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Optimization of uptake and analysis of mycotoxin by irradiated functionalized branched polymer using response surface methodology (RSM).

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ABSTRACT

An acrylamide polymer beads were assembled by gamma irradiation followed by interacting with ethylene diamine and cyanuric chloride to enrich the polymer by functional groups. This branched polymer was used as solid-phase extractor (SPE) to uptake mycotoxins in food. The interacting effects of various uptake parameters, such as pH, concentration ofcitrinin, methanol and NaCl(ionic strength) in sample solutions, as well as the mass of the polymer on the uptake efficiency were studied and optimized by response surface methodology method with central composite design. The effect of acetonitrile concentration on citrinindesorption was also studied. The uptake of citrinin was detected by UV Spectrophotometric analysisat 331 nm with acetonitrile - phosphoric acid (0.25 N)- isopropanol (55/35/10) as a solvent. The results showed that the optimum conditions of uptake were recorded at pH 6, 0.048 mMol/L citrinin, 3.0% methanol and 2.5% NaCL, whilethe maximum desorption was at 100% acetonitrile. This work proved the ability of this irradiated branched polymer to uptake citrininmycotoxin and could be applied for purification of infected foods in farther studies.

Keywords: gammairradiation, citrinin,response surface methodology (RSM), citrininUVSpectrophotometric analysis.

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INTRODUCTION

Mycotoxins was detected in food chain as a result of fungal infection. Due to their resistance to decomposition, they remain in meat and dairy products [1]. The studies show that the temperature treatments such as cooking and freezing cannot destroy some mycotoxins. [2]. Citrinin"CIT"(4, 6-dihydro-8-hydroxyl-3, 4, 5-trimethyl-6-oxo-3H-2-benzopyran-7-carboxylic acid) **Figure 1** one of the major groups of mycotoxins, is very toxic, carcinogenic metaboliteandmutagenic.CIT was first isolated from Penicilliumcitrinum and it is a causative agent in human hepatic and extra hepatic carcinogenesis [3],[4].The contaminations of CIT was found in many food such as cheese,fermented maize,wheat, barley, corn, apples, brewed beer and red yeast rice, have been reported [5, 6]. In addition, the ingestion of CIT is harmful effects to animals. It causes serious health problems such as kidney and liver diseases, carcinogenicity and nervous system damage [7].

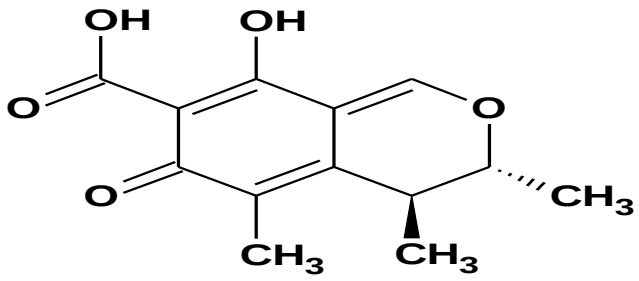

Figure 1: **Citrinin (4, 6-dihydro-8-hydroxyl-3, 4, 5-trimethyl-6-oxo-3H-2-benzopyran-7-carboxylic acid)**

Weiping Wong (2014) stated that is no standard method for extraction and analysis of citrinin in red fermented rice [8] the commonly used method Were high performance liquid chromatography (HPLC) [9], liquid chromatography-mass spectrometry (LC–MS) [10] thin-layer chromatography (TLC) [11] enzyme immunoassays (EIA) [12, 13] and micellar electro kinetic capillary chromatography (MEKC) [14] have been reported for determination of CIT. However these methods need very expensive equipment and include time consuming extraction steps to eliminate contaminants. These problems can easily be solved by: UV technique [15].

Polyacrylamide (PAA) is one of common used synthetic polymers as "water soluble polymers"[16]. The major advantage is that it can be polymerized either chemically or by using radiation. Advantages of gamma ray polymerization against chemical polymerization is that the polymerization can be carried out even under frozen conditions thus allowing the matrix to be molded to any form such as beads or membranes[17, 18].
The word optimize (from the Latin word Optimus = best) is to make the best use of resources in order to make something, such as a method or process, as effective, perfect, or functional as possible. The term optimization is often used in chemistry to provide a deep understanding and an ability to explore ranges of experimental and reactions variables [19].

Response surface methodology is a process and product optimization using designed experiments. The more number of variables has in a given system, the more complicated becomes the job of optimization. But regardless of the number of variables, there will be a relationship between a given response and the independent variables. Once this relationship is known for a given response, it defines a response surface. It is this surface that must be evaluated to find the values of the independent variables, which give the most desirable "optimal" level of the response [20].

The aim of this work is the use chemically modified polyacrylamide prepared by gamma irradiation to qualitative and quantitative determination of citrinin using a rapid and cheap method (UV spectroscopy) and using the response surface methodology method central composite design to optimize the extraction process.

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EXPERIMENTAL

Chemicals and Reagents

Acrylamide (AAm) (GC 99% purity) was purchased from BDH(pool,UK),Acetone, Ethanol (gradient HPLC),monylsulphate 99 % (surfactant) from alfaaesar china, Methanol (gradient HPLC), Isopropanol (gradient HPLC), Phosphoric acid (99%) and Ethyl acetate (99%), from Merck, Germany, Ethylene Diamine from Lobachemie, India, Cyanuric chloride (2,4,6-trichloro-1,3,5-triazine) and Citrinin 98% powder (8-Hydroxy-3,4,5-trimethyl-6-oxo-4,6-dihydro-3H-isochromene-7-carboxylic acid) were purchased from Alfa Aesar Chemical Co. (Alfa Aesar is a part of Thermo Fisher Scientific) Karlsruhe, Germany and Acetonitrile (gradient HPLC) from scharlab, Spain.

Radiation polymerization

Acrylamide monomer was dissolved in distilled water containing 5% surfactant and this solution were placed in a glass test tube and irradiated in Co^{60} gamma cell Canadian type with dose rate of 2.18 kGy/h installed in national center for radiation research and technology (NCRRT), Atomic energy authority (AEAE), Egypt

Chemical Treatment of polyacrylamide

A specific function group that was used to increase interaction with citrinin was introduced to the polymer beads by chemical treatment of the polymerized function group with boiling "100 C^{o}" ethylenediamine as method described by Sharma N[21].

