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ARTICLE

A Bacteriophilic Resin, Synthesis and *E. coli* Sequestration Study.

Cite this: DOI: 10.1039/xoxxoooooox

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Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

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The poly-cationic pyridinium-Merrifield resin **I** was prepared and characterized by elemental analysis, infrared spectroscopy and SEM, with a loading of 2.96 10^{-3} mol of pyridinium chloride groups per gram. Its sequestering properties towards bacteria were evaluated, using *E. coli* as a model. The capture of *E. coli* was followed by Capillary Electrophoresis (CE) that showed the effectiveness and the celerity of the capture, *i.e.* around 90% of cells after 3 h of contact. The bacterial loading was evaluated at 7 mg bacteria/g resin. The bacteriophilic behaviour of resin **I** was confirmed by confocal microscopy which evidenced the presence of bacteria at the surface of the beads. Attempts to correlate CE capture results to bacterial counting at the surface of beads are presented.

1. Introduction

Sanitary events related to bacteria, such as community and hospital-borne infections, can be fought, in parallel to the design of new antibacterial therapies,[1] by the development of strategies targeting them in their natural reservoirs, for example by elimination/control of their waterborne or airborne forms.[2-4] This could pass through the genesis of bacteriophilic materials designed to trap the bacteria from these media, for example by means of "electrostatic" interactions between poly-cationic polymers and the negatively charged bacterial surface. As reported by Rotman,[5] the study of cationic or anionic resins in the field of microbiology begins in the middle 50's; most contributions are directed towards viruses, but some literature is dedicated to their interactions with bacteria, essentially *Escherichia coli*, *Salmonella schottmuelleri* and *typhosa*, *Shigella dysenteriae* and *Staphylococcus aureus* strains. Since this period, with respect to the assumption of bacterial surfaces displaying a global negative charge, many advances have been reported in terms of water bacterial disinfection by means of cationic polymers, e.g. flocculation[6] or clustering[7] of bacteria, dispensation of bactericidal triiodide with insoluble ammonium resins,[8] adhesion of bacteria on insoluble poly(vinyl-N-alkylated-pyridinium chlorides or bromides) beads[9] or clothes.[10] These approaches, highly interesting for health and environmental purposes, have generated numerous contributions related to polymeric materials displaying an antimicrobial activity; they have been reviewed recently by Mũnoz-Bonilla and Fernàndez-Garcia.[11]

Among them, polymers displaying heterocyclic units have been investigated, mostly derivatives of imidazole or pyridine. For the latter, mainly poly(vinylpyridine), pure or co-polymerized with styrene and divinylbenzene, or with hydrophilic methacrylates have been studied. The quaternization of the pendant pyridine subunits generates the antibacterial activity, which depends on the nature of the quaternizing substituent and from the nature of the counter anion.[12-14]

In the course of our investigations on new poly-cationic antibacterial agents, we have developed a strategy related to a rapid grafting of these compounds on the easily accessible poly(4-chloromethylstyrene) or Merrifield Resin.

This synthetic strategy involving the formation of a pyridinium link directly attached to the benzyl group of polymer, we have prepared the corresponding "blank" derivative **I** by reacting pyridine with the Merrifield resin. This reaction initially developed by Merrifield in order to quantify chlorine in its polymers *via* a modified Volhard's titration process,[15,16] was also employed by Lemaire and coll. to access supported ionic liquids prone to trap nitrogen-containing substances from fuels,[17,18] by Maki et al. to prepare supported boronic acid catalyst for amide condensation,[19] and by Luis and coll. who developed Merrifield resins derivatives incorporating Rose bengal and pyridinium groups as solar photocatalysts.[20]

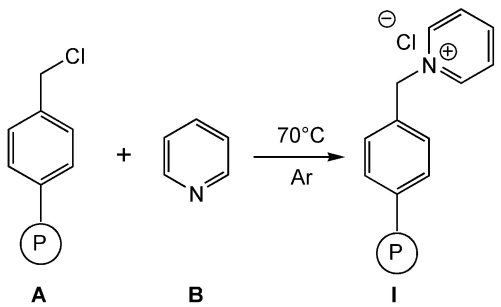
As far as we know, this type of pyridinium resins has not been subjected to biological investigations, notably as bacteria sequestering agents, attractive as materials for aqueous media disinfection.

