPR chip the adhesion between gold layer and glass is only physical. Pirhana was not made to clean gold surface. So, acetone and ethanol were used,

2. Grafting of SAM on the gold surface: the sensor is immersed in 15 mM of cysteamine during two hours,

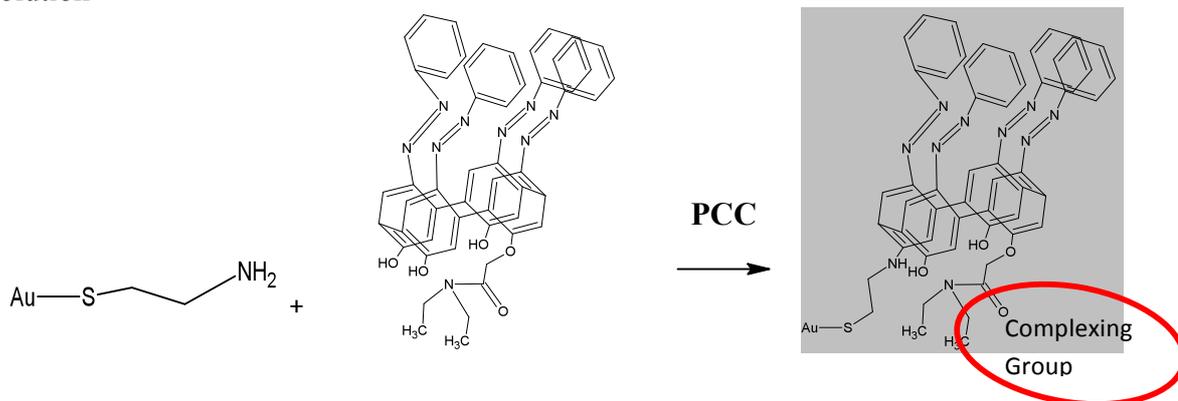
Diagram 1 Grafting of SAM on the gold surface



3. Cleaning of SAM by ethanol for few minutes and drying by nitrogen gas,
4. Deposition of Calix[4]arene onto SAM of cysteamine in presence of PCC solution. The PCC was made to protect oxygen provider group and catalyse the reaction between the SAM and alcohol group of calixarene (Halouani et al.,

2002; Corey and Suggs, 1975; Shervedani and Mozaffari, 2005). Deposition solution was prepared by mixing 1 ml of calixarene solution with 0.5 ml of PCC for six hours at room temperature (cf. figure 3). After, 30 μ l of mixture solution was deposited onto modified sensor by thin film method.

Diagram 2 Deposition of Calix[4]arene onto SAM of cysteamine in presence of PCC solution



3. RESULT AND DISCUSSION

All tests were made using SPR method. The influence of pH on ions detection was studied and optimized for each ion. The regeneration of the sensor was made by injection of 10 mM EDTA.

3.1. Characterization of modified sensor by electrochemical impedance spectroscopy (EIS)

The EIS is a powerful technique for characterization and studying electrical and electrochemical properties of a large variety of systems.

All electrochemical measurements were carried out using VOLTALAB 40 analyzer (PGZ301 & VoltaMaster 4). A three-electrode electrochemical cell was used, with the chemically gold electrode as the working electrode; A calomel electrode was used as the reference electrode and a platinum was used as the auxiliary electrode. Measurements were

used in buffer solution at adjusted pH for about 7.3.

Concerning the use of EIS to characterize thin films in contact with electrolyte solutions, three different contributions, bulk, interfacial and electrolytes may be determined (Sun et al., 1998; Xiao et al., 1999; Delvaux and Demoustier-Champagne, 2003). From an electrochemical point of view, when a metal is placed in contact with an electrolyte, a potential is generated due to the unequal distribution of charge across the interface, in addition, hydrated ions will not be able to approach indefinitely close to the interface.

In order to confirm immobilisation of calixarene onto modified SAM gold surface faradaic impedance spectra were presented as Nyquist plots (Z_{im} vs. Z_{re}) for gold, SAM of cysteamine and SAM-Calixarene (cf. Figure 4).

We show that the covalent attachment of each layer increases the charge transfer resistance. The bare Au electrode exhibits an almost straight line (curve a) that is characteristic of a mass diffusional limiting electron-transfer process. Assembly of the cysteamine monolayer on the electrode surface (curve b) generates a layer on the electrode that introduces a barrier to the interfacial electron-transfer. The deposition of Calix[4]arene onto SAM cysteamine layer results in an increase of the electron-transfer resistance (curve c).

3.2. pH optimization

SPR method was used for pH optimization and tests sensors. pH detection of the sensor was optimized for each ion at 1 μM concentration. The pH of buffer solution was adjusted by using 0.1 M of HCl or NaOH solution. Figure 5 shows that ions detection will be made in basic solution at about 8. This is in agreement with proton/ion exchange (Hudlicky, 2005; Hunsen, 2005). At low pH, quinone protonated and ion complexation does not take place. At $\text{pH} > 7$, oxygen was deprotonated making the sensor surface become negatively charged. Thus, the incorporation of ion by the Calix[4]arene-SAM also can be favored by electrostatic interactions (Sato et al., 1996). Such coupling of favorable characteristics is probably the key to achieving the remarkable sensitivity to ions of the proposed sensor.

3.3. Sensor detection

For all ions, we observed that output refractive index increases with increasing ions concentration. As shown in (Hunsen, 2005) ions are coordinated with the oxygen atoms of the amide group and its complexation takes place.

As shown in figure 8, detection limit reached are lower than 10-5 μM for the most ions used. Detection limits

reached are 9 10^{-5} , 10-4 and 5 10^{-3} μM for Na^+ , K^+ and Ca^{+2} ions, respectively (cf. figure 6).

Three zones of detection were observed between 0 and 1 μM : high concentration zone from 0.1 to 1 μM (figure 7 a)), second zone between 10-3 and 10-2 μM (figure 7 b)) and third zone between 10-4 and 10-5 μM (figure 7 c)). In order to determine the sensitivity of sensor, linear smoothing curves were plotted for each zone detection. In figures 7 a), b) and c), a linear variation of signal was showed for each ions in all detection zones. The slopes of straight lines represent the sensitivities of sensor in μM^{-1} . A recapitulative table which gives sensitivity of sensor for each zone was drawn (table 1). The results obtained in table 1 show different sensitivities of sensor for alkali ions. In the zone of high concentration, low sensitivities were observed 10-2, 1.4 10^{-3} and 2.4 10^{-3} μM^{-1} for Na^+ , K^+ and Ca^{+2} , respectively. In the second zone, the sensitivities increase and the values obtained are 0.122, 0.034 and 0.0039 μM^{-1} for Na^+ , K^+ and Ca^{+2} , respectively. In the range between 10-4 and 10-5 μM , high sensitivity values were observed for alkali ions in which the border of detection limits were reached. As shown in table 1, the sensitivities obtained are 0.992 and 0.742 μM^{-1} for Na^+ and K^+ .