Preparation of branched polymer

We have chosen to use a Cyanuric chloride"trivalent triazine" as a basic molecule. This small molecule has been previously utilized as basic molecule for synthesizing dendrimer structures and conjugates folic acid and methotrexate with defined ratio [22]. Details of the synthesis procedure are given below:

Dissolve 5.4 g(0.03Mol)Cyanuric chloride in 50 mL of acetone in a 250 mL flask, and irradiated polymerized functionalized polymer in acetone "50 mL" was added drop wise with stirring. After the addition was finished, the reaction mixture was continuously stirred at room temperature for 24 h. Then the precipitated product was collected by filtration and washed with acetone, ethanol and distilled water. Finally, it was dried at room temperature overnight, yielding the branched polymer.

FTIR spectroscopic analysis

IR analysis was carried out using an FT-IR 8400 spectrometer (Schimadzu, Japan) over the range 400-4000 cm^{-1} at a resolution of 4 cm^{-1}. High signal to noise spectra were obtained by the collection of 100 scans for each sample.

Instrumentation

The analytical system was performed at room temperature on a UV/VIS spectrophotometer (Jasco V-630, Japan)

Solid-phase extractor(uptake) Procedure

The standard solution of citrinin was prepared by appropriate dilution of stock solutions of citrinin with buffer solution (pH - 6) according to the preparation of Britton-Robinson buffer. Solid-phase extractor"SPE" cartridges were prepared by packing with 100.0 mg of branched polymerinto special glass syringe had 0.5 µm sieving plate the polymer was conditioned with 2 ml methanol/ water mixture (50%/50%) prior to sample loading. Then 5.0 mL of above prepared citrinin standard solution (pH 6) with 2.5% NaCl (w/v) was passed through the SPE cartridges. After uptake, the extracted citrinin in 2.75 mL acetonitrile were collected effluent,

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then add 1.75 mL of 0.25 N H_3PO_4 and 0.5 mL of isopropanol. 4 mL of this filtered solution was measured by UV/VIS spectrophotometer against blank.

Experimental Design

A central composite design of four factors each at five levels was then constructed in order to generate the response surface map for the effects of the experimental variables on the uptake. The matrix of the design including the experimental factors and their coded levels is shown in **Table 1**, while the actual values of each level are shown in **Table 2**.The design consists of a central core of randomized two-level fractional factorial design "denoted as +1 and –1", five central points "denoted 0" and 8 axial points "denoted + α and – α, where α =1.68". Design Expert® software "State Ease Inc., USA" was used for the generation and statistical evaluation of the design.

Preparation

Citrinin standard solution was prepared by appropriate dilution of stock solutions of citrinin (200µg/ml).the polymer mass was fixed at 100.00 mg and loading sample solution volume was fixed at 5 ml. The values of the other experimental factors were varied according to the central composite design "CCD" as shown in **Table 1**. After prediction of the optimum conditions, the citrinin were prepared under these conditions as a check point batch to validate the optimization process.

RESULTS AND DISCUSSION

Spectrophotometric analysis

Researchers had utilized different solvents and techniques for extraction and analysis of citrinin from RFR (red fermented rice). Although ultrasonic and shaking extraction techniques were mostly used in reported articles [23, 24], these methods differed significantly in their extraction solvents and procedures. Ethanol / water, acetonitrile / potassium chloride / water were used repeatedly in extraction and analysis of citrinin. However the UV absorbance of citrinin in these solvents were low. In this method we used two solvent systems A and B where solvent A is: methanol - phosphoric acid0.25 N - ethyl acetate (55/35/10) and Solvent B is acetonitrile- phosphoric acid0.25 N - isopropanol (55/35/10) for amplification of citrinin absorbance at wavelength of 331 nm as shown in **Figure 2**.The detection wavelength of 331 nm was chosen for citrinin in solvent A system [15]The relationship between peak heights and concentration of citrinin was used to construct calibration curve at wavelength 331 nm as shown in **Figure 3.**

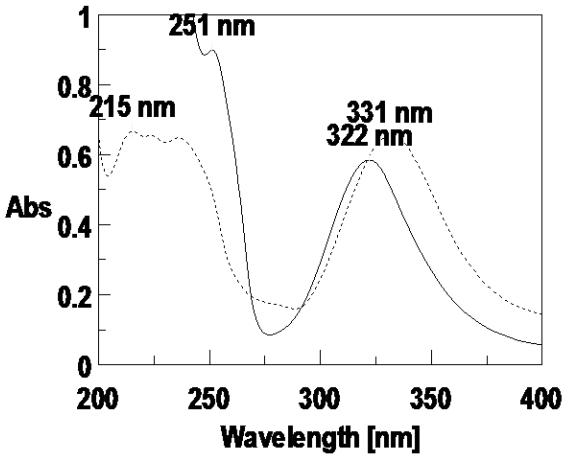

Figure 2: **UV absorbance spectra of citrinin (solvent A) (——) & (solvent B) (------)**

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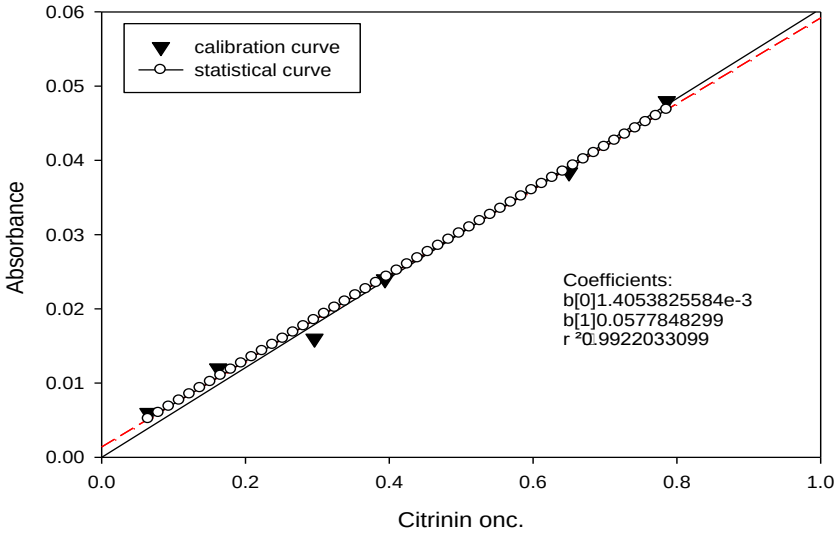

Figure 3: standard curve for citrinin / acetonitrile -0.25 N phosphoric acid - isopropanol (55/35/10) at wave length 331

A statistical test was adopted to compare the y-axis intercept to zero[25] that is to discover if zero concentration could correspond to a reading of zero. The test is two-sided t- test with (n - 2) degrees of freedom of the following form:

$$t = \frac{|a - 0|}{\sqrt{S_a^2}}$$

Where (a) is the intercept and (S_a^2) is the variance estimate of the intercept.

