



Enhanced pro-coagulant hemostatic agents based on nanometric zeolites



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ABSTRACT

Micro- and nanometric *faujasite* zeolites were tested as hemostatic coagulant agents. The as-made zeolites and their calcium ion exchanged derivatives were characterized by XRD, SEM, AFM, TGA, and DSC. Zeta potential measurements of the micro and nanometric zeolites, as a function of pH (1–12.5), revealed the hemostatic potential of the materials. The isoelectric point for FAU, FAU/Ca, Nano-FAU, and Nano-FAU/Ca were measured at pH 2.0, 1.9, 3.2, and 2.5, respectively. The hemostatic activity was confirmed by the TEG technique for both micro and nanometric zeolites, however a superior activity were observed for the nanometric materials. FAU and FAU/Ca exhibited a reduction of the R parameter (defined as period of time of latency from the start of test to initial fibrin formation for the formation of a clot of an amplitude of 2 mm) from 8.6 ± 0.7 min (control) to 3.2 ± 0.7 min and 2.3 ± 0.1 min, respectively. On the other hand, for the Nano-FAU, and Nano-FAU/Ca the observed decrease were to 2.4 ± 0.6 min, and 1.1 ± 0.2 min, respectively. The amounts of heat released were 105.60 J/g (FAU), 65.8 J/g (FAU/Ca), 85.48 J/g (Nano-FAU), and 78.21 J/g (Nano-FAU/Ca). Statistical analysis using one-way analysis of variance showed a global value of $p < 0.0001$. Tukey-Kramer multiple comparisons revealed significant ($p < 0.0001$) differences for FAU, FAU/Ca, Nano-FAU, and Nano-FAU/Ca, relative to the control. Nano-FAU/Ca showed the most important reductions of the R with significant differences compared to the control ($p < 0.0001$ and $p < 0.0001$, respectively) and FAU ($p = 0.0071$ for the R parameter).

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1. Introduction

Hemostatic coagulant agents are materials capable of reducing bleeding and can be grouped into three classes, depending on their mechanism of action: factor concentrators [1], mucoadhesive agents [2], and procoagulants [3,4]. They can also be grouped according to their forms and types: solid sheets, normally known as hemostatic dressings [5]; solid particles, or powders and fibers [3]; hydrogels [6]; liquid tissue sealants [7]; dispersions made from natural [8] or synthetic polymers [9], ceramics [10] clays such as smectite and kaolin [11–13]. Several of these materials have been

reported to be used as hemostatic agents both in their pure forms or combined with other materials [10,14]. Among the inorganic classes of materials, zeolites have attracted the attention of the scientific and medical communities for their potential use as efficient hemostatic agents.

Hemostatic agent employing dehydrated zeolites as powder dressing was employed during the Iraq war 2003 under the trademark QuikClot[®] (Z-Medica, Wallingford, CT) [3,15–17]. In principle it acts as a molecular sieve, adsorbing water molecules at the wound site and increasing the concentrations of clotting factors, platelets, and erythrocytes in order to stimulate hemostasis [18,19]. Other authors have studied different classes of composites based on zeolites with the purpose of finding more efficient hemostatic agents. Zhang et al. [9] developed an antibiotics-loaded zeolite/polymer composite for hemostatic applications, while

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Mortazavi et al. [20] showed that a mixture of bentonite and zeolite minerals could dramatically reduce bleeding and the volume of blood loss, as well as the clotting time, and observed a control of exothermic reactions, which eliminated the risk of burning in the wound. Alavi et al. [21] studied *in vivo* the effects of a new impregnated sterile gauze containing bentonite and halloysite minerals on blood coagulation and the wound healing rate in male Wistar rats, and it was observed that topical application of this material decreased the clotting time and accelerated the wound healing rate.

Despite the beneficial effects of zeolites in accelerating blood coagulation and shortening the clot formation time, the main drawback of their use is related to the exothermic reaction, which can reach temperatures higher than 100 °C (212 °F) and cause full-thickness tissue damage [22,23]. Several methods have been tested to attenuate this adverse effect, such as ion exchange treatment of the as-made zeolites using K^+ , Ba^{2+} , Sr^{2+} , or Ag^+ . A heat reduction from 680 J/g to 420 J/g was reported for ion exchange in Linde Type 5A zeolite using Ag^+ ions [24].

Reduction of particle size from the micrometric to the nanometric scale leads to substantial changes in the properties of the materials, which in turn affects the performance of zeolites in the traditional applications areas such as catalysis and separation [25]. A survey of the literature will show that the hemostatic agents based on zeolitic materials that have so far been tested appear to be on the micrometric scale. To the best of our knowledge no study concerning the behavior of nanometric zeolites used as hemostatic coagulation agents has until now been reported. Therefore, the questions raised in this study are: a) Does zeolite size (micrometric or nanometric) affect the coagulation cascade? b) Does it affect the collateral damage of burning? With the aim of answering these questions, systematic studies were undertaken with micrometric and nanometric *faujasite* zeolites hydrothermally synthesized, ion exchanged with calcium, characterized by several spectroscopic techniques, and tested as hemostatic agents using *in vitro* thromboelastography assays to observe the acceleration in clotting time.

