Reference medium for blood coagulation ultrasonic studies

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Introduction

Quality control is a general requirement for every scientific equipment, including ultrasound medical. For this purpose reference liquids are used in general. Most popular reference liquids for ultrasound velocity measurements is distilled degassed water. It fits to check the equipment only at one velocity point, experiments being provided at 37^0 C. Other known standard liquids with different velocities commonly used for calibration are physiological solution, water-ethyl alcohol mixtures [1]. Water-glycol mixtures have valuable properties because by varying the proportions ultrasound velocity can be changed from 1450 m/s to 1800 m/s. Such an interval is typical for biological liquids and soft tissues. At certain mixture proportions ultrasound velocity is independent on temperature [2].

Quality control procedures to ensure reliable operation in blood coagulation studies, when the regularities of ultrasound velocity variations are investigated as the media structure changes, are more complicated. For example, manufacturers of whole blood analyzers operating on various principles offer bovine blood plasma as a reference because it has clotting constituents similar to those of humans. All blood should be taken from the same herd to ensure standardization and accurate reproducibility of results [3].

In this article an alternative proposal is investigated. A chemical reaction with similar ultrasound velocity variation as in blood coagulation was searched. For this purpose the polymerization reaction of acrylamide has been chosen. Acrylamide $CH_2=CHCONH_2$ is a white water-soluble crystalline solid, melting at 84.5^0 C. The polymerization form of acrylamide and hence the growing radical is also water soluble. This property is very important in experimentation. Moreover, the same peptide groups –CO-NH- are included in the composition of polyacrilamide as in final product of blood coagulation, e.g. fibrin, with the difference that structure of fibrin is based on α-amino acids.

Experimental

The studies of polymerization reaction of acrlylamide were provided with the same modified pulse time-of-flight technique that was used for the investigation of blood coagulation process [4]. The system operates at 5 MHz and follows the variation of ultrasound velocity with the uncertainty ± 3 cm/s. The cell volume was 0.3 ml, the temperature of the medium 36.6^0 C, maintained within $\pm0.01^0$ C. The generalised character of blood coagulation curve observed in numerous ultrasonic measurements is shown in Fig.1. The curve in every separate case may differ, sometimes significantly, but it reflects the main phases of ultrasound velocity changes – the beginning and the end of clotting, latent and main retraction [5]. It is characterised in principle by reduction and later increasing of an ultrasound velocity.
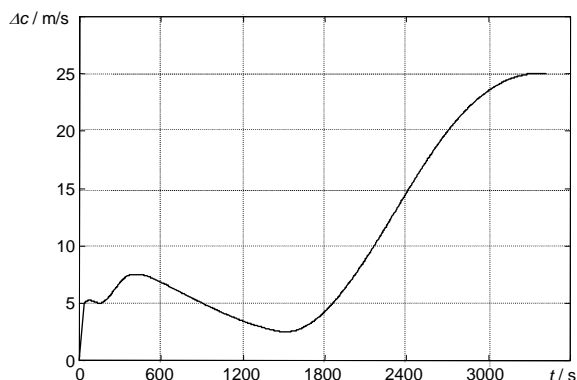

Fig. 1. **Generalised blood coagulation curve**

The minimal aim of this work is to get the change of velocity in chemical reaction only in one direction, the magnitude being alike. The optimal case is the change of a velocity in both directions. The solutions were prepared from bidistilled water and commercial acrylamide. The inorganic salts used were of analytical grade and were not purified. The ammonium persulfate as a simple free-radical initiator for the polymerization of acrylamide was used at first. Fig.2 presents the course of polymerization reaction at initial monomer concentration 5 % on mixture weight and at concentration of $(NH)_2S_2O_8$ 0.008 moles/liter.
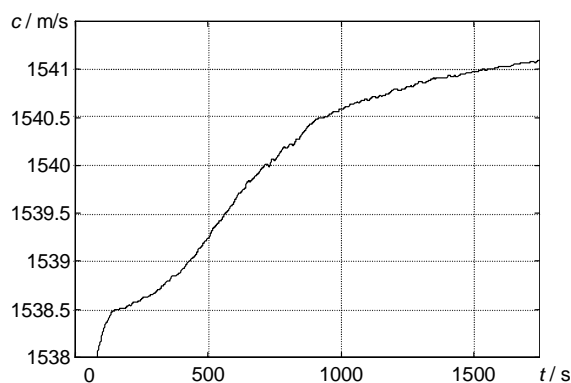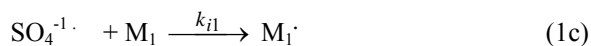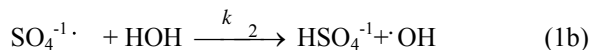