The calculated t-values were (0.0165). Since the calculated t-values are less than their corresponding tabulated value (2.571) at the 0.05 level, so the intercept was not significantly different from zero. This would be a reasonable assumption if the absorption versus concentration relationship was a straight line from zero to the highest concentration tested. Therefore, spectrophotometric assay within the concentration range of (0.006-0.048 mMol/L) could be taken as a valid method to assess citrinin in the mentioned solution.

Chemical modified PAAm

FTIR spectroscopy was used to describe the chemical and physical changes that may occur in polymeric materials upon gamma irradiation and copolymerization of branched polymer and PAAm **Figure 4(A)**, showed The IR spectrum of acrylamide we see the absorption bands at 3012 cm^{-1} (broad band), 2916 cm^{-1} (broad band),2872cm biforked and 1701 cm^{-1}. These bands were characterized the (OH, NH_2) group stretching (O=C-NH_2HO-C=NH), (=CH) and (–CH_2), and (C=O, C=C) stretching. **Figure 4(B)**, showed The IR spectrum of PAAm treated with ethylenediamine showed the peaks at 3307 cm^{-1} bi-forked, 3230 cm^{-1} (broad band), 2918 cm^{-1}, 2848 cm^{-1}(broad band), 1645cm^{-1} and 1568 cm^{-1} . These peaks were attributed to NH_2, (OH, NH) group stretching (O=C-N HO-C=N), alkyle stretching, NH group and C=O stretching, respectively **Figure 4(C)** showed the IR spectrum of PAAm treated with ethylenediamine then treated by Cyanuric chloride (2,4,6-trichloro-1,3,5-triazine), The figure showed the peaks at 3211 cm^{-1}, 3419 cm^{-1} (broad band), 1600 cm^{1}, 1463 cm^{1} 2918 cm1,2711 cm^{1} these peaks were attributed to (OH, NH) group stretching (O=C-N HO-C=N), C=Oof amide stretching, C=N of triazine and CH respectively **Figure 4(D)**showed the IR spectrum of PAAm treated with ethylenediamine then treated Cyanuric chloride (2,4,6-trichloro-1,3,5-triazine) and uptake of citrinin(4,6-dihydro-8-hydroxyl-3,4,5-trimethyl-6-oxo-3H-2-benzopyran-7-carboxylic acid) The figure showed the peaks at 2375 cm^{-1}, 3625 cm^{-1} (broad band), 1550 cm^{1}, 1750 cm^{1} (broad band), these peaks were attributed to (OH) carboxylic and phenolic group and (C=O) carboxylic and ketonic group

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Optimization of operating conditions

To achieve optimal uptake %, various parameters were investigated and discussed.

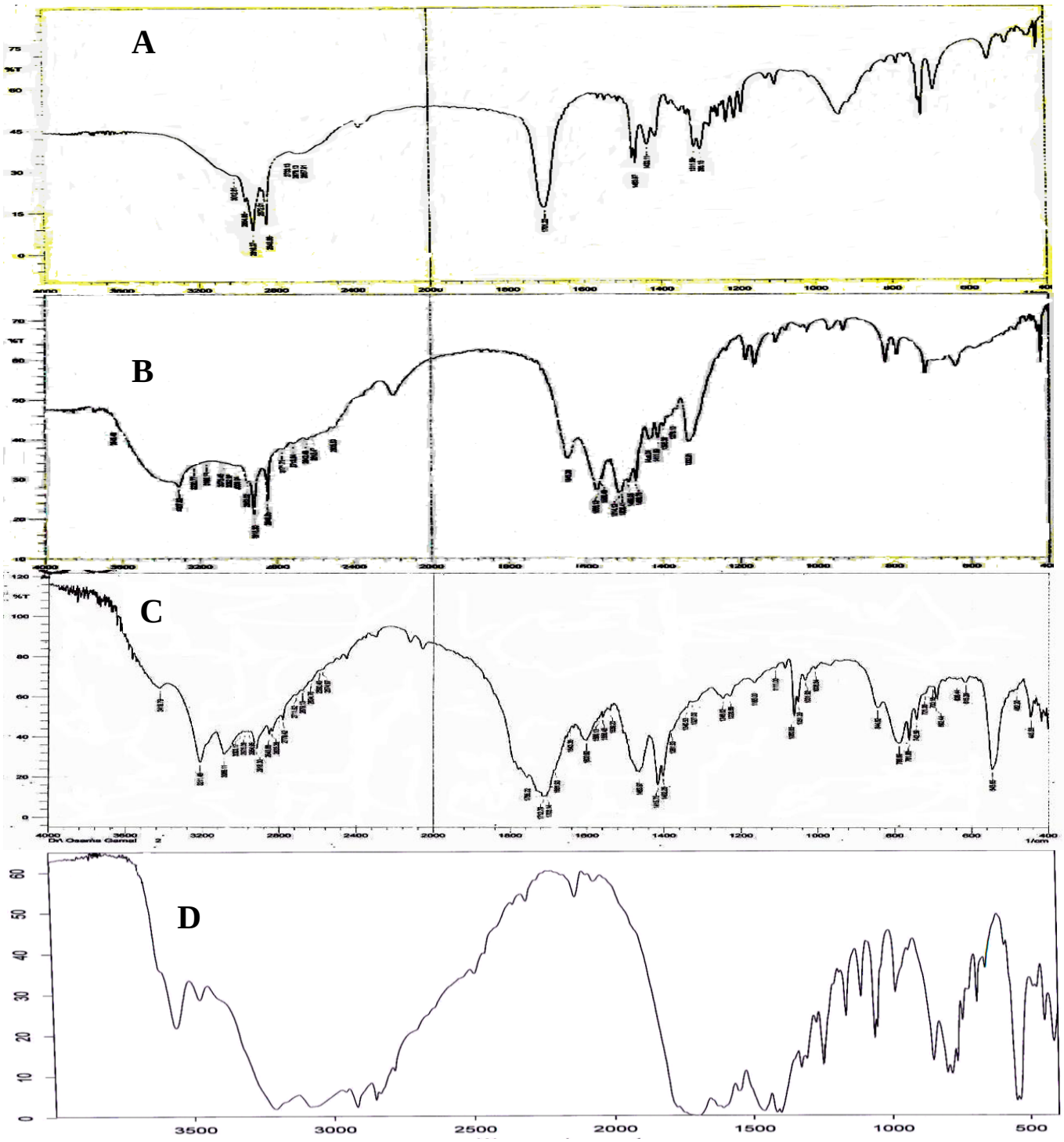

Figure 4: **The IR spectrum (A) irradiated polymerized acrylamide, (B) PAAm treated with ethylenediamine, (C) PAAm treated with ethylenediamine then treated by Cyanuric chloride, (D) branched polymer after uptake of citrini**

