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The application of polymerized lipid vesicles as colorimetric biosensors for real-time detection of pathogens in drinking water

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Abstract The inadequate treatments given to the served waste water which are disposal to the rivers and sea coast are the major sources of faecal Microorganisms and enteric bacterial pathogens. They are among the most serious effects of water pollution bringing risks on public health. None of the current methods for detection of pathogens offer real-time on site solutions, are capable of delivering a simple visual detection signal, or can be easily instrumented as an indicator of the presence of a pathogen in water. The use of lipid vesicles incorporating Polydiacetylenes (PDAs) for the development of biosensors for "real-time" detection of pathogens has become an alternative, due to its potential for simple colorimetric response against harmful environmental effectors. However, its actual application in the field has been complicated because lipid vesicles are unable to respond specifically to environmental changes. In this paper, we report several experimental trials leading to improved response in the detection of flagellated pathogens in drinking water. Chromatic biomimetic membranes of TRCDA/DMPC and TRCDA/DMPC/Tryptophan were used in agar and liquid media, which were challenged with different amounts of *Escherichia coli* and *Salmonella typhimurium*. In addition, the effect of some divalent cations on the interaction with vesicles TRCDA/DMPC was investigated. The results indicated an improvement in the response times, both visually and quantitatively, through the use of TRIS-EDTA and proper growing conditions for *E. coli and Salmonella*. With the application of both conditions, it was possible by incubation at 35ºC to promote bacterial growth, therefore avoiding a dramatic effect on the colour change over control samples which may invalidate the test. Our experiments indicated that the minimum bacterial concentration necessary to produce the transition from blue to red on the vesicles as biosensor approaches 10^8 CFU/ml within 4 hrs, faster than traditional methods such as MPN or plate count agar.

We present here incubations of samples of contaminated water at 35ºC, in agar plates containing chromatic biomimetic membranes of TRCDA/DMPC. A measurable colour transition is obtained within a reaction time of four hrs, which compares favourably with detection times between seven to 24 hrs corresponding to available tests.

Keywords: bacterial detection, biosensors, lipid vesicles, PDA

INTRODUCTION

Faecal pollution of water resources such as surface waters (Hörman et al. 2004), stormwater (Jiang et al. 2005), rainwater (Ahmed et al. 2010), and supply water (Cabral, 2010) from point and non-point

sources poses public health risks due to exposure to pathogenic bacteria, viruses and protozoa, causing millions of deaths worldwide, as well as economic losses even in developed countries.

The problem is even more acute in developing countries (Ivnitski et al. 1999; Venter, 2000; Gleick, 2002; Leonard et al. 2003). The knowledge about microorganisms involved in enteric diseases has increased due to the development of new and more sensitive methods leading to the better taxonomic classification. Common water microbiological and chemical contaminants include microorganisms such as *Escherichia coli, Giardia* spp, *Vibrio* spp, *Salmonella* spp. and *Shigella* spp., (Hörman et al. 2004) metals such as lead, arsenic, copper, cadmium and mercury, organic pollutants such as toluene, pesticides, herbicides and several agents based on chlorine and asbestos (Yang et al. 2005; Zawala et al. 2007). Public health and environmental protection agencies require safer drinking water; this means that drinking water should be free of pathogenic bacteria, and other chemicals and pollutants.

Bacterial detection protocols recognized by the Health authorities recommend diagnosis through isolation and identification of the pathogens after growth from the source. A standard procedure includes plate count method using enriched culture on selective medium and subsequent specific identification of presumptive colonies (Deisingh and Thompson, 2002) In some cases such as *Salmonella* and *Escherichia coli* O157:H7, *enterodiarreica*, and other pathogens of higher incidence, identification is performed by biochemical tests also including the use of specific sera (Hajra et al. 2007) and phage typing (Hickman-Brenner et al. 1991).

Since pathogens bacterial detection requires three to four days and complex procedures, it is convenient to develop rapid and sensitive tools for early detection (Hobson et al. 1996; Ertl and Mikkelsen, 2001; Canhoto and Magan, 2003; Gfeller et al. 2005; Simpson and Lim, 2005; Oczkowski et al. 2007; Mata et al. 2010).

