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**DETECTION OF DENATONIUM BENZOATE (BITREX) REMNANTS  
IN NONCOMMERCIAL ALCOHOLIC BEVERAGES  
BY RAMAN SPECTROSCOPY**

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**ABSTRACT:** Illegal alcoholic beverages are often introduced into market by using cheap technical alcohol which is contaminated by denatonium benzoate (Bitrex) of very small concentration. Bitrex is the most bitter chemical compound and has to be removed before alcohol consumption. The home-made methods utilize sodium hypochlorite to disintegrate particles of denatonium benzoate in alcohol and to remove bitter taste before trading. In this experimental studies we propose a novel method which detects in a fast way remnants of denatonium benzoate in dubious alcohol samples by Raman spectroscopy. This method applies a portable Raman spectrometer of excitation wavelength 785 nm and utilizes effect of Surface Enhanced Raman Spectroscopy (SERS) to recognize the suspected alcoholic beverages. High effectiveness (over 98%) of YES/NO classification of the investigated samples was observed when the non-linear algorithm Support Vector Machine (SVM) was exploited at carefully adjusted detection parameters.

**KEYWORDS:** forensic science, Raman spectroscopy, denatonium benzoate, Bitrex, illicit alcohol, support vector machine algorithm

The excise on spirits is important source of state income tax. The income losses in Poland caused by unpaid taxes on alcoholic beverages are estimated over a few hundred millions USD each year. This amount can be reduced by introducing more popular and fast alcohol tests which wouldn't require bulky laboratory equipment or excessive waiting time. In this experimental study we propose a fast procedure of determining if the investigated samples of alcoholic beverages contain some denatonium benzoate (Bitrex) remnants. Such test points out alcoholic beverages which were composed of noncommercial alcohol.

Bitrex is an odorless compound forming a white crystalline powder and was introduced to the Guinness Book of World Records as the “bitterest substance known to man” (1, 2). Its chemical structure is shown in Figure 1. This extremely bitter taste and nauseating effects during consumption predestined denatonium benzoate to be used as a denaturing agent for many products worldwide. In more than 40 countries it is added to alcohol (3, 4), household products (1, 5), cosmetics and other personal care products (2, 6), garden products (1, 7), etc. to protect against its consumption on purpose or by accident. Under the Polish law denatonium benzoate can be used as a denaturant for ethanol, either alone or mixed with other compounds. The minimal permitted amount is laid down by the directive of the Ministry of Agriculture and Rural Development. In 2003 it was 0.3 g of Bitrex per 100 liters of 100% ethanol (8), but in 2008 this value was increased to 2 g per 100 liters (9).

One of the problems with denatured alcohol detection, however, is that it contains less than the recommended amount of Bitrex or contains its remnants only because technical alcohol was purified by addition of sodium hypochlorite to remove bitter taste. Although methods for the quantitative analysis of denatonium benzoate in alcohol products are known (3), the procedure for determining its degradation products is very rarely examined. Zuba et al. (10) investigated the effectiveness of removing denatonium benzoate from denatured spirit preparations with sodium hypochlorite using HPLC with DAD detector and mass spectrometry in both positive and negative ionization modes. They showed that the addition of 50  $\mu\text{L}$  sodium hypochlorite to one liter of spirit denatured by 3 mg/L denatonium benzoate reduced the number of denatonium ions, while 200  $\mu\text{L}$  of NaOCl per liter was enough to remove these ions altogether which were responsible for the bitter taste. On the basis of mass spectra, those authors gave an indication of the possible product of the reaction between Bitrex and sodium hypochlorite, but they did not determine the chemical structure of this compound (10). While this method is satisfactory for tracking the decrease in Bitrex concentration in alcohol, it is insufficient to reveal the presence of denatonium benzoate if sodium hypochlorite is used. Matyjasek (11) therefore applied GC-MS. A mixture of ethanol



denatured by Bitrex and sodium hypochlorite was first warmed to 50°C, after which it was extracted with chloroform; the extract was analyzed by GC-MS. Several compounds were identified (including *N,N*-dibenzylamine) on the basis of the mass spectra. Sodium chloride is one of the reduction products of sodium hypochlorite, but it is too common substance for use as an indicator (11).