The analysis of bacteria, which display a negative global surface charge, by capillary electrophoresis (CE)[21] has gained considerable interest during the last ten years.[22] Numerous CE modes have been employed to evaluate microorganisms, such as capillary zone electrophoresis and capillary isoelectric focusing,[23] or capillary electrochromatography.[24] More recently, mobilization of bacteria was successfully demonstrated by isotachophoresis with borate and chloride acting as terminating and leading ions respectively.[25]

We present here the synthesis of the bacteriophilic resin **I**, its characterization by elemental analysis, ATR-IR spectroscopy and SEM, and the preliminary study by means of Capillary Electrophoresis combined to Confocal Microscopy of its bacteria sequestration ability against *E. coli*. The latter, due to its commensal and potentially pathogenic nature, was chosen as model.

2. Results and discussion

2.1. Synthesis and characterization of resin I



Scheme 1: Synthesis of pyridinium resin **I**.

The reaction of pyridine with Merrifield resin **A** (scheme I) was performed at 70°C under argon, under soft stirring to avoid mechanical disintegration. The rinsing process involving solvents of various polarities was also carefully carried out and with control of filtrates; this was completed by a drying step under vacuum until a constant mass is obtained. This results in a gain of mass of 45% that, if attributed to the grafting of pyridine alone, should correspond to 0.566 moles of the latter for 100 g of resin **A**. This gives, with respect to the initial substituable chlorine (0.5 mole *per* 100 g), an abnormal yield of 113%, and implies the presence of supplementary substances, we suspected to be polar solvents associated to pyridinium groups and issued from rinsing process.

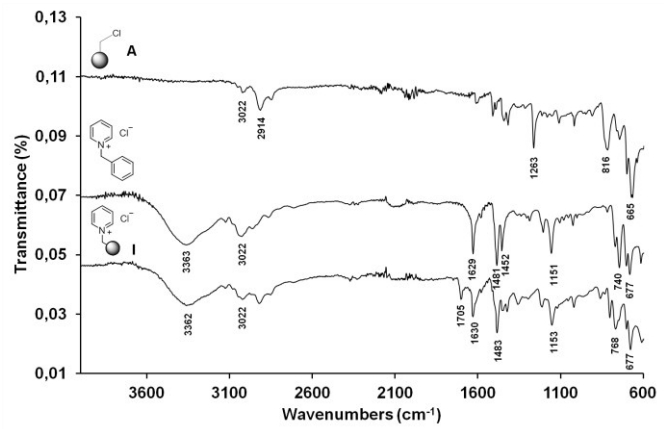
Combustional elemental analysis of resin **I** was thus performed, giving an amount of 4.69 % of nitrogen. This value corresponds to 0.335 mole of N or pyridine in 100 gr of resin, *i.e.* a content of 26.5 % of pyridine in mass; the residual 73.5 g, considered as starting resin **A,** contain 0.368 mole of chloromethyl groups on which pyridine has partially reacted. The grafting ratio was calculated as 0.335/ 0.368 = 91 % with respect to chlorine.

The elemental analysis results were checked with different compositions involving reactants and solvents employed during the synthesis. The best result showed that each pyridinium was accompanied by 1.4 molecule of water.

In this sense, the gain of mass of 45% corresponds to 0.43 moles of the couple pyridine:1.4 H_2O (MW 104.32) for 100 g of starting resin **A**, giving a grafting ratio of 0.43/0.50 = 86%. The new resin **I** diplays thus a formel anion exchange capacity of 2.96 10^{-3} mole per gram. A preliminary evaluation of its anion exchange capacity was performed against sodium picrate in water by UV-visible spectroscopy, giving, with respect to pyridinium chloride groups, an exchange ratio of 69%.[26]

The resin **I** was analysed by ATR FT-IR and compared to the starting Merrifield resin **A** and the model benzylpyridinium chloride (**Figure 1**). The main modifications of the spectrum of **I** *vs* **A** are the shift of the band of **A** at 1263 cm^{-1} (CH_2Cl groups[27]) to 1153 cm^{-1}, attributed to CH_2-N^+ groups, and the apparition of two relatively intense bands at 1483 and 1630 cm^{-1}, attributed to C=C and C=N^+ groups of pyridinium subunits. The strong similitude between IR spectra of **I** and of the benzylpyridinium chloride suggests the formation of the expected pyridinium groups within resin **I**.

Figure 1: ATR-IR spectra of resins **I**, **A**, and model benzylpyridinium chloride.



The morphology of the beads of resin **I** was analysed by Scanning Electron Microscopy (**Figures 2** and **3**).