Fig.2. **Course of acrylamide polymerization initiated by ammonium persulfate**

It's known that the thermal decomposition of persulfate yields two species capable of initiating polymerization: the sulfate radical-ion and the hydroxyl radical [6].

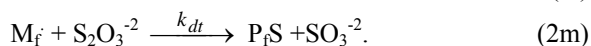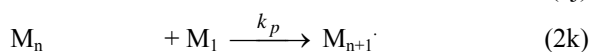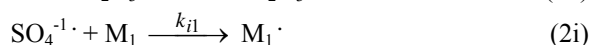In the presence of monomer the following reactions would be suitable for a scheme:

$$S_2O_8^{-2} \xrightarrow{k_1} 2\ SO_4^{-1}\cdot \qquad (1a)$$

$$SO_4^{-1}\cdot\ + HOH \xrightarrow{k_2} HSO_4^{-1} + \cdot OH \qquad (1b)$$

$$SO_4^{-1}\cdot\ + M_1 \xrightarrow{k_{i1}} M_1\cdot \qquad (1c)$$

$$\cdot OH + M_1 \xrightarrow{k_{i2}} M_1\cdot \qquad (1d)$$

$$M_n\cdot + M_1 \xrightarrow{k_p} M_{n+1} \qquad (1e)$$

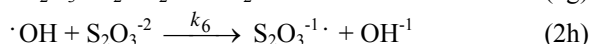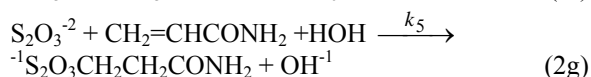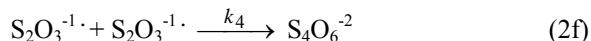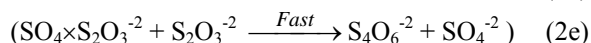$$M_f\cdot + M_k\cdot \xrightarrow{k_t} P_{f+k}, \qquad (1f)$$

where the letter subscripts denote the degree of polymerization of radical M$\cdot$ and polymer P species. Reaction (1b) and (1c) compete for the sulfate radical-ion. For the persulfate decomposition authors [6] report that $k_1=3.18\times10^{-6}$ min and activation energy $E_1=33.5$ kcal/mole. It is the classical pattern for free-radical polymerization with the first-order dependence of rate on monomer concentration.

The persulfate-tiosulfate redox couple has been investigated also as an initiation system in the polymerization of acrylamide.The stoichiometry is following by the reaction (2):

$$S_2O_8^{-2} + 2\ S_2O_3^{-2} \rightarrow S_2O_6^{-2} + 2\ SO_4^{-2} \qquad (2a)$$
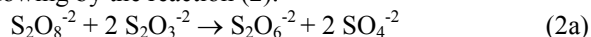
The kinetic picture is proposed in the scheme of equations (2b÷2m):

$$S_2O_8^{-2} + S_2O_3^{-2} \xrightarrow{k_1} SO_4^{-1}\cdot + SO_4^{-2} + S_2O_3^{-1}\cdot \quad (2b)$$

$$SO_4^{-1}\cdot + HOH \xrightarrow{k_2} HSO_4^{-1} + \cdot OH \qquad (2c)$$

$$SO_4^{-1}\cdot + S_2O_3^{-1}\cdot \xrightarrow{k_3} SO_4\times S_2O_3^{-2} \qquad (2d)$$

$$(SO_4\times S_2O_3^{-2} + S_2O_3^{-2} \xrightarrow{Fast} S_4O_6^{-2} + SO_4^{-2}\ ) \quad (2e)$$

$$S_2O_3^{-1}\cdot + S_2O_3^{-1}\cdot \xrightarrow{k_4} S_4O_6^{-2} \qquad (2f)$$

