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Recognition and Adsorption Characteristics of Tramadol Hydrochloride Molecules in Molecular Imprinted Poly(MAA-co-EGDMA)

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Abstract

A simple method for adsorption and recognition of tramadol-HCl in molecular imprinted poly (methacrylic acid-co-ethylene glycol dimethacrylate); poly (MAA-co-EGDMA), is described. Seven tramadol imprinted polymers (TIPs) were synthesized based on tramadol hydrochloride as the template molecule, methacrylic acid (MAA) as a functional monomer, ethylene glycol dimethacrylate (EGDMA) as a crosslinker, chloroform as a porogenic solvent and benzoyl peroxide (BP) as an initiator. Other seven non-imprinted polymers (NIPs) were prepared with the same compositions as the TIPs without the template molecule. A simple method for determination of tramadol concentration by optical absorption method was described. The effects of crosslinker and functional monomer concentration, soaking time and the pH on the adsorption efficiency of tramadol by the polymers were studied. TIPs exhibited more adsorption affinity than their NIPs counterparts as they acquired recognition sites to capture the tramadol molecules. The TIP polymer with 15 mmol EGDMA and 1.15 mmol MAA stirred for 3h at 7 pH tramadol solution showed 90% adsorption capability for the tramadol, while the corresponding NIP sample showed 55.6% adsorption. SEM images showed the presence of (micro/meso/macro) pores in such TIP sample.

Keywords: Tramadol determination; imprinted polymer; tramadol adsorption; optical absorption; crosslinker.

1. Introduction

Molecular imprinting is considered as an engineering technique to synthesize receptors for a certain type of other molecules known as template molecules. This technique is fully described in several publications [1-3] which is based upon the formation of a three-dimensional polymer network around a template (analyte). After the template is removed, specific recognition sites are left as pores, which comply in shape, size and chemical functionality. The process for molecularly imprinted polymers (MIPs) requires template molecules, functional monomers, crosslinkers, a porogenic solvent, and an initiator [4,5]. Template molecules include both biological and chemical molecules such as amino acids and proteins [6-8], nucleotide derivatives [9], pollutants [10,11], drugs and food [12,13]. Functional monomers, such as acrylic acid, methacrylic acid (MAA), acrylamide, 4-vinylpyridine, 2-hydroxyethylmethacrylate, interact with the template molecules to form a pre-polymerization complex that is later locked in place during the crosslinking reaction. The most common crosslinkers used are ethylene glycol dimethacrylate (EGDMA) and divinylbenzene [14]. A solvent, such as acetonitrile, chloroform, and toluene, is used in the

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imprinting process to dissolve the template molecules and monomers and to impart porosity to the final polymer [15]. Therefore, it is known as a porogen solvent. Azobisisobutyronitrile benzoyl peroxide (BP), ammonium persulfate and potassium persulfate are used to initiate the free radical polymerization reaction [16-19]. Non-imprinted polymers (NIPs) are synthesized in the same way as MIPs, but there are not template molecules used. Usually, NIPs allow for the assessment of non-specific interactions between the template and the polymer [20].

MIPs are applied in separation and purification techniques where chromatography is one of their most traditional applications [21,22]. Their applications are extended to chemical sensors and biosensors [23], catalysis [24] and drug delivery [25]. MIP is considered a promising technique for the recognition of biological and chemical molecules [26,27] including amino acids and proteins [6,8], pollutants [10] and drugs [12].

Tramadol is a chemical material approved for the treatment of moderate to moderately severe pain in adults [28]. However, it is considered as a central analgesic with opioid and non-opioid receptors affinity [29]. Nevertheless, high doses of tramadol produce a signal of abuse potential such as, tramadol dependence which may occur when used for prolonged periods of time and can produce opioid-like effects [30]. Such effects cause many problems particularly when truck drivers deal with this material as non-medical use, to overcome the feeling of tiredness caused by long-distance driving. In such a case, those drivers cause several catastrophic along the traffic roads. Therefore, there is a need to check that the drivers are not driving under the effects of the tramadol material. The most recently developed methods for the determination and recognition of tramadol include chromatography [31], spectrophotometry [32], mass spectrometry, electrophoresis, and potentiometric method [33].

In the present work, a simple spectrophotometric method based on the optical absorption technique is used for tramadol estimation. The recognition, determination, and adsorption of tramadol molecules were performed in MIP and NIP poly(MAA-co-EGDMA). The sorbents were prepared based on MAA as a functional monomer, EGDMA as a crosslinker, chloroform as a porogenic solvent and BP as an initiator. The adsorption capacity, recognition sites, and effects of time and pH on the adsorption were recorded.

2. Experimental

2.1. Material

Methacrylic acid (MAA) was obtained from Merck Co. (USA). Ethylene glycol dimethacrylate (EGDMA) and benzoyl peroxide (BP) of reagent grade were purchased from Sigma–Aldrich (Germany). Tramadol HCl, manufactured by Grunenthal Co., Germany, was kindly supplied by Minapharm Pharmaceuticals (MIPH), Egypt. Chloroform of a chemical grade was used.

2.2. Methodolog

2.2.1. Synthesis of tramadol imprinted (TIPs) and non-imprinted polymers (NIPs)

Seven samples of tramadol imprinted polymers (TIPs) were prepared following Azodi et al. procedure [34]. Poly(MAA-co-EGDMA) was prepared as a polymer matrix and tramadol-HCL as a template molecule. Tramadol-HCL was firstly dissolved in chloroform in a glass test tube. Then, a predetermined amount of MAA as a functional monomer and EGDMA as a cross-linker was added to the glass tube. Finally, BP dissolved in chloroform was added to initiate the free-radical polymerization reaction. The reactants in the glass tube were subjected to ultra-sonication for 30 min, purged with nitrogen for 2 min, and then, sealed. The glass tube was placed in a water bath at 80^{o}C for 7h to allow the initiation of the polymerization reaction. After this period, a solid and rigid bulky material was obtained in the tube which was left overnight to cool at room temperature. The rigid material was extracted from the tube, dried and ground into fine powders using mortar and pestle. The crushed powders were sieved to below 212 μm, washed with 10% acetic acid and methanol solutions to remove the tramadol and then with distilled water.

