# SYNTHESIS OF MOLECULARLY IMPRINTED POLYMERS (MIPS) USED FOR ESTIMATION OF BETAMETHASONE DISODIUM PHOSPHATE (BMSP) USING DIFFERENT FUNCTIONAL MONOMERS

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**ABSTRACT**

**Betamethasone sodium phosphate (BMSP) selective molecularly imprinted polymers(MIPs) were based on ion-pair by prepared four polymers(MIPs) using BMSP as the template a well as (Acryl amide) (AAM), 2-Acrylamido-2-Methyl-1-Propane sulphonic Acid (2-AAMMPSA as monomer, used N,N-ethylenebismethacrylamide (EBMAA) ,ethylene glycol dimethacrylate ethylene glycol(EGDMAC), N, N-methylene bisacrylamide (NNMBAAM)) as cross linker and used benzoyl peroxide as initiator . NIPs prepared by using the same composition of MIPs except the template (BMSP). The MIPs were prepared using variation ratio of monomer and cross linker .These MIPs applicate as solid phase extraction for determination BMSP in pharmaceutical preparation used UV as detector .the results gave good response, where the reconstruction percentage (Rec%) value of BMSP drug took the range (99.058149 % - 101.887004 %), and the relative standard deviation (RSD%) value took the range (0.224149 % - 0.743651 %) for standard solution and Rec% took values of (98.400035 - 99.404218) %, and RSD% took values of (0.572589 - 1.012777) % of BMSP drug for the Betamethasone sodium phosphate pharmaceutical.**

**Keywords: Betamethasone sodium phosphate, Spectrophotometric, Scanning Electron Microscopy.**

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# تحضير البوليمرات االطبعة الجزيئية (MIPS) لتقدير بيتاميثازون ثنائي الصوديوم فوسفيت عن طريق مونومرات وظيفية مختلفة

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**المستخلص**

استندت تحضير بوليمرات فوسفات الصوديوم بيتاميثازون (BMSP) ذات الطبعة الجزيئية البوليمرية (MIP) على الزوج الايوني باستخدام أربعة بوليمرات (MIPs) محضرة وباستخدام BMSP كقالب وكذلك (أكريل أميد) (AAM، 2 – أكريلاميدو 2– ميثيل 1–بروبان سولفونيك حامض (2–AAMMPSA كمونومر ، يستخدم N، N– إيثيل بنيسميثاكريلاميد (EBMAA)، الإيثيلين كليكول ثنائي ميثاكريلات إيثيلين كليكول ( (EGDMAC، N، N– ميثيلين بيس اكريلاميد. تم تحضيرها باستخدام نفس تكوين MIPs باستثناء القالب (BMSP). تم تحضير MIPs باستخدام نسبة مختلفة من مونومر ورابط التشابك. تم تطبيق هذه MIPs كا طوار صلبة من اجل تعيين BMSP في المستحضرات الصيدلانيةباستخدام الأشعة فوق البنفسجية ككاشف. أعطت النتائج استجابة جيدة، حيث أخذت قيم المدى تتراوح (Rec٪) لعقار BMSP أخذ النطاق (99.058149٪ – 101.887004٪)، وقيمة الانحراف المعياري النسبي (RSD٪) النطاق (0.224149٪ – 0.743651٪) للمحلول القياسي وأخذ Rec٪ قيمًا (98.400035 – 99.404218)٪ ، وأخذ RSD٪ قيم (0.572589 – 1.012777)٪ لعقار بيتاميثازون فوسفات الصوديوم.

الكلمات المفتاحية: بيتاميثازون فوسفات الصوديوم، الطيفية، المسح الإلكتروني المجهري.

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