Terahertz time-domain spectroscopy for studying the kinetics of tissue adhesives

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ABSTRACT

This study deals with the kinetics of tissue adhesives used for supporting the hemostasis and wound closure during surgical intervention. There are several types of adhesives of different composition which is closely related with their application. The time of curing plays an important role because some applications may require very fast glue for prompt vessel or wound closure; conversely some situations need slower solidification because of longer manipulation with the glue during surgery. Here, the terahertz time-domain spectroscopy is used for studying the kinetics of the glues. To slow the reaction rate, an oily substance is added to the glue samples. The technique of attenuated total reflection is used in this application; the defined amount of glue sample or its mixture is applied on the silicon crystal and the terahertz response is measured in time. This time dependences are analyzed to find time constants for mathematical description of the glue kinetics.

Keywords: glue kinetics, oil, terahertz time-domain spectroscopy, tissue adhesive.

1. INTRODUCTION

Terahertz time-domain spectroscopy is a modern method suitable for studying variety of materials. Therefore, it finds useful applications in many technical areas. The techniques using terahertz radiation have number of advantageous properties that are very important for biological and medical sciences. This spectroscopic method is non-invasive, non-destructive, and usually contact-free. The measurement is quite fast and no special procedure of sample preparation is needed. This study is focused on the kinetics of tissue adhesives.

Tissue adhesives are used in surgery for supporting the hemostasis and closure of wounds, fistulae and anastomotic leaks [1]. They can be sorted into three groups according to the main compound they include – cyanoacrylate glue, fibrin and thrombin glue. Effect of each type of glue is based on different mechanism. Fibrin and thrombin glues are usually derived both from human or bovine plasma. Their effect is based on their participation in the physiologic clotting cascade but there are some potential risks of transmitting viral infections [2]. Cyanoacrylates are of synthetic origin and they are known as superglues used for fast bonding of variety of materials in industry and household. Because of toxicity of the cyanoacrylates themselves, there are used their derivatives. The mechanism of action is based on rapid solidification when in contact with weak bases, such as water or in this case – blood [3].

The other research of the tissue adhesives is needed for better insight of their mechanism of action. The kinetics of each type of glue can be different and the time of curing can be also influenced artificially when needed. The time of curing is very important because some applications require the fast wound closure, but some of them need slower solidification because of longer manipulation with the glue during surgery or possible damage of the surgical instrument during injection of the glue (adherence to catheters and endoscopes) [4], [5].

The aim of this work is to study the kinetics of the curing reaction of cyanoacrylate adhesive using the terahertz time-domain spectroscopy. Because of the rapidity of reaction of glue itself, some mixtures with appropriate substance were used to slow the reaction and to allow better characterization of the curing process.

2. MATERIALS AND METHODS

Instrumentation

To monitor the polymerization kinetics of cyanoacrylate glues, we have used the terahertz time-domain spectroscopy system TPS Spectra 3000 by TeraView Ltd. This spectrometer is capable of measuring in several modes. For our purpose, the attenuated total reflection (ATR) mode was chosen as the most appropriate method. The ATR measurement is quite quick, it has no special demands on sample preparation and uses small sampling area, and therefore small amount of investigated material is enough. The principle of ATR is based on the Goos-Hänchen effect. The sample is placed directly on the ATR crystal which has significantly higher index of refraction than sample. In this case, the silicon crystal with refractive index 3.42 is used. The terahertz signal enters the crystal at the angle of 35° and is totally reflected at the crystal-sample interface. The formed evanescent wave penetrates into the sample to a depth which depends on wavelength, the refractive index of sample and crystal, the angle of incidence and polarization. There must be fulfilled one important requirement of full contact of the sample with the ATR crystal because the evanescent wave penetrates only a few microns into the sample.

Samples

N-butyl cyanoacrylate (Histoacryl®), a homologue of cyanoacrylate adhesive, was used in this study. Generally, the cyanoacrylate-based adhesives are synthetic glues with low-viscosity and typical rapid curing. They are usually known as “superglues” used in household and industry, but they have found some useful applications also in medicine. The curing is relatively fast and takes a few minutes. This is the main advantage, because the rapid wound closure is usually desirable but there are also applications that require delayed reaction. For this purpose, some special substances can be added to the glue to slow the reaction [4], [5], [6]. The curing mechanism relates also to the content of water. The biological tissue consists of high percentage of water; consequently, the reaction is almost immediate.

There were prepared four samples – pure N-butyl-2-cyanoacrylate and its mixtures with poppy seed oil at a ratio of 3:1 (75 vol% of glue, 25 vol% of oil), 1:1 (50 vol% of glue, 50 vol% of oil) and 1:3 (25 vol% of glue, 75 vol% of oil). Poppy seed oil is the main content of Lipiodol® that is used as a contrast agent in surgery. There was found its ability to regulate the polymerization time of the glue when mixed together. Here, the oily substance in various ratios is used to slow the reaction and for better characterization of the process.

Experimental design

Samples of cyanoacrylate glue were prepared for preliminary transmission measurement. The defined amount of cyanoacrylate sample or its mixture was applied on the cellulose underlayer and the optical parameters were measured in time. Some problems arose during this measurement. It was associated with the glue consistency and measurement geometry. The spectrometer allows transmission measurement only in vertical position of the sample but the glue flows over the underlayer because of thin liquid consistency. Therefore it is not possible to ensure the same thickness of the sample during the whole measurement.

Finally, ATR technique was chosen as an appropriate method. The parameters of the spectrometer were set up for fast measurement because of the glue curing reaction rate. The measurement was carried out at the laboratory temperature of 28 °C and relative humidity of 25 %. Every act during the measurement was timed precisely. First, the measurement on the spectrometer started, then the exact amount of glue sample was put directly on the silicon crystal and it was covered by aluminum foil. The time interval of approximately 5 seconds was kept between each two following acts. The experiment was repeated for more samples with the same preparation.

3. RESULTS

In this section, the data processing is described and the results are discussed. The measured terahertz response is shown in Figure 1 left. The spectrometer was scanning each second to catch as many changes as possible during the measurement. The result of one measurement was a data set of around 600 samples (terahertz time domain signals taken in each second) but only a part was really useful for other processing. The curing time was usually not longer than 3 minute thus 200 samples were enough. We identified the minimal value of terahertz pulse amplitude for description of the investigated process. This area of interest is highlighted in Figure 1 left as a marked window. The window is shown in detail in Figure 1 right. There are caught changes of the minimal peak amplitude in time. Here, the response in specified time interval after each 10 seconds after foil covering is presented only. A slow descending is obvious and we assume this is connected with the curing process.

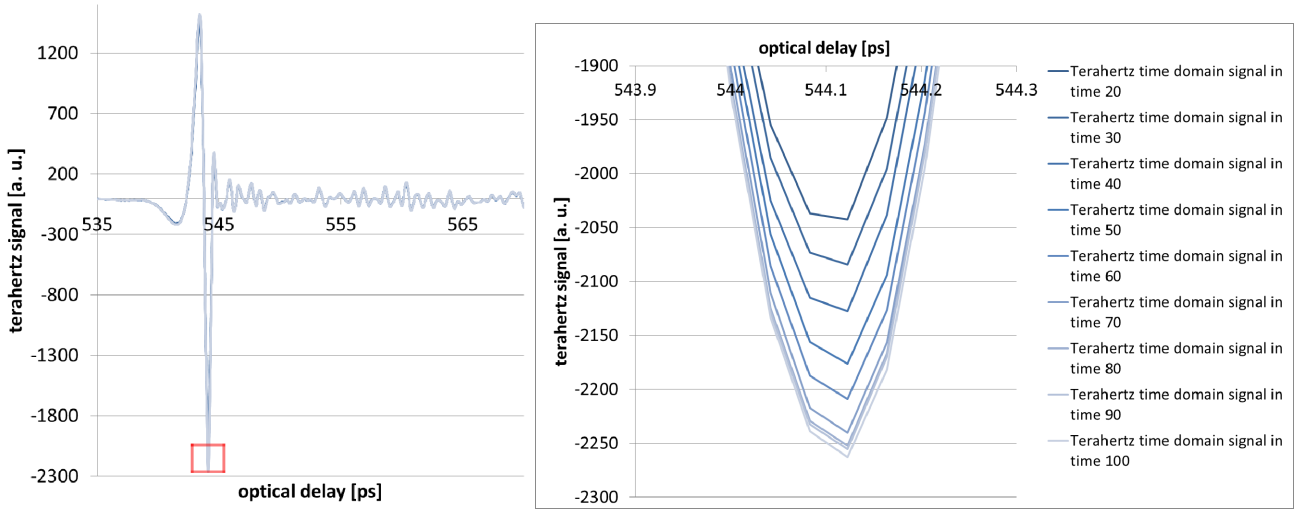

Figure 1. The terahertz time domain signal measured in time 20, 30, 40, …, and 100 seconds after the measurement starts (left). A marked window highlights an area of interest where the changes of minimal value of amplitude are analyzed. This area is extracted in the right.

Figure 2 shows the time course of the whole ATR measurement as the time dependence of minimal peak value of terahertz pulse as described before. Points A, B and C define the sequence of all executed acts and divide the graph practically into three sections; reference measurement of clean crystal (A-B), adhesive application on the crystal (B-C) and the reaction of the adhesive after the aluminum foil covering (C-end). Cyanoacrylate-based glue joins two faces only when the glue layer is thin enough; this is the reason of foil usage. After that, the spontaneous curing reaction occurs between these two faces. Naturally, this last part of the graph is the most important for other detailed analysis and the fast transients are neglected.

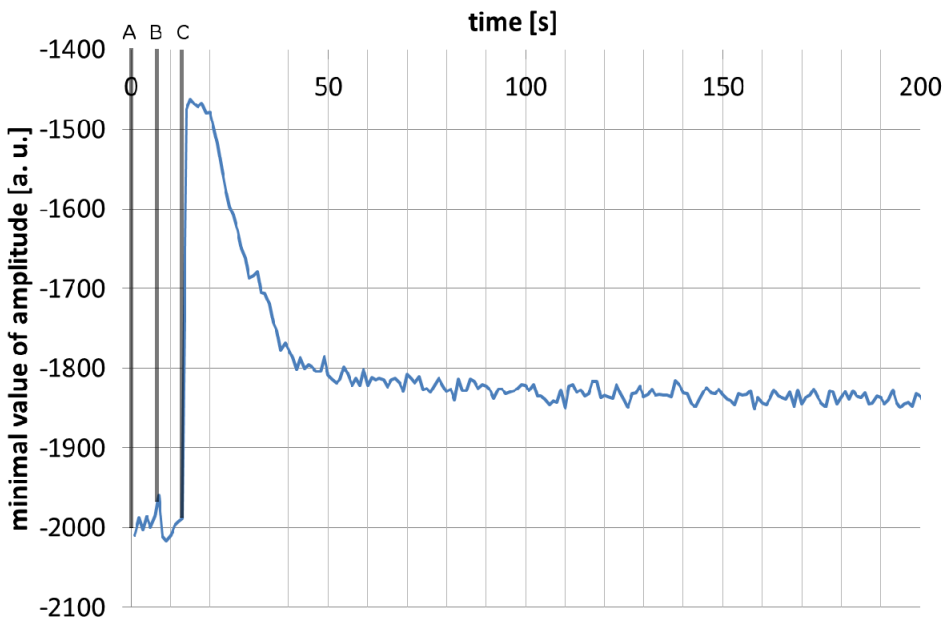

Figure 2. The graph of dependence of minimal value of amplitude on time represents the time course of the whole experiment. The measurement starts at point A (clean ATR crystal). The glue sample was applied on the crystal (point B) and covered by the aluminum foil (point C). A time lag was set between these acts.

As we are focused on the dynamic system description, the exact value of investigated parameter is not important at all as its change corresponding with the kinetics of curing. Therefore, we monitor the relative change of minimal value of amplitude during the curing reaction that starts after the foil covering. Consequently, only the part of the signals after the foil covering (starting at point C in Figure 2) were extracted from measured data and further processed. For better visualization and signal comparison, the measured signals *n(t)* were normalized by scaling between 0 and 1. The normalized value (*normalized n(t)*) for minimal value of amplitude *n* in the time *t* was calculated according to the Equation 1:

$$normalized\ n(t) = \frac{n(t) - n_{\min}}{n_{\max} - n_{\min}}, \tag{1}$$

where n_{min} (resp. n_{max}) is the minimum (resp. maximum) value of variable *n*.

As we can see in Figure 2, the parameter *n(t)* was changing which should correspond to the changes in the glue structure during the polymerization reaction. There is obvious exponential decreasing trend of the whole measured data set; consequently, the decreasing form of exponential decay was chosen for modelling the system. The mathematical approximation of *model n(t)* in time *t* is given by Equation 2:

$$model\ n(t) = norm.\ n_{\min} + (norm.\ n_{\max} - norm.\ n_{\min}) \times e^{-\frac{t}{\tau}}, \tag{2}$$

where *norm.* n_{min} (resp. *norm.* n_{max}) is minimum (resp. maximum) value of *normalized n(t)*, τ is the time constant of the system.

After the model is formed, the goal is to adjust the parameter τ of a model function to best fit the data set. This is solved by the generalized reduced gradient method. An optimization criterion for estimated parameters' selection is based on the least squares method. Our data set consists of *m* data pairs sampled in time *t* (*model* n_i, *norm.* n_i), i = 1 up to *m*, where *norm.* n_i is the independent variable and *model* n_i is a variable dependent on the input parameters. The optimum value is found when the sum of squared residuals is minimal. A residual is defined as a difference between the actual value of the *norm.* n_i and the value *model* n_i predicted by the model.

The results are shown in Figure 3, Figure 4, Figure 5, and Figure 6. All graphs compare the original data and its mathematical approximation for each investigated sample. The blue line is the representative curve of measured data. A red line marked with the suffix "_model" represents data of the calculated model as described before. The mixtures of Histoacryl with oily substance are entitled according to the percentage of the glue (e. g. mix75 consists of 75 vol% of Histoacryl and 25 vol% of oil).

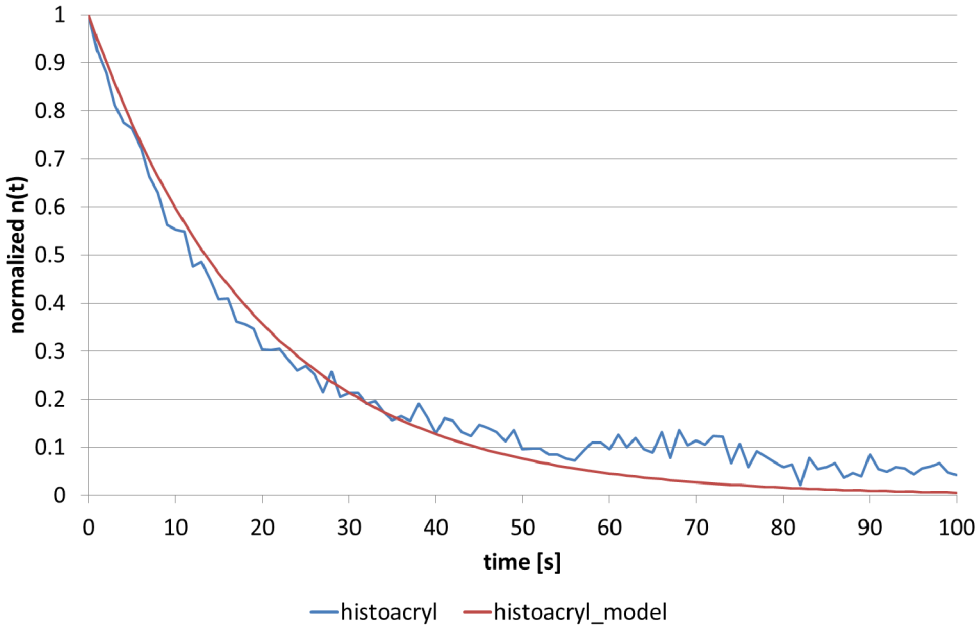

Figure 3. The comparison of original measured (blue) and model (red) data of pure Histoacryl sample

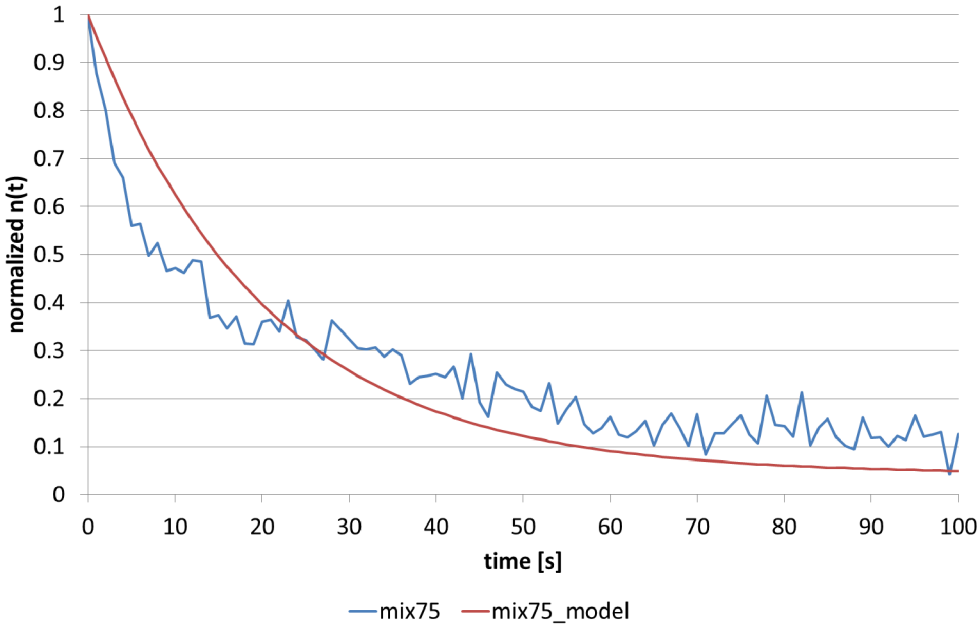

Figure 4. The comparison of original measured (blue) and model (red) data of mix75 sample (75 vol% of Histoacryl, 25 vol% of oil)

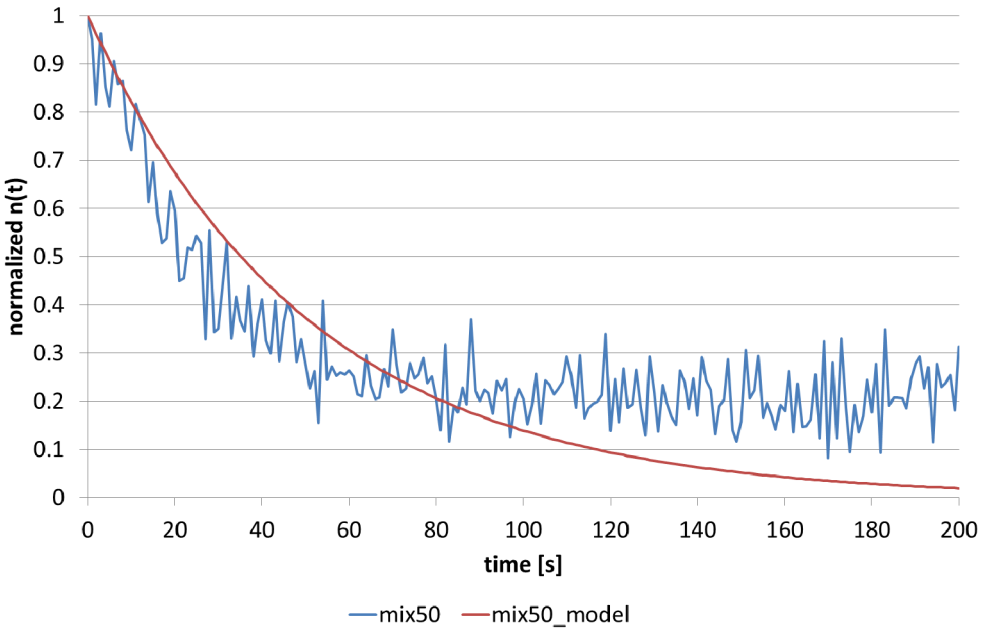

Figure 5. The comparison of original measured (blue) and model (red) data of mix50 sample (50 vol% of Histoacryl, 50 vol% of oil)

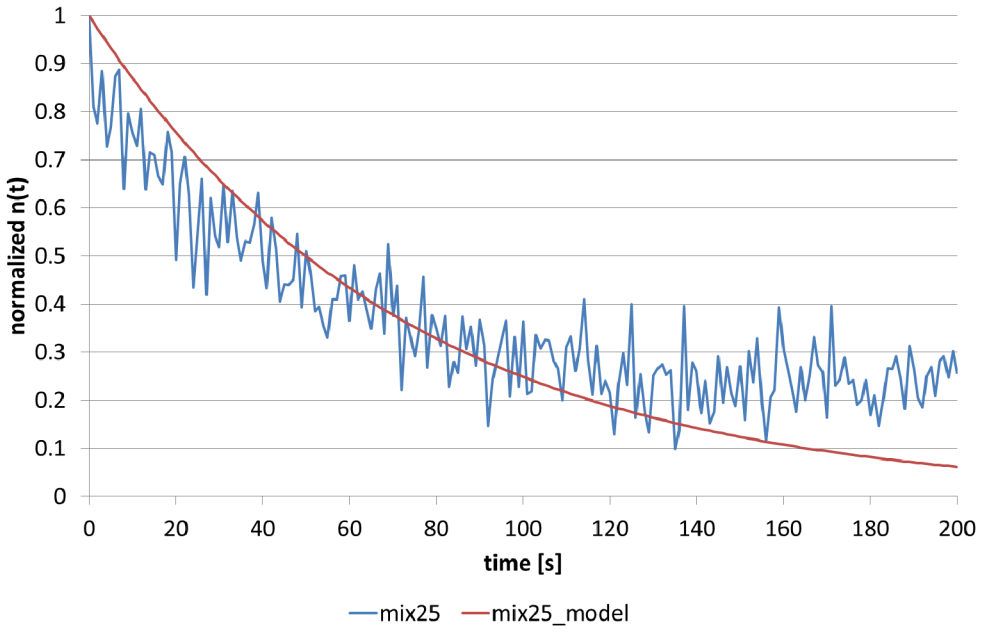

Figure 6. The comparison of original measured (blue) and model (red) data of mix25 sample (25 vol% of Histoacryl, 75 vol% of oil)

Figure 7 shows approximation curves for all investigated samples. As we can see, the curing process can be described by exponential decay function. The reaction speed varies depending on the content of the glue in the sample and can be good described by its time constant. The time constants for all samples are summarized in Table 1. All processes were approximated by the exponential decay for a simple first-order system, thus the time constant is defined as a time it takes

for the *normalized n(t)* to reach 0.37 of its maximal value [7]. The sample of pure glue (100 vol% of glue) and its mixture called mix75 (75 vol% of glue and 25 vol% of oil) show the fastest process. But we cannot suggest that the pure glue react faster than its mixture with the lowest content of oily substance, as it was expected. The curves are very similar and also time constants for both systems do not differ significantly: (20 ± 9) seconds for pure Histoacryl, (22 ± 6) seconds for mixture mix75. Considering the mixtures mix50 and mix25, where the oily content grows at the expense of glue, the time delay is obvious. The shapes of the curves also change; they lose their exponential character and gently approach to linear function. The time constants extend as expected; it is almost double for mix50, (51 ± 20) seconds, compared to pure glue and mix75, and it takes around (72 ± 10) seconds for mix25.

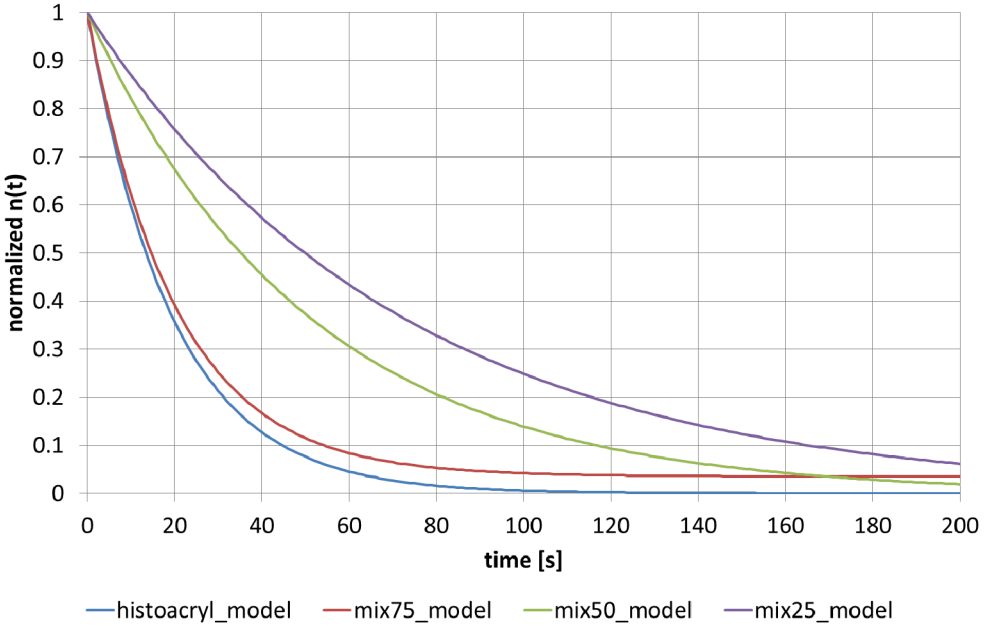

Figure 7. Approximation curves of measured data of pure Histoacryl and its mixtures with oil in various mixing ratio (mix75, mix50, mix25).

Table 1. Summary of the calculated time constants for all investigated samples.

Sample	Time constant [s]
Histoacryl (100 vol%)	20 ± 9
Mix75 (75 vol% of Histoacryl)	22 ± 6
Mix50 (50 vol% of Histoacryl)	51 ± 20
Mix25 (25 vol% of Histoacryl)	72 ± 10

There is evident that the time constant of curing grows with the increasing content of oil in the sample. The shape of the curve refers to changes in the glue structure and its solidification. When the glue in a liquid form is applied on the crystal, the signal attenuation is the strongest. After the foil covering, the signal attenuation is getting weaker; the glue changes its structure and cures. Based on the assumption that the material shows no dispersion, we can consider that the detected signal amplification is connected with the decreasing absorption of the sample during curing reaction. According to Figure 7, the curing process ends after approximately 60 - 80 seconds for pure glue and its mixture mix75. This time extends up to 150 seconds for mix50 and around 200 seconds for mix25.

4. CONCLUSION

Results of the presented study prove the capability of terahertz time domain spectroscopy to study the kinetics of fast processes that the tissue adhesive curing indisputably is. The description of the curing kinetics was derived from measuring the changes of minimal peak value of the terahertz signal amplitude of prepared glue samples. The mathematical approximation of the investigated system was designed based on the simple exponential function that could be further improved.

ACKNOWLEDGMENTS

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