The other seven non-imprinted polymers (NIPs) were prepared following the same procedure as mentioned above without the addition of tramadol. The NIPs assist in the verification of the molecular recognition sites in TIPs. The NIPs are considered to adsorb tramadol through non-specific bindings (such as H-bonds), however, TIPs adsorb tramadol through non-specific and specific sites. The compositions of the TIPs and NIPs polymers are listed in **Table 1**.

2.2.2. Spectrophotometric measurement

The estimation of tramadol-HCl concentration dissolved in distilled water is performed using a spectrophotometric technique. The optical absorption spectra of tramadol solutions were measured in the range 190-1100 nm, by using a double beam spectrophotometer (JASCO corp. V-570, Rel-OO, Japan). Covets of quartz glass were used for holding the tramadol solution and the distilled water as a reference sample. The obtained data were drawn in the range of 190-375 nm as no significant changes are noticed above 375 nm. The absorbance at maximum absorption peak that obeys Beer's law is followed to draw a calibration curve which will be used to estimate the unknown concentration of tramadol.

2.2.3. Preparation of tramadol standard and stock solutions

Four tramadol standard aqueous solutions were prepared with tramadol concentrations of 2.5, 5, 7.5, and 10 (mg/L). **Fig.1** shows the absorption curves of these solutions in the UV-region. The absorbance at the intense band (197 nm) was considered for constructing the calibration curve from which the concentration of the unknown tramadol in solutions can be estimated by knowing the corresponding absorbance at 197 nm.

A stock of tramadol solution that will be used as an immersing solution for soaking the polymeric sorbents (TIPs) and (NIPs) is prepared. A solution with low tramadol concentration, to be sensitive for any change in its concentration, is chosen for this purpose. An aqueous solution of 1 mg/L tramadol (C_i) is prepared to be the initial concentration before the immersion of the polymer samples.

2.2.4. Determination of adsorption % (A%) and adsorption capacity (AC) of tramadol by TIPs and NIPs

The capability of the prepared TIPs and NIPs to adsorb tramadol was determined by soaking 0.25 g of TIPs or NIPs polymers in 10 mL stock tramadol solution of concentration (C_i= 1 mg/L) then magnetically stirred for 3h. The remained tramadol concentration (C_f) after soaking the polymers was estimated by recording the absorbance at 197 nm, then correlating this absorbance with the calibration curve to estimate the corresponding concentration (C_f). Equation (1) was formulated to calculate the percentage of the tramadol adsorbed by TIPs and NIPs (*A%*).

$$A\% = \frac{C_i - C_f}{C_i} \times 100 \quad (1)$$

C_i: is the initial concentration of tramadol solution (1 mg/L) before soaking the sorbents in it. C_f: is the final concentration of tramadol solution after soaking the sorbents in it.

The adsorption capacity of tramadol by TIPs and NIPs could be calculated by the formulated equation (2) :

$$AC = \frac{C_i - C_f}{0.25} \quad (2)$$

where 0.25 is the weight of the soaked TIPs or NIPs in grams.

2.2.5. Determination of the effect of soaking time and pH on (A%)

The effect of soaking time on the percentage of the tramadol adsorbed (A%) by the prepared TIPs and NIPs was determined as described above, yet for different soaking time intervals. However, for studying the effect of pH on the (A%), buffer solutions of pH 3.6, 5.6, 7, 8.5 and 9.8 were prepared. The adsorption % of the prepared TIPs and NIPs at different pH was determined by stirring 0.25 g TIP or NIP in 1 mg/L tramadol buffer solutions for 3 h. Then, the polymers were removed and A% was estimated for the remaining tramadol in solution by UV-vis spectroscopy.

2.2.6. Determination of the template sites in the TIPs

The template sites or recognition sites (the specific sites) can be calculated by the formulated equation (3).

$$\text{Template sites } \% = \frac{A\%_{TIP} - A\%_{NIP}}{A\%_{TIP}} \times 100 \quad (3)$$

$A\%_{TIP}$: is the percentage of the tramadol adsorbed by TIP which is due to the specific and non-specific sites.

$A\%_{NIP}$: is the percentage of the tramadol adsorbed by NIP which is due to the non-specific sites.

TABLE 1. Molecular compositions of the prepared TIPs and NIPs

MIP	MAA (mmol)	EGDMA (mmol)	Tramadol (mmol)	BP (mmol)	Chloroform (ml)
TIP1	1.00	1	0.5	0.41	8
TIP2	1.00	5	0.5	0.41	8
TIP3	1.00	10	0.5	0.41	8
TIP4	1.00	15	0.5	0.41	8
TIP5	1.15	15	0.5	0.41	8
TIP6	1.25	15	0.5	0.41	8
TIP7	1.40	15	0.5	0.41	8
NIP1	1.00	1	---	0.41	8
NIP2	1.00	5	---	0.41	8
NIP3	1.00	10	---	0.41	8
NIP4	1.00	15	---	0.41	8
NIP5	1.15	15	---	0.41	8
NIP6	1.25	15	---	0.41	8
NIP7	1.40	15	---	0.41	8

2.2.7. Scanning electron microscopic analysis

A SEM instrument of Quanta FEG-250 SEM, Ametek Holland, was used to obtain the electron micrographs to study the internal morphology of the prepared polymer samples.

3. Results & discussion

3.1. The standard calibration curve

In order to determine the concentration of tramadol in unknown solutions through the optical absorption technique, four solutions with different concentrations of tramadol 2.5, 5, 7.5, and 10 (mgL^{-1}) were prepared. These solutions are given the codes T2.5, T5, T7.5, and T10, respectively. Distilled water was used as a reference sample. **Fig.1** shows the UV spectra of the four standard solutions of tramadol hydrochloride prepared in aqueous medium of pH=7. The spectra show three characteristic absorption bands of tramadol hydrochloride at wavelengths of 197 nm, 218 nm, and 273 nm. The present results are in agreement with published data [35]. The absorbance at λ_{max}= 197 nm was selected as the maximum wavelength that could be used to draw a calibration curve (**Fig.1 inset**) as Beer's law was obeyed in the concentration range of 0–10 mg/ml.

