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Anion effects on anti-microbial activity of poly[1-vinyl-3-(2-sulfoethyl imidazolium betaine)]

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ABSTRACT

Recent investigations in the anti-microbial properties of the functional polymers are predominantly focused on the structure of the cationic moieties. In the present study, we investigated that the nature of the anion present in polysulfobetaines affects activity against certain microorganisms and their anti-microbial properties have been rationalized in terms of the structure–activity relationship. Vinyl imidazolium-based polysulfobetaines were prepared by the quaternization of poly(*N*-vinyl imidazole) with sodium salt of 2-bromo ethanesulfonic acid. The bromide counter anion of the resulting polymer was exchanged with different anions to generate a series of polymers. These were characterized by FTIR, DSC, XRD, SEM, elemental analysis (C, H, N and S) and viscosity measurements. The anti-microbial activity studies were carried against three fungi (*Aspergillus niger, Byssochlamys fulva* and *Mucor circenelliods*) and two bacteria (*Bacillus coagulans BTS-3* and *Pseudomonas aeruginosa BTS-2*). The nature of the anion affects the structure of polysulfobetaine by realignment of polymer chains. The anion-dependent anti-microbial properties of polysulfobetaines result from the interaction of the microbes at the polymer interface.

1. Introduction

Sulfobetaine zwitterions are reported to resist non-specific protein adsorption and cell adhesion [1–3]. These have been grafted on the surface of various polymers to tailor or improve their blood compatibility and biocompatibility [4–8]. The biocompatibility of sulfobetaine zwitterions is attributed to the hydration of the zwitterionic surface by the formation of a dynamic boundary layer [9]. Polysulfobetaine has interesting chemical structure with an anionic sulfonate and a cationic quaternary N^+ group on each repeating unit. The net result is electrical neutrality that renders these isoelectric at the neutral pH [10,11]. In the salt free solution their physical properties are dependent on the electrostatic interactions, and any change in their microenvironment significantly changes their physical properties. Polysulfobetaines exhibit stimuli-responsive phase transition behavior in the neutral media [12]. The inter and intra-chain ion-pairing interactions in polysulfobetaines change on variation of the solution temperature and these strongly influence their physical properties [13,14]. The super-collapsed sulfobetaine brushes can change to a more hydrophilic form at higher temperature [15]. Polysulfobetaines exhibit anti-polyelectrolyte behavior in the presence of ions as their polymer chains tend to expand in aqueous solutions [16,17]. The anti-polyelectrolyte behavior affects solubility and rheology of the linear polysulfobetaines.

This means that the ion-pair is defined by the covalently attached spacer and these two ions are not labile, and the properties of the polymers can be affected by changing the length of the spacer between the N^+ centre and the sulphonate (SO_3^-) anion end of the repeating unit. The change in the property profile of polysulfobetaines as a function of the variation of the length of the alkyl spacer group has been reported [18]. However, there are two limiting factors to prepare a series of polysulfobetaines using this strategy. One, the choice of the alkylating reactants with variable alkyl chain length to generate N^+ and also to have sulphonate at the other end is limited. Two, the alkyl chain length does not necessarily change the properties of polysufobetaine thus obtained with a useful, rich and differentiated property profile to merit such synthesis. On the other hand, there have not been many attempts to affect changes in the property profile of polysulfobetaines by the change of the anion component at the N^+ centre. A lot of changes in the technologically desirable properties can be obtained by targeting an anion as the technicophore [19]. It can exhibit a high potential for the tuning of the technological properties such as solubility and viscosity, useful in the biotechnological applications. The anion exchange involved is a simple process that affects many useful properties of the polymers [20]. There are reports

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where the effect of the nature of the anions present in the solution influences, the properties of the polysulfobetaines as in the case of poly[1-vinyl-3-(3-sulfoethyl imidazolium betaine)] hydrogels, more strongly than the nature of the cations with higher concentrations of the salt exhibiting the anti-polyelectrolyte effect [21]. The polysulfobetaines have been reported to exhibit anion-dependent properties when used in chromatographic applications [22,23]. However, there are no reports in literature where the anion exchange at the N^+ centre was used to tailor the properties of polysulfobetaines for use in the biomedical and biotechnological applications.

In view of the above, in this study, we thought of the counter anion (Br^-) at the N^+ of a polysulfobetaine, poly[1-vinyl-3-(2-sulfoethyl imidazolium betaine)], as a technicophore that can be exchanged to tune the anti-microbial properties of the polysulphobetaine. The anti-bacterial activity of the quaternary N^+ polymers is largely driven by the alkyl chain branching and hydrophobicity of the cation [24–26]. The alkyl chain length of the substituent in pyridinium, imidazolium, and quaternary ammonium salts largely affects the anti-bacterial activity [27,28]. We attempted to change it by the anion exchange and obtained novel polysulfobetaines derived from poly[1-vinyl-3-(2-sulfoethyl imidazolium betaine)]. The latter was obtained by quaternization of poly(*N*-vinyl imidazole) [*N*-VIm] with sodium salt of 2-bromoethane sulfonic acid ($Br\_CH_2CH_2SO_3^-Na^+$). Br^- from thus obtained polysulfobetaine was replaced with Cl^-, F^-, OH^-, SH^-, SCN^-, NO_3^-, BF_4^- and CH_3COO^- to get a series of polysulfobetaines of [poly[1-vinyl-3-(2-sulfoethyl imidazolium betaine)] having different anion at the N^+ centre. The evidence of the anion exchange was obtained by the characterization of different polysulfobetaines by Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), elemental analysis (C, H, N and S) and viscosity measurements. The key question of this study was, whether the anion as technicophore of a polysulphobetaine can influence its anti-microbial properties. Anti-microbial properties of the polysulfobetaines series were studied against three fungi (*Aspergillus niger, Byssochlamys fulva MTCC 505* and *Mucor circenelliods*) and two bacteria (*Bacillus coagulans BTS-3* and *Pseudomonas aeruginosa BTS-2*). The anti-microbial activity was calculated by minimum inhibitory concentration (MIC) method [29].