2. Experimental section

2.1. Chemicals

The preparation of the *faujasite* zeolite and the ion exchange experiments employed the following chemicals: sodium hydroxide pellets (NaOH, 98%, Sigma-Aldrich, Steinheim, Germany), sodium aluminate ($NaAlO_2$, Sigma-Aldrich, Steinheim, Germany), fumed silica (SiO_2 , 99.8%, Sigma-Aldrich, Steinheim, Germany), and calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$, Merck, Darmstadt, Germany). All the chemicals were used as received. *In vitro* assays were performed with human blood samples. All the patients who participated in this study gave informed consent before blood donation. The study was approved by the Research Ethics Committee of FAMERP (São José do Rio Preto Medical School) under the protocol number CAAE 48358215.9.0000.5415.

2.2. Preparation of materials

2.2.1. Synthesis of as-made micrometric and nanometric *faujasite* zeolites

The nanometric *faujasite* zeolite was synthesized by hydrothermal crystallization, following the procedure reported by Zhan et al. [26]. Aluminosilicate gel was prepared by mixing freshly prepared aluminate and silicate solutions at a molar ratio of 5.5 $Na_2O:1.0 Al_2O_3:4.0 SiO_2:190 H_2O$. In a typical synthesis, sodium hydroxide solution (5.34 g of NaOH/50 mL of H_2O) was freshly prepared, followed by addition of 2.42 g of sodium aluminate

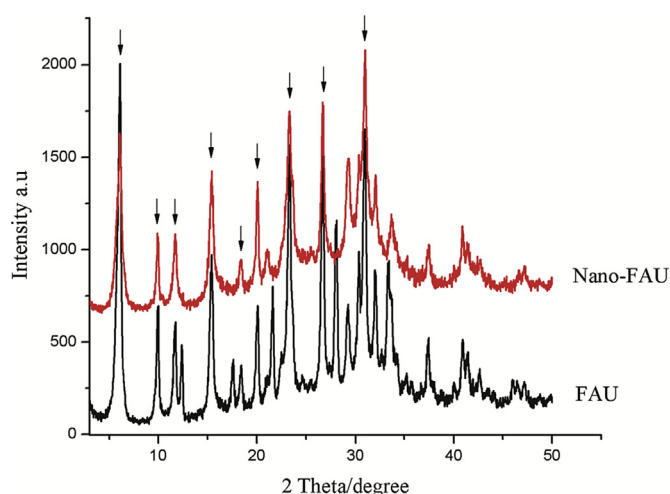


Fig. 1. XRD patterns of the as made micrometric (FAU) and nanometric *faujasite* zeolite (Nano-FAU).

($NaAlO_2$), with stirring for 15 min at room temperature. Fumed silica (SiO_2 , 3.43 g) was then added to the solution, with stirring until a homogeneous gel was obtained. The gel was immediately transferred to a temperature-controlled shaker and agitated at 250 rpm for 2 days at 60 °C. The powdered product was recovered by centrifugation at 13,400 rpm (CR22N centrifuge, Hitachi, Tokyo, Japan), washed with deionized water until reaching pH < 8, and dried in an oven at 40 °C for 16 h. The micrometric *faujasite* zeolite was synthesized following the same procedure described above, except that crystallization was conducted at 90 °C for 4 days in a static reactor (Parr Instruments Co., USA). The micrometric and nanometric zeolites were denoted FAU and Nano-FAU, respectively.

2.2.2. Ion exchange of the zeolite

In a typical ion exchange experiment [27], 1 g portions of as-made FAU and Nano-FAU were added to several Teflon-lined digestion reactors (Parr Instruments Co., USA), followed by the addition of 30 mL of 0.5 M $CaCl_2$ solution to each reactor. The ion exchange reactions were conducted at 90 °C for 12 h. The solid products were subsequently collected by centrifugation at 13,400 rpm, washed three times with deionized water, and dried in an oven at 40 °C for 16 h. The ion exchanged zeolite derivatives were denoted FAU/Ca (micrometric) and Nano-FAU/Ca (nanometric).

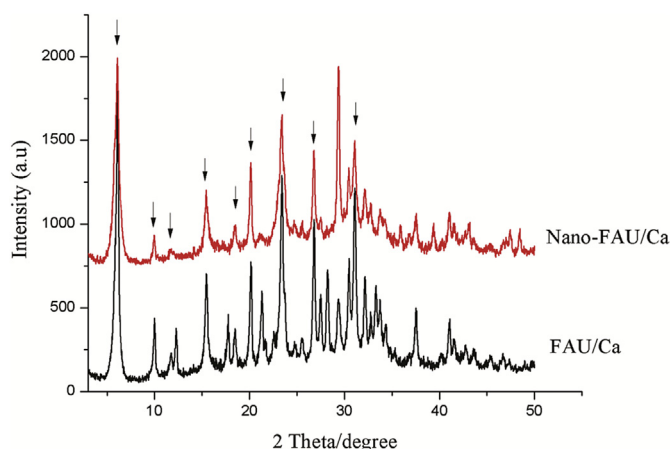


Fig. 2. XRD patterns of the calcium-exchange derivatives (FAU/Ca; Nano-FAU/Ca).

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