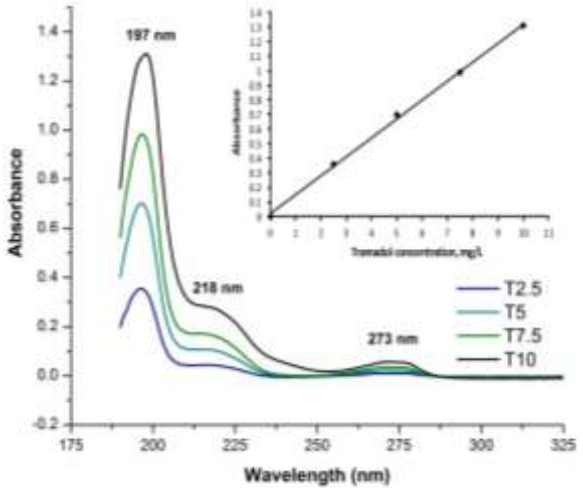

Fig.1. The optical absorption spectra in the UV region of the four standard solutions with tramadol concentration of: 2.5, 5, 7.5 and 10 (mg/L). The inset shows the calibration curve of tramadol hydrochloride at 197nm.

3.2. Crosslinker and functional monomer effects on A%

The amount of the crosslinker along with the functional monomer is expected to affect the tramadol adsorption behavior of either TIPs or NIPs. To study these effects on the efficiency of tramadol adsorption, seven TIPs and another seven of NIPs were prepared by changing the molar ratio of the crosslinker (EGDMA) and the functional monomer (MAA) **(Table 1)**. A specific amount of TIPs or NIPs (0.25 g) were immersed in (10 mL) of tramadol stock solution of 1 mg/L and pH 7. After stirring for 3h, the absorption curves of the solutions are measured **(Fig.2)** followed by estimating the absorbance at λ_{max} = 197nm and the corresponding tramadol

concentration (C_f). **Table 2** lists the variation of absorbance and tramadol concentration in accordance with the amounts of crosslinker and functional monomer. The concentration (C_f) represents the tramadol retained in the solution after the soaked polymers adsorbed part of the original concentration (C_i). The smaller the concentration of the retained tramadol, the greater is the adsorption behavior of the soaked polymer.

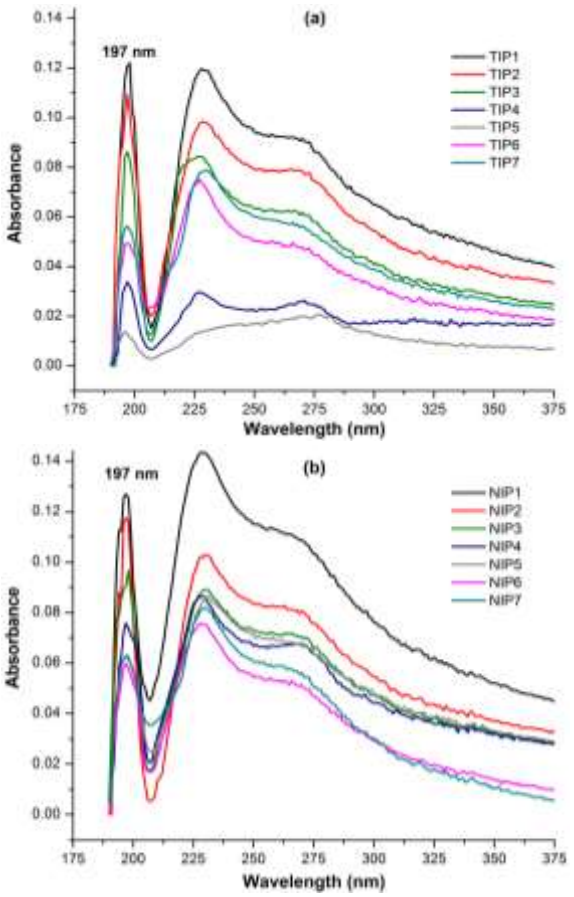

Fig.2. UV-vis absorption spectra of 1 mg/L tramadol solution of pH 7, after immersion of te polymers for 3h and increasing content of the functional monomer (TIPs and NIPs from 1 to 4) and increasing content of the crosslinker (TIPs and NIPs from 4 to 7) for TIP and NIP samples (a) and (b) respectively.

It can be seen from **Table 2** that for imprinted polymers (TIP1 – TIP4) with MAA equals 1.0 mmol, the retained tramadol decreases as the crosslinker was increased from 1 to 15 mmol, which was further decreased upon a slight increase in the functional monomer (1.15 mmol for TIP5). Similar observations are valid for the corresponding non-imprinted samples, yet the amounts of the retained tramadol are much higher in the present case. For NIP polymers, NIP6 sorbent showed the least retained tramadol, i.e., maximum adsorption for the dissolved tramadol.

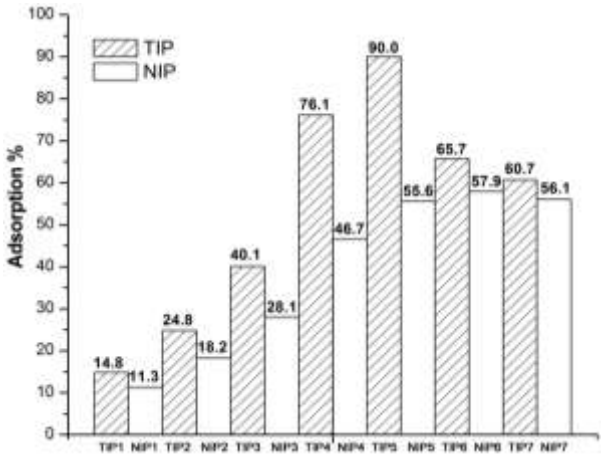

Fig.3. The adsorption percentage (*A%*) of tramadol by TIPs and NIPs in a 1 mg/L tramadol solution of pH 7 for 3 h.