2. Materials and methods

2.1. Materials

N-vinyl imidazole [Aldrich Schuchardt, Germany], Sodium salt of 2-bromomo ethane sulfonic acid, NaSH (Acros Organics, Belgium), Penicillin (Hi Media, Mumbai), [Carbendazim (12%) and Mancozeb (63%)] (Insecticides India Limited, India) NaF, KCl, NaOH, $NaNO_3$, $NaBF_4$, KSCN and CH_3COOK (S.D. Fine Ltd., Mumbai), were used as received. The test organisms for anti-microbial studies, i.e., *A. niger*, *M. circenelliods*, *B. fulva* (*MTCC 505*), *B.* coagulans BTS-3 and *P.* aeruginosa BTS-2 were obtained from the Department of Biotechnology, Himachal Pradesh University, Shimla, India.

2.2. Synthesis of polysulfobetaines

Polymerization of *N*-vinyl imidazole (*N*-VIm) was carried out by the irradiation method by using γ rays at a dose of 12.96 KGy on Gamma chamber 900 (BARC Mumbai, India). The polymer was washed with water and dried at 70 °C. Quaternization of poly(*N*-VIm) was carried out by treating it with sodium salt of 2-bromo ethane sulfonic acid in 1:3 weight ratio in a minimum amount of water to ensure maximum quaternization. The reaction mixture was heated at 100–105 °C for 18 h. The product was designated as polysulphobetaine poly[1-vinyl-3-(2-sulfoethyl imidazolium betaine)] and used as such without purification. Br^- was replaced from the precursor polysulphobetaine with F^-, Cl^-, OH^-, SH^-, SCN^-, NO_3^-, BF_4^-, and CH_3COO^- by anion exchange reactions to get a series of polysulphobetaines. The anion replacement with Br^- was carried by dissolving precursor polysulphobetaine in a minimum amount of the distilled water to make a saturated solution and the saturated solution of different salts such as NaF, NaBr, NaOH, NaSH, $NaNO_3$, $NaBF_4$, KSCN and CH_3COOK were separately prepared in the distilled water. The solution of the precursor polysulphobetaine was added to each of the salt solutions, allowed to stand for 30 min and the complete precipitation was affected by the addition of distilled acetone to precipitate the polysulphobetaines [20]. The polymers thus obtained were dried in vacuum desiccator. To ensure complete removal of any trace of salts from the polymers after anion exchange, the dried materials were re-dissolved in the double distilled water and stirred for 2 h to ensure total removal of the salts. The precipitation and drying process was repeated. For the sake of brevity and simplicity, the resultant polysulfobetaines are named as $[PSB]^+Br^-$, $[PSB]^+NO_3^-$, $[PSB]^+{-}OH$ $[PSB]^+SCN^-$, $[PSB]^+F^-$, $[PSB]^+BF_4^-$, and $[PSB]^+CH_3COO^-$, where $[PSB]^+$ stands for [poly[sodium 1-vinyl-3-(2-sulfoethyl imidazolium)].

2.3. Characterization of polysulfobetaines

The polysulfobetaines obtained by the anion exchange as reported above were characterized by DSC on Metller Toledo 851e in nitrogen atmosphere, FTIR on Nicollet 5700, element analysis (N and S) on Carlo Erba Instrument 1150 analyzer and on Elementar Vario El, respectively, SEM on FE-SEM (Philips XL30 S FEG), XRD on SIMENS D5005 X-ray diffractometer, and viscosity measurements at 25 °C. The density of each PSB solution under study was measured by density and sound analyzer (DSA, Anton Paar).

2.4. Anti-microbial activity of polysulfobetaines

The anti-microbial activity of different polysulfobetaines was studied against fungi (*A. niger, B. fulva MTCC 505* and *M. circenelliods*) and bacteria (*B. coagulans BTS-3* and *P. aeruginosa BTS-2*). The anti-microbial activity was calculated by minimum inhibitory concentration (MIC) method, i.e., the lowest concentration of the compound that inhibited the visible growth of microbes. The determination of MIC involves a test procedure, which gives an approximation to the least concentration of an anti-microbial agent to prevent microbial growth. The polymers were screened against the test organisms. The anti-microbial activity of the polymers has been assayed at concentrations ranging from 16 to 0.0313 mg/mL in comparison to the standard drug. The susceptibility testing was carried out by the conventional method of serial dilution and finding the MIC in the respective nutrient medium. The protocol involves the addition of sample compound to the medium following the method of serial dilutions.

The medium used for the *A. niger*, and *B. fulva* (*MTCC 505*) included potato dextrose agar (4%, 40 g in 1 L) which was added to 1 L of distilled water and the pH was maintained to 3.5. For *M. circenelliods* peptone (5 g/L), yeast extract (1 g/L), beef extract (3 g/L) were dissolved in the distilled water (1 L), while the pH was maintained at 3.5. About 2.5 mL of the media was taken in different test tubes and these were sterilized by autoclaving at 121 °C and 15 psi pressure for 20 min. Then, known amounts of different polysulfobetaines were added to the test tubes, and were inoculated in laminar flow with 25 μL spore suspension of the given fungus [*A. niger, M. circenelliods*, *B. fulva* (*MTCC 505*)]. Then, the test tubes were incubated at 30 °C for 5 days. The minimum amount of the compound that inhibits the growth of fungus, i.e., MIC was calculated and compared with the standard fungicide [Carbendazim (12%) and Mancozeb (63%)].