Comparative results show that signal is proportional to the capacity of adsorbed ions on the surface of sensor which is in agreement with literature (Muzart, 1992; Macdonald, 1987).

Different limits detection observed for various ions can be explained by several parameters: adsorption potential and formation of various metal hydroxides which not the same for different heavy metals ion, oxygen deprotonation making the sensor surface become negatively charged (Hsu et al., 2001; Ding et al., 2005; Norlin et al., 2002; Profumo et al.,

2006; Parsons et al., 1995). So, the incorporation of ion by the Calix[4]arene-SAM can be favored by electrostatic interactions which influences directly the stability of complex formed and its association binding (Sato et al., 1996; Karlsson and Falt, 1997). Although the ionic force in aqueous medium and interactions between host and guest such as π -stacking, dipolar-dipolar interactions have a strong influence on the stability of the complexes (Paarmann et al., 2002; Ma et al., 2001; Muñoz and Palmero, 2004; Farghaly, 2003; Tsukube, 1997).

4. Conclusion

In conclusion, this work demonstrates that alkali ions can be detected using an SPR sensor elaborated by immobilization of Calix[4]arene onto modified SAM gold surface and open the way to biosensor for ions detection and to the use of Calix[4]arene in biochemistry field. SPR sensor developed shows a high sensitivity and a low limit of detection for different alkali ions. Since the magnitude of the SPR response is proportional to the molecular masses of the interacting species, the system is ideally suited to study complexation of small molecules.

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FIGURE CAPTIONS

- Figure 1: SPR sensing principle
- Figure 2: Reaction sequence for the synthesis of p-tetrakisphenylazocalix[4]arene tetra-amide derivative
- Figure 3: SAM-Calix[4]arene scheme for ions detection
- Figure 4: EIS measurement of sensor in buffer solution: a) Gold b) SAM
c) Calixa[4]rene-SAM
- Figure 5: pH optimization for each ion
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- Figure 7: Behavior of alkali ions adsorption on calixarene-SAM in different zones of detection: a) zone 1 b) zone 3 c) zone 3
- Table 1: Sensitivities of SPR sensor for different detection zones

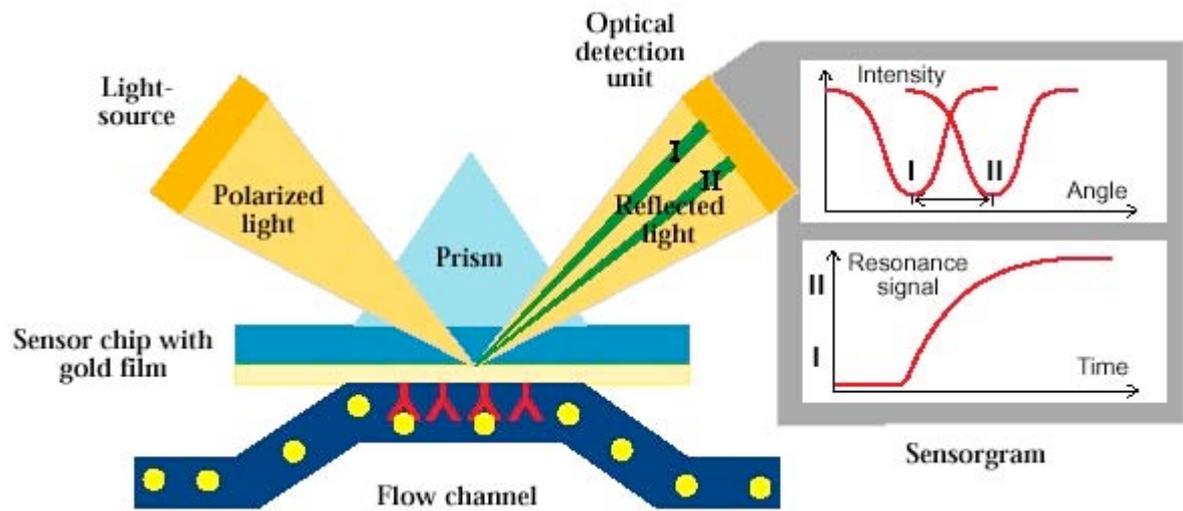


Figure 1. SPR sensing principle

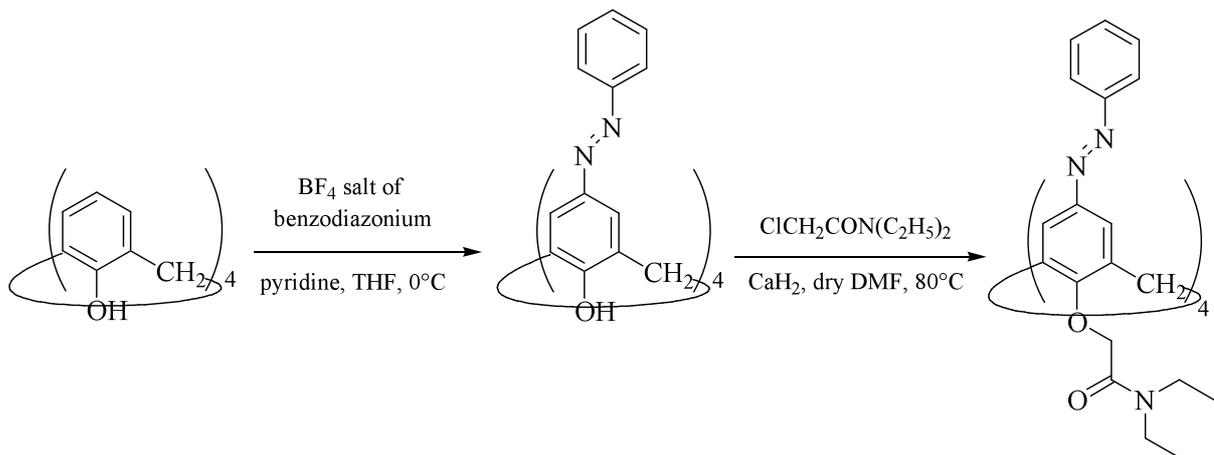


Figure 2. Reaction sequence for the synthesis of p-tetrakisphenylazocalix[4]arene tetraamide derivative