The adsorption percentage of tramadol (*A%*) by TIPs and NIPs sorbents was calculated using eq.1 and plotted as shown in **Fig.3**. From this figure, it can be noticed that all TIPs sorbents have higher adsorption efficiency than those of their corresponding NIPs sorbents. This observation can be attributed to the presence of interstitial pores in TIPs samples representing the recognition sites of the tramadol templates. These imprinted cavity sites increased the surface area and the adsorption efficiency of the TIPs over NIPs.

Fig.3 shows that the TIP1 to TIP4 sorbents with the same amount of functional monomer and increasing content of the crosslinker exhibit a pronounced increase in tramadol adsorption (*A%*). On the other hand, in samples (TIP4, TIP5, TIP6, and TIP7) where the functional monomer is gradually increased in presence of the same amount of crosslinker, a continuous increase in (*A%*) is observed until reaching a maximum of 90% for TIP5 followed by a decrease in (*A%*). These findings prove that the adsorption efficiency of tramadol by TIPs sorbents is directly connected with the crosslinker and functional monomer content. For NIPs, the same trend is observed as in TIPs where the highest adsorption (*A%*) is observed with the highest amount of crosslinker (NIP5 to NIP7). However, the adsorption (*A%*) in NIP samples is generally lower than that of the corresponding compositions of TIP polymers.

The general increase in adsorption of tramadol with increasing the crosslinker can be attributed to the contribution of the ester groups in EGDMA through physical interactions (non-specific affinity) with OH and -N- groups in tramadol. The adsorption preference for TIPs compared to their counterparts in NIPs can be assigned to the recognition sites that originated in the TIPs. These tramadol recognition sites exist in the form of meso and micro pores that have increased with the crosslinker. Therefore, the very small increase in the adsorption (*A%*) of TIP1 (14.8%) compared to NIP1 (11.3%) can be attributed to the formation of very few template sites due to the lack of high network structure required for their fixation. The significant increase of *A%* in TIP4 (76.1%) compared to NIP4 (46.7%) can be assigned to the increase of these specific tramadol sites originated by the increased crosslinker plus the non-specific adsorption sites.

The increase in adsorption that occurred with the increase of MAA and the fixation of the EGDMA in TIP5 or NIP5 was due to the increase in functional groups capable of non-specific adsorption. However, with the increase of the functional monomer relative to the crosslinker in TIP6 and TIP7, the network structure began to collapse, which is the guarantor of template sites.

The adsorption capacity, AC, (the adsorption of tramadol/gram sorbent) of TIPs and NIPs can be expressed using eq.2. **Fig.4** shows that the adsorption capacity increases gradually with increasing the crosslinker in both TIPs and NIPs. The adsorption capacity reaches the maximum at TIP5 (3.6 mg tramadol for each 1g TIP5) with the highest amount of EGDMA. It can be seen that the adsorption capacity is in accordance with that of the adsorption % where the capacity difference between each TIP and its NIP counterpart increases with the crosslinker up to TIP5/NIP5. Such increment in both (AC) and the capacity difference is accompanied by the increase of the crosslinker up to maximum values in samples TIP5 and NIP5. After which, the capacity difference is observed to decrease with increasing the content of MAA while the crosslinker remains constant in samples TIP6 and TIP7 and their corresponding NIPs. Therefore, we can conclude that the recognition sites are increasing by increasing the network structure and this is the reason for the steady increase in the efficiency of adsorption between each TIP and its NIP counterpart. However, with the persistence of the crosslinker and the increased concentration of the functional monomer, the polymer chains began to elongate and gradually lose the network shape, thus the identification sites begin to destroy. Therefore, the difference in adsorption has been significantly reduced between TIP6/NIP6 and TIP7/NIP7 because the adsorption is greatly due to the non-specific sites.

TABLE 2. The absorbances at 197 nm and their corresponding tramadol concentration (C_f) retained after soaking of TIPs or NIPs sorbents for 3 h in tramadol solution of 1 mg/L (abs. = 0.144) and pH =7 in accordance with the amounts of the functional monomer and crosslinker.

Amount of		Sorbent code	Imprinted Polymer		Sorbent code	Non-imprinted Polymer	
MAA (mmol)	**EGDMA (mmol)**		Abs. at 197nm	(C_f) (mg/L)		Abs. at 197nm	(C_f) (mg/L)
1.00	1	**TIP1**	0.122	0.85	**NIP1**	0.127	0.89
1.00	5	**TIP2**	0.109	0.75	**NIP2**	0.117	0.82
1.00	10	**TIP3**	0.086	0.60	**NIP3**	0.091	0.72
1.00	15	**TIP4**	0.034	0.24	**NIP4**	0.076	0.53
1.15	15	**TIP5**	0.014	0.10	**NIP5**	0.063	0.44
1.25	15	**TIP6**	0.049	0.34	**NIP6**	0.060	0.42
1.40	15	**TIP7**	0.056	0.39	**NIP7**	0.062	0.44

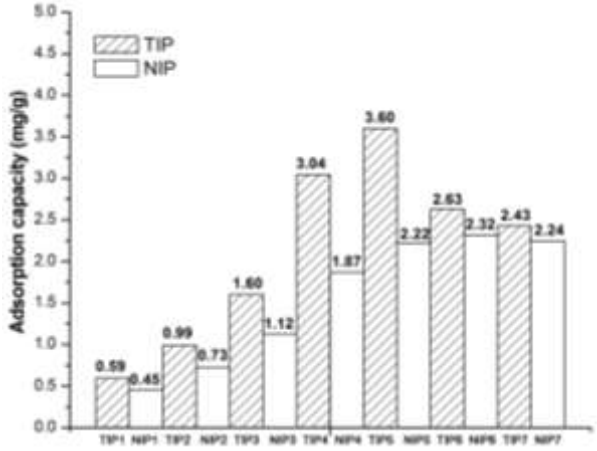

Fig.4. The adsorption capacity of TIPs and NIPs measured in a 1 mg/L tramadol solution of pH 7 for 3 h.