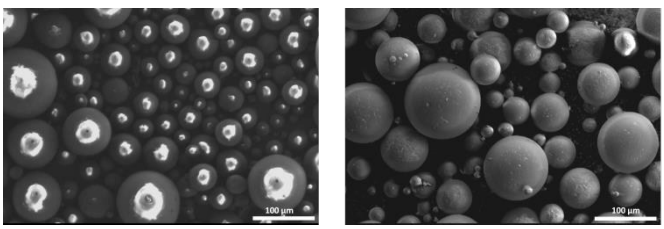


Figure 2: SEM Secondary Electrons Images 3 kV: left) Merrifield Resin **A**, right) Pyridinium resin **I**.

In the same time, the native Merrified resin was also analysed (**Figure 2**, left), showing a size dispersion more important than proposed (74 - 37 µm), in a range of 105 - 7 µm. In addition, this Merrifield resin was found unstable at 10 and even 3 kV, visible through the white spots generated at the top of beads

during scanning. At 10 kV, the dry pyridinium resin **I** (**Figure 3**) displayed a much better behaviour, without visible degradation under scanning. The bead size was found distributed between *ca.* 7 and 150 µm.

The beads obviously enlarged upon incorporation of pyridine, and quaternization seems to stabilize them during the scanning process. The mechanical stirring generates some slight deterioration of the polymer (fragments), but the **Figure 3** shows nevertheless a neat surface of the beads.

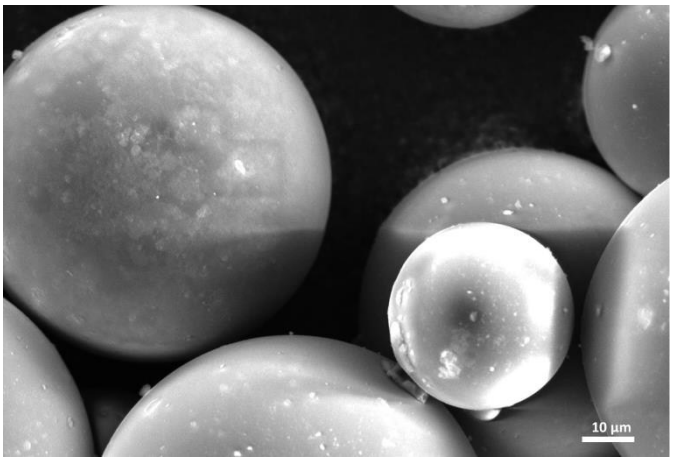


Figure 3: SEM Secondary Electrons Images 10 kV: Pyridinium resin **I**

2.2. Bacterial sequestration study

2.2.1 Demonstration by capillary electrophoresis

A preliminary sequestration kinetic study was carried out over a 24h period, using 50.0 mg of pyridinium resin **I** added to a 0.05 mg/mL *E. coli* suspension (5.0 mL) and kept under stirring for 24h (sample R). A bacteria standard control suspension (sample T, 0.05 mg/mL) without the resin was also agitated in the same time. Bacteria concentrations of both samples (R and T) as a function of the stirring time were evaluated with the capillary electrophoretic method (**Figure 4**).

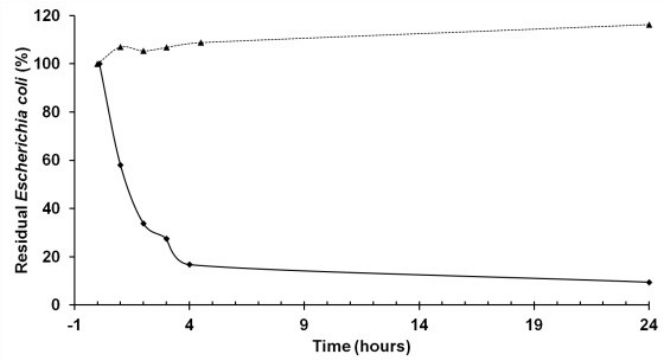


Figure 4: Residual *Escherichia Coli* concentration after contact with the pyridinium resin **I** (solid line, sample R) and without resin (dot line, sample T).

A very fast decrease of the residual *E. coli* peak after mixing with resin **I** is observed, with 40% of bacteria captured in the first contact minutes. A plateau around 10% residual bacteria in suspension (90% captured) was obtained after three hours. In the same time, the bacteria concentration of the standard sample T remained stable in this medium with values around 100 – 120%. A comparison of electrophoregrams of the two samples R and T under study after 3 hours of stirring is shown in **Figure 5**.

