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Effect of cationic polyelectrolytes on the performance of paper diagnostics for blood typing

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ABSTRACT

We investigated the effect that two common types of cationic polyelectrolytes used in papermaking might have on the performance of paper diagnostics using blood typing as an example. The results were analyzed in terms of red blood cells (RBC) retention and antibody–antigen specificity. Two questions were addressed: (1) can poly(amido-amine) epichlorohydrin (PAE) typically used for paper wet strength affect the diagnostic performance? (2) can high molecular weight cationic polyacrylamide (CPAM) employed as retention aid enhance or affect the selectivity and sensitivity of paper diagnostics?

A series of paper varying in type of fibers and drying process were constructed with PAE and tested for blood typing performance. Residual PAE has no significant effect on blood typing paper diagnostics under normal conditions. Positives are unaffected with PAE, while negatives lose slight sharpness as some RBCs are unselectively retained.

CPAM, the most common retention aid, can also be used to retain cells and biomolecules on paper. Paper towel was treated with CPAM solutions varying in polymer concentration and charge density and tested for blood typing. We found that CPAM dried on paper can retain RBC. CPAM affects the negative tests by retaining non-specifically individual RBC on fibers. RBC retention increases non-linearly with the CPAM charge density and concentration. As expected, wet CPAM retain RBCs at concentrations higher than 0.1 wt%.

As paper diagnostics are becoming a reality, more realistic papers than the Whatman filter paper will be engineered. This study provides guidance on how best use the required polymeric wet-strength and retention agents.

1. Introduction

Bioactive paper has emerged as a new generation of products combining a biomolecule as specific agent with a cellulose fiber/surface used as functional substrate. Pelton [1] provided a comprehensive review on bioactive paper. A rapidly expanding application of bioactive paper is for biomedical applications; these were reviewed by a few research groups [2–7]. A paper-based diagnostic consists of a cellulose fiber based substrate on which an analytical system is deposited. This paper diagnostic is usually wetted with a biofluid to detect and quantify a biomolecule such as an enzyme, antibody, a protein or a hormone – and a response is read. As paper is used wet, and samples are often very valuable, a wet-strength agent and retention aid will be required for mass producing paper diagnostics. However, little is known on the effect such polymeric agents – typically cationic polyelectrolytes – might have on the behavior of the paper diagnostic.

A new generation of low cost and easy to use paper diagnostics has recently been developed for blood typing [8–13]. The concept relies on specific antibody–antigen interactions to selectively agglutinate red blood cells (RBC) on paper, to separate the agglutinated RBC (positive) from the non-agglutinated RBC (negative), to directly communicate results; the paper test can also be retained as a document.

Red blood cells (RBC) agglutinated from antibody-specific haemagglutination reactions transport differently on paper compared to non-agglutinated blood [8]. The basis of immunohaematology used in blood typing diagnostics is nicely reviewed elsewhere [14–16]. The non-agglutinated RBCs can wick paper and transport by capillary flow; agglutinated RBC cannot transport on paper and become retained, forming a visual indicator of a positive reaction. This concept laid the foundation for developing paper-based blood typing devices. Subsequently, Al-Tamimi et al. [9] developed a paper-based assay for rapid blood typing.

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The new method involved fixation of RBCs on paper treated with blood typing antisera and an elution step. RBCs in contact with the specific antibody agglutinated and fixed onto the paper while non-agglutinated RBCs were eluted through capillary action.

For a sensitive and reliable test, it is critical that a positive response yields strong agglutination and retention of the red blood cells (RBC) on paper upon elution, therefore providing a clear visual signal; for a negative test, all RBC need to be completely washed off the paper, leaving it pristine white. While filter paper (Whatman mostly) has been the preferred substrate for bioactive paper [17–22], this material cannot be translated into product development and commercial process. Filter paper is not only too expensive but in most cases shows poor performance especially for blood typing [10]. Tissue and light weight paper substrates of controlled structures and porosity have been shown to be more effective [8–11]. However, two types of polymeric additives are required to develop colloids retention and strength of these lightweight non-woven fibrous composites typically used wet: retention aids and wet strength agents. These polymeric additives, such as cationic polyacrylamide (CPAM) and polyethyleneimine (PEI) retention aids are typically cationic; wet-strength agents, such as poly(amido-amine) epichlorohydrin (PAE) are able to bind covalently with cellulose when heated at 100 °C. The chemical structures of these two polyelectrolytes are shown in Fig. 1. A critical issue is whether or not these polymers can affect the retention or desorption of the red cells and antibody used on the paper diagnostics. Of particular concerns are the false positive, in which red blood cells are indiscriminately retained on paper, or false negative, for which positive and normally retainable cells are eluded [2]. Paper diagnostics for blood typing rely on two mechanisms: elution/chromatography in the plane of paper and filtration through paper [11].

We previously observed that filter paper with surface treatments behaved differently than those without which affected typing clarity of the positive and negative tests [10]. We also reported that potentiators often used in the formulation of antibody solution could contribute to false positive [11]. Potentiators such as dextran and polysaccharides are sometimes added to the formulation of the commercial antibodies to enhance the RBC coagulation when using antibodies with low avidity resulting in weak blood group reactions [15]. However, potentiators and retention aids can also be needed to improve the strength and size of RBC aggregates, especially in the case of weak or secondary blood groups.