The mentioned methods of Bitrex remnants detection require bulky laboratory machinery and highly qualified staff to perform detailed chemical analysis. These factors determine that such technology can't be popularized to increase volume of alcoholic beverages tested on state borders or by the selected teams during routine checkups. Therefore we propose another method which utilizes Raman spectra to produce reliable detection results. Raman spectroscopy is a very fast and reliable technique which can be done by a portable device and by a staff without excessive learning. Raman spectrum identifies vibrating modes, characteristic for chemical bonds of the compound irradiated by laser light and visible as intense lines in the Raman spectra. The selected classification algorithm can perform YES/NO detection which does not require adjusting of various parameters at measurement process, as in other applications of Raman spectroscopy (12). The method seems to be a promising way of confirming presence of Bitrex in alcoholic beverages, even if sodium hypochlorite has been used but some questions have to be addressed before its effective usage. We propose to collect Raman spectra when the investigated sample is excited by a laser of wavelength 785 nm. This wavelength can be generated by semiconductor laser diodes of low power consumption (below a few Watts) and can be built into a portable unit, together with CCD detector which registers Raman spectrum (13). Such systems are offered by a few independent companies and therefore the proposed method of illicit alcohol identification can be easily spread between numerous potential users. The Raman spectrometer can be characterized by spectra line resolution of app. 10 cm<sup>-1</sup> which is typical for portable Raman spectrometers and much worse when compared with laboratory equipment. Therefore we can assume that the detection results were representative for a class of portable Raman systems and should be better for laboratory spectrometers when the identified spectra lines should be narrower and should be less affected by background noise.

By means of Raman spectrum of crystalline powder Bitrex it is easy to identify vibration modes (FIG. 2) but Raman spectra of liquid samples of alcoholic beverages are dominated by spectra lines of ethanol because of very low concentration of Bitrex remnants. Therefore, we propose to modify a way of Raman spectra acquisition by applying surface enhanced Raman spectroscopy (SERS). This is a surface-sensitive technique that amplifies



Raman scattering by molecules adsorbed on rough metal surfaces. Amplification of the Raman scattered light can reach tens of thousands and therefore we can expect that even very small concentrations of Bitrex remnants can be identified. Additionally, this method warms the investigated liquid sample by a laser light and induces fast evaporation of ethanol which characteristic spectra lines vanish from the recorded spectra.

Another problem which have to be solved properly is a detection algorithm. We applied nonlinear detection algorithm called support vector machine (SVM). This is supervised learning model that analyze experimental data (Raman spectra) and recognize pattern used for classification into two separate groups.

## **MATERIALS AND METHODS**

Three main points describe this experimental study:

- investigated alcoholic beverages composition,
- Raman spectrometer and procedure of spectra recording,
- applied SVM algorithm and method of its parameters optimal adjustment.

These points are addressed below to explain details of the experimental study which characterize application of the proposed method to determine its efficiency when applied for the samples of practical meaning.

### *Preparation of the investigated alcoholic beverages*

The studies were done by purchasing selected commercial alcoholic beverages, available in Poland (“Sobieski” vodka, Gin, “Flic-Flac” sweet liqueur, Hennessy cognac, “Żubrówka” Bison grass vodka). Only some of these alcoholic beverages comprised of clear ethanol and water only. We decided to use not only transparent alcoholic beverages but other which compose of sugar or some additives to make them more tasty.

The separate batch of purchased alcoholic beverages was denatured with 20 mg/L of denatonium benzoate, after which 1.33 mL/L sodium hypochlorite was added. To remove any possible precipitate, all samples were centrifuged (10 min., 4000 rpm). Standards of denatonium benzoate (CAS: 3734-33-6) and sodium hypochlorite at a concentration of 10÷15% available chlorine (CAS: 7681-52-9) were purchased from Sigma-Aldrich and applied during the experiment.

### *Measurement setup*

The measurement system consisted of four main elements (FIG. 3). The first was the Multimode Spectrum Stabilized Laser Subsystem made by Ocean Optics. This laser emits

785 nm wavelength at controlled optical power up to 400 mW. The second element was Ocean Optics spectrometer (type QE6500), equipped with Peltier cell to cool CCD detector (up to  $-15^{\circ}\text{C}$  to reduce background noise) and resolution of 1024 pixels. The next element was a fiber-optic Raman probe made by InPhotonics company. This probe was designed to work with the 785 nm laser and works backward scattering configuration (called backscattering). The focal length (distance between the probe and the tested sample) of this probe is about a few mm and the distance was set between the probe's edge and the investigated sample. The drop of alcoholic beverage was placed on the Klarite® substrate made of golden rough structure to assure SERS effect (14). Recording of a single Raman spectrum was proceeded by a lag of 2 min. when the laser beam of 400 mW irradiated the investigated sample to vaporize ethanol. After that the Raman spectrum was recorded within 1 min. at the same optical power of the laser beam.