The bacterial sequestration efficiency of pyridinium resin **I** was compared to those of two commercial quaternary ammonium-type anion exchange resins, cholestyramine (Questran®, trimethylbenzylammonium functional group, chloride form, 2% DVB; oral biliary acid sequestering agent) and Amberlite IRA-400 (trimethylbenzylammonium functional group, chloride form, 8% DVB). Those two models were preliminary washed and dried, and their ammonium loading was evaluated by elemental analysis, giving respectively 4.98 and 3.77% of N, that correspond to 3.56 and 2.59 mmol of ammonium chloride groups *per* gram, respectively.

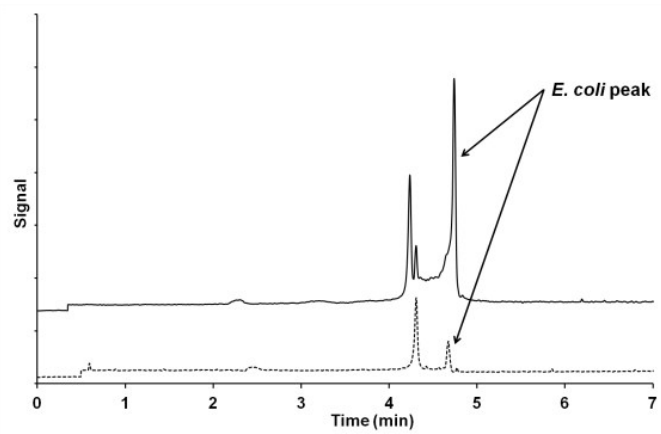


Figure 5: Electrophoregram of bacterial samples after 3 hours of stirring, with (dot line; sample R) and without (solid line; sample T) addition of the pyridinium resin **I**. The first peaks at 4.2 min were supposed to be bacteria residues resulting from freeze-drying process.

The contact time between bacteria suspension and resin samples was fixed at 3 hours, as deduced from the sequestration kinetic study. After this mixing step, the suspension was allowed to settle for 3 min, then supernatant were injected and quantified by capillary electrophoresis with the method described above, leading to the amount of residual *E. coli*.

The results presented in **Table 1** show the potency of resin **I** to capture *E. coli*, with a yield of *ca.* 7 µg per mg, much more than Amberlite IRA 400 that fixes only 1.17 µg of bacteria per mg and cholestyramine that appears inactive, as expected from its therapeutical use. No description of such effect is described in USP, but cholestyramine has been proposed for fixing toxins in case of intestinal infections by *Clostridium difficile*; it is also proposed in the treatment of some types of diarrheas, without more precisions on the mechanism.[28]

The removal coefficient of each resin was calculated according to Kawabata and coll.[9,29] The formula developed by these authors is given in Equation 1 :

$$\text{Removal coefficient} = (V/W \cdot t)\log(N_0/N_t) \qquad \text{(Eq. 1)}$$

where V is the volume of cell suspension (mL), W the dry weight of resin (g), t the contact time (h), N_0 the initial number of cells and N_t the terminal one. For our purpose, N_0 and N_t were replaced by the corresponding mass of bacteria.

The results displayed in **Table 1** show a removal coefficient of 20.68 for resin **I**, 1.87 for IRA 400 and null for cholestyramin. They are in accordance with the results of Kawabata who conclude in the marked advantage of cross-linked poly(N-benzyl-4-vinylpyridinium halides) with regards to alkylammonium derivatives of Merrifield resin (among which IRA-400) in terms of bacteria sequestration ability.

Table 1: Typical results of bacteria sequestration on resins **I**, IRA-400 and cholestyramine after 3 hours of stirring.