$$S_2O_3^{-2} + CH_2=CHCONH_2 + HOH \xrightarrow{k_5}$$
$$^{-1}S_2O_3CH_2CH_2CONH_2 + OH^{-1} \qquad (2g)$$

$$\cdot OH + S_2O_3^{-2} \xrightarrow{k_6} S_2O_3^{-1}\cdot + OH^{-1} \qquad (2h)$$

$$SO_4^{-1}\cdot + M_1 \xrightarrow{k_{i1}} M_1\cdot \qquad (2i)$$

$$\cdot OH + M_1 \xrightarrow{k_{i2}} M_1 \qquad (2j)$$

$$M_n\quad + M_1 \xrightarrow{k_p} M_{n+1}\cdot \qquad (2k)$$

$$M_f\cdot + M_k\cdot \xrightarrow{k_t} P_{f+k} \qquad (2l)$$

$$M_f\cdot + S_2O_3^{-2} \xrightarrow{k_{dt}} P_fS + SO_3^{-2}. \qquad (2m)$$

There are many competing reactions what may predict various courses of polymerization. In this scheme the basic assumption is made that the thiosulfate radical-ion is unreactive in initiating polymerization and that at certain concentrations of this radical reaction (2d) dominates in the competition with reaction (2i) making the hydroxyl radical essentially the only initiating species. At very low thiosulfate concentrations, the increase of the rate of polymerization over that for the polymerizations initiated by persulfate stems from the rapid production of sulfate

radical-ions by reaction (2b). As the initial thiosulfate concentration is increased two changes occurs: the concentration of thiosulfate radical-ion reaches a level where reactions (2d) and (2f) become important, and degradative termination by reaction (2m) starts to compete effectively with the normal quadratic termination. Fig.3 shows various courses of polymerization with time resulting from the different concentration of thiosulfate when persulfate is present in the same amount: 3a - concentration of thiosulfate 1×10^{-4} moles/liter, 3b - 5×10^{-4} moles/liter. The accelerating effect of thiosulfate on the rate is very evident.
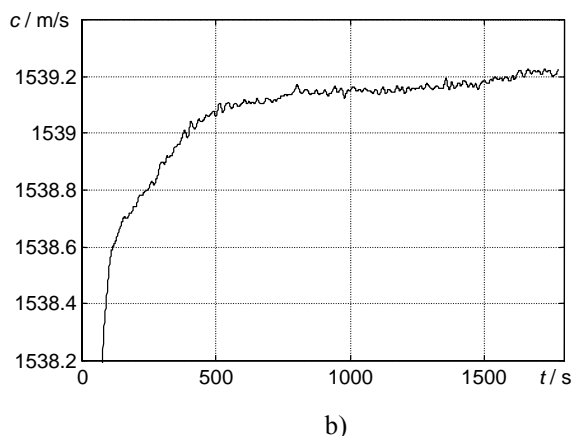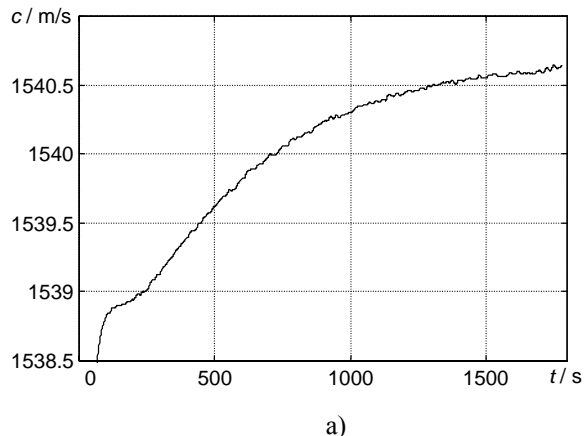

a)

b)

Fig.3. **Acrylamide polymerization initiated by the persulfate-thiosulfate redox couple at different concentrations of thiosufate: a) C=1×10^{-4} moles/liter, b) C=5×10^{-4} moles/liter**

So, increasing the thiosulfate concentration increases the rate of reaction (2b) and (2m) proportionately. The polymerization at high thiosulfate concentration can be understood by considering the competition introduced by reactions (2g) and (2h) and perhaps (2e). These steps are initially oppose each other in the terms of their effect on the rate. The consumption of thiosulfate in reactions (2g) and (2e) would increase the rate by competing with reaction (2m), and the reaction with hydroxyl radical in reaction (2h) would decrease the concentration of initiating species thus decreasing the rate of reaction (2j).