The fourth element of the measuring system was a PC computer. Its task was to acquire data but not perform any preliminary data processing to adapt the recorded Raman spectra to the SVM detection algorithm. We assumed that any data preprocessing (e.g., background removal, spectra smoothing (13)) could have influenced efficiency of the SVM algorithm in unpredictable way. The recorded Raman spectra were saved as ASCII files for further processing.

#### *Support Vector Machine Algorithm of illicit alcoholic beverages detection*

Chemical compounds at their low concentration can be identified by Raman spectroscopy when the right detection algorithm is selected. Various detection algorithms were developed to determine presence of the selected chemical compound or its concentration in the investigated sample. The input data are formed by abundant vectors (e.g., Raman spectra of independent 1024 points) which have to be reduced to YES/NO answer or to get a concentration of the estimated chemical compound which is a singular value. Usually the Raman spectral lines caused by investigated components have low intensity and can be masked by much more intense spectra lines induced by other dominating components. In recent years, the non-linear SVM classification algorithm has been developed and was found to be more efficient than the earlier proposed one (linear method of principal component analysis – PCA) (15, 16).

The SVM detection algorithm was applied to separate the data into two sets (categories) by a gap that was as wide as possible. One of the sets was Raman spectra of the samples which contained Bitrex remnants, while the second group was Raman spectra of pure



reference samples or clean Klarite® substrates which amplified recorded Raman scattered light by SERS effect. The SVM algorithm gave good results even when the non-linear dependence exists between concentration of the detected chemical compound and intensity of Raman spectra lines induced by this compound (FIG.4) (17). The detailed mathematics of the SVM detection algorithm was presented elsewhere (17–19) and therefore it has not been now enclosed.

Using the SVM algorithm requires selection of an appropriate kernel functions, applied in the classifier and its parameters. The SVM algorithm can use a kernel function which is linear, polynomial, hyperbolic tangent or gaussian function (radial basis function – RBF). Gaussian function was applied as the kernel function of the SVM detection algorithm because this function can be adjusted to the experimental data to establish model parameters and gave reliable results in numerous practical applications (19, 20).

## **DISCUSSION ON EXPERIMENT DETAILS AND DETECTION RESULTS**

In this experimental study we recorded numerous Raman spectra of various alcoholic samples from two distinct batches:

- the set of five different commercial alcoholic beverages,
- the set of the same types of five alcoholic beverages after performing the above described procedure of their denaturation by adding Bitrex and then sodium hypochlorite to remove bitter taste.

A number of 1040 independent Raman spectra was recorded for the mentioned batches together. Samples of five commercial alcoholic beverages were investigated to record 640 spectra. Each type of the alcoholic beverage and three clear Klarite® substrates were used to measure 80 Raman spectra for each sample type, gathering together the mentioned number of 640 spectra. The next 400 spectra were recorded by investigating the samples contaminated with Bitrex, which were subjected to purification by sodium hypochlorite. Each of five illicit alcoholic beverage type was used to record a set of 80 independent Raman spectra.

The number of 13 Klarite® substrates were used to measure 1040 spectra (5 for illicit alcoholic samples, 5 for commercial alcoholic samples and 3 for recording spectra of clean Klarite® substrates). Raman spectra measured for each type of the mentioned sample were repeated by irradiating randomly selected and different point of active substrate area of size 5x5 mm<sup>2</sup> (FIG. 3).

In the set of 400 spectra some of the spectral lines of Bitrex remnants were expected to be present at the same Raman shifts as the main spectral lines observed for crystalline powder



Bitrex (FIG. 2, FIG.5) even if their intensity was strongly reduced. Intensity of these fragmentary spectra lines should be sufficient to detect (by applying the SVM algorithm) which alcoholic beverages were made of illicit ethanol.