	I	IRA-400	Cholest.[a)]
Ammonium	pyridinium	trimethyl	trimethyl
DVB(%)	1	8	2
%N	4.69	3.77	4.98
Cation loading (mol/mg)	$2.96\ 10^{-6}$	$2.69\ 10^{-6}$	$3.56\ 10^{-6}$
Sample mass (W, mg)	2.3	2.8	2.3
Moles of available cationic charges	$6.81\ 10^{-6}$	$7.53\ 10^{-6}$	$8.19\ 10^{-6}$
Initial mass of bacteria (M_0, µg)	20.00	20.00	20.00
Residual mass of bacteria (M_t, µg)	3.88	16.72	20.00
Mass of captured bacteria (M_c, µg)	16.12	3.28	0.00
% residual bacteria	19.4	83.6	100.0
% captured bacteria	80.6	16.4	0.0
Mass of captured bacteria / mass of resin (µg /mg)	7.01	1.17	0.00
Removal coefficient $= (V/W{\cdot}t)\log(M_0/M_t)$ [b)]	20.68	1.87	0
Number of captured bacteria (N_{high}) [c)]	$1.47\ 10^{8}$	$2.98\ 10^{7}$	0
Number of captured bacteria (N_{low}) [d)]	$1.71\ 10^{7}$	$3.45\ 10^{6}$	0
Loading Capacity (C_{high})[e)]	$6.39\ 10^{10}$	$1.06\ 10^{10}$	0
Loading Capacity (C_{low})[e)]	$7.43\ 10^{9}$	$1.23\ 10^{9}$	0

[a)] cholestyramine (Questran®)
[b)] in $mL{\cdot}g^{-1}{\cdot}h^{-1}$; V = 0.2 mL; t = 3 h; W in grams.
[c)] hyp mass *E coli* = $0{,}11\ 10^{-12}$ g
[d)] hyp mass *E coli* = $0{,}95\ 10^{-12}$ g
[e)] (N_{high}) or (N_{low}) /sample mass

The evaluation of the average weight of the *E. coli* cell is a subject of sustained interest in the literature. It is currently proposed at relatively different values comprised between 0.1 and $5.0\ 10^{-12}$ g by unit; for example, a calculated mass of $0.95\ 10^{-12}$ g is reported in Neidhardt et al.[30] while Kubitschek[31] proposes a mass of $1.126\ 10^{-12}$ g, as collected in BioNumbers database.[32] More recent evaluations involving "nanobalances" such as, for example, the suspended microchannel resonators developed by Burg and coll. resulted in a proposed cell mass of $0.11\ 10^{-12}$ g for *E. coli*.[33] This last mass evaluation is in accordance with the calibration method developed in our laboratory for capillary electrophoresis experiments,[34] and was chosen for our calculations, instead of the former.

2.2.2 Evidencing by confocal microscopy

In order to verify if and how bacteria are associated to the beads, a study of these adducts by fluorescence microscopy was engaged, using Sybr Green II as stainer. Each element of the study, native beads, native bacterial suspension, buffer and loaded beads was treated with Sybr Green II, showing the presence of fluorescent satellites at the surface of the beads, and presuming the presence of bacteria. Nevertheless, the ball-shape of the beads rendered the focus uneasy, inhibiting the obtention of good images and necessitating the use of confocal microscopy.

The images obtained with this technique are given in **Figure 6**. They show clearly the effective sequestration of bacteria by beads of resin **I**. The **Figure 6a** shows the noticeable presence of bacteria as green individual structures at the periphery of two close beads, some of them appearing associated together into a hillock. The **Figure 6b** shows that all the beads, whatever their size, are engaged in bacteria sequestration. The **Figure 6c** represents a tomography of the skullcap (18.1 µm radius, 6.3 µm height; surface = 1084 μm^2) of a 27.35 µm radius bead (surface = 9400 μm^2). The number of well-defined bacterial objects on this surface (70 units) was extrapolated to the sphere, giving an amount of 610 bacteria for this bead.

The average bacterial loading capacity was thus estimated to $6.41\ 10^{10}$ cell units/m^2, (N_{conf}) (**Table 2**) *i.e.* $7.1\ 10^{-3}$ g following the hypothesis of $0.11\ 10^{-12}$ g/cell unit,[33] or $61.3\ 10^{-3}$ g following the hypothesis of $0.95\ 10^{-12}$ g/cell unit.[30]

The poly-dispersity of beads in size (7 to *ca.* 150 µm) and distribution, makes uneasy the precise correlation between these values and the mass of resins employed. Nevertheless, the approximate number of beads per gram of resin can be calculated with the following equation[35]:

$$N = 1/[(4/3\ \Pi)(1/2\ D)^3(d)] \qquad \text{(Eq. 2)}$$

where D is the mean bead diameter in cm and d the density of the resin in g/cm^3.

The mean bead diameter was evaluated from SEM results at a value of 37.4 µm, and the density of dry resin **I** was evaluated at 0.66 g/cm^3 (see experimental part). This afforded a number of $5.5\ 10^7$ beads of 37.4 µm mean diameter (surface of $4.4\ 10^{-9}$ m^2), by gram of **I**.