The specific or the template sites percentage of the TIPs were calculated using eq. 3 and plotted in **Fig.5**. From which a gradual increase in the template sites from TIP1 (23.6%) to TIP4 (38.6%) can be observed. Then there is a slight reduction in these sites in TIP5 which is accompanied by a collapse of these sites in TIP6 and TIP7.

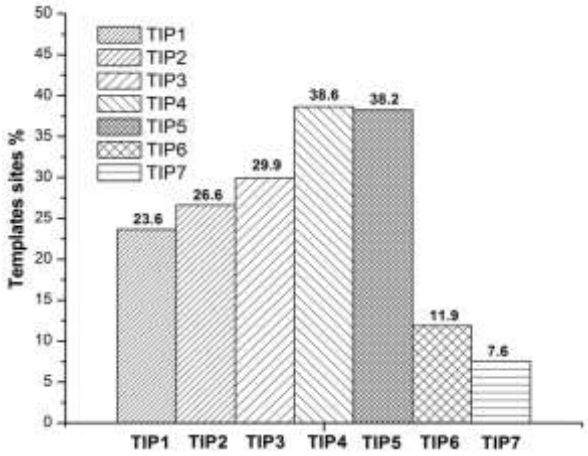

Fig.5. Variation of the template site % of TIPs samples with increasing EGDMA (TIP1 – TIP4) and with increasing MAA (TIP4 – TIP7).

An illustrative figure of the effect of the crosslinker and functional monomer on the specific and non-specific adsorption sites is drawn in **Fig.6**. It is expected that large spacing gaps between the matrix chains are formed when using low ratios of the crosslinker to the monomer from 1 (TIP1) to 5 (TIP2). These large interfacial gaps couldn't be considered as template sites for tramadol molecules. The adsorptive sites are mainly represented by the functional monomer. However, by increasing the crosslinking agent (as represented by TIP3 and TIP4, a more compact and rigid network structure is expected to be formed which increases the chance for the polymeric chains to host the tramadol molecules between its matrix. Therefore, as the compactness (brought by crosslinking) of the polymeric matrix increases, the opportunity to generate templates for the tramadol molecules also increases. Therefore, the adsorptive sites increase and higher binding and selectivity to the tramadol molecules can be obtained. When the MAA is increased while the concentration of crosslinker is fixed (as in TIP4 - TIP5), the physical non-specific adsorptive sites is increased as a result of increasing the functional groups. However, with the continuous increase of MAA relative to EGDMA (in TIP6 and TIP7), the polymeric chains lengthen and the compactness of the polymer is expected to decrease resulting in low recognition sites formation as represented by TIP7 in **Fig.6.** Accordingly, the adsorption percentage of tramadol decreases.

3.3. Effect of soaking time on the adsorption A%

As shown by the previous results, TIP5 and TIP4 showed the best results in the adsorption of tramadol from the solution. Therefore, the effect of soaking time of TIP5 and TIP4 and their counterparts of NIP5 and NIP4 on their ability to adsorb tramadol was studied. Specific weights of the polymers were immersed in tramadol solution of initial concentration 1 mgL^{-1} (C_i) under stirring for 1h, 2h, 3h, and 4h. **Fig.7** shows the UV-vis absorption spectra of the tramadol solutions at the end of the soaking times. The absorbance values at 197 nm were followed after each soaking time and their corresponding concentrations of tramadol (C_f) were estimated from the standard calibration curve followed by A% calculation. **Tables 3 and 4** list the effect of soaking time on the adsorption % of (TIP5 and NIP5) and (TIP4 and NIP4), respectively. The intensity of the bands at λ_{max}= 197 decreased with the time of immersion. This means greater adsorption is obtained over time. The absorbance values of all samples were correlated with the calibration curve to determine the concentration of tramadol in solutions (C_f). A% was determined by eq. 1 and drawn against time in **Fig.8**. The figure shows that the adsorption of TIP4 and TIP5 exceeds that of NIP4 and NIP5 at all time intervals. Also, the adsorption percentage (*A%*) gradually increases with the soaking time until the third hour.