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Effect of polymer mass

The amounts of polymer that was used ranged from 25.0 to 200.0 mg "25, 50, 100 and 200 mg". It was found that the uptake % was 98 ± 2.00%, **Figure 5** when 50.0 mg of polymer was used as the Solid-phase extractor "SPE" and there was no difference when 100 and 200 mg of polymer were used. This indicated that 100.0 mg of polymer was sufficient for the extraction of these investigated citrinin. The uptake was performed with 100.0 mg.

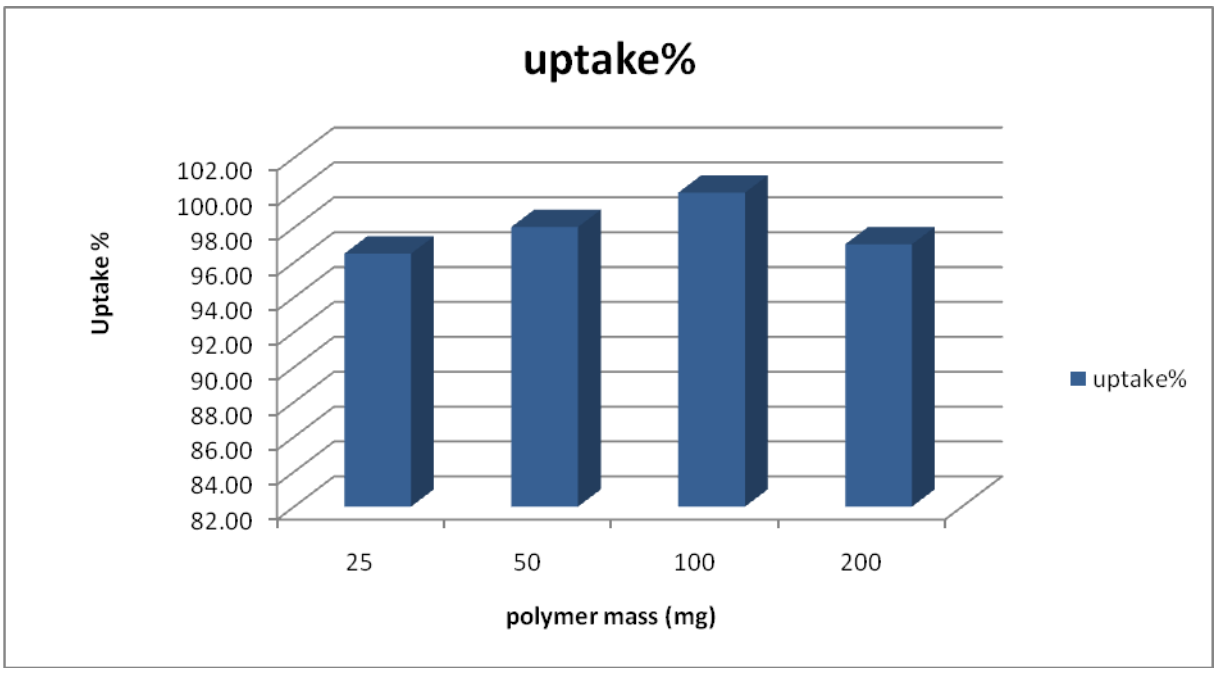

Figure 5: **Effect of polymer mass on uptake %**

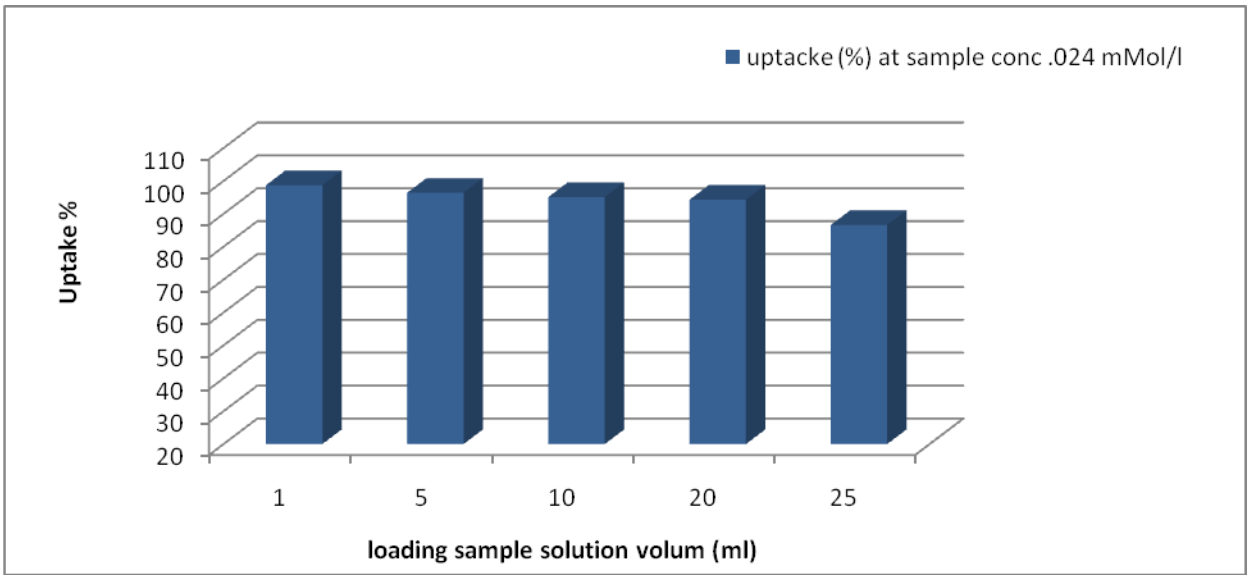

Figure 6: Effect of loading sample solution volume on uptake %

Effect of loading sample solution volume

Citrinin are always at trace levels in the matrices, so that in this study, 1.0, 5.0, 10.0, 20.0 and 25.0 mL of sample were investigated(all samples have 200µgcitrinin). The results showed that the uptake % decrease as the sample volume increased **Figure 6**. This meant that the uptake % would increase as the citrinin

concentrations increase. As a result, for a certain sample, a small sample loading volume "with high citrinin concentrations" was helpful for the uptake. In this experiment, a sample volume of 5.0 mL was selected.