The corresponding mean surface accessible in theory to bacteria was evaluated to 0.242 m^2 by gram of **I**. The average bacterial loading capacity of $6.41\ 10^{10}$ cell units/m^2 (N_{conf}), obtained from confocal microscopy experiments, allowed to estimate, depending of the mass of unit cell available in the literature, and, by this way, of the resulting number of bacteria sequestered by samples of resin, the specific surface of resin **I** (**Table 2**).

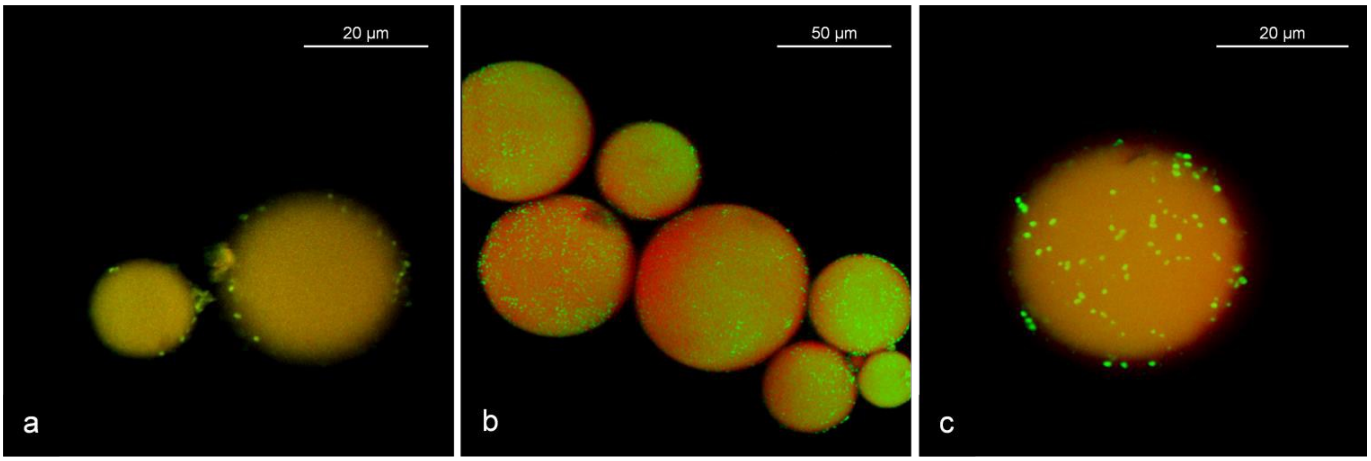


Figure 6: Confocal microscopy images of *E. coli*/pyridinium resin **I** adducts. a) Direct visualisation at the periphery of two close beads; b) Tomographic visualisation of a group of beads; c) Tomographic visualisation of the 18.1 µm radius and 6.3 µm height skullcap of a 27.35 µm radius bead.

Table 2: Evaluation of specific surface of resin **I**.

Sample mass of **I** (g)	Mass of sequestered E. coli (g)	Number of cells per m^2 (confocal) N_{conf}	Loading capacity C_{high}* ($0.11\ 10^{-12}$g/cell) (N bact/g)	Specific surface C_{high}/N_{conf}	Loading capacity. C_{low}* ($0.95\ 10^{-12}$g/cell) (N bact/g)	Specific surface C_{low}/N_{conf}
$2.30\ 10^{-3}$	$16.22\ 10^{-6}$	$6.40\ 10^{10}$	$6.39\ 10^{10}$	1.00 m^2/g	$7.43\ 10^{9}$	0.116 m^2/g

*See Table 1

For that point, the specific surface in m^2/g was calculated by reporting the loading capacity (number of bacteria per g), in the low (C_{low}) and the high (C_{high}) hypotheses, to the number of bacteria per m^2 (N_{conf}). Following the hypothesis of $0.11\ 10^{-12}$ g/cell unit,[33] (N_{high}) a surface of 1.00 m^2/g was obtained, while the hypothesis of $0.95\ 10^{-12}$ g/cell unit[30] (N_{low}) afforded a surface of 0.116 m^2/g.

It is interesting to note that the specific surface obtained from the "mean surface approach" lays between these two values.