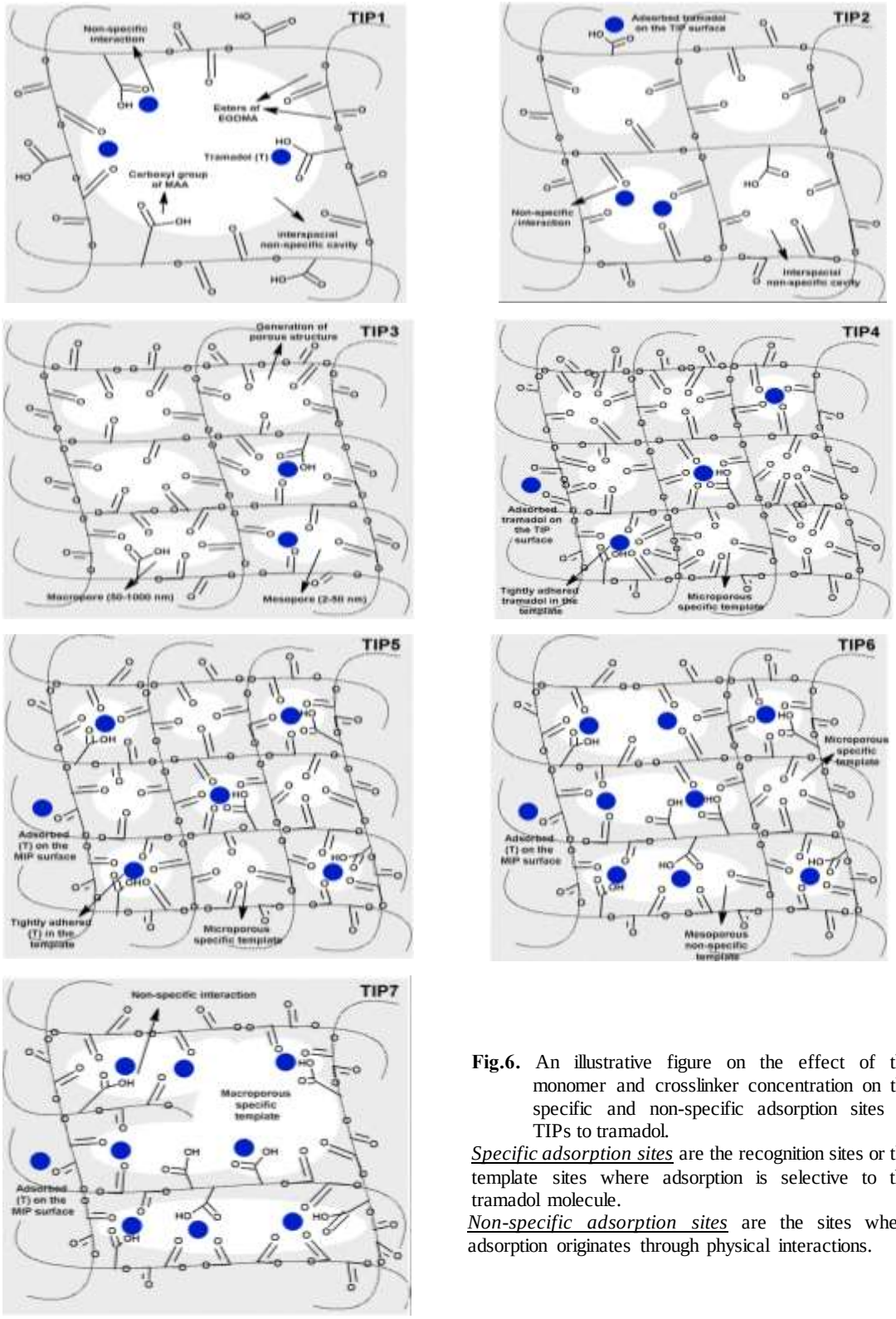

Fig.6. An illustrative figure on the effect of the monomer and crosslinker concentration on the specific and non-specific adsorption sites of TIPs to tramadol.

Specific adsorption sites are the recognition sites or the template sites where adsorption is selective to the tramadol molecule.

Non-specific adsorption sites are the sites where adsorption originates through physical interactions.

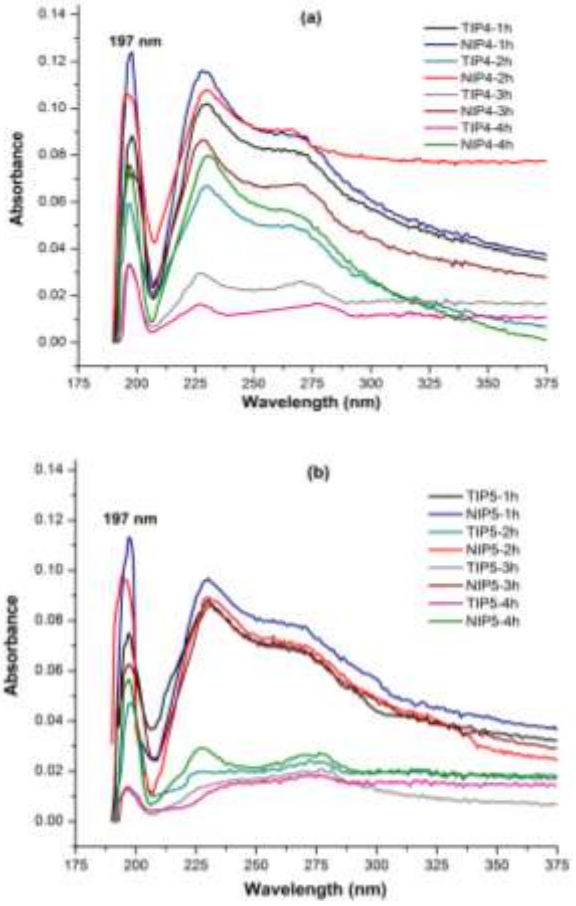

Fig.7. UV-vis absorption spectra of 1 mg/L tramadol solutions of pH 7, for samples TIP4 and NIP4 (a) and TIP5 and NIP5 (b) at different soaking times.

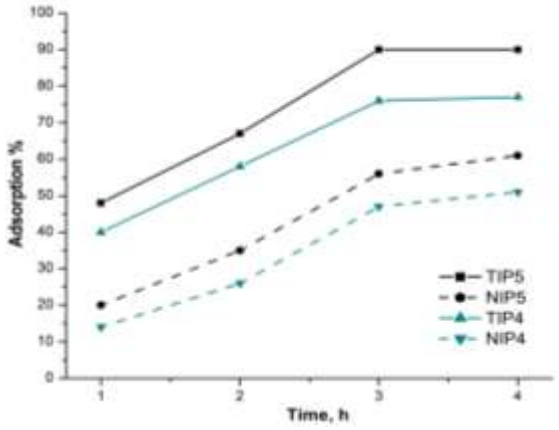

Fig.8. Tramadol adsorption % by TIP4, TIP5, NIP4, and NIP5 measured in a 1 mg/L tramadol solutions of pH 7 and at different time intervals.

Saturation adsorption is observed after which the form of adsorption in TIP4 and TIP5 differs from that of NIP4 and NIP5. It seems that the adsorption of TIP4 and TIP5 is constant from the third hour (76% and 90%, respectively) to the fourth (77% and 90%, respectively) while it still growing in NIP4 and NIP5 but in slow rate (47% to 51%) and (56% to 61%), respectively. The high adsorption rate in the first 3 h is due to the preferential and fast adsorption of tramadol onto the specific sites in the pores of TIPs [34]. After the occupation of the recognition sites, it becomes difficult for tramadol to implant in the TIPs.

TABLE 3. The absorbances at 197 nm and their corresponding tramadol concentration retained after soaking TIP5 and the corresponding NIP5 for different times in tramadol solution (pH 7) of 1 mg/L (abs. = 0.144).