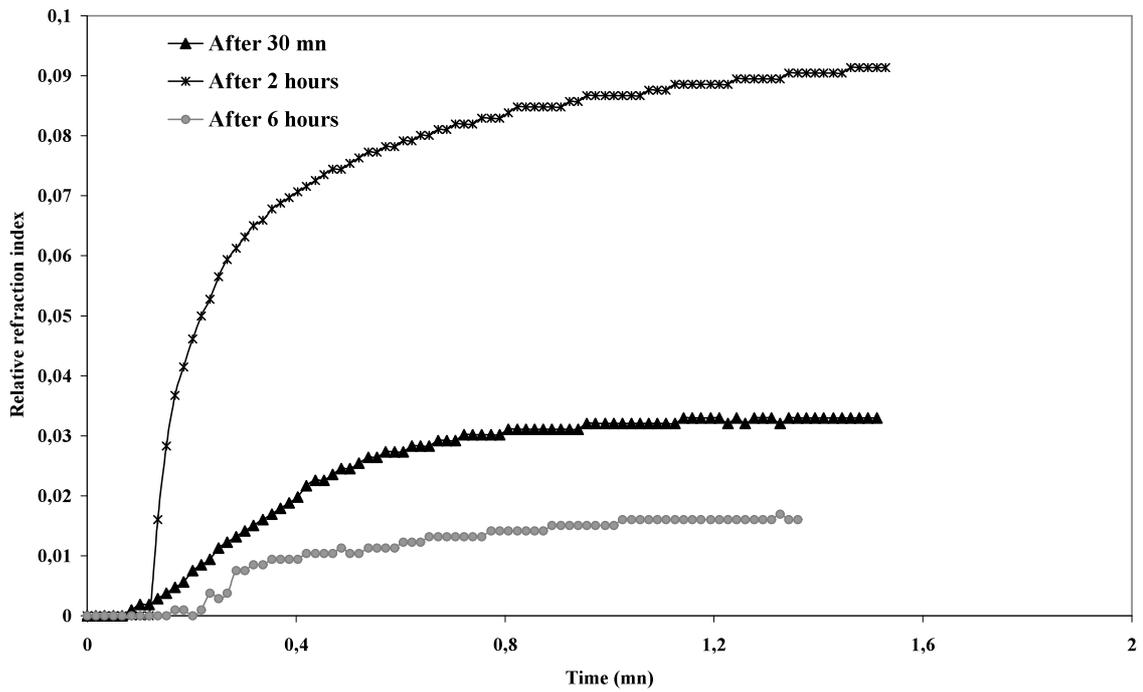


Figure 3. Optimization of time

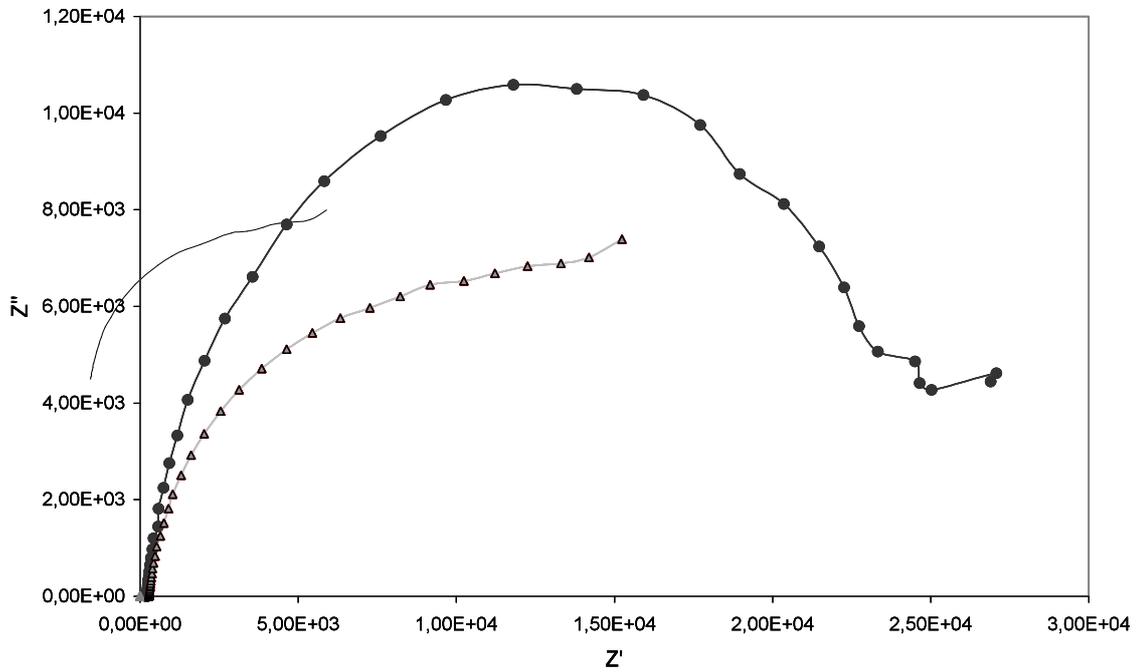


Figure 4. EIS measurement of sensor in buffer solution:

