



Application of Nano-sized Poly (N-methyl pyrrole-pyrrole) Fiber to the Headspace Solid-Phase Microextraction of Volatile Organic Compounds from Yogurt

Abdolkarim Saidfar, Mohammad Alizadeh and Sajad Pirsas*

Department of Food Science and Technology Faculty of Agriculture, Urmia University, P. O. Box 57561-51818, Urmia, Iran

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ABSTRACT

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A new poly (N-methyl pyrrole-co-pyrrole) (P-NMPy-co-Py) coated flexible polyester fiber was provided by chemically-deposition of P-NMPy-co-Py on the surface of polyester fiber in the mixed electrolytes of sodium dodecyl sulphonate (SDS) and FeCl₃ (as an oxidant). The Scanning Electron Microscopy (SEM) and Fourier Transform Infrared spectroscopy (FTIR) were used for characterization of morphology, size, porosity and composition of synthesized copolymer. The nanosized and spherical P-NMPy-co-Py particles are observed according to the SEM results. The P-NMPy-co-Py fiber was employed to extraction of volatile organic compounds (VOCs) in yogurt samples as an extractor. An experimental design was utilized to optimize operational parameters that affect the analysis of VOCs in yogurt samples using headspace solid phase microextraction (HS-SPME) in the pre-concentration step. Some parameters including, sample pH, temperature, ionic strength (NaCl percent W/W %) were optimized. Gas chromatography-flame ionization detection (GC-FID) was used for separation, detection and quantitation of VOCs. Results show that P-NMPy-co-Py modified polyester fiber is provided fast and easily by chemical method and is suitable for the successful extraction of the VOCs from yogurt samples.

1. Introduction

In recent years conducting organic polymers, copolymers and their composites have been studied intensively as electroactive material, filter and membrane [1-3]. Conducting polymer films and membranes have received considerable attention for generating modified fibers and electrodes with analytical utility, like as an absorbant [4-6]. These polymers can be used also as polymeric batteries, membranes, light emitting diodes, sensing devices such as muscle like actuators, or as transistor circuits. Polypyrrole, polyaniline and other conducting polymers can be functionalized by different dopants or other organic polymers to provide special films, membrane and electrodes to enhance determination, filtration or absorption ability of these polymers [7-9]. The functionalization of conducting polymer films can be achieved by electrostatic or chemical incorporation of anionic complexing ligands during the chemical or electrochemical polymerization step [5-11]. Among conducting polymers polypyrrole and its copolymers

and derivatives like poly N-methyl pyrrole, pyrrole-N-methyl pyrrole copolymer and so have more attention in research studies [5-11]. Poly (N-methyl pyrrole) and pyrrole-N-methyl pyrrole copolymer could be obtained on mild steel or other fibers successfully from acid medium in the presence of oxidant agent. N-methyl pyrrole has some advantage in contrast to pyrrole, methyl group in N-methyl pyrrole which can create the hydrophobic effect of pyrrole ring. On the other hand, this group could also increase the adhesive strength of organic compounds to the polymer surface [9-11]. Copolymerization is also very useful for tailoring diverse properties of coatings. The obtained polymer will have different properties depending on the ratio of monomer concentrations [8-11].

Solid-phase microextraction (SPME) has been successfully used as a universal tool for isolation and preconcentration of pollutants, volatile and other organic compounds from water, solid and air samples [12 and 13].