Currently, the Food Drug Administration (USA), the agency responsible for health in the United States, is working on various sophisticated techniques and methods that together give an idea of presence of contaminant microorganisms in a few hrs. Some detection methods include agglutination tests (Chapman et al. 1997), ELISA, PCR (Waage et al. 1999), hybrid (Fitts et al. 1983; Datta et al. 1988), and the direct use of antibodies (Taitt et al. 2004), bioconjugated nanoparticles (Zhao et al. 2004), bioelectrical devices (Lazcka et al. 2007; Skottrup et al. 2008; Yang and Bashir, 2008). The main challenges facing these techniques not only requires long incubation times to obtain results, but also highly trained personnel or specialized equipment that make them impractical applied to field conditions. On the other hand, the use of PCR or ELISA are unable to discriminate between live and dead cells, which potentially can lead to false positive results. Other technologies under development rely upon the metabolism to discriminate among alive and dead organisms. ATP assays (Deininger and Lee, 2001) and ethidium monoazide bromide (EMA)-PCR (Nogva et al. 2003; Rudi et al. 2005) are examples.

The 10, 12-Tricosadiynoic acid (TRCDA) produces conjugated polymers - polydiacetylenes - that have attracted great attention in recent decades. Its main feature is that exhibits colour properties (Charych et al. 1993; Ma et al. 2002; Jelinek and Kolusheva, 2001). The polydiacetylene is formed by adding aligned diacetylene monomers initiated by ultraviolet radiation. The molecular mechanism for the colour change is believed to be irreversible where stress-induced structural transition of the conjugated polymer backbone takes place. The structural features of the TRCDA, *i.e.* hydrophobic tail (methyl group) and hydrophilic polar group (carboxylate) result in the formation of vesicle aggregates in aqueous solutions (Reppy and Pindzola, 2007; Chen et al. 2011).

The TRCDAs polymers may turn from blue to red on exposure to heat, mechanical stress, pH change, or binding to biological agents. Extensive studies of polymerizable diacetylene have been carried out in vesicles. The optical properties of color transitions of polydiacetylene are suitable for many applications in optical devices and biosensors (Su et al. 2004; Su et al. 2005).

Many recent scientific reports have years illustrating biosensor applications for TRCDA vesicles (Charych et al. 1993; Reichert et al. 1995; Spevak et al. 1995; Charych and Nagy, 1996; Pan and Charych, 1997; Sarkar et al. 2005; Pires et al. 2011). Most of these applications involve the modification of the head groups of the TRCDA by incorporation of functional groups and biomolecular recognition units on the surface of TRCDA.

Experiments described in the literature have shown various alternatives for colorimetric detection of proteins, nucleic acids, and microorganisms, all through their interaction with the units of specific receptors on the surface of TRCDA vesicles. In their work, Rangin and Basu (2004) and West et al. (2009) have demonstrated the colorimetric recognition of lipolysaccharides from different bacteria using TRCDA derivatized with L-tryptophan. These reports show a precedent for the application of this receptor in interaction with bacteria. The detection of influenza viruses was achieved through sialic acid residues located on the surface of the vesicles (Reichert et al. 1995).

Despite the versatility of applications assigned to the TRCDA, very few experiments dealt with lipid-TRCDA vesicles tested with genuine samples or field applications. The nature of lipids and their ability to generate attraction to different biological and chemical components leads to multifactorial experiments, because the vesicles are susceptible to many different factors. The use of TRCDA materials for the production of biosensors has been complicated by their lack of selectivity to environmental conditions such as ions, osmotic pressure, temperature changes, solvents and pH (Reppy and Pindzola, 2007).

In this paper, we describe various experiments oriented towards achieving an accelerated response of bacterial detection using TRCDA/lipid vesicles for the development of biosensors, mainly associated with the contamination in drinking water. The application of biosensors in field tests through a simple and direct signal should give information on the presence of pathogens to promote the reduction of illness and deaths caused by this far-reaching problem.