Analysis of the Experimental Design:

For the central composite design "CCD", a total of 21 experimental runs were proposed by Design-Expert® software 'Stat-Ease Inc., USA" including four independent factors each at five levels. According to this experimental proposal, various citrinin solutions were prepared. The effects of independent variables "factors" upon the measured dependent variables "uptake" were investigated as optimization response parameters in this study. Overview of the experimental plan is presented in **Table 1&Table 2.**Design-Expert® software generated cubic polynomial models for precise evaluation of the effects of the independent variables on the uptake %. Lack of fit and R^2were analyzed to evaluate the model fitting **Table 3**. The values of $R^2$and F-values for lack of fit revealed that the model generated was fitting the experimental data accurately and hence suitable for describing the effects of the independent variables on the uptake.

Table 1: Coded levels of experimental factors for the central composite design

Factor				
Run	Factor(A)	Factor(B)	Factor (c)	Factor(D)
	pH	NaCl conc.	Sample conc.	Methanol
		%	mMol/L	%
R-1	1	1	-1	-1
R-2	-1	1	1	1
R-3	0	0	0	0
R-4	-1	-1	1	-1
R-5	0	0	-1.68	0
R-6	1	-1	-1	1
R-7	1.68	0	0	0
R-8	-1	1	-1	1
R-9	0	0	0	1.68
R-10	1	1	1	-1
R-11	0	0	0	0
R-12	1	-1	1	1
R-13	-1	-1	-1	-1
R-14	0	0	0	0
R-15	0	0	0	0
R-16	0	-1.68	0	0
R-17	0	0	0	0
R-18	0	0	0	-1.68
R-19	0	1.68	0	0
R-20	0	0	1.68	0
R-21	-1.68	0	0	0

Table 2: Actual values for the coded levels of experimental factors for the central composite design

level	Factor(A) pH	Factor (B) NaCl conc.	Factor (c) Sample conc.	Factor(D) Methanol
		%	mMol/L	%
1.68	6.68	2.93	0.056	36.8
1	6	2.25	0.048	30
0	5	1.88	0.036	20

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-1	4	1.25	0.024	10
-1.68	3.32	0.8	0.0158	3.18

Table 3: R-squared values of the fitted regression model for uptake %

Std. Dev.	**0.92**	**R-Squared**	**0.99**
Mean	**80.26**	**Adj. R-Squared**	**0.99**
C.V. %	**1.14**	**Lack of fit F-value**	**0.19 NS***

*NS: Non-significant (critical F-value = 6.59 at p < 0.05).

The generated model was:

$$y=\beta_O+\beta_A.A+\beta_B.B+\beta_C.C+\beta_D.D+\beta_{AB}.A.B+\beta_{AC}.A.C+\beta_{AD}.A.D+\beta_{BD}.B.D+\beta_{AA}.A^2+\beta_{BB}.B^2+\beta_{CC}.C^2+\beta_{ABD}.A.B.D. + \beta_{AAC}.A^2.C$$

Where: y is the response, $\beta o$is the intercept, βA, βB, βC, $\beta D$are the regression coefficients for the individual effects of the independent factors A, B, C, and D respectively, βAB, βAC, βAD, are the regression coefficients for the interaction effects, βAA, βBB, βCC, are the regression coefficients for the quadratic effects,and β_{ABD},β_{AAC} are the regression coefficients for the cubic effects, that were used to simulate the curvature in the design space.

One-way ANOVA was applied for estimate the significance ($p < 0.05$) of the model and individual response parameters. The ANOVA results revealed that the model was significant for the response.
The 3-D response surface plots and the corresponding 2-D contour plots were analyzed to investigate the effects of independent factors on the measured response

The uptake %

The uptake % of irradiated functionalized branched polymer for citrininwhich prepared according to the CCD ranged from **45.8**% in R-21 to **92.1**% in R-20 **Figure 7 &Table4**. The model F-value of **517.14**implied thatthe model was statistically significant ($p < 0.0001$). The 'R-Squared' of 0.99 was in reasonable agreement with the 'Adjusted R-Squared of 0.99 the linear correlation between the predicted and the observed responses indicated **excellent fitting of the model.**

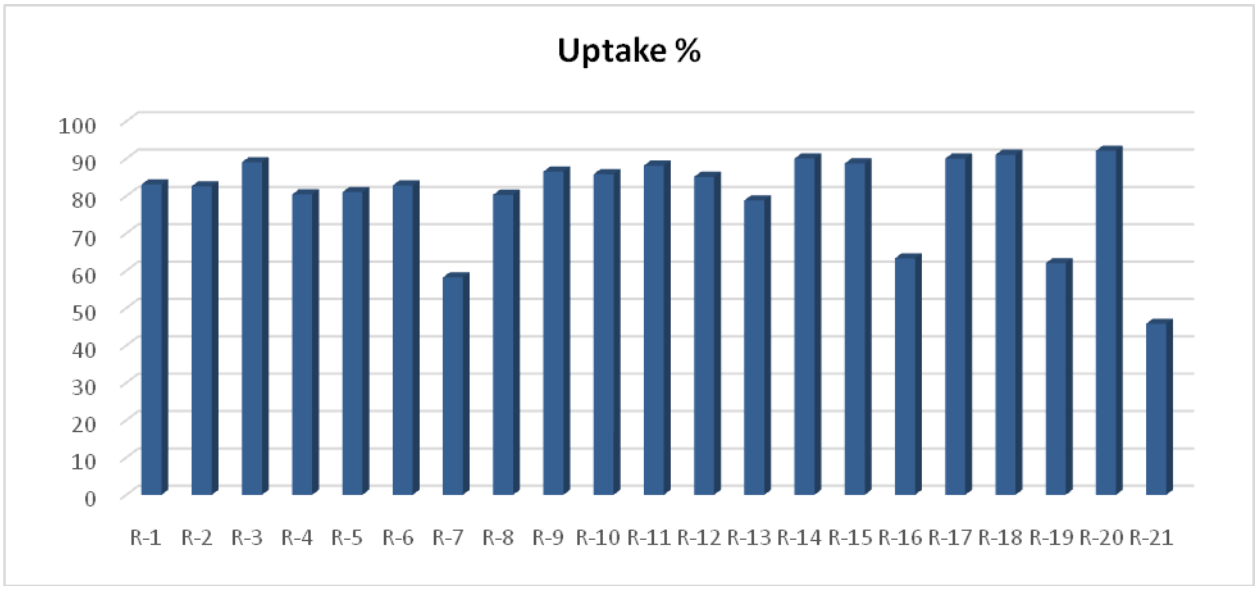

Figure 7: The uptake % for the 21 optimization runs by irradiated functionalized branched polymer for citrinin