In the SPME analytes is partitioned between the sample matrix and the extraction medium (polymeric

* Corresponding author. Tel.: +989145454914; e-mail: Pirsas7@gmail.com; s.pirsas@urmia.ac.ir

fibre) subsequently. The characteristics of the sorbent and the type of sorbed organic compounds affect adsorption efficiency [13-15]. In the SPME technique by a polymer film analytes are extracted from aqueous or gaseous samples. Then, the fibre is inserted directly into the injector of a gas chromatography system and the extracted analytes are thermally desorbed and analyzed. SPME is employed for the preconcentration and extraction of volatile compounds at trace levels in a variety of matrices [15-19]. In the many researches the analysis of VOCs using solid phase micro extraction coupled with headspace is used [12-16]. For example Pirsá and Alizadeh [20] on VOC determination in textiles showed that toluene, xylene and styrene can be qualitatively analyzed by headspace combined with SPME [13-16]. Statistical design of experiments (DOE) is superior to the traditional change-one-at-a-time approach, when different factors need to be optimized. If the factors in the design are correlated, that is if the change in response to a change in a factor level depends on the level of another factor, then it is unlikely that the optimum will be discovered and more experiments than necessary will have been done [12-20].

This paper focuses on the chemical polymerization of P-NMPy-co-Py on the polyester fibers surface in the mixed solution of sodium dodecyl sulphate (SDS) with FeCl₃ and its application as a SMPE fiber to extraction of VOCs. Finally, use of the statistical design of experiments (DOE) for the optimization of used method for the extraction of volatile organic compounds from yogurt samples, using headspace-solid phase microextraction and gas chromatography-Flame Ionization detector (GC/FID).

2. Material and methods

2.1. Reagents and Chemicals

Sodium dodecyl sulphate (SDS) (99%) as a dopant, ferric chloride (FeCl₃) as oxidant and N-methyl pyrrole (95%) (as copolymer with pyrrole) were used from Aldrich. Pyrrole (99%) was provided from Fluka, Switzerland and stored in a refrigerator in the dark after distillation.

The yogurt samples and commercial polyester fibers were purchased from market Urmia, Iran. All other organic compounds to examination were purchased from Merck.

2.2. Apparatus

Chemical polymerization method was used to coat P-Py-NMPy copolymer (P-Py-co-NMPy) on the surface of polyester fibers at room temperature and atmosphere condition very fast and simply.

A SPME syringe with fiber holder was used to manually sampling. This SPME syringe was fabricated in Urmia University (Iran) by Dr. Sajad Pirsá and Dr. Mohammad Alizadeh research group [20]. An Agilent 7890

(Wilmington, DE, USA) GC instrument was used for separation, detection and analysis of volatile organic compounds in yogurt samples. The scanning electron microscopy (SEM) from Philips XL30 (Holland) was used for analysis of morphology and size of coated copolymers. FT-IR spectrum of the P-Py-co-NMPy was recorded at 4 cm⁻¹ resolution with a Nicolet 100 Fourier transform infrared (FT-IR) spectrometer.

2.3. Preparation of polyester fiber modified by P-NMPy-co-Py

The chemical polymerization at room temperature in a water solution containing of NMPy, Py, and FeCl₃ as an oxidant and SDS as dopant was used for polymerization of P-NMPy-co-Py-DS on the surface of polyester fibers. The concentrations of 0.1 mol L⁻¹ NMPy, 0.1 mol L⁻¹ Py, 0.2 mol L⁻¹ FeCl₃ and 0.2 mol L⁻¹ SDS were used to polymerization. Processing of polymerization was done as following: 10 mL distilled water containing SDS and FeCl₃ was prepared and 50 cm of polyester fiber submerged to it, then another 10 mL distilled water containing NMPy and Py gradually added to above solution and reaction for 2 h was done under stirring atmospheric condition. After coating of P-NMPy-co-Py-DS on the surface of polyester fiber, it was taken from reaction vessel and washed with deionized water for 30 min at 70 °C in oven.

