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# Liposome-based sensor for the detection of bacteria

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ABSTRACT

Identification and quantification of bacteria affecting human life, directly causing diseases and indirectly contaminating natural ecosystems, are mostly carried out by expensive and time-consuming methods. There is the need for rapid and economic ways for detecting bacteria in the environment and in clinics. We propose the use of engineered liposomes for detecting bacteria in drinking water. Our approach exploits cationic liposomes functionalized with a surface potential-sensitive fluorophore, 4-heptadecylumbelliferone (C17-HC). The interaction between liposomes and bacteria involves a change in the surface potential experienced by C17-HC and switches on an optical signal.

We investigated, by DLS, zeta-potential and fluorescence experiments, a large number of cationic liposomes formulated with a natural phospholipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) or 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), C17-HC, and one of three synthetic cationic components, differing from each other for the number of unsaturations on the polar ammonium head, two of which ad hoc synthesized. Then we evaluated the ability of liposomes to produce a fluorescent signal upon interaction with three bacterial strains, *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus faecalis*; moreover, we analyzed the fluorescent response of each liposome formulation in the presence of the three bacterial strains at the same time, in order to simulate a real scenario. We found that interaction with bacteria triggers an optical signal in six of the evaluated formulations, resulting responsive down to $10^2$ CFU/mL of bacteria suspended in pipeline water coming from the water main of Rome (Italy).



## 1. Introduction

In recent years, sensor systems for the detection of pathogenic bacteria have received improved interest, especially in food safety, clinical diagnostics and germs warfare fields. This is due to the increasing number of pathologies caused by bacteria, which account for as many as 40% of the 50 million annual deaths worldwide [1].

For decades, the identification and quantification of bacterial strains, affecting human life both directly causing diseases and indirectly contaminating natural ecosystems, were based on traditional bacteriological culture and biochemical identification techniques [2,3]. These conventional methods [4] are based on nonselective and selective enriched culture media, followed by biochemical and serological identification. The main goal for the newest non-conventional systems has been to encompass a rapid alternative to the time-consuming culturing methods, which usually require several days to give a result.

Many approaches [5,6] for the detection of bacteria and for the construction of sensors for their non-specific or specific identification have been explored; among others, polymers, nanoparticles and liposomes have been used to meet this challenge and have produced nanosensor detection systems, such as carbohydrate-functionalized fluorescent polymers [7], magnetic nanoparticles

*Abbreviations:* C17-HC, 4-heptadecylumbelliferone; MGNPs, magnetic nanoparticles; ELISA, enzyme-linked immunosorbent assay method; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; NMR, nuclear magnetic resonance; TMS, tetramethylsilane; DLS, dynamic light scattering; PBS, phosphate buffered saline; $T_m$, transition temperature; MLVs, multilamellar vesicles; BHI broth, brain heart infusion broth; LB broth, Luria-Bertani broth; TSS, trypcase soy agar + 5% sheep blood agar; MCK, Mac Conkey agar; SEM, scanning electron microscopy; $D_h$, hydrodynamic diameter; EC, *Escherichia coli*; EF, *Enterococcus faecalis*; SA, *Staphylococcus aureus*.

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(MGNP) [8] and polydiacetylene (PDA) liposome sensors [9]. These systems have been shown to be very attractive also for the future design of more efficient detectors for pathogens, but criteria such as detection limit, sensitivity, time required to obtain a result, and laboratory outlays (including skill, labour and cost) remain often unsolved.

Functionalized polymers, displaying a versatile scaffold for the attachment of a variety of different carbohydrates, have the advantage of providing the simultaneous detection of more than one pathogen, unfortunately the detection limit of these methods is quite high (~$10^4$ CFU/mL) [7].

MGNPs could be very advantageous for the detection of bacteria because they are characterized by a high surface/volume ratio that offers a larger contact surface for attaching targeting molecules to capture pathogens [10]. Targeting molecules can be carbohydrates, peptides or antibodies. In the case of antibodies, MGNPs can display a detection limit considerably lower than that of enzyme-linked immunosorbent assay method (ELISA) [8]. Glyco-MGNPs are functionalized with sugar moieties and successfully exploit the well known ability of bacteria in recognizing carbohydrates, usually exposed on mammalian cell surface, to infect the cells [11,12]. However, surface modification of MGNPs with targeting ligands might induce aggregation and agglomeration of nanoparticles, thus influencing their toxicity and behaviour in the biological environment [13,14].