- a) — Gold b) \triangle SAM c) \bullet Calixa[4]rene-SAM

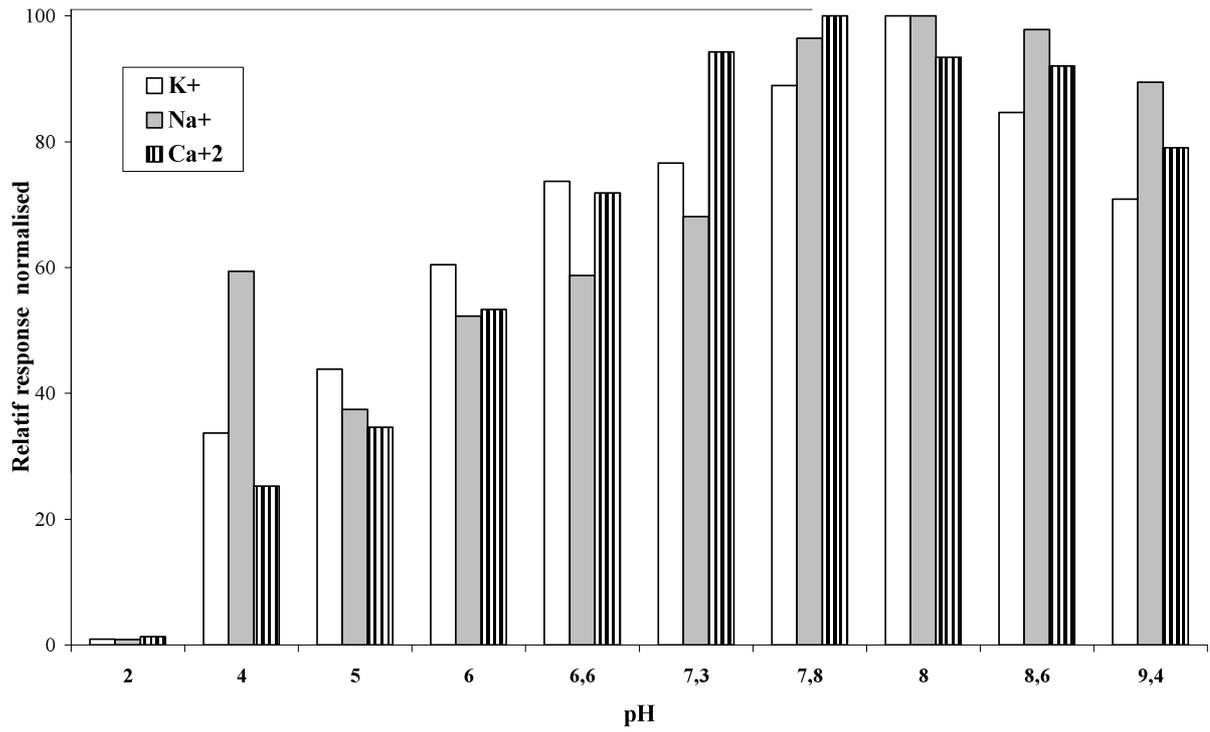


Figure 5. pH optimization for each ion

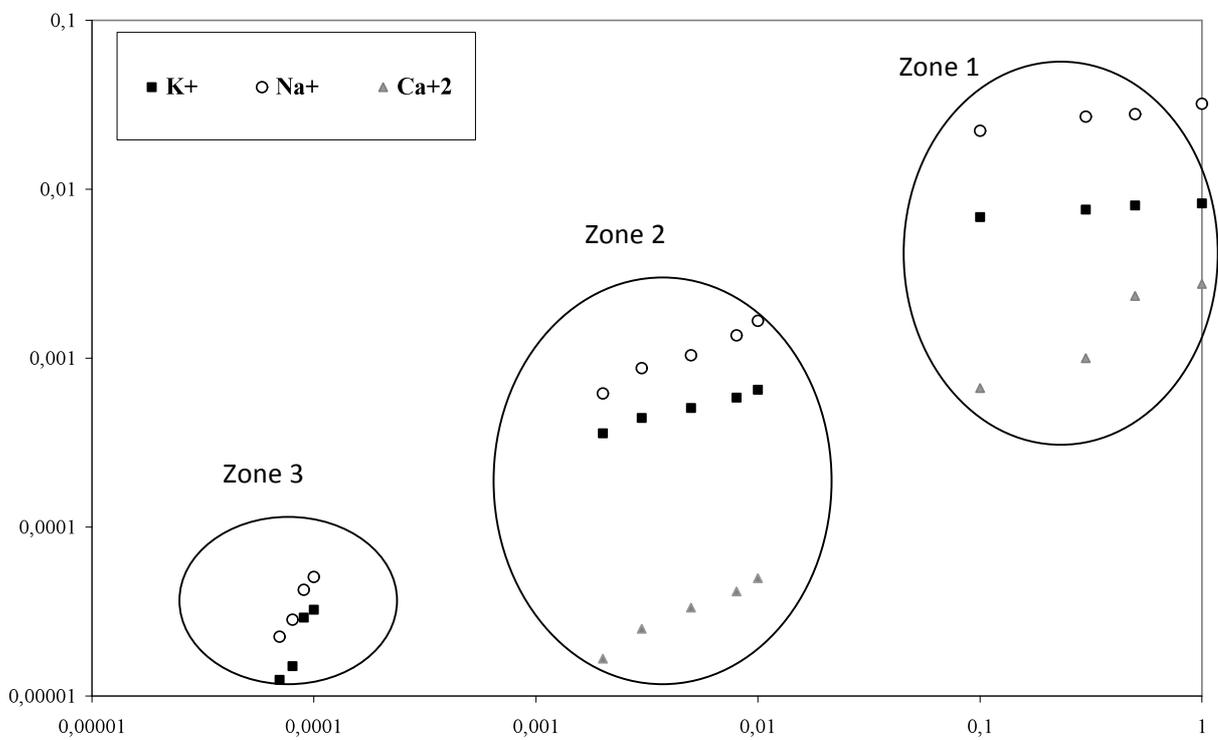
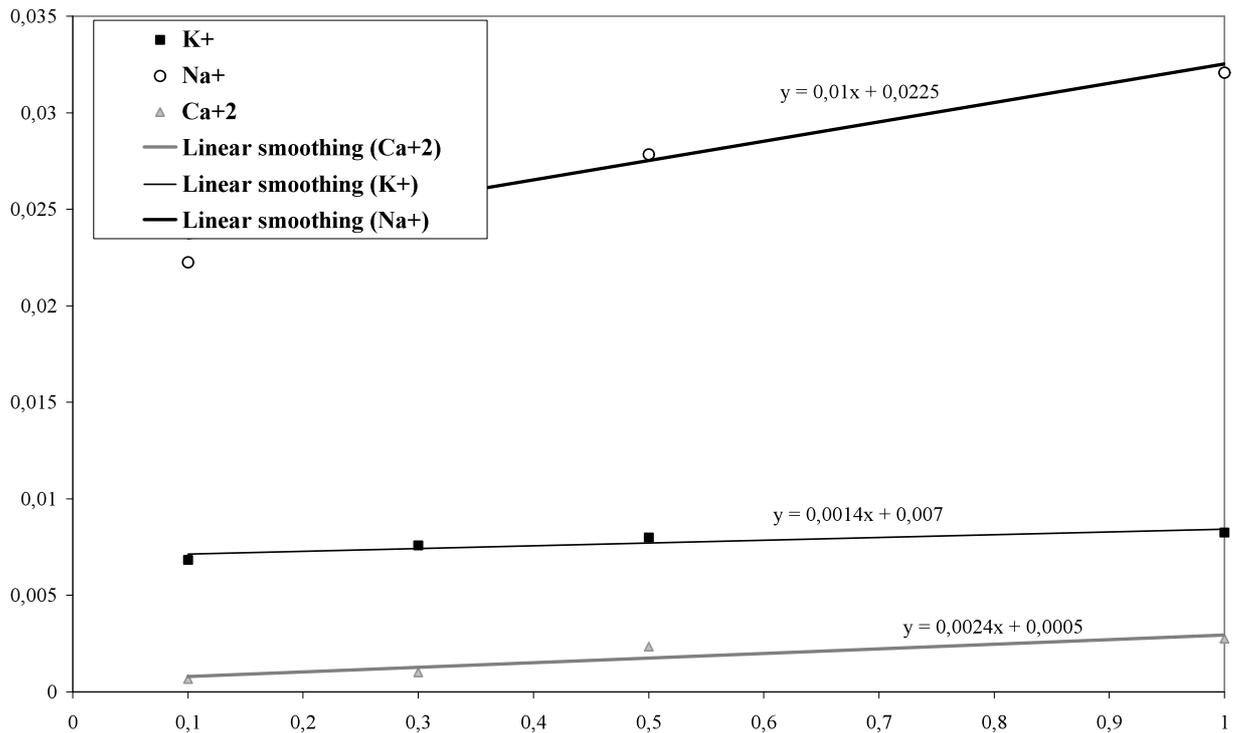
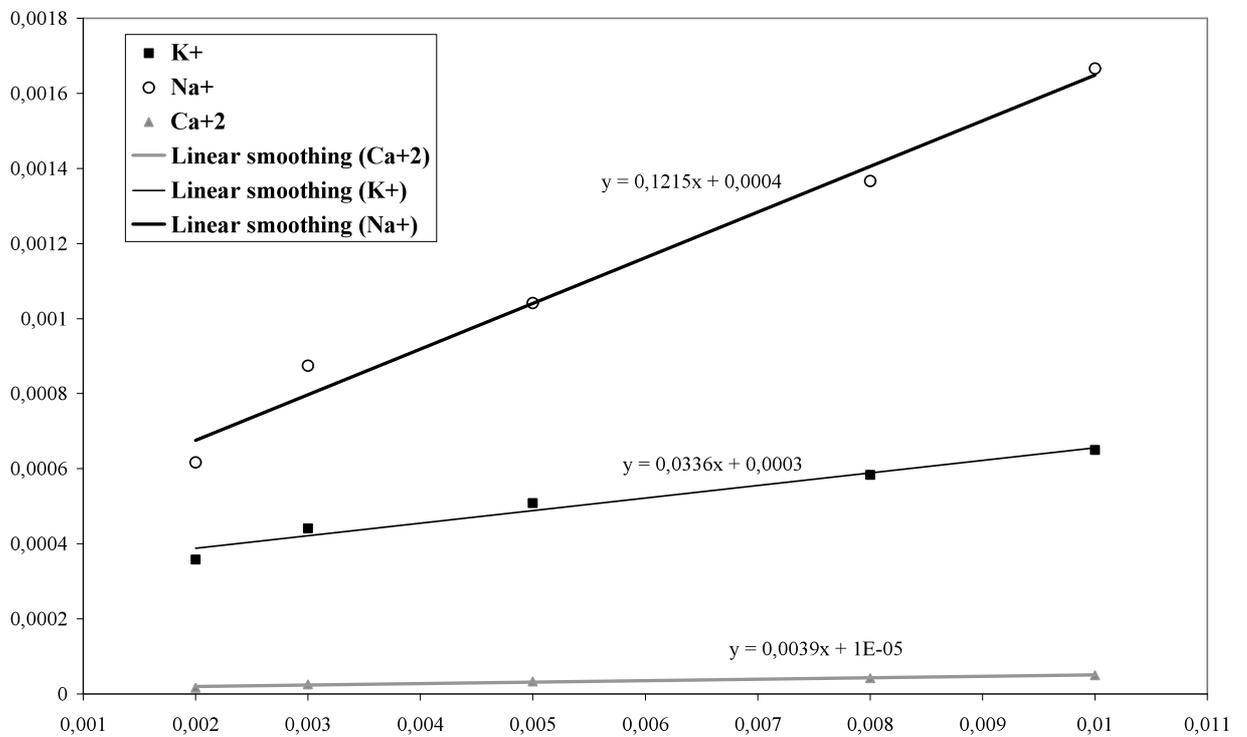