2.4. Chromatographic Conditions

The operating condition of gas chromatography (Agilent7890 A, Wilmington, DE, USA) with flame ionization detector (GC-FID) is reported below: fused silica capillary column (HP-5), 30 m length, 0.25 μm phase thickness and 320 μm i.d was used to separation of VOCs. Experimental optimum conditions were obtained as follows: N₂ as carrier gas with a flow rate of 2 mL/min, injection port of GC in the splitless mode was 50 mL/min splitting ratio in 0.75 min. The initial temperature of the column was 50°C and then the temperature was increased to 80°C at 2°C/min and kept in 80 °C for 5 min. The detector temperature was 250 °C. Heater temperature 200 °C, H₂ flow 27 mL/min, air flow 20 ml/min and make up gas flow rate 20 mL/min were used.

2.5. Headspace Extraction Procedure

The yogurt sample (5 mL) was extracted with P-NMPy-co-Py fibre using headspace solid phase microextraction (HS-SPME). Yogurt sample (5 mL) was transferred to a 10 mL glass vial with a polytetrafluoroethylene silicon septum. Then a magnetic stir bar was added, the vial was tightly sealed with an aluminum cap to prevent sample loss due to evaporation. During the extraction process, the vials were heated by using a hot plate accommodated in a glass beaker contained some water. Thus, the

samples were heated indirectly, with temperature controlled by a thermometer. The yogurt sample fiber was exposed to the headspace over the stirring liquid sample for 10 min. After the sampling step, the fiber was withdrawn into the needle and removed from the vial. The fiber was then immediately inserted into the

injection port of the GC. To use SPME to measure spiked analytes, the compound was extracted with a laboratory-made P-NMPy-co-Py fibre with an absorbent from the headspace of the samples and measured by GC. The chromatographic separation is shown in Fig. 1.

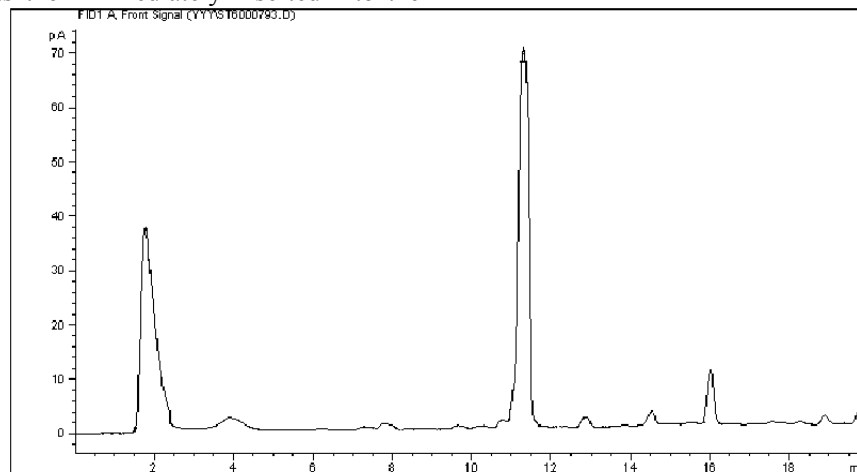


Fig. 1. GC-FID chromatograms for VOCs by HS-SPME from yogurt sample

2.6. Experimental design and statistical analysis

The independent variables were extraction pH, temperature, extraction time and NaCl concentration. The actual values of the factors are given in Table 1. Twenty eight yoghurt samples were evaluated according to a Box–Behnken design with the four variables and three levels for each variable. Data were fitted to second-order polynomial equation (1) for each dependent Y variable, through a stepwise multiple regression analysis using Design-Expert version 7 (Stat-Ease, Inc., Minneapolis, USA) statistical software.

$$Y = \beta_0 + \sum_{i=1}^n \beta_i x_i + \sum_{i=1}^n \sum_{j=i+1}^n \beta_{ij} x_i x_j + \sum_{i=1}^n \beta_{ii} x_i^2 \quad (1)$$

Where: Y = predicted response, β_0 = a constant, β_i = linear coefficient, β_{ii} = squared coefficient and β_{ij} = interaction coefficient.