3. Conclusions

The bacteriophilic pyridinium-derived Merrifield resin **I** was prepared and characterized by IR, elemental analysis, anion sequestration study and SEM. This poly-cationic resin, which appears highly poly-dispersed (7 to 150 µm), exhibits anion exchange properties that were thought to be useful face to negative surface charges of bacteria in view of trapping them for environmental purpose. In parallel to IRA-400 and cholestyramine polyammonium analogues, its high bacterial sequestration ability, evaluated to 7 mg bacteria/g resin, has been confirmed by capillary electrophoresis. The sequestration of bacteria was visually demonstrated by confocal microscopy, that showed clearly the presence of bacterial objects at the surface of the polymer beads, and allowed a preliminary counting of $6.4\ 10^{10}$ cell units per m^2. The latter was employed to determine specific surface of the resin **I**, with respect to two unit cell mass values given in the literature for *E. coli*. This resulted in two specific areas, 1.00 m^2/g and 0.116 m^2/g, that were found consistent with the value of 0.242 m^2/g obtained through standard methods employed for determining mean surface value of a poly-dispersed material.

Further experiments, particularly related to the preparation of beads samples with a narrow poly-dispersity, the refinement and the understanding of bacteria-beads interactions notably by modification of pyridine subunits, as well as the study of cyclability of the sequestration process are on the way. This allows us to expect the access to a platform of materials able to disinfect aqueous media, for environmental and therapeutical purposes.

4. Experimental

4.1. Synthesis

ATR Infrared analyses were performed on a Bruker Vector 22 FT-IR apparatus using the PIKE MIRacle™ Single Reflexion Horizontal ATR accessory, crystal of germanium, or on a

Shimadzu FT-IR Affinity 1 apparatus, using the PIKE GladiATR accessory (diamond) (ν in cm^{-1}). Elemental analyses were performed at the Service de Microanalyse, Nancy. UV spectra were recorded on a SAFAS UV mc2 apparatus. All commercially available products were used without further purification unless otherwise specified. Merrifield resin *ca.* 5 mmol Cl/g, 200-400 mesh, 1% DVB, was from Fluka, ref 53873.

4.1.1 Pyridinium resin I

The Merrifield resin **A** (2.12 grams, 0.0106 mole of chlorine), was treated under argon by an excess of pyridine **B** (20 mL) at 70°C for 24 h (**scheme 1**). The material was recovered by filtration, and was subjected to a careful cleaning process involving multiple washings with CH_2Cl_2, MeOH, H_2O, then Me_2CO, and was finally dried under vacuum at 40°C until a constant mass was obtained. The resin **I** was recovered as clear-light yellow beads (3.07 g). Anal. Calc. For $C_{18.727}H_{18.727}Cl_{1.087}N_{1.000}$, 1.4 H_2O (321.57): C 69.95, H 6.75, N 4.36; found: C 69.58, H 6.53, N 4.69. IR (ATR, pure beads): 1630, 1483 (C=C and $C=N^+$); 1153 ($-CH_2-N^+$). Chloride/picrate anion exchange capacity (UV-visible; H_2O): 69 %. Density (dry): 0.66 g/mL. Volumic expansion in water: 270%; dead volume: 35 %; density (wet but drained): 0.763 g/mL.

4.2. Capillary Electrophoresis (CE)

4.2.1 Materials

Escherichia coli (ATCC 11303) was received as freeze-dried from Sigma Aldrich (Saint Louis, USA). In this work, *E. coli* was considered as a negatively charged molecular object, concentrations used or determined were described in mass/volume units. Boric acid ≥ 99.5% was obtained from Carlo Erba (Val de Reuil, France), Tris ≥ 99.9%, benzyl alcohol, hydrochloric acid 1.0 M and sodium hydroxide standard solutions were purchased from Sigma Aldrich (Saint Louis, USA). Ultrapure water was obtained in a Purelab Ultra MKII system from Elga (Le Plessis Robinson, France).

Bacteria samples: *E. coli* stock suspensions were prepared in the LBE buffer at the concentration of 1.0 mg/mL and stored at 4°C (renewed each day). They were diluted at the desired concentration before injection. All samples were vortexed 0.5 minutes to avoid sedimentation.

4.2.2 Apparatus

All experiments were performed on a Beckman P/ACE 5500 model (Beckman, Fullerton, USA) equipped with a UV detector (λ = 280 nm). Capillary temperature was controlled by coolant at 25.0°C. An untreated fused-silica capillary (Polymicro Technologies, Caudebec les Elboeuf, France) 75 µm I.D. (Internal Diameter) with a total length of 37 cm (effective length 30 cm) was employed. All samples were injected in the hydrodynamic mode (10 s under pressure) at 10 kV. The experimental set-up, data acquisition, and processing were governed using Beckman P/ACE Station software.