On the contrary, liposomes do not display this problem while having many advantages such as sensitivity, rapidity, wide dynamic range, relatively simple and low-cost manufacturing equipment. PDA liposome-based systems are the most extensively studied liposome-based sensors; PDA, variously embedded in liposome membrane, can undergo a simply detectable colorimetric transformation upon interaction with whole bacteria or with bacterial secreted molecules [15,16]. In order to improve PDA liposome performances in terms of specificity and sensitivity, a lot of efforts have been made either by decorating PDA liposomes with different specific "bacterium labels" [17,18], or by modifying lipid formulation [19]. The majority of these systems allow detecting and identifying different Gram-negative bacteria strains, whereas only few papers report on the successful detection of Gram-positive bacteria by PDA liposomes, in addition with long response times [20]. Further, it is well-known that cationic liposomes can interact with both Gram-positive and Gram-negative bacteria, although in a non specific manner, by adsorbing on the negatively charged cell wall [21] and, in fact, electrostatic attraction is largely exploited in the development of drug delivery systems for non-specific targeting of bacteria [22,23] and biofilms [24].

Here we propose a liposome-based approach for the detection of bacteria in drinkable water, exploiting cationic liposomes functionalized with the fluorophore 4-heptadecylumbelliferone (C17-HC), a probe sensitive to surface potential [25]. The approach relies on the induction of a fluorescent signal upon interaction of liposomes with bacteria, due to the change of surface potential experienced by C17-HC. We explored a number of cationic formulations in their ability to produce a fluorescent signal upon interaction with three bacterial strains, *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus faecalis*. We investigated the interaction of each liposome formulation both with single strain and with a mixture of the three bacterial strains, in order to simulate a real pollution scenario. To this end, all the experiments were performed suspending both liposomes and bacteria in pipeline water coming from the water main of Rome (Italy). This procedure was adopted in the frame of the project AQUALITY "Online industrial water quality analysis system for rapid and accurate detection of pathogens" (funded by EU FP7 2007–2013). In fact, at the beginning of the project, in each of the involved countries, a questionnaire was distributed to some target industries to get deeper inside in the application scenario of AQUALITY project. One of the industries that contributed to the questionnaire was LaboratoRI SpA, an Acea Group Company, the largest Italian group utility in water sector, operating on the Roman aqueducts. Liposomes were formulated with a natural phospholipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) or 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), the fluorescent probe C17-HC and one of three synthetic cationic amphiphiles, namely *allyldimethylhexadecyl ammonium iodide* **1**, *diallylhexadecylmethylammonium iodide* **2**, *triallylhexadecyl ammonium bromide* **3**, reported in Chart 1.

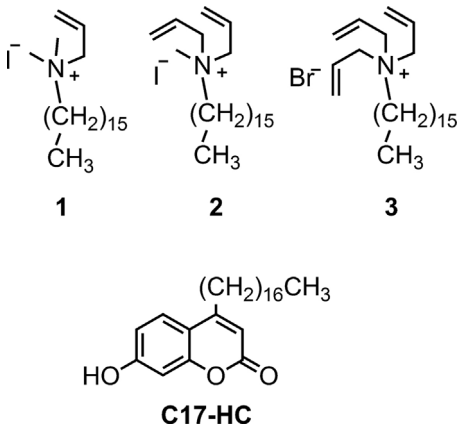


**Chart 1.** Cationic surfactants **1**–**3** and the fluorescent probe C17-HC.

The overall cationic charge of liposomes has the role of promoting electrostatic interaction with bacteria that display an overall negative charge on their cell wall. The three synthetic cationic components differ from each other for the number of unsaturations on the polar ammonium head. We chose these particular structures because they allow investigating the influence of headgroup polarity on liposome/pathogen interaction, on fluorescent probe topology in the lipid bilayer, and hence, on its optical response. Moreover, we suppose that allylic moiety, being more rigid with respect to a flexible propyl residue, might have a larger effect on bilayer reorganization upon the interaction with bacteria, and, therefore, on fluorescent probe response.

## 2. Experimental

### 2.1. Instrumentation

NMR spectra were recorded on a Bruker Avance II 300 spectrometer operating at 300.13 and 75.47 MHz for $^1$H and $^{13}$C, respectively, equipped with a sample tube thermostating apparatus. Signals were referenced with respect to TMS ($\delta$ = 0.000 ppm), used as internal standard in $CD_3OD$.

Steady state fluorescence spectra were recorded at room temperature ($T$ = 25 °C) on a Horiba Jobin-Yvon FLUOROMAX 4 spectrofluorometer. Spectra were corrected by means of a built-in program in order to counterbalance the decay in sensitivity in the near infrared region. All fluorescence experiments were carried out on solutions with optical density lower than 0.05 to minimize the inner filter effect.

Dynamic light scattering (DLS) and zeta-potentials measurements were performed with Zetasizer Nano ZS (Malvern, Herrenberg, Germany) equipped with a 633-nm He–Ne laser. Solvent-resistant micro cuvettes (ZEN0040, Malvern, Herrenberg, Germany) have been used for DLS experiments with a sample volume of 1 mL. Measurements were made at a fixed position with an automatic attenuator and at a controlled temperature. Obtained count rates were then corrected for the attenuator used.