Table 1. Box-Behnken design for optimization of HS-SPME of yoghurt volatile compounds

un	Factor 1 A:pH	Factor 2 B:Te (°C)	Factor 3 C:Time (min)	Factor 4 D: NaCl (%)
1	7	60	25	10
2	5.5	60	45	10
3	5.5	30	5	5.63
4	7	60	45	5.63
5	5.5	90	45	5.63
6	4	30	25	5.63
7	5.5	60	25	5.63
8	5.5	60	25	5.63
9	7	90	25	5.63
10	5.5	60	25	5.63
11	5.5	30	25	10

12	4	60	45	5.63
13	5.5	90	25	10
14	5.5	60	25	5.63
15	4	60	25	1.25
16	4	60	5	5.63
17	5.5	60	5	10
18	7	60	25	1.25
19	5.5	90	5	5.63
20	7	30	25	5.63
21	4	60	25	10
22	5.5	90	25	1.25
23	5.5	30	25	1.25
24	5.5	60	45	1.25
25	5.5	60	5	1.25
26	7	60	5	5.63
27	4	90	25	5.63
28	5.5	30	45	5.63

2. 7. Optimization of SPME

Response surface methodology was performed to optimize the extraction condition. To identify the overall optimal processing condition, the models obtained for different responses (peak number, total height of peaks and total areas of peaks) were subjected to the numerical optimization. Simultaneous consideration of multiple responses involves first building an appropriate response surface model for each response and then trying to find a set of operating conditions that optimizes all responses or at least keeps them in desired ranges [22]. For this purpose, the use of desirability functions is one of the useful approaches to the optimization of multiple responses. In this method, the general approach is to first convert each response Y_i into an individual desirability function d_i that varies between 0 and 1, where if response Y_i is at its target value, then $d_i = 1$, and if it is outside an acceptable region, $d_i = 0$. Then the

design variables were chosen to maximize the overall desirability as:

$$D = \sqrt[m]{d_1 \times d_2 \times \dots \times d_m} \quad (2)$$

Where: m is the number of responses.

3. Results and discussion

3.1. Morphology

Polyester fiber coated by P-NMPy-co-Py is analyzed by scanning electron microscopy and the morphology and size of P-NMPy-co-Py is reported in Fig. 2. SEM figures show P-NMPy-co-Py doped DS-powder (A) and P-NMPy-co-Py coated on the surface of polyester fiber (B). The morphology of P-NMPy-co-Py doped DS-coated on the polyester fiber is relatively spherical shape (~60–180 nm).