#### *Raman spectra of crystalline powder Bitrex and its remnants*

Raman spectrum of crystalline powder Bitrex (FIG. 2) exhibits the strong bands at  $992.5\text{ cm}^{-1}$  and  $1590.4\text{ cm}^{-1}$  due to aromatic ring vibrations, and there are also bands at  $2918\text{ cm}^{-1}$  and  $3015.3\text{ cm}^{-1}$  corresponding to aliphatic groups ( $\text{CH}_2$ ,  $\text{CH}_3$ ). Strong Raman peaks at the position  $992.5\text{ cm}^{-1}$  and  $1590.4\text{ cm}^{-1}$  were observed also in Raman spectrum of Bitrex and ethanol solution, which was recorded applying Klarite® substrates (FIG. 5). Zuba et al. demonstrated that, after the addition of sodium hypochlorite in an amount sufficient to remove denatonium ions, benzoate ions were still present in the solution. But the concentration of sodium hypochlorite required to remove the benzoate anions was so high that it made the alcohol unsuitable for consumption because of its high pH (10). GC-MS studies of Bitrex oxidation products indicated the presence of several compounds containing aromatic rings (11). It was observed that both present in the Bitrex Raman spectrum bands that are the consequence of the aromatic ring vibrations and aromatic C=C stretching were still present indicating contamination in the Raman spectrum of the sample obtained after the addition of sodium hypochlorite ( $994.9\text{ cm}^{-1}$ ,  $1591.6\text{ cm}^{-1}$ ). The positions of these bands seemed to be the most characteristic ones for indicating the presence of Bitrex and its degradation products after oxidation with sodium hypochlorite. When we considered some exemplary Raman spectra of noncommercial alcoholic beverage recorded by applying the Klarite® SERS substrate we could claim a presence of tiny bands in vicinity of Raman shifts  $1000\text{ cm}^{-1}$  and  $1600\text{ cm}^{-1}$  (FIG. 6) which corresponded the above enumerated values and induced by vibrations of aromatic C=C stretching. In order to better visualization of mentioned spectral lines the authors propose simple mathematical operation. It consists in subtraction the spectrum of clean SERS substance from purifying noncommercial alcohol (FIG. 7). Unfortunately, due to etaloning effect present in the applied CCD detector and optical characteristic of clean Klarite® SERS substrate the recorded Raman spectra displayed numerous bands of similar intensity as at two earlier mentioned values of Raman shifts. Therefore, some other popular detection algorithms (e.g., correlation algorithm, peak search algorithm (20)) failed completely to detect the illicit alcoholic beverages.

#### *Optimization of SVM detection algorithm*





At the learning stage of SVM algorithm, when the experimental data (Raman spectra) of two sample sets was investigated, the RBF required selection of the kernel parameter  $\sigma^2$  (sig2). At the same stage a regularization parameter  $\gamma$  (gam) had to be selected as well. Both parameters influence robustness of the model and have to be selected carefully to get high rate of illicit alcoholic beverages detection. As in other reported applications of SVM algorithm we selected values of  $\sigma^2$  and  $\gamma$  by repeating necessary computations for various pairs of  $\sigma^2$  and  $\gamma$  belonging to the ranges:  $0.1 < \sigma^2 < 14$  and  $0.1 < \gamma < 20$  (both parameters were modified by the step of 0.1) which assured identification of detection rate maximum for the applied experimental data – the set of 1040 spectra used to learn the SVM detection algorithm. The necessary computations were performed in Matlab software, by applying LS-SVMlab toolbox where all functions for SVM-based detection are available (18).

Each time, after selecting  $\sigma^2$  (sig2) and  $\gamma$  (gam), the detection model of SVM algorithm was created to check classification quality within the set of 260 Raman spectra which were recorded independently from the previously mentioned set of 1040 spectra used at the learning stage. The Raman spectra considered for detection efficiency estimation, comprised of two subsets: 160 spectra recorded for six commercial alcoholic beverages (20 independent spectra of each studied alcoholic beverage) and 100 Raman spectra of the samples contaminated by Bitrex remnants (20 spectra recorded for each type of contaminated alcoholic beverage). The results of the experiment are presented as percentage of the correctly detected samples (Fig. 8). The detection efficiency averaged within a set of all beverage sample types was over 98% when the adjusted parameters belonged to the plane limited by the approximated sets of values  $\sigma^2 \in \{2.5 \ 4.5\}$  and  $\gamma \in \{1.5 \ 3\}$ . Within the set of the investigated five different beverages the samples of "Gin" exhibited slightly lower detection efficiency (app. 95%). Thus, we can conclude that the proposed method of illicit alcohol detection is highly efficient. Additionally, the established wide set of  $\sigma^2$  and  $\gamma$  values when detection rate exceeded 98% suggest that high efficiency would be observed when other learning Raman spectra are utilized.

## CONCLUSIONS

In summary, Raman spectroscopy permits fast and simple detection of alcoholic beverages which were polluted by some Bitrex remnants. The applied SVM detection algorithm assure at carefully adjusted parameters  $\sigma^2$  (sig2) and  $\gamma$  (gam) high detection rate (over 98% within a selected and wide range of  $\sigma^2$  and  $\gamma$  values) of illicit alcoholic beverages.

The proposed method can be performed by a portable Raman spectrometer and requires a few minutes only to run the complete analysis and get the final result without any alcoholic sample preparation.

The present method requires application of the Klarite® SERS substrates what makes the method more expensive and less user-friendly. It can be supposed that intense ethanol evaporation from the investigated sample (induced by sample heating or by flow of dry nitrogen during decompression) would increase concentration of the Bitrex remnants to secure correct detection. This problem is currently under in-depth study and will be reported later.

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## CAPTIONS TO FIGURES

FIG. 1. The chemical structure of Bitrex = denatonium benzoate = THS-839 = *N,N*-diethyl-*N*-[(2,6-dimethylphenylcarbamoyl)methyl]benzylammonium benzoate = benzyldiethyl(2,6-xylylcarbamoylmethyl)ammonium benzoate.

FIG. 2. Raman spectrum of crystalline powder denatonium benzoate (Bitrex).

FIG. 3. Measurement setup applied for Raman spectra recording of samples of the investigated alcoholic beverages; the integrated Raman probe (top) was applied and the Klarite® substrate (bottom) to observe SERS effect with visible alcoholic beverages remnants after ethanol evaporation on golden plate.

FIG. 4. Illustration of support vector machine (SVM) classification rules for experimental data which belong to two classes: group A and group B.

FIG. 5. The spectrum of Bitrex solution in ethanol at different concentrations (exposure time was 120 s and the laser power was 350 mW).

FIG. 6. The averaged spectra of 100 recorded Raman spectra of: SERS substrate (dotted red line), “Sobieski” vodka (blue line) and contaminated “Sobieski” vodka after Bitrex removal by sodium hypochlorite (black line with black arrows); the spectra are shifted to their better visibility.

FIG.7. The averaged spectra of 100 recorded Raman spectra of: “Sobieski” vodka (dotted line) and contaminated “Sobieski” vodka after Bitrex removal by sodium hypochlorite (solid line); the Raman spectrum of clear SERS substrate was subtracted to remove influence of the applied substrate; additionally, the spectra were shifted to their better visibility

FIG. 8. Detection efficiency of alcoholic beverages with Bitrex remnants by Raman spectra versus parameters of detection algorithm: a regularization parameter  $\gamma$  (gam) and kernel parameter  $\sigma^2$  (sig2) of RBF function.

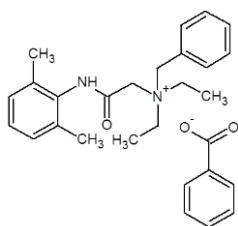


Fig. 1

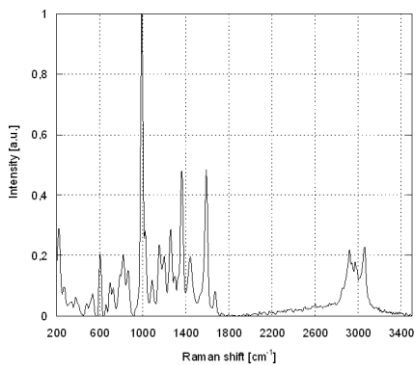


Fig. 2

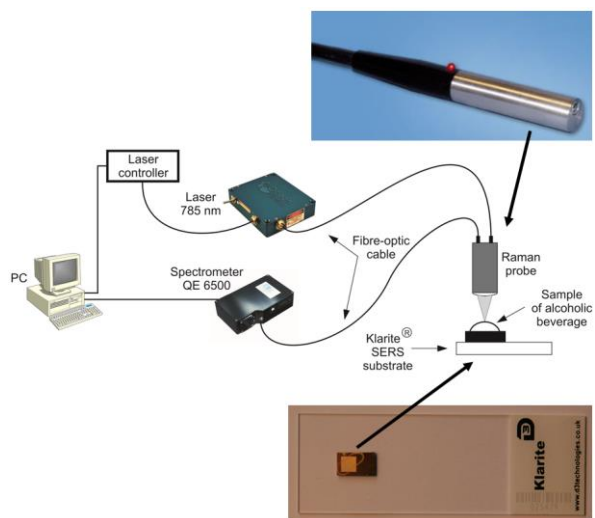


Fig. 3

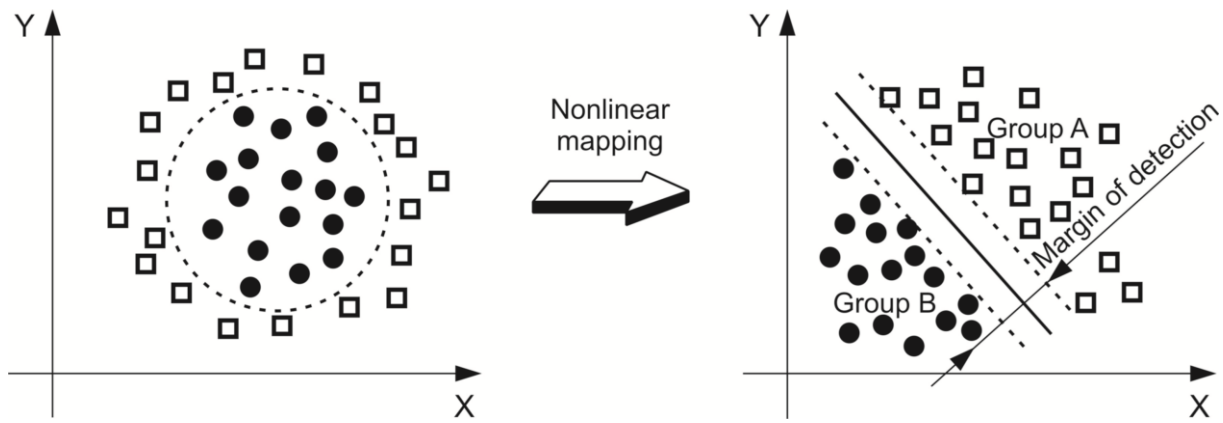


Fig. 4

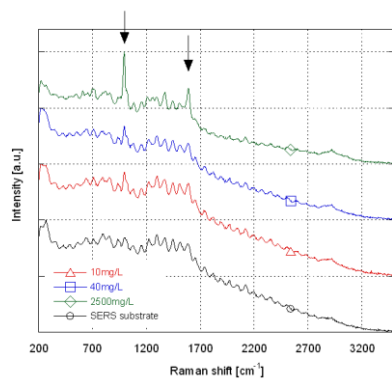


Fig. 5

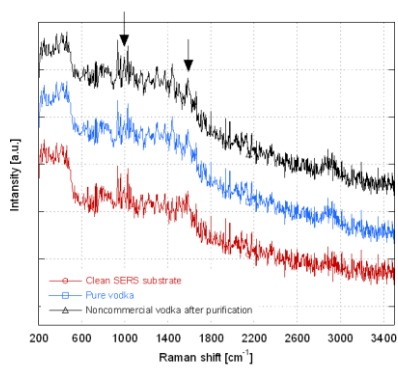


Fig. 6

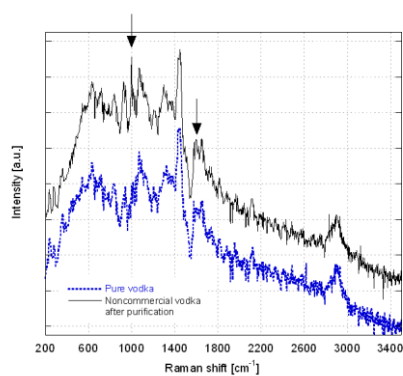


Fig. 7

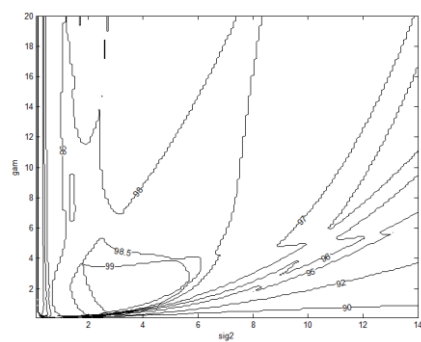


Fig. 8