MATERIALS AND METHODS

Reagents

10, 12-Tricosadiynoic acid (TRCDA) monomer was purchased from Sigma-Aldrich (USA). Cleaning of TRCDA was carried out by dissolution in chloroform and filtered using a nylon membrane of 0.45 µm of pore size. The supernatant was removed by rotary evaporator.

The lipids 1, 2-sn-glycero-Dimyristoyl-3-Phosphocholine (DMPC), 1,2-Dimyristoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] (sodium salt) (DMPG); 1,2-Dimyristoyl-sn-glycero-3-Phosphoethanolamine (DMPE) were obtained commercially from AvantiLipids (USA). Tri-(hydroxymethyl) aminomethane (TRIS-amino) and EDTA were purchased from Merck Chemical (Germany).

Bacterial strains

Several bacterial strains were used several enteric bacterial strains: *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028) and *Shigella sonnei* (ATCC 29930). Strains were cultured at 37ºC on solid medium using Luria-Bertani (LB) broth (Beckton Dickinson, USA) supplemented with 1.5% of microbiological agar (Beckton, Dickinson). For bacteria free of culture medium, they were recovered from the agar and washed three times with phosphate buffer solution. In each washing, the cells were recovered by centrifugation at 5,000 use g not rpm for 5 min at 4ºC.

Minimal medium was used in the experiments with derivatized TRCDA-tryptophan vesicles prepared using the following reagents on the bases of concentrate solutions: 10x (g/L) of dipotassium phosphate, 7.0; Phosphate monopotassium 2.8, sodium chloride, 0.5. 100x (mg/l) of thiamine-HCl; 0.1, folic acid, 0.04; nicotinamide, 0.2; pyridoxine-HC l, 0.2; 500x (g/l) of $MgSO_4 \bullet 7H_2O$, 0.1; $FeCl_3 \bullet 7H_2O$, 0.01; $MnSO_4 \bullet H_2O$, 0.005; $ZnCl_2 \bullet H_2O$, 0.0064; $CaCl_2 \bullet 2H_2O$, 0.00066; $CuSO_4 \bullet 5H_2O$, 0 , 00036; $CoCl_2 \bullet 6H_2O$, 0.0003; H_3BO_3, 0.0065; EDTA, 0.01 (g/l) and 0.146 ml of HCl 37% ($^v/_v$); glucose, 1(g/l) and ammonium sulphate, 1 (g/l).

Synthesis of derivatized TRCDA

The production of derivatized TRCDA was carried out following the procedure described in previous studies (Rangin and Basu, 2004; West et al. 2009). The synthetic reaction of derivatized TRCDA is shown in the Figure 1. The procedure included two synthetic steps. First synthesis corresponds to a

Steglich esterification, where both reactants TRCDA and N-hydroxysuccinimide (NHS) are converted to N-succinimidyl-10, 12-pentacosadiinato where the reaction is catalyzed by the compound 1 (3-dimethylaminopropyl) -3-ethylcarbodiimide hydrochloride (EDC). After this reaction, TRCDA is converted in an ester (compound A in Figure 1). This reaction had a yield of 83%.