Soaking time (h)	TIP5			NIP5		
	Abs. at 197nm	(C_f) (mg/L)	A%	Abs. at 197nm	(C_f) (mg/L)	A%
1	0.075	0.52	48	0.113	0.80	20
2	0.047	0.33	67	0.093	0.65	35
3	0.014	0.10	90	0.063	0.44	56
4	0.014	0.10	90	0.056	0.39	61

TABLE 4. The absorbances at 197 nm and their corresponding tramadol concentration retained after soaking TIP4 and the corresponding NIP4 for different times in tramadol solution (pH 7) of 1 mg/L (abs. = 0.144).

Soaking time (h)	TIP4			NIP4		
	Abs. at 197nm	(C_f) (mg/L)	A%	Abs. at 197nm	(C_f) (mg/L)	A%
1	0.085	0.60	40	0.123	0.86	14
2	0.060	0.42	58	0.105	0.74	26
3	0.034	0.24	76	0.076	0.53	47
4	0.033	0.23	77	0.070	0.49	51

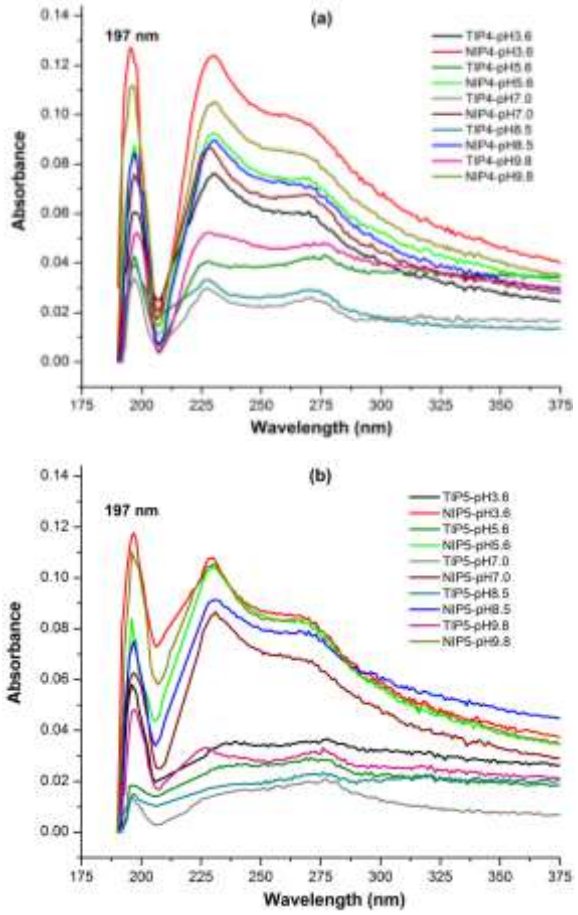

Fig.9. UV-vis absorption spectra of 1 mg/L tramadol solutions after immersion of TIP4 and NIP4 (a) and TIP5 and NIP5 (b) at different pH values.

3.4. Effect of pH on the tramadol adsorption A%

The effect of pH of the medium on the adsorption of tramadol by TIP5 and TIP4 and their counterparts of NIP5 and NIP4 was studied. 0.25g of each polymer is immersed with stirring for 3hs in solutions of 1 mg/L tramadol at different pH ranges.

Fig.9 shows the UV-vis absorption spectra of the tramadol solutions at the end of the soaking time at different pH solutions. The absorbance at 197 nm was measured and the corresponding concentrations of tramadol were estimated from the calibration curve (C_f), then the percentage of tramadol adsorbed A% was calculated using equation 1. The results are listed in (**Tables 5 and 6)** and drawn in **Fig.10.** From the figure and these tables, it can be seen that the least absorbance occurs in the neutral solutions and the absorbance begins to increase with acidity or alkalinity in the solution. This is attributed to the deprotonation of the carboxylic groups of the functional monomer (MAA) which is responsible for the physical non-specific adsorption of tramadol. On the other hand, a decrease in the adsorption capability of TIPs and NIPs is observed with increasing the acidity of the solution. This is due to the protonation of the hydroxyl and tertiary amine groups in tramadol [34].

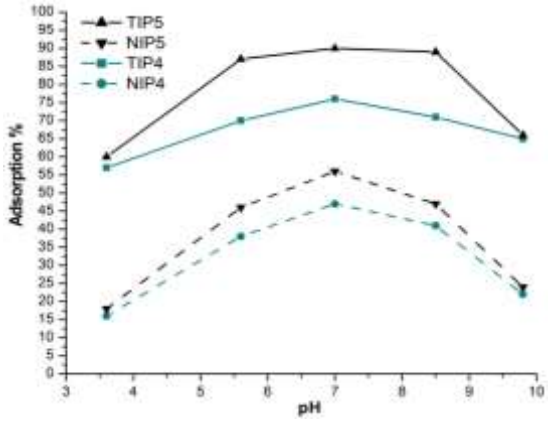

Fig.10. Effect of pH of 1 mg/L tramadol solution on the adsorption % of TIP4, TIP5, NIP4, and NIP5 immersed for 3 h.

TABLE 5. The absorbances at 197 nm and their corresponding tramadol concentration (C_f) retained in solution of 1 mg/L tramadol after immersion of 0.25g of TIP5 and NIP5 for 3 h at different pH.

pH of solution	TIP5			NIP5		
	Abs. at 197nm	(C_f) (mg/L)	A%	Abs. at 197nm	(C_f) (mg/L)	A%
3.6	0.057	0.40	60	0.118	0.82	18
5.6	0.018	0.13	87	0.077	0.54	46
7	0.014	0.10	90	0.063	0.44	56
8.5	0.015	0.11	89	0.075	0.53	47
9.8	0.048	0.34	66	0.108	0.76	24

TABLE 6. The absorbance at 197 nm and their corresponding tramadol concentration (C_f) retained in solution of 1 mg/L tramadol after immersion of 0.25g of TIP4 and NIP4 for 3 h at different pH.