Figure 6. Calibration Curve of SPR calix[4]arene SAM sensor for alkali ions detection



a) Zone 1



b) Zone 2

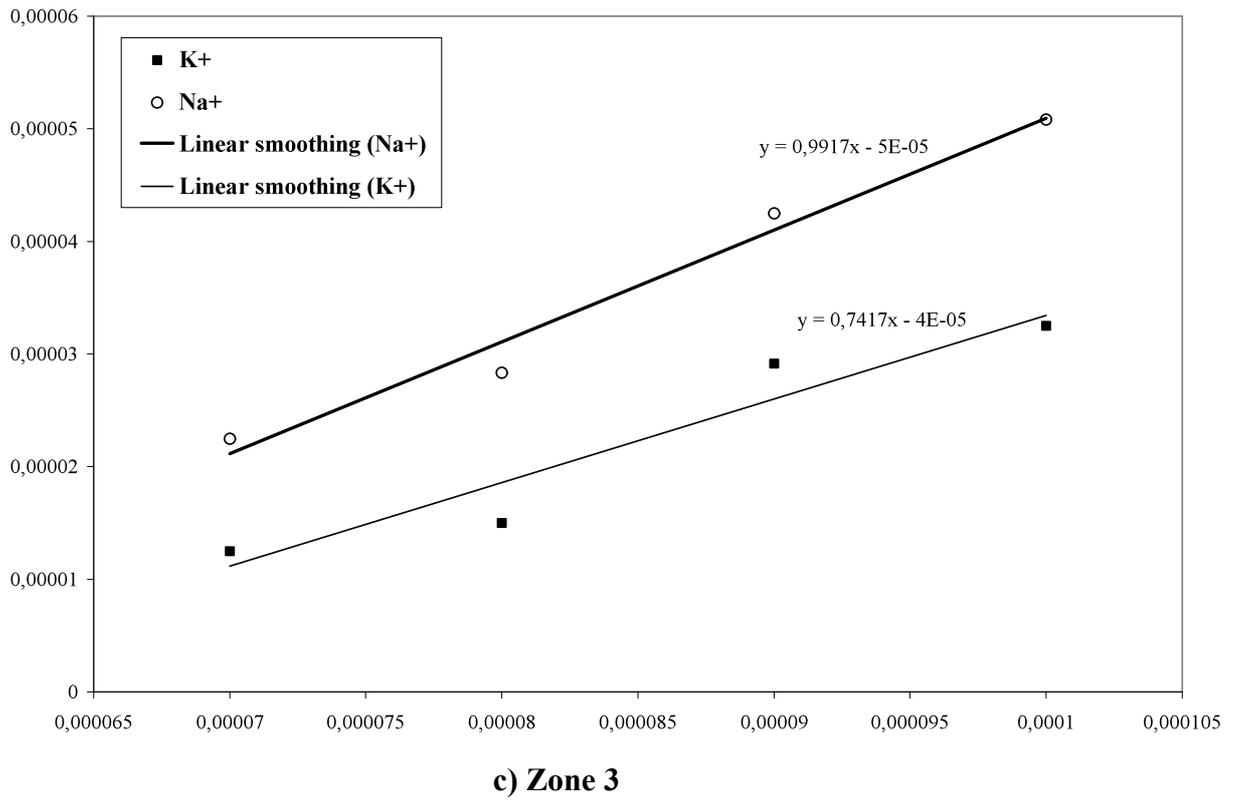


Figure 7. Behavior of alkali ions adsorption on calixarene-SAM in different zones of detection: a) zone 1 b) zone 2 c) zone 3