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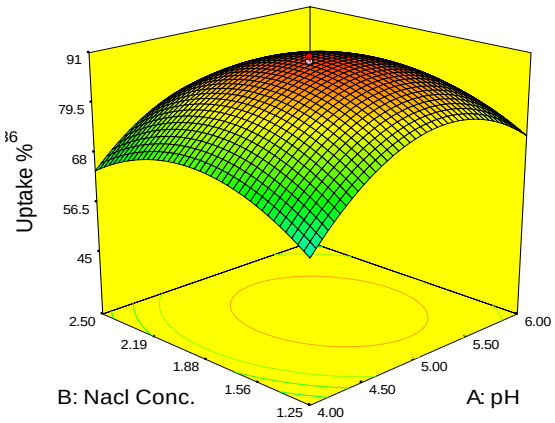

Figure 8: **The surface plot showing the effect of factors A & B on the uptake % by irradiated functionalized branched polymer for citrinin**

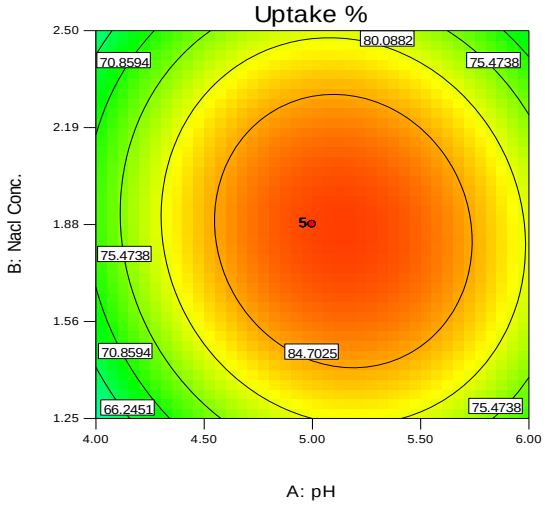

Figure 9: The contour plot showing the effect of factors A & B on the uptake % by irradiated functionalized branched polymer for citrinin

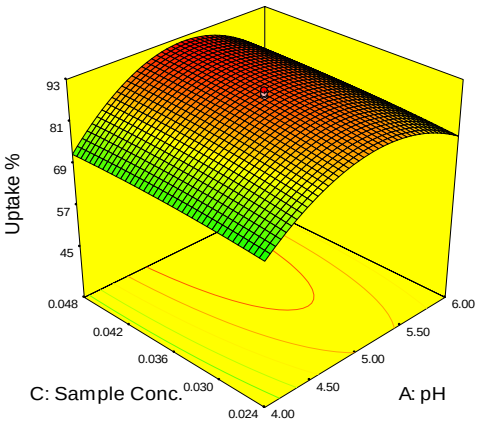

Figure 10: The surface plot showing the effect of factors A & C on the uptake % by irradiated functionalized branched polymer for citrinin

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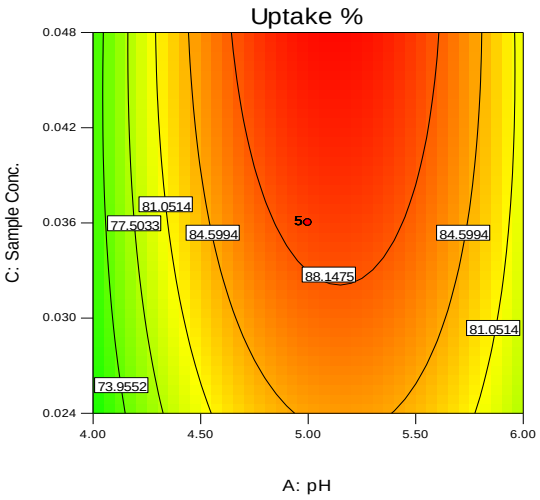

Figure 11: The contour plot showing the effect of factors A & C on the uptake % by irradiated functionalized branched polymer for citrinin

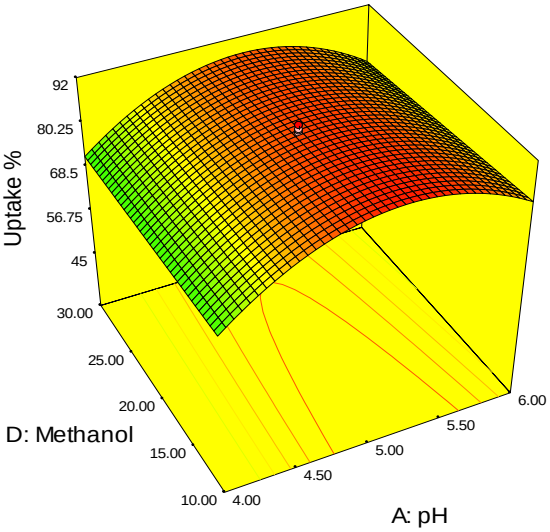

Figure 12:The surface plot showing the effect of factors A & D on the uptake % by irradiated functionalized branched polymer for citrinin

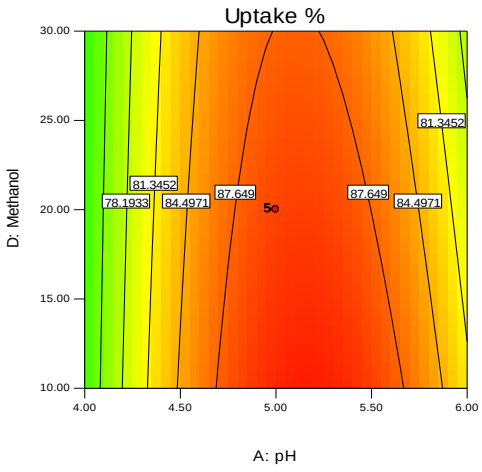

Figure 13: The contour plot showing the effect of factors A & D on the uptake % by irradiated functionalized branched polymer for citrinin