pH of solution	TIP4			NIP4		
	Abs. at 197nm	(C_f) (mg/L)	A%	Abs. at 197nm	(C_f) (mg/L)	A%
3.6	0.061	0.43	57	0.121	0.84	16
5.6	0.043	0.30	70	0.088	0.62	38
7	0.034	0.24	76	0.076	0.53	47
8.5	0.039	0.27	71	0.085	0.59	41
9.8	0.051	0.35	65	0.111	0.78	22

3.5. SEM micrographs of TIPs and NIPs

Fig.11 shows the SEM micrographs of NIP4, TIP4, NIP5, and TIP5. This figure shows a significant difference in the surface morphology of these polymers. NIP4 and NIP5 appear as massive blocks with large spaces and cavities between them. These cavities may be formed during the polymerization where phase separation is generated between the porogen and the growing polymer. No interstitial pores appear nested within these blocks. TIP4 and TIP5 appear as smaller masses with many small pores in the blocks. The micropores (< 2 nm), mesopores (2-50 nm) and macropores (> 50 nm) formed within the surface structure of TIPs are considered to be responsible for its higher adsorption affinity than NIPs where the surface area and the specific sites increased. While the functionality in NIPs is the only factor responsible for adsorption of tramadol.

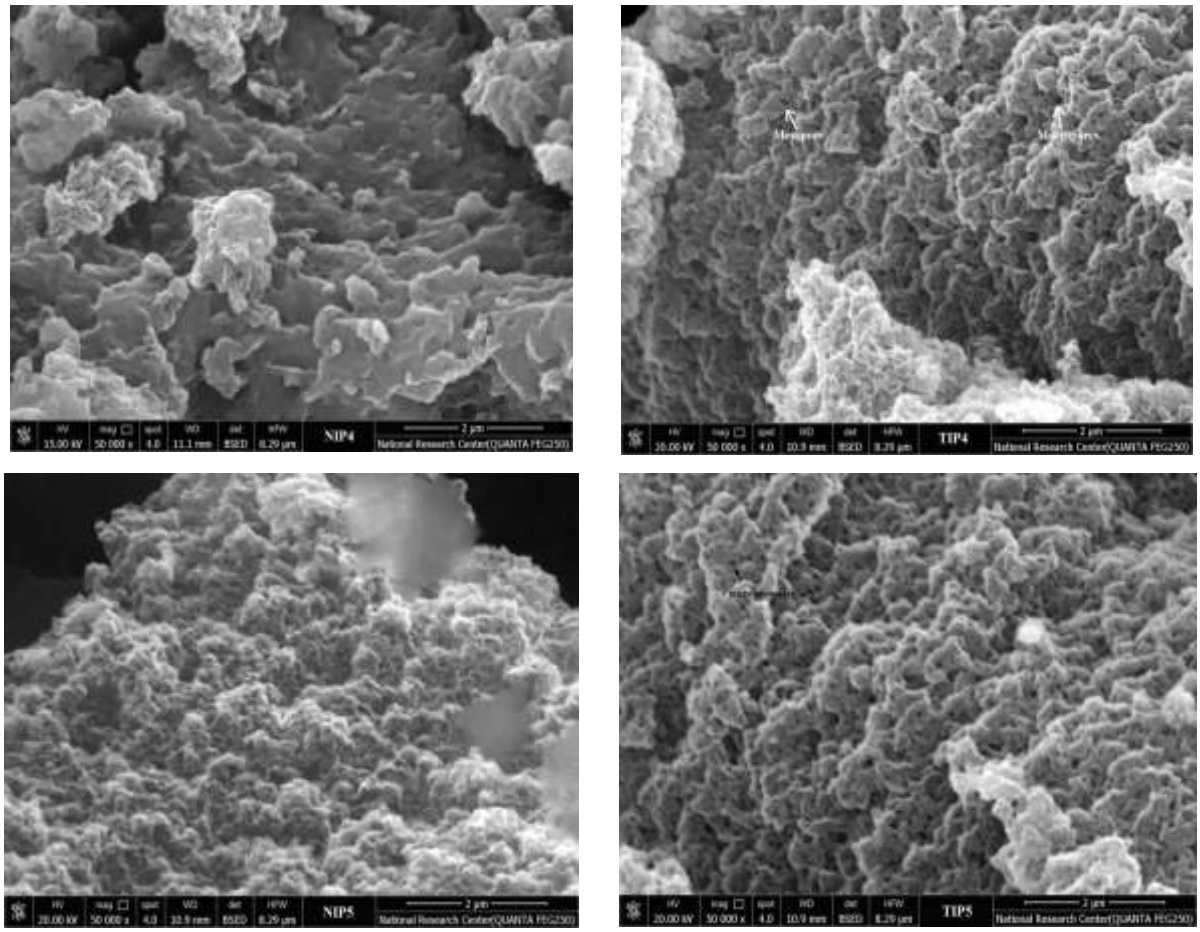

Fig.11. SEM images of NIP4, TIP4, NIP5, and TIP5.

Conclusion

Tramadol imprinted polymers (TIPs) and non-imprinted polymers (NIPs) were synthesized and used as sorbents for tramadol molecules. Both polymeric sorbents showed increased adsorption affinity with increasing the crosslinking agent in the matrix. The higher adsorption efficiency of TIPs over NIPs is due to the presence of specific porous sites within the polymeric matrix. The adsorption capacity reaches the maximum at TIP with the highest amount of EGDMA as the recognition sites increased by increasing the network structure. While increasing the amount of the functional monomer to the optimum amount, the efficiency of TIPs increased and began, with the continuous increase in monomer, to suddenly drop. SEM micrographs of TIP at high crosslinker showed (micro/meso/macro) pores in the polymer matrix while no interstitial pores are present in NIP. TIPs samples exhibited a higher adsorption rate in the first 3 h than NIPs due to the preferential and fast adsorption of tramadol onto the specific sites. After the occupation of the recognition sites, the adsorption rate becomes very low. The polymers showed high uptake of tramadol in the neutral medium and their efficiency decreased in alkaline or acidic media due to deprotonation or protonation effects, respectively.

Conflicts of interest

There are no conflicts to declare.

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