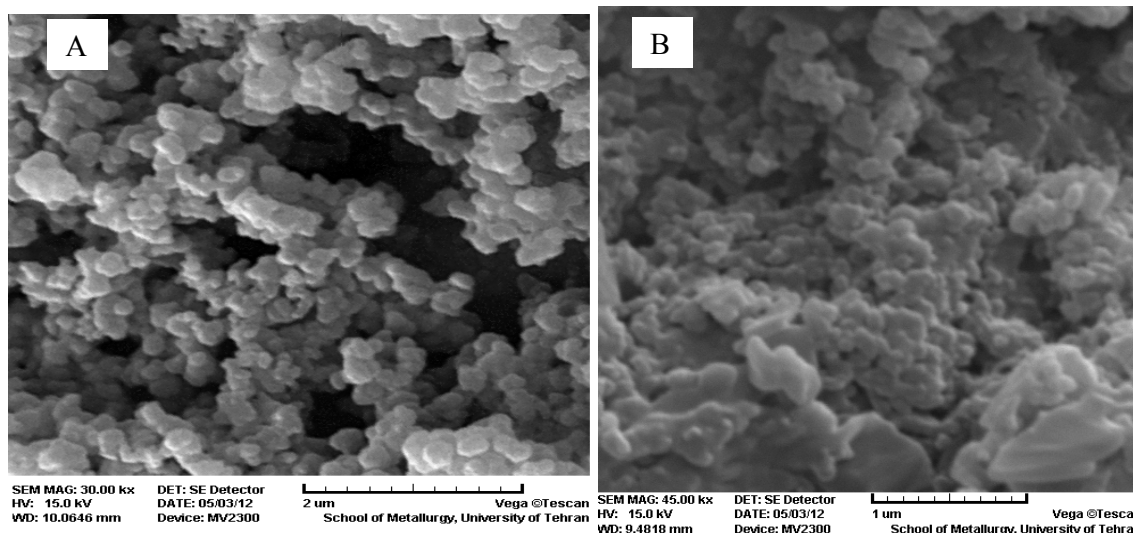


Fig. 2. SEM images of the *P-NMPy-co-Py* powder doped DS^- (A) and *P-NMPy-co-Py* coated on the surface of polyester fiber (B)

3.2. FT-IR spectra

P-NMPy-co-Py was analyzed by FT-IR spectroscopy and formation of copolymer and different bands in copolymer structure was confirmed.

In the *P-NMPy-co-Py* FT-IR spectrum C–H stretching bands is shown at: 3124 cm^{-1} ; asymmetric and symmetric methyl C–H stretching band at 2954 and

2923 cm^{-1} ; carbonyl groups stretch at 1697 cm^{-1} ; intra ring C=C and inter ring C–C vibrations at 1565, 1542 cm^{-1} ; C–N vibrations and C–H deformations bands at 1450, 1381 and 1326 cm^{-1} ; and C–H deformations and CH_3 rocking bands at 763, and 717 cm^{-1} are observed [10, 11 and 21].

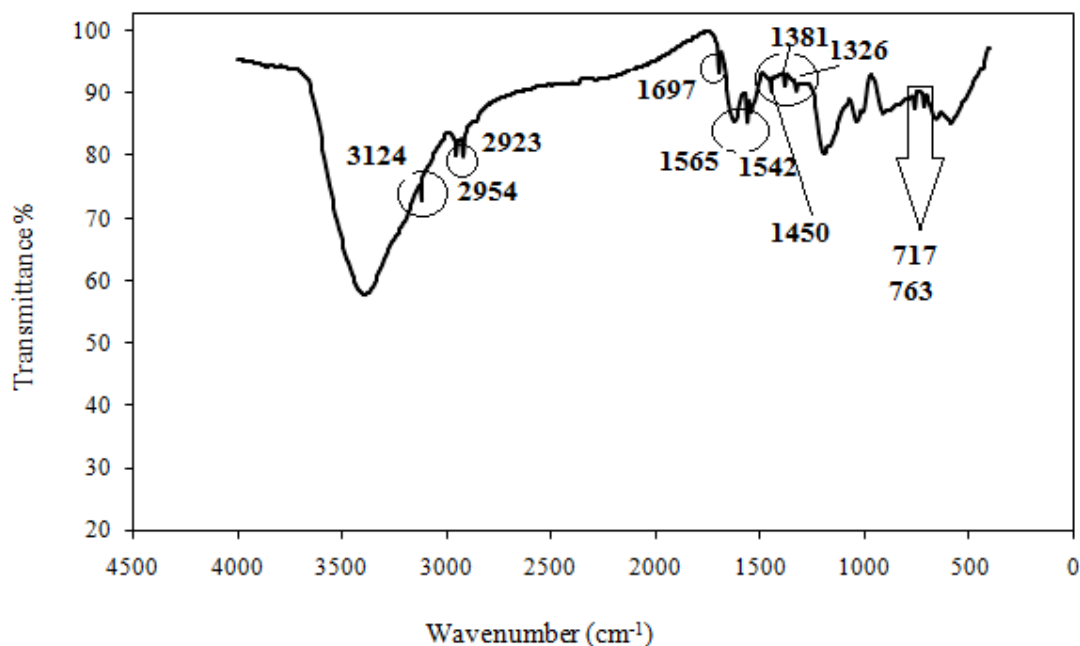


Fig. 3. FT-IR spectra of *P-NMPy-co-Py* doped DS^-

3.3 Total peak numbers

Data analysis and using of regression approach, the

following model was used to estimate total peak numbers:

$$\text{Peak number} = 189.33 - 7.75A + 8B - 0.83D - 3.25AB - 7.78A^2 + 3.1D^2 \quad (3)$$

Where: A =pH, B=temperature and D=NaCl concentration.