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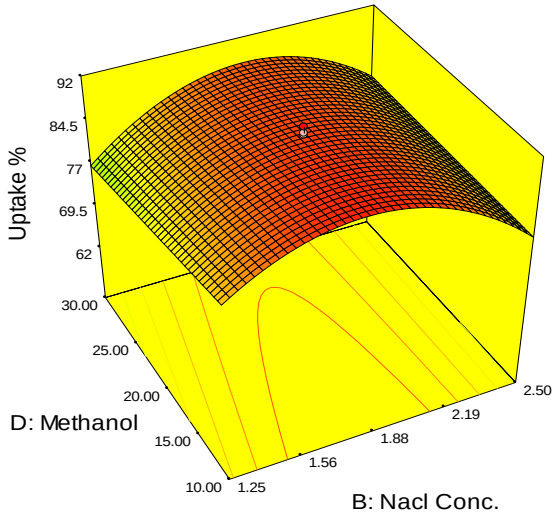

Figure 14: The surface plot showing the effect of factors B & D on the uptake % by irradiated functionalized branched polymer for citrinin

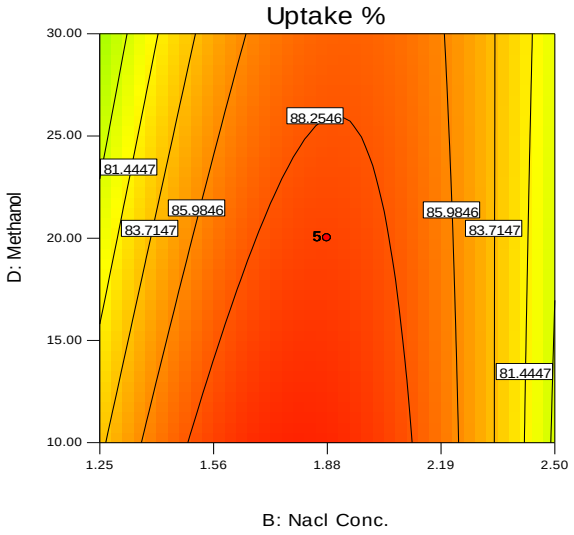

Figure 15: The contour plot showing the effect of factors B & D on the uptake % by irradiated functionalized branched polymer for citrinin

ANOVA results also revealed that all the coefficients of the main effects, interaction effects and cubic effects were statistically significant ($p < 0.05$) except factor B (NaCl conc.), and the interaction AC (pH and Sample Conc.)**Table 6**The sign of the regression coefficients revealed that all the significant effects were negative except factorA (pH), C (sample conc.) main effect, and the interaction AC (pH and Sample Conc.) and BD(NaClconc. And Methanol %) which were positive. While the magnitude of the regression coefficients revealed that the most influential factors affecting the uptake % were the pH, sample Conc., the $(pH)^2$ and the $(NaCl\ conc.)^2$ and the interaction between the pH, NaCl Conc. and Methanol %**Table 5**.The 3-D surface plots and the corresponding 2-D contour plots relating the uptake % are presented in**(Figure 8-Figure 15)**The three-dimensional response surface plot is very useful in learning about the main and interaction effects of the independent variables, whereas the two-dimensional contour plot gives a visual representation for the values of the response [26].All plots indicated non-linear relationships between the independent variables and the uptake %.

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Table 4: Uptake % by irradiated functionalized branched polymer for citrinin obtained from the central composite design runs

Run	Uptake %	Run	Uptake %
R-1	83.06	R-12	85.1
R-2	82.6	R-13	78.8
R-3	89.02	R-14	90.01
R-4	80.4	R-15	88.7
R-5	81.01	R-16	63.3
R-6	82.8	R-17	90
R-7	58.2	R-18	91.02
R-8	80.3	R-19	62
R-9	86.5	R-20	92.1
R-10	85.8	R-21	45.8
R-11	88.1		

Table 5: Regression equations for uptake % in terms of coded and actual factors

Response	Regression equation
Final Equation in Terms of Coded Factors: UPTAKE% =	89.19 + 3.65 A - 0.38649 B + 3.29 C - 1.34 D -1.68 AB + 0.14 AC - 0.96 AD + 1.85 BD - 13.14 A^2 - 9.38 B^2 - 0.93 C^2 - 16.63 ABD - 2.17 A^2C
Final Equation in Terms of Actual Factors: UPTAKE% =	285.5739- 23.8753 A - 169.418 B - 3884.35 C -25.14 D + 50.49416 AB + 1828.189 AC + 4.889874 AD + 13.59471 BD - 6.56042 A^2 -23.8946 B^2 - 6125.8 C^2 - 2.65961 ABD - 181.631 A^2 C

Table 6: ANOVA table for the uptake by response surface cubic model

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob> F	
Model	3062.84	13.00	235.60	517.14	< 0.0001	significant
A-pH	76.88	1.00	76.88	168.75	< 0.0001	
B-NaCl Conc.	0.84	1.00	0.84	1.85	0.22	
C-Sample Conc.	61.49	1.00	61.49	134.98	< 0.0001	
D-Methanol%	10.21	1.00	10.22	22.42	0.0021	
AB	9.42	1.00	9.42	20.68	0.0026	
AC	0.16	1.00	0.16	0.36	0.57	
AD	3.11	1.00	3.11	6.83	0.03	
BD	11.39	1.00	11.39	25.00	0.0016	
A^2	2135.32	1.00	2135.32	4686.97	< 0.0001	
B^2	1084.16	1.00	1084.16	2379.71	< 0.0001	

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C^2	9.68	1.00	9.68	21.25	0.0025	
ABD	882.71	1.00	882.71	1937.52	< 0.0001	
A^2C	15.74	1.00	15.74	34.55	0.0006	
Residual	3.19	7.00	0.46			
Lack of Fit	0.41	3.00	0.14	0.19	0.90	not significant
Pure Error	2.78	4.00	0.70			
Cor Total	3066.04	20.00				

Table 7: Criteria for the uptake % by irradiated functionalized branched polymer for citrinin

Experimental Factors		Uptake %	
Factor	Optimum	Range	Goal
A-pH	6	45.8%: 92.1 %	maximize
B-NaCl Conc.	2.50%		
C-Sample Conc.	0.045 mMol		
D-Methanol	3%		

Table 8: Predicted and observed values of the uptake % by irradiated functionalized branched polymer for citrinin

Response	Predicted	Observed	Residual*
uptake %	97.71 %	95.1 %	-2.61 %

From above tables and figures the following item can be discussed:

Effect of NaCl concentration

The effects of NaCl concentration "ionic strength", which was modified by adding NaCl, were also studied. As can be seen in **Table 5**the quadratic term of sodium chloride conc. is one of the most influential factors that affect the uptake (coefficient = 9.3). In general, the presence of NaCl will reduce the solubility of citrinin in water, since the affinity of organic compounds to water layer would decrease in salt solution, making hydrophobic interaction between citrinin and polymer easier. However, when a certain amount of salt was added (2.93 %, R-19 in table 4) in to the solution, the complexion of electronic subunits of polymer with cations may be formed through cation interaction [27]. And partial active centers would be occupied, leading to less uptake for the investigated citrinin.