	Sensitivity μM^{-1}		
	Zone 1	Zone 2	Zone 3
Na⁺	0.01	0.1215	0.992
K⁺	0.0014	0.0336	0.742
Ca⁺²	0.0024	0.0039	-

Table 1. Sensitivities of SPR sensor for different detection zones

Full Length Research Paper

A Systematic Approach: A Solution to Algerian Higher Education

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Accepted May 6 2011

Since the attainment of Algeria's independence (1962) and more particularly since the 1971 reform of higher education, various national year Plans, commissions and national studies have considered the development of education at this level. Although all of them have demonstrated that there has been a subsequent growth (in numbers) at this level, most of them have proposed models of growth in the form of statistical projections and estimations for the 2000s. But until now, no attempt has been made to revise the system's weaknesses and deficiencies in a rational way, or to introduce a comprehensive overview which could help the government and higher educational leaders to pursue higher educational objectives.

Keywords: Higher education; system's weaknesses; deficiencies; rational way; systematic approach, feedback.

INTRODUCTION

The problem, at the higher education level in Algeria is not whether this system can train a cadre of manpower for national development, but, whether it has an adequate capacity to respond to the requirements of the new generation. It would seem that the question of self-reliance and the fulfillment of university functions as discussed in contemporary literature are still adversely affected by many pressures and factors in the Algerian university system. One of the central elements of the higher education sector is its relationship to society. The main problem is how the present system can respond purposefully to the development and needs of Algerian

society itself, and promote the direct participation of universities in economic, social and cultural level. The analysis of Algerian tertiary education suggests that there are shortcomings in the system. Firstly, the increasing shortage of educated manpower and cadres in all spheres in the 1960s led the Algerian government and educational ministries to take into consideration the quantitative rather than the qualitative side of education. Manpower scarcity has become a real problem in the 2000s. Policies have focused on the supply of university students, rather than trying to relate the supply to demand, thereby closing the gap between education and employment needs. Since the reform of higher education in 1971, it would seem that little has been done in Algeria, towards advancing modern

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industrial training. This lack of national system of university education designed to control and orient the necessary manpower in relation to all economic sectors, has been accentuated by past government decisions to allow over production of graduates within the humanities and literary fields. It is also due to students themselves selecting non-technological studies. This has resulted in a serious scarcity of national manpower in scientific and technological fields.

It is thus recognized that Algerian universities have to date not been organized effectively to further national development. Many deficiencies characterise their past and present development. It is now proposed to offer an approach designed to help overcome these problems.

THE SYSTEMATIC APPROACH

Education and training systems are highly complex. What might be solution-seeking at one time could be problematic at another. At this point it is important to recall the previous experience of some industrialised western countries which, in the long-run, convinced developing countries that education and training systems most commonly fail to achieve certain goals due to the absence of adequate scientific planning. It is therefore useful to mention, that most of these countries have attempted to apply a systematic approach, beyond the bounds of electronics, engineering, military services and ecology, to the service of education and training. This new approach seems to be potentially successful in solving a system's problems if it is utilized in a comprehensive and rational manner. In practical dimensions, it can offer a solution to many technical, social and economic as well as educational problems. The writer

proposes to use this systematic approach in relation to Algerian higher education. This is in order to introduce an element of re-thinking in respect to some selected problems.

In the following discussion the term systematic approach is preferred to the term systems approach. This is because the latter has connotations of management theory and methodology, not appropriate to the present discussion of Algerian higher education.

It is first proposed to define a successful system. A successful system can be identified by its simplicity and acceptability. The systematic approach embodies the concept of applying common sense to problems and situations. Mood (1975, p.284) argued that it is simply the idea of viewing a problem or a situation in its entirety with all its ramifications, with all its exterior connections and with full cognizance of its place and its content. Harlow (1971, p.146) defined it as: '... An alternative to traditional approaches to the design of courses of training and education'. Romiszowski (1970, p.72) indicated a system to be 'Any entity, conceptual or physical that consists of independent but, interrelated parts.'

From the above, it may be inferred that the systematic approach should be one feature of the 'planning process'. Planning, in this sense, means the process of selecting one alternative instead of others. As the use of the systematic approach is important in the educational field, it will be useful to describe two types, in using a systematic approach, there can be two bases. Firstly, the feed-forward mode and secondly, the feedback mode. In the feed-forward mode, the emphasis is on objectives. It relies on explicit procedures being used both to design and put the system into operation. In the feedback system, emphasis is less ambitious and less explicit. It is,

however, safer than the feed-forward system because it relies on the process of testing and recycling successive adjustments. This latter mode is felt to be more appropriate in the Algerian case. In any systematic approach, consideration has first to be given to the several steps required by it. Failure to do this can lead to the omission of key steps and a subsequently inadequate performance. The use of such a systematic approach involves goals, a database, and predictive procedure.

In the earlier section, it was stressed that the use of a systematic approach as a means of ensuring a re-thinking of the development process of Algerian universities could be one way of improving the several shortcomings of higher education in Algeria. In order to be comprehensively and rationally used, the Algerian university system, as it is now, must expand its limited academic, traditional programmes beyond its present bounds. This is because there is no hope of introducing effective planning or re-organization of the system if political, economic, social and cultural dimensions are neglected.

The following two phases are felt to be appropriate for the introduction of basic suggestions for the development of the Algerian university system. There would be little hope of bringing about a successful outcome considering what has been done up to the present. Phase one could be termed as the phase of 'General Awareness and Definition of the Problems of the University System in Relation to its General Surroundings'.

The first notion to establish is whether higher educational objectives are well understood and well defined. Government policies at this stage should be working towards creating more systematic communication flows between the different organizations and people in higher educational development in

Algeria Information, for example, about specialities needed for national development could be discussed in this first phase. The first step could make students, teachers, employers and the public aware of the kind of job opportunities and abilities required. Thus these new educational objectives and procedures have to be studied together.