The Adj-R2 of the above equation was equal to 0.92 which indicates good predictivity of the model. Among the studied variables, pH and extraction temperature had the maximum effect on peak numbers and the quadratic effect of pH was also significant ($p < 0.01$). Extraction time was not significant factor in peak numbers ($p > 0.05$). As shown in Fig.4, peak numbers increased by decreasing pH from 7 to 4.8 and then increased. This figure also shows the positive effect of extraction temperature on peak numbers.

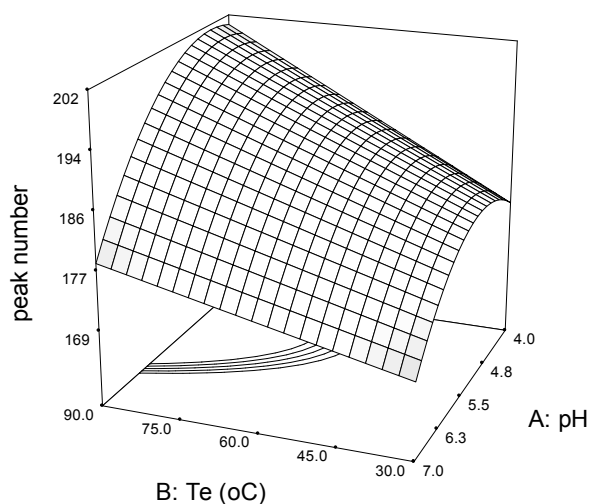


Fig. 4. Response surface plot of peak numbers; extraction temperature vs. pH, NaCl concentration and extraction time kept in central levels.

3.4 Total peak heights

The regression analysis showed that the linear effect of extraction time and NaCl concentration was not significant ($p > 0.05$) but interactive effect of these two factors on total peak heights dominate over the other variables. The quadratic effect of the pH on the total peak heights was significant ($p < 0.01$). Reaction temperature had positive influence on total peak heights. Total peak heights, as shown in Fig. 5, increased by increasing extraction time at high level of salt concentration and decreased at low level of this factor.

The following model was used to relate studied independent factors to total peak heights:

$$\text{Peak height} = 97.53 - 2.53A + 25.41B + 21.29C - 6.89D + 37.67BC + 66.08CD - 41.89A^2 \quad (4)$$

Where: A =pH, B=temperature, C=extraction time and D=NaCl concentration.

The R2 of the above equation was equal to 0.73 which indicates acceptable predictivity of the model.

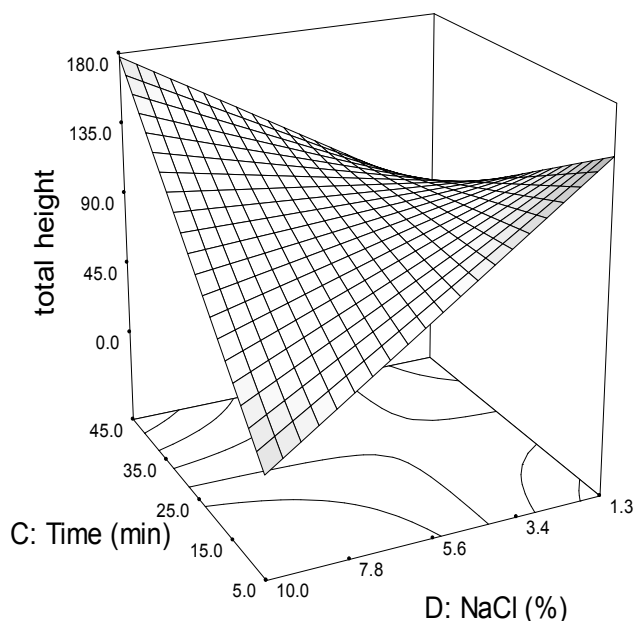


Fig. 5. Response surface plot of peaks total height; extraction time vs. NaCl concentration, pH and temperature kept in central levels.