Effect of pH

The pH of the solution used during the uptake procedure can influence the polarity of the citrinin and/or the polymer. In the case of citrinin, since the pKa is equal to 2.3 [28]. So in the pH working range (4 – 6) citrinin will be mainly ionized to the anionic form. When pH increases to 6.0, the concentration of the anion form of citrinin increases [1]. While the polymer containing secondary amine groups (Scheme 1)will be positively charged at the same pH range. This can allow for the formation of electrostatic interactions between the polymer and citrinin. Consequently, the dependence of the uptake% in the investigated pH range may be also due to the differences in hydrogen binding and hydrophobic effects between the polymer and citrinin. As can be seen in **Table 5**the main effect of pH and its quadratic term are of the most influential factors that affect the uptake (coefficient = 3.65, 13.14 respectively).It may be easy to form complexes between the polymer and citrinin based on electrostatic interactions, weak hydrogen binding and hydrophobic interactions at this pH range.

Effect of methanol concentration

Since the most common solvents used for citrinin extraction in solid food samples are methanol and water[10],[29],[14],the effects of methanol in the sample solution on the uptake of citrinin were examined over the range of 10–30%methanol according to the central composite design "CCD". Due to the good solubility of citrinin in methanol ,the uptake efficiencies of citrinin for the designed polymer decreased as the methanol concentration increased in the sample solution. This can be also explained by the deterioration of π-electron stacking and hydrogen binding interactions (Section 3.3.1).As can be seen in **Table 5 &Table 6**the citrinin concentration has a significant ($p<0.05$), negative effect on the uptake.

Effect of sample (citrinin) concentration

The effects of citrinin concentration in the sample solution on the uptake% of citrinin were examined over the range of 0.024-.048 mMol/L according to the central composite design "CCD". The uptake % of citrinin for the designed polymer increase as the citrinin concentration increased in the sample solution. As can be seen in **Table 6**the sample concentration has a significant ($p<0.05$),also in **Table 5**the main effect of sample concentrationis of the most influential factors that affect the uptake (coefficient = 3.29).

The optimal parameters for citrinin uptake

The results in **Table 7**Shows uptake % by irradiated functionalized branched polymer for citrinin at different pH values, NaCl %, sample concentration and methanol % are varied from (45.8 - 92.1%),but at pH 6,NaCl 2.5 %, sample concentration 0.048 mMol/L and methanol 3% the Predicted and observed values of the uptake % were 97.71% and 95.1% respectively**Table 8**.

Effect of acetonitrile concentration on desorption of citrinin

The effect of the desortion solvent "acetonitrile" was evaluated in acetonitrile/water mixtures (70:30; 80:20; 90:10 and 100:0, v/v).As shown in **Figure 16**higher response was obtained with a mixture of 100:0 v/v acetonitrile/water. The formation of hydrogen bonding was achieved by means of π-electron stacking effects in the aqueous media "it was confirmed that π-electron stacking effects were present by other aromatic compounds". For the sake of preferable solubility of citrinin in acetonitrile, the π -electron stacking effects between the polymer and citrinin were destroyed in the acetonitrile desorption solvent. Under these conditions, hydrogen bonding that was still in the interior hydrated layer of polymer would also deteriorate. Certainly, π -electron stacking effects "hydrophobic interactions" were diminished as the acetonitrile concentration increased. This may be the reason that acetonitrile was an effective desorption solvent[30].

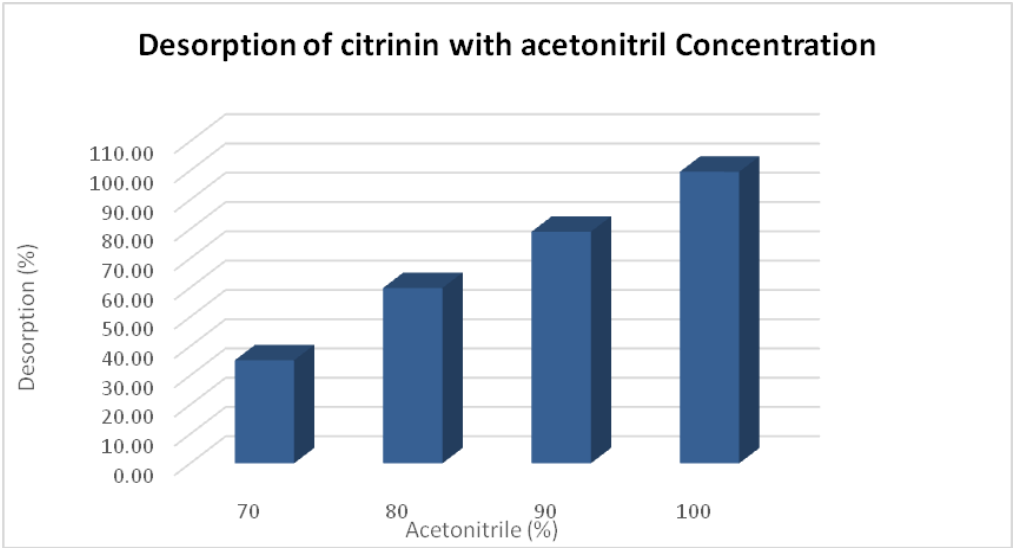

Figure 16: **Effect of acetonitrile concentration on desorption of citrinin for branched polymer**

CONCLUSION

In this work we used modified acrylamide polymer to uptake and analyses citrininmycotoxin by simple tecknique (UV spectrophotometric analyses). Many factors affecting (single and inter acting with each other) the uptake % were studied; polymer mass, pH, concentration of citrinin, methanol and NaCl(ionic strength) in sample solutions. This factor were optimize using response surface methodology method by central composite design The results showed that the optimum conditions of uptake were recorded at pH 6, 0.048 mMol/L citrinin, 3.0% methanol and 2.5% NaCL, while the maximum desorption was at 100% acetonitrile. This work proved the ability of this irradiated branched polymer to uptake citrininmycotoxin and could be applied for purification of infected foods in farther studies.

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