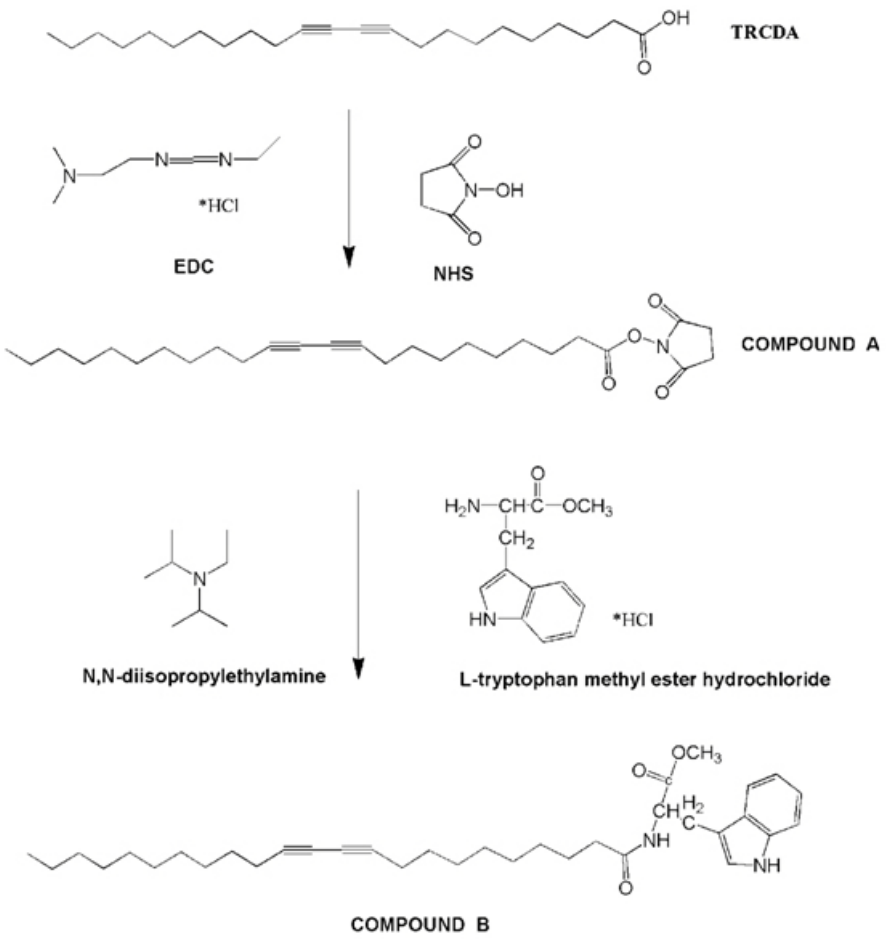

Fig. 1 Synthesis reaction for the production of compound A: N-succinimidyl-10,12-tricosadiinato and compound B: acid 3 - (1-H-indole-3-methyl)-2-10, 12-diinoilamino-propionic acid methyl ester. The latter compound corresponds to derivatized TRCDA.

In the second synthesis, the ester (compound A) is converted to an amide (compound B) which is obtained by association of compound A and with L-tryptophan methyl ester using N, N-diisopropylethylamine to give the basic medium, pH 9.0. The yield of this reaction is 35%.

Colorimetric vesicle preparation

Both types of vesicles were prepared at a concentration of 1 mM using TRCDA/DMPC in a molar ratio (3:2) and TRCDA/DMPC/Tryptophan (2.5:2.0:0.5). The lipids were dried under a stream of nitrogen for 5 min. After evaporation, the vesicles were diluted in Milli Q water and the suspension was sonicated for 3 min at 70ºC, using a probe sonicator (Sonics Vira Cell, Model VCX 130, Series No. 53341 R) at a power of 130W, a frequency of 20 kHz and amplitude of 30%. Resulting vesicles solution was cooled to 4ºC overnight. Then, they are irradiated at 254 nm using a Cross-linker UV (Vilber Lourmat, Model BLX 254, Series No. 996 444) to a power of 0.8 J for 0.4 min.

Preparation of lipid vesicles in agar

After sonication, the solution of vesicles TRCDA/DMPC and TRCDA/DMPC/Tryptophan (concentration 5 mM) was added to LB agar plates at a temperature of 45-50ºC. The mixture was cooled to room temperature and poured into six-well plate (Cellstar, Greiner Bio-One). After solidification of the agar, the plate was kept at 4ºC overnight, after which it was irradiated at 254 nm for 0.4 min. Challenge tests of the vesicles with bacteria was carried out inoculating 5 mL of a solution of *Escherichia coli* and *Salmonella typhimurium,* at different concentrations (10^{11}, 10^{10} and 10^{8} UFC/mL) on chromatic vesicles. The vesicles were incubated at a temperature of 35ºC.