In Fig.4 the curve represents the course of reaction at concentration of persulfate twice higher as compared with the data which are showed in Fig.3a and 3b. Increasing of the persulfate concentration increases the rate of consumption of thiosulfate by reaction (2b) over the course

of polymerization and, hence, reduces the fraction of total thiosulfate consumed in the termination reaction. As seen, the curve shows a lower rate as the ratio of persulfate to thiosulfate and consequently the rate of consumption of thiosulfate is increased. This considers with the conclusion that in termination of the reaction thiosulfate is a dominant agent.
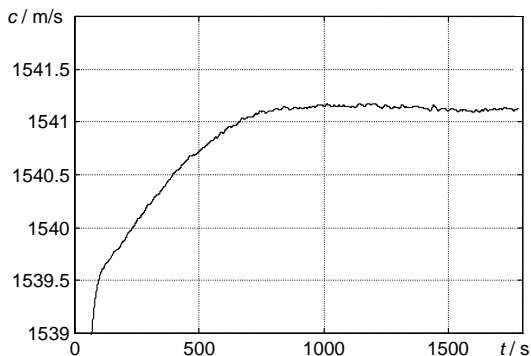

Fig.4. **Polymerization in the presence of K$_2$S$_2$O$_8$, C=0.08moles/liter and of Na$_2$S$_2$O$_3$, C=1×10^{-4} moles/liter**
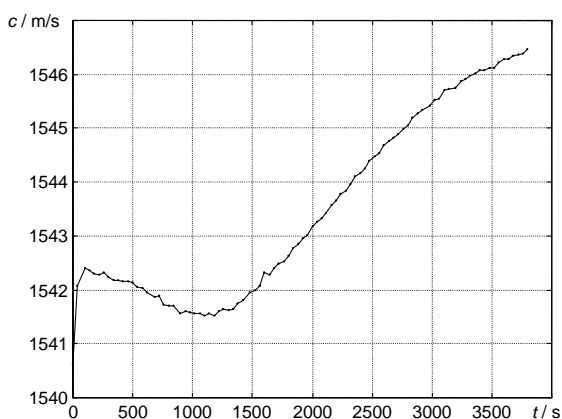

Fig.5. **Optimal polymerization reaction**

Fig.5 presents the polymerization reaction curve what fits the best the blood clotting process. In common case ultrasound velocity depends on a molecular weight. As seen, polymerization reaction demonstrates a wide range of effect on molecular weight dependence resulting from the generation of different initiator species. The length of chain reaction depends on the ratio of amounts of active particles that's are generated in time unit to numbers of

these particles which are produced for growing radical. The amount of thiosulfate ions in this composition of mixture is twice lower than in Fig. 3. The reaction between thiosulfate and monomer would be expected to affect the final molecular weight.

Conclusions

A chemical polimerization reaction of acrylamide initiated by a certain ratio of persulfate and thiosulfate is characterized by the similar ultrasound velocity changes as in blood clotting. This reaction can be used for a quality control of ultrasonic coagulometer.

Acknowledgement

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Terpė kraujo krešėjimo ultragarsinių tyrimų kokybei kontroliuoti

Reziumė

Ištirta akrilamido polimerizacijos reakcija, inicijuota amonio persulfato, taip pat kalio persulfato ir natrio tiosulfato poros. Parodyta, kad, esant tam tikroms reagentų proporcijoms, ultragarso greičio kitimų pobūdis polimerizacijos metu analogiškas, kaip ir krešant kraujui. Svarbu, kad polimerizacijos produktas yra tirpus vandenyje, o reakcijoje dalyvauja peptidinės grupės -CO=NH-, esančios ir galutiniame krešėjimo produkte fibrine. Akrilamido polimerizacijos reakciją siūloma taikyti ultragarsinio kraujo koagulometro kokybės kontrolei.