3.3. Optimization

Optimum condition for the extraction of yoghurt volatile compounds was determined to obtain maximum peak numbers and total peak heights. Second-order polynomial models obtained in this study were utilized for each response in order to determine the specified optimum conditions. By applying the method of desirability function, optimum extraction condition was determined as follows: 4.4 for extraction pH, 90 °C extraction temperature, 10% for salt concentration and 44.7 min for extraction time. Fig. 6 shows the overly plot for optimization (maximization) of peak numbers and total peak heights. Extraction time and the salt concentration are varied during construction of the overly plot while keeping the amount of pH and the extraction temperature at their optimum (4.4 and 90 °C, respectively) in order to obtain peak numbers more than 190 and total peak heights more than 150. The zone of optimization as shown in the overly plot depicts the extraction time to be in the range of 33-45 min, and NaCl concentration between 4.64 and 10%.

4. Conclusion

A copolymer of pyrrole and N-methyl Pyrrole is synthesized by chemical polymerization on the polyester surface fibers by soft chemical polymerization at

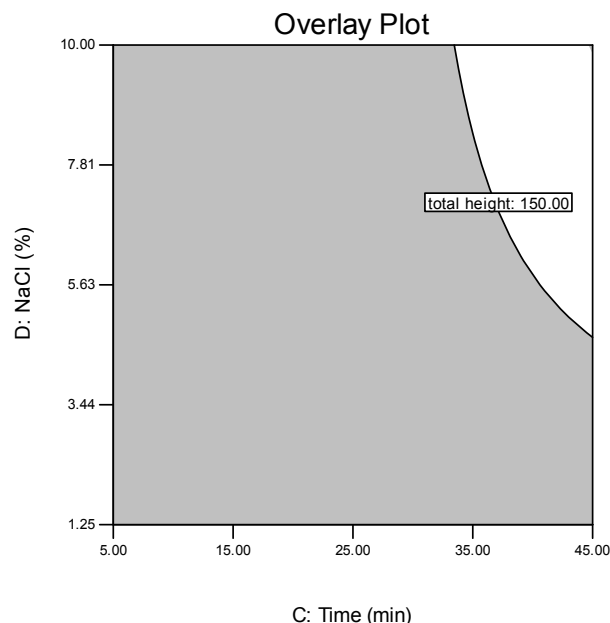


Fig. 6. Optimum region (white zone) identified by overlaying plots of the two responses (Peak number and total height) as functions of extraction time and salt concentration.

atmospheric condition in the mixed solution of pyrrole and N-methyl Pyrrole monomers and in the presence of sodium dodecyl sulphonate (SDS) as a dopant and FeCl_3 as an oxidant. Surface coating is done simple and fast. The size, morphology and copolymer formation of P-NMPy-co-Py are analyzed by Electron Microscopy (SEM) and Fourier Transform Infrared spectroscopy (FTIR) techniques. The P-NMPy-co-Py modified polyester fiber is used a HS-SMPE fiber to extraction of VOCs from yogurt samples and analysis by GC-FID. Statistical design of experiments (DOE) was successfully used for the optimization of a method for analysis of VOCs in yogurt samples, using headspace-solid phase microextraction and GC-FID. According to results, P-NMPy-co-Py modified polyester fiber coupled HS-SPME-GC-FID and design of experiments method can easily use to extraction, optimization and analysis of VOCs in yogurt samples.

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