Measurement of colour change of the vesicles by UV-VIS spectroscopy

The testing tubes were prepared by the addition of 400 mL of bacterial samples to 200 mL of vesicle solution 1 mM and 400 mL of TRIS buffer pH 8.0, 25 mM plus 5 mM EDTA. All measurements were performed in a spectrophotometer UV/ VIS model UNICAM UV-4 (American Instruments, USA) following the protocol described by West et al. (2009). Colorimetric response (CR) was evaluated as follows:

$$CR = \frac{[(\mathrm{PB}\,0 - \mathrm{PBF})}{\mathrm{PB}\,0} \times 100\%$$

[Equation 1]

where PB = A blue / (A red + A blue), A is the absorbance of either the blue component (≈ 640 nm) in the UV-VIS or the red component (≈ 540 nm), PB_0 is the initial percentage of vesicle solution before addition of the sample, and PBF is the final percentage of the solution of vesicles after the addition of the sample. Each experiment was repeated 3 times in order to acquire an average data.

RESULTS

Bacterial detection using agar chromatic vesicles

Bacterial assays were carried out using chromatic biomimetic membranes TRCDA/DMPC and TRCDA/DMPC/Tryptophan dispersed in agar, which were challenged with different concentrations of *E. coli* and *S. typhimurium*. The colour transitions from blue to red caused by bacterial growth at 35ºC of *Escherichia coli* on agar are shown in Figure 2. The results indicate that vesicles TRCDA/DMPC/Tryptophan react in 4 hrs using a concentration of 10^{11} CFU/mL of *E. coli* and *Salmonella typhimurium*. Red colour became intense and expanded in the plate as time passed, indicated spread of bacterial biomass. In the case of vesicles TRCDA/DMPC, we observed the same phenomenon, but the colour change occurred 30 min later, suggesting that the tryptophan receptor favours the response time in those chromatic vesicles. This phenomena associated to colour change occurring during bacterial growth has also being described by other authors (Silbert et al. 2006; Pires et al. 2011).

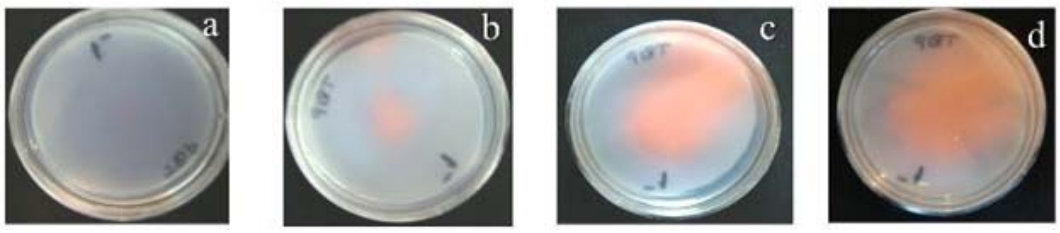

Fig. 2 Transition of colour induced during the growth of bacterial colonies. Vesicles plate images TRCDA/DMPC/Tryptophan in agar at a concentration of *E. coli* 10^{11} CFU/mL. (a) Bacterial growth on plates at time zero; (b) After 4 hrs; (c) after 5 hrs and; (d) after 7 hrs. Incubation was carried out at 35ºC.

A bacterial concentration of 10^{10} CFU/mL was detected 5 hrs after inoculation a+nd a concentration of 10^{8} CFU/mL was detected 7 hrs after inoculation. All these results would indicate that it is possible to accelerate a response from chromatic agar plates incubated at 35ºC. Figure 3 shows different bacterial numbers grown on agar, which reinforce the fact that the minimum bacterial concentration needed to induce visible colour change is 1 x 10^{8} CFU/ml in LB agar.