This study has two objectives. The first is to elucidate whether wet-strength agents such as poly(amido-amine) epichlorohydrin (PAE) and common papermaking retention polymers, such as cationic polyacrylamides (CPAMs) can affect blood typing analysis and retain RBC or antibodies on paper. A requirement of the antibody physisorbed on paper is its ability to desorb and diffuse within the blood droplet, therefore agglutinating the biospecific RBC. The second objective is to quantify the effect of cationic polyelectrolyte charge density and concentration on the retention of RBC and antibody and to analyze how these affect the sensitivity of positive and negative tests. Three studies are performed. In the first, PAE is adsorbed on pulp fibers prior to papermaking and dried. The polymer effect on antibody, RBC retention and diagnostic performance is then evaluated. In the second study, a series of CPAM at various charge densities is investigated as retention aid for antibody on paper and tested for positive and negative bio-specific test with RBC. The effect of CPAM charge density on antibody and RBC bridging ability and the consequences are analyzed. In the third study, the retention ability of wet CPAM adsorbed on paper is analyzed in the context of paper based blood typing analysis. It is the objective of this study to investigate cationic polyelectrolytes to improve paper based blood typing analysis performance.

2. Experimentals

2.1. Materials

The cationic polyacrylamides (CPAMs) were kindly supplied by AQUA + TECH (Switzerland) and used as received. These are copolymers of uncharged acrylamide with cationic dimethylaminoethylacrylate methyl chloride having 5%, 10%, 20%, 30%, 50% of charge monomer and MW 13 MDa. The 80% charge CPAM had MW 8 MDa as defined by the manufacturer. Poly(amido-amine) epichlorohydrin (PAE) was supplied from Nopco, Australia. The standard Professional Kleenex paper towel from Kimberly–Clark, Australia was used as substrate for most experiments. It has a basis weight of 26.4 g/m^2 and is a trilayer sheet. A Filter paper grade 1845 having a pore size 25 μm and a basis weight of 70 g/m^2 was purchased from Filtech and used as comparison. Three EDTA stabilized blood samples, one of each group A, B and O, stored at 4 °C, were supplied by Australian Red Cross Blood Service (Sydney) and were used between 10 and 14 days post collection. Blood typing antisera used was FFMU (For Further Manufacture Use) anti-A and anti-B purchased from Alba Bioscience, Edinburgh, United Kingdom. The washing solution used was PBS (Phosphate Buffered Solution) prepared with MilliQ water and PBS tablets supplied by Sigma–Aldrich.

2.2. Methods

2.2.1. PAE paper preparation

Paper handsheets were prepared according to the Australian/New Zealand Standard Method 203s. Basically the dry pulp was thoroughly wetted by soaking in deionized water for about 12 h. The pulp was transferred to a disintegrator (Model MKIIIC, Messmer Instruments Ltd.), diluted to 2 L with deionized water and disintegrated for 75 000 propeller revolutions. If required, the PAE solution was added to the pulp slurry prior to handsheet forming and stirred for 5 min. The pH of the pulp slurry mixture was not adjusted and the value was about 5. The addition quantity of PAE (20 mg/g fiber) was based on oven dry grammage of 60 g/m^2. After manual couching and wet-pressing at 0.4 MPa for about 15 s, the sheets were either air dried 24 h at 23 °C and 50% relative humidity (RH) or cured in a SEMMAR Auto Dryer Type MR-3 at 112 °C for 10 min, in order to activate covalent bonds between the PAE and cellulose. Each paper type was cut into 40 individual 3 cm × 3 cm squares. Anti-A and anti-B were each dispensed on to 20 squares of all the prepared papers. Positive test used A cells and B cells, respectively, while O cells was the negative test with 10 replicates of each using the same blood testing method as below.

2.2.2. CPAM treated papers

CPAM solutions were prepared as follows. Six differently charged CPAMs were dissolved at 1 g/L in deionized water and vigorously mixed for 48 h, followed by two serial one in five dilutions, in deionised water and mixed for 24 h after each dilution. This resulted in three concentrations: 1 g/L, 0.2 g/L and 0.04 g/L, for the CPAM treated paper testing. Likewise, two serial one in ten dilutions of 30% charged CPAM were made for the wet CPAM testing resulting in 1 g/L, 0.1 g/L and 0.01 g/L solutions with a minimum 24 h mixing for each step.

Paper toweling was prepared by drawing a dozen 6 cm × 6 cm squares onto 72 sheets. Each CPAM solution (100 μL) was dispensed onto the middle of all 12 squares on 4 sheets of the prepared paper towel, ensuring no contact between paper towel and bench. The paper towel with CPAM was subsequently dried in a SEMMAR Auto Dryer Type MR-3 (112 °C for 10 min) prior to testing. Once dry, the sheets of paper towel were all tested in the same manner.

In addition, an extra 24 sheets of paper towel were prepared with a dozen 6 cm × 6 cm squares. CPAM 30% charge solution