There should be a wider general awareness of the problems of higher education and job opportunities. The general public should be informed and feel the urgency of these new educational objectives. When such groundwork has been achieved it will then be time for the government to prepare itself for implementation.

It is advisable, too, that sudden changes of educational policy which have characterised past Algerian governmental policy, should cease and only take place after prior discussion. It is suggested that there is a need to create a special team or network of small committees made up of academics from each university in Algeria. Their role will be informative and explanatory, in the hope that this will help eradicate problems that may emerge in the early phase of planning. This could involve university educational staff, economists, sociologists, members from the ministry of labour and work, parents and students. This wide coverage is necessary because the ranges of problems are numerous at this phase. The involvement and awareness of universities alone in national problems cannot guarantee the success of national development. It would thus be important that the public and diverse organisations also become involved, as their opinions will aid university development.

For a better systematic diagnosis of higher education demand and employment needs, it is suggested that it is necessary to study overall regional and local problems, such as geographical

disparities between the North and the South. This needs to be done in a systematic way in order to introduce the type of higher educational programmes related to job requirements for these regions. Again, this will involve the views of local wails, rectors, parents, teachers, students and employers.

An equally important consideration at this proposed first phase is the support and participation of university students. If a greater contribution is expected from universities to help solve national needs, student involvement is essential. Their participation in university affairs will increase their awareness and consciousness of local problems and realities. In this case, it is proposed that specialised staffs are provided to guide students and provide special systematic training for local development. It should therefore be possible to develop responsible, non-alienated mature students.

One basic principle that has to be adopted in this first phase is the continuous study of the effects of investment on the higher education system, and the deployment of financial provision throughout the different components of the system. A plan to distribute these resources should be designed in advance during phase one. This is in keeping with a systematic approach and process. Systematic early arrangements are important to overall implementation. It must be borne in mind that sometimes the availability or otherwise of finance for small-scale operations may have effects on large ones.

Phase two consists of introducing 'A Systematic Service-Oriented Approach' to the University Role in Algeria'. This second phase would help promote one of the objectives of the national five-year plan, which aims to solve local problems related to food, sanitation, health problems and manpower shortages.

The principles to be observed at this phase are the side effects which occur when a programme is implemented for selected areas. A more systematic approach would ensure that when assessing small or large experiments or projects, careful attention would be given, at an early stage, to possible, unpredictable changes. If, for example, the decision is made by government and educational leaders to expand the supply of university students in technological and scientific fields: it will not be sufficient merely to start estimating the number of students in these fields and building the necessary technological and scientific sections. What is needed is a systematic estimate as to whether the people and resources currently operating would still be available in the years ahead.

At this second phase, higher educational objectives which were discussed in phase one, must begin to be related to rural/urban and local projects. They should be adjusted and made to support the specific conditions of regional and national development. In this, the writer suggests the creation of national departments of geology, agronomy, and mines (including north and south Sahara regions) in each university centre. These would probably promote and extend the awareness and the role of university education to national needs. These steps would allow this system to offer its assistance to small and large projects. It could also create new forms of university curriculum, based on the development and utilisation of local skills.

To bring the university into contact with the surrounding environment at phase two, the Algerian educational ministry should devote its efforts, not to prestigious architecture (as with the buildings of the university of Constantine), but to the systematic provision of more commodities and

facilities, which are indispensable elements for the developing of mature students and better staff. The improvement of the maintenance of existing university facilities such as the provision of drinking water are not matters of complex engineering, but of provision control and revision. So little systematic attention has been given to the provision of such facilities that it will be necessary to form a special welfare service section in each department to deal with these deficiencies and to resolve them.

Furthermore, too little systematic attention has been given to a university's role in developing Algerian culture and religious forms of education. In Algerian universities, a comprehensive plan to integrate cultural and religious programmes at a higher level should be implemented. This could help to provide ways of identifying culture, values, traditions and religion. Systematic planning to university staff could help students, as well as other primary and secondary institutions and local communities, by providing adequate books, brochures and other materials to develop their knowledge, it is suggested that a theological-centre or department be established in each university, as well as centres of music and art, to help develop creative ways to educate students and people about their country.

To encourage the participation of parents, employers and others, a systematic extension of evening class provision, already in existence, could provide further access to universities. To alleviate the pressure from the increasing number of qualified secondary students enrolling at university and to reduce the number of full-time students, an increase in part-time schemes for students could be implemented. This would also make more use of part-time teachers.

University staffs are rapidly becoming Algerian's, but in order that the university system can continue to produce more productive graduates and resolve the problem of quality of teaching staff, the university should implement a systematic staff training programme to produce more Algerian teachers. Firstly, universities must develop an advanced level programme to produce people with PhD's and other high-level professorial qualifications. More international co-operation and greater systematic use of highly qualified Algerians could facilitate such programmes. Secondly, there is a need to develop the role and activities of university teachers, in respect to rural/urban work. Thirdly, it is important to increase research opportunities and raise the motivation of university teachers to work on development programmes. Fourthly, postgraduate research facilities and salaries have to be improved to reduce the 'brain drain and related problems.

To encourage and improve the university teaching staff, a systematic -problem-centred approach to methods of teaching and research will have to be developed. It is necessary to decentralise and decrease differences within regions in Algeria . It is suggested that to attract university teaching staff and students to work or study in the different university centres and towns, incentives should be offered such as increased salaries and better accommodation for those willing to do so.

The above suggestions cannot be implemented if a rigid university system and structure still dominates. The role of educational and university planners and policy makers in this second phase is important in terms of demonstrating the necessary systematic capacity for change. In order to complete this second, 'service-oriented' phase for the development of the 'university's role in Algeria, policy-

makers must maintain the following systematic links, if manpower needs and social demands are to be balanced:

- Links between secondary and higher education;
- Links between higher education and employment;
- Links between higher education and overall national planning;
- Links between higher education and scientific research;
- Links between higher education and society

The above suggestions are not exhaustive, but they should help universities play a more significant and effective role in national development. These steps would best be developed if a more systematic approach would be adopted in relation to higher education in Algeria.

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Full Length Research Paper

THE CONTRIBUTION OF LANGUAGES TO CULTURE

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Accepted May 6 2011

This article discusses three separate topics of interest. Firstly, it gives a general historic and modern discussion of some important views in languages and culture. The distinctive relationship between them is explained and discussed; a particular attention is being paid to the contribution of globalization in the development of languages and culture.

Secondly, it deals with the position of English language in the world today. The increased awareness and the reasons for teaching it are discussed.