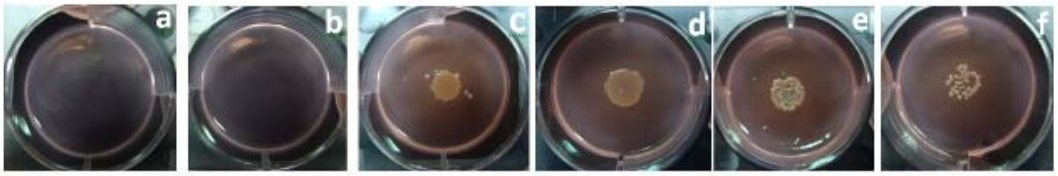

Fig. 3 Transition of color induced by the growth of *E. coli*. Agar plates without inoculation (a) and inoculated with bacteria-free buffer (b) correspond to experimental targets. Concentrations were 10^{11} (c), 10^{10} (d), 10^{9} (e) and 10^{8} (f) CFU/mL. The plates were incubated at 35ºC.

Use of TRIS-EDTA buffer in the measurement of drinking water

As mentioned earlier, substances secreted during bacterial growth diffuse through the agar matrix and interact with phospholipid-TRCDA nanoparticles, and thus induce colorimetric transitions resulting in an indication of bacterial presence.

To address the experiments toward the design of a biosensor using chromatic biomembrane it is necessary to consider the sensitivity of TRCDA/DMPC vesicles to different environmental water conditions or the presence of trace compounds, such as the presence of divalent metals, which obstruct the direct detection bacteria in drinking water. Indeed, preliminary experiments performed to test water samples with different types of vesicles, produced high colorimetric responses due to the interaction of pathogen-free water at a concentration of 1 mM after 3 hrs of incubation at 27ºC, pH 8.0. The colorimetric transition induced by drinking water on vesicles were 46.5, 77.7 and 89.1% for the vesicles prepared with TRCDA/DMPC (molar ratio 3:2), TRCDA/DMPG/DMPE (molar ratio 3:1:1) and TRCDA, respectively. The RC larger obtained with TRCDA/DMPG/DMPE than TRCDA/DMPC indicate membranes are weaker when they are prepared with DMPE lipids. Also, these results indicate a strong response from the water, without the presence of bacterial, which would invalidate the uses of chromatic vesicles as method of detection.

The content of metal ions and anions to guarantee the quality of drinking water is established by the Chilean Drinking Water Standard (NCh, 2005). Some of the species present in drinking water include among others calcium, magnesium and free residual sodium hypochlorite (chlorine). The effect of these substances on the interaction with vesicles TRCDA/DMPC was investigated. Also we tested phosphate, which is frequently used in laboratories. Colorimetric responses are shown in Table 1. The results indicate that divalent metals cause greater interference in the chromatic vesicles.

Table 1. Colorimetric responses of 1 mM TRCDA/DMPC (3:2) vesicles exposed to the interaction with chlorine, phosphate, calcium magnesium. Reaction time: 3 hrs at 27ºC.

Compound	%CR
Chloride 2 ppm	0,2
Chloride 30 ppm	0,1
Phosphate 50 ppm	0
Phosphate 400 ppm	8,0
Magnesium40 ppm	13,6
Calcium 40 ppm	29,6

Tris-EDTA (TE buffer) buffer is commonly used in molecular biology, especially in procedures involving manipulation of DNA or RNA. Tris is a common pH buffer, and EDTA, a chelating molecule for cations such as Mg +2. We incorporated the use of this buffer in the measurement of drinking water to neutralize the effect of calcium and magnesium. The proportions used were 25 mM Tris and 5 mM EDTA. Previous experiments determined that the colorimetric response to water and vesicles TRCDA/DMPC was 46.6%, while when using the Tris-EDTA buffer, the observed response fell sharply to 0.7% suggesting the formation of a complex of divalent metal-EDTA, which prevents interaction with the chromatic vesicles. This is a major step forward toward possible use of lipid-based vesicles TRCDA/DMPC for detecting bacterial contamination in drinking water.

Bacterial detection with lipid vesicles solutions

From the results described above and in studies published by other authors (Ma et al. 2002; Silbert et al. 2006), we learned that the transition from blue-red colour observed in the agar matrix, is related directly with the diffusion of substances secreted by the bacteria during growth. Since the aqueous medium allows a more direct contact between the environmental elements and bacteria, tests were conducted to evaluate the TRCDA/DMPC vesicles challenged with bacteria in aqueous phase. The trial incorporated TRIS-EDTA buffer at pH 8.5 in vesicles TRCDA/DPMC and samples were incubated at a temperature of 35ºC for 1 hr. Colorimetric responses of *Escherichia coli* (EC), *Shigella sonei* (SS) and *Salmonella typhimurium* (SE), added to vesicles are shown in Table 2. When bacterial was added directly from the growth medium to vesicles solution they showed a CR of 15.2%; 0.3% and 25.6% for *Escherichia coli* (EC), *Shigella sonei* (SS) and *Salmonella typhimurium* (SE), respectively. The CR remains practically at the same levels when vesicles were exposed to the bacterial supernatant, free of bacteria after centrifugation and subsequent filtration (filtered sample). By contrast, in the case of washed bacteria, the colorimetric response is low indicating that the colour change is not caused by bacteria but by what they excrete into the environment during growth.

Table 2. Colorimetric responses (CR) of the solutions of bacteria added to the vesicles TRCDA/DMPC, using TRIS-EDTA buffer at pH 8.5 using a concentration of 10^9 CFU/ mL of each bacterial type. The samples were incubated at 35ºC for 1 hr.

Bacteria	Direct Sample	Filtered Sample	Washed Sample
Escherichia coli	15,2	14,8	0
Shigella sonei	0,3	0,5	0
Salmonella typhimurium	25,6	25,0	0

Use of culture medium and chemical receptor

Tryptophan has been described as an amino acid capable of interacting with Lipopolysaccharides (LPS) of different bacterial groups. Tryptophan can be joined to the TRCDA molecule by a derivatization reaction (Rangin and Basu, 2004). In their assays with pure commercial LPS, the CR obtained with tryptophan derivatized TRCDA was twice that those obtained from TRCDA. To assess the possibility of improving the sensitivity of the vesicles against bacterial presence vesicles were prepared with TRCDA-tryptophan and DMPC. The new vesicles were challenged with *Escherichia coli* at concentrations of 10^{11} CFU/mL in Tris-EDTA buffer pH 8.0 and defined minimal medium. CR results indicate that when using vesicles prepared with TRCDA-tryptophan, the CR increased from 15% to 20.2% showing a better sensitivity to the presence of tryptophan. This CR more than doubled when introducing the minimum medium in the trials. While these responses improve with build-tryptophan, the use of minimal medium favours the analysis to the naked eye, since CR in the container looks red, demonstrating in dramatic colour change due to the presence of bacteria (Figure 4).

Fig. 4 Vesicles of TRCDA/DMPC/Tryptophan challenged with *Escherichia coli* using culture medium MMD as a support matrix. Blue tubes (left) correspond to control samples (MMD without bacteria). Red tubes (right) correspond to samples containing bacteria.

DISCUSSION

Water pollution is one of the most serious and far-reaching problems in the world. Even in developed countries, access to clean uncontaminated water is not absolute, and infectious diseases occur (Fenwick, 2006; WHO, 2009).

Although in recent years there has been substantial progress in the control of drinking water quality, access to safe drinking water is still a latent problem in public health. Even in developed countries, the number of cases of disease outbreaks spread by contamination of pathogenic bacteria in drinking water has proven to be a significant and present problem (Hodgson and Manus, 2006). The methods validated by the health authorities whom are based on the detection of pathogenic indicators are currently being revised since enteric disease outbreaks shows relevant statistics. Alternative methods have emerged to address two main problems: (i) accelerate the response so that the detection becomes an early alert below the minimum bacterial infective doses, and (ii) availability of appropriate high tech devices currently proposed to be applied in the field are impractical.

The use of chromatic vesicles in liquid samples for the detection of bacteria is very difficult because the vesicles are not specific and react with many chemicals, including detergents (Kolusheva et al. 2000a; Kolusheva et al. 2000b; Jelinek and Kolusheva 2001; Kolusheva et al. 2001; Orynbayeva et al. 2007; Groysman et al. 2008; Sokolovski et al. 2008).

The TRCDA is a molecule that can be incorporated into artificial lipid vesicles adding the possibility of exhibiting a visible and rapid colorimetric transition based on the interaction of the vesicle with a variety of biological compounds (Ma et al. 2000; Silbert et al. 2006).

The experiments presented in this study describe several tests using TRCDA/DMPC for bacterial detection focused on drinking water. Our experiments of bacterial detection in which TRCDA/DMPC was included with nutrient agar indicated the minimum bacterial concentration that is necessary to produce the colour transition in the vesicles from blue to red. These results were consistent with similar experiments described by Ma et al. (2000), Silbert et al. (2006) and Pires et al. (2011). Although Ma et al. (2000) succeeded in developing a prototype biosensor to detect bacteria in no more than 70 minutes the procedure is too complex to perform in field samples, since it is based on the measurement of isotherms by FACE pressure gauge. The experiments described by Silbert et al. (2006) are simpler, using particles TRCDA/DMPC included in agar. In their experiments with *Escherichia coli, Bacillus cereus* and *Salmonella typhi* detection times of at least 18 hrs at 26ºC are being reported. We present here incubation temperatures with agar expanded at 35ºC which allows obtaining colorimetric responses in 4 hrs.

Several studies on the integration of specific receptors on glycolipid vesicles for the detection of bacteria have been conducted (Ma et al. 2000; Guo et al. 2005; Su et al. 2005; Guo et al. 2007; Boullanger, 2008). Where good results were obtained under laboratory condition using a FACE surface pressure meter HBM applied to bacteria. Pires et al. (2011) incorporated ribonamide a carbohydrate derived surfactant (TDER) detecting a *Staphylococcus aureus* and *Escherichia coli* bacterial concentration of 10^8 UFC/mL. In our simple tests on agar we were also able to detect bacterial presence with a detection time of 4 hrs that is reduced in approximately 30 min when vesicles include the L-Tryptophan receptor. This times response can be comparable to times response obtained by PCR. However, colorimetric vesicles are not requiring sophisticated equipment neither highly trained personal and suggesting a potential use in samples field.

The change of colour in the chromatic vesicles are due to the presence of active compounds that are released by the bacteria to their environment during growth, a process that often has a functional role essentially as defence mechanism or bacterial communication facilitator that allow the proliferation of colonies (Prescott et al. 2002). The interactions of these compounds with the vesicles are related with electrostatic attraction, formation of hydrogen bonds or the insertion of peptides/proteins in the lipid layer.

This study is oriented towards the detection of pathogenic bacteria in liquid matrices such as clean drinking water, groundwater or well water. The presence of divalent cations in these types of water, make the application of this kind of vesicles problematic due to their ability to react with TRCDA, also temperature and pH show interferences (Reppy and Pindzola, 2007). The addition of Tris-EDTA buffer into the colorimetric vesicles solution makes possible the use of TRCDA/DMPC in detection of bacteria, since EDTA forms complexes with divalent cations such as calcium and magnesium, therefore stabilizing the colorimetric signal. It also allows incubation at 35ºC, permitting bacteria to proliferate more quickly, according to their metabolism, which favours shorter incubation times.

Another suggestion offered in this study is to add culture medium to promote growth of bacteria. There are several culture media generally containing sugars, salts and amino acids. Some of these components react with the chromatic vesicles making difficult an appropriate medium choice. In our work we used a minimal medium MMD, which contains essential compounds for bacteria to grow. The use of this medium with Tris-EDTA gives better results, both visually and quantitatively, as colorimetric response was increased.

CONCLUDING REMARKS

The colorimetric model TRCDA/DMPC can be applied to detect the presence of pathogenic bacteria in water for the range over 10^8 UFC. When tryptophan is added to the membrane model the time responses improved in 30 min.

The use of the buffer Tris-EDTA as a carrier for the vesicles makes possible colorimetric detection of bacteria in water in the presence of cations, and also allows for larger temperatures ranges up to 35ºC. In addition, the results are suggesting that buffer Tris-EDTA could be tested in slightly more complex matrices such food matrices. However, additional trials are needed to improve the sensitivity and specificity of these structures.

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