Leading and terminating background electrolytes (LBE and TBE respectively) were prepared as described by Oukacine *et al.*[25] The LBE was composed of 4.5 mM Tris + 50 mM boric acid + 3.31 mM HCl and the TBE was composed of 4.5 mM Tris + 50 mM boric acid. The pH values of LBE and TBE were 6.7 and 7.2, respectively.

Before each injection, the capillary was successively rinsed with 0.1 M sodium hydroxide (2 min), ultrapure water (2 min) and LBE (2 min). All solutions introduced in the capillary were preliminary filtrated with an Alltech (Tampleuve, France) regenerated cellulose filter (0.45 µm pore size).

Before use, the electrophoretic method was evaluated with respect to specificity, linearity, precision and limit of quantification (LOQ).

Calibration curves were prepared with bacteria suspensions ranging from 0.01 to 0.70 mg/mL (5 concentrations, n = 3). A standard curve of *E. coli* peak corrected area against concentration was plotted and calibration curves were fitted using least squares linear regression. A good linearity was observed with correlation coefficients > 0.99.

The specificity of the method was investigated by injecting blank samples (resins without bacteria and background electrolyte alone) in order to confirm that there were no interferences. The sensitivity of the method was determined by means of LOQ (RSD < 20%). The LOQ, evaluated using corresponding diluted samples, was $2.8.10^{-4}$ mg/mL.

The repeatability was evaluated by assaying triplicates samples of blinded concentrations representing low, middle and high bacteria concentrations (0.01, 0.10 and 0.70 mg/mL respectively) and satisfactory results were obtained (RSD < 6.0 %).

4.3. Bacteria sequestration

As typical experiment, 20 µg of *E. coli* in 0.2 mL LBE buffer was mixed with *ca.* 2.5 mg of resin in 1.5 mL Eppendorf tube. The suspension was mixed for 3 h before titration of residual bacteria by capillary electrophoresis.

4.4. Scanning Electron Microscopy

Scanning Electron Microscopy was performed on a JEOL JSM 6010LA Microscope; samples were observed in Secondary Electrons and/or in Back Scattered Electrons mode with an acceleration voltage from 3 to 10 kV.

4.5. Confocal laser scanning Microscopy

Samples were observed with an inverted microscope (Nikon TE 2000U Eclipse) equipped with Biorad Radiance 2100 Rainbow AGR3Q/BLD laser scanning confocal head.

The bacteria-beads adducts samples were prepared according to bacteria sequestration study, *i.e.* 3 h of contact. They were rinsed with the LBE buffer and stained appropriately with Sybr Green II 1 x for 15 min. in the dark. They were then recovered on 0.2 µm filters and the latter were mounted directly on the glass slide, using CITIFLUOR AF 87 (medium viscosity, refractive index *ca.* 1.52 at 20°C) from Biovalley. The slides were observed with 60X-Apo-DIC-oil objective lens. All the

experiments were performed at ~20° C. An Argon Gaz laser source was employed at 457 nm and 488 nm to discriminate autofluorescence of beads and fluorescence of bacteria stained by Sybr Green II. The fluorescence was detected with a spectral bandwidth of 530-540 nm for bacteria.

For **Figure 6a**, data were stored in raw format consisting of 1024 X 1024 pixel images (corresponding pixel size, 72.624 X 72.624 µm) on x and y axes. For **Figure 6b** (tomography), data were stored in raw format consisting of 512 X 512 pixel images (corresponding pixel size, 196.085 X 196.085 µm) on x and y axes, and with 79 stacks of 0.410 µm along z axis. For **Figure 6c** (skullcap tomography), data were stored in raw format consisting of 1024 X 1024 pixel images (corresponding pixel size, 60.616 X 60.616 µm) on x and y axes, and with 17 stacks of 0.410 µm along z axis. All data are in 8-bit color map.

Acknowledgements

We thank the French Ministère de la Recherche et de l'Enseignement Supérieur, particularly F. Lemée for a PhD grant, the CNRS and the Région Lorraine for financial support. We also thank Dr L. Mathieu and Mrs E. Angel from LCPME, (UMR 7564 CNRS - Université de Lorraine; Nancy) for preparing fluorescent treatments of bacterial/resin adducts, and Dr. C. Mustin (L.I.E.C; UMR 7360 CNRS - Université de Lorraine; Nancy) for confocal microscopy experiments.

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