The third part of this article is to supply the readers with some useful recommendations for teaching additional languages to develop the cultural aspect of a population with special reference to the 'Aures' region of Algeria.

Keywords: History; language; English; globalization; teaching, Aures, Algeria.

INTRODUCTION

The idea of teaching languages to culture is not a new one. In the historic period, the teaching of foreign languages was confined to the classical teaching of Latin and Greek. Around 500 BC, Greek literature was considered as the apogee and the origin of knowledge in the Western world. Famous theologians such as Plato and Aristotle advanced different new theories and reasoning about the creation of the universe.

Plato's view is very interesting in terms of mobility and change. He established a school of philosophy called "Academia" and with his pupil Aristotle advocated that in physical terms the mobility of every form is inevitable. In linguistic terms the implications are similar in the

sense that every human being is progressing and therefore progress comes along with them. Language is learnt automatically element by element; without memorizing rules.

During the renaissance, educators such as the French Montaigne Michel Eyquem (1533-1592) introduced a new literary essay on the knowledge of humanity in general. He asserted that "Each man bears the complete stamp of the human condition". The natural order means each one of us learns by himself at his own pace, with no dictation.

In "Prodromus Pansophia", Johann Amos Cornemius" (1592-1670) views on language acquisition were similar to Montaigne's. His teaching approach advised a gradual exposure of the pupils to the world because he thought that learning results from the inner desire of the human and not of what it is imposed

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on them. He advised what we call today: The “contextual approach” (teaching by images) in Prevot. J., (1981).

In the late 18th century, authors such as Wilhelm von Humboldt (1762-1835) considered the importance of the addition of teaching culture into languages.

In his theory of “human education” (1793), he mentioned that ‘...self-education can only be continued ...in the wider context of development of the world’ (p.33).

In 1789, he stressed that ‘the education of the individual requires his incorporation into society and involves his links with society at large’ (p.155). Humboldt’s educational ideas are entirely in connection with the new trends of globalization of today’s change. In reality educational progress can only be possible if social considerations are considered locally, nationally and internationally.

Similarly, Jean Jacques Rousseau’s (1772-1778) philosophy of education in the “Emile” was not a major point of controversy in the education per se, but it was interesting in terms of language and its relationship to society in the sense that someone who is properly educated cannot return to primitive life and ignore his relationship with society.

In Africa alone there are about 1500 out of 6000 universal spoken languages.

The question to ask here is which language do people across the world choose to communicate with this diversity of languages?

The model of English preferred and established as a prestigious international language has evolved since the second war as a result of the industrialized revolution which created new scientific and technological language terms linked to the new inventions. According to the writer, this position of the English language as the most preferred in the world could have been better in terms of progress if another language other than

English had had the opportunity to develop like it and expand itself across the World! Of course this “so-called language’ has to be as much richer and easier in terms of its vocabulary, phonetics and written transcript as the English Language. The writer thinks that other nations did not have the chance to progress and expand their languages as international languages as a result of their difficult geographical positions. Therefore; this inhibited their languages to be established across the world like the English Language did.

From personal perspective, the author’s views about the success of a language depends on many factors not found in old and contemporary literature. From a scientific point of view, our ancestors’ human nature may have played a determinant factor in the essence of each language. The writer means by that if people of a given language have, for instance, been more relaxed, nervous or stressed in their way of life, their temperaments affect the whole composition of their languages (the structures, forms and lexis). This could explain why a given language’s lexical dictionary is more developed and rich than another! The genetic disposition to acquire more than one language could also play a major factor.

The nature of work activity and stability of a country could also be considered as a main vector in the success of its language. I mean, if our ancestors had an interesting varied, business activity; they would have invented more appropriate business terminology adopted to sell their products. But external factors like wars, colonization, dictators and religions were additional vectors influencing the success, stagnation or destruction of local languages. Certain types of daily media which are considered as a window to develop some languages throughout the

world, such as the daily exposure to some well known Television channels are to blame and may lead to a kind of isolation in some neglected areas and can be a determinant factor in decreasing the contact in oral communication between people around the globe.

Authors such as Sir Ernest Gowers (1987) see the demand of the English language originating from residential reasons. With over 300 million people speaking English, most of them live in the United States of America and England (222 million and 56 million respectively). There are other significant numbers elsewhere, mainly Canada (17 million), Australia (14 million). In addition, English is the official second language in many countries where it is used as a means of education. Among these countries we name former British colonies such as India (700 million) Pakistan (85 million).

Paradoxically, to this big demand of English, the facilities and conditions for learning it are still very poor, especially in the African continent.

If we take the example of Algeria, the country has been colonized many times. The French one lasted for more than a century (1830-1962). After such a long period, the contribution of Algerian languages (estimated to be 18 with the predominant ones are: Arabic, Chaoui, Kabyle, Tachelhit and Tamazight's, Acalan, 1986) to culture had decreased significantly due to the French policy of assimilation of our languages. This resulted in the disintegration of Algerian culture and favoured the spreading of the monolingual learning of French. Today, most people in Algeria are still limited to the learning of only one foreign language, namely French.

CONCLUSION

With the expansion of hydrocarbons and the geographical position of some underdeveloped countries like Algeria, Emirates Arab's... English language will continue to be a considerable tool of linking cultures, ideas and customs worldwide. The Daily Telegraph (2008,p1) advocated that "The increased mobility and mass migration across borders, culture is becoming more homogenized and, some might consider, less meaningful as it gets easier to feel free at home in many countries".

On one hand, if we don't make an effort to preserve our national languages in Algeria, Africa and the world, by the next decade many languages and dialects will disappear taking with them hundreds of culture. On the other hand, if we want to become civilized citizens in a multicultural world, we must have contact with other cultures through the learning of languages. Because languages often bring change. This change can be social, economical and cultural.

RECOMMENDATIONS

Additional languages are a vital source to enrich the school curricular and the local culture. In order to bring the Algerian Universities, especially, the University Center of Khenchela closer to its community (schools and parents), to remove the existing linguistic and cultural barriers and to build a special multilingual and multicultural Algerian society, the introduction of at least two 'Additional Languages' (A.L) is advised for children aged 5-15 years old in the school curricula like English and Berber. Berber dialect, in our geographical context "Chaouia" is part of our identity and our cultural heritage. As part of a nation we must encourage its use in

schools. For the Algerian Berber to prosper, one must make sure that young people in this region of 'Auras' have the opportunity to learn it. Schools are the only vector for language change. Over centuries different colonizers have directly or indirectly oppressed and assimilated our local languages and culture." Berber" language should be valued as any other spoken and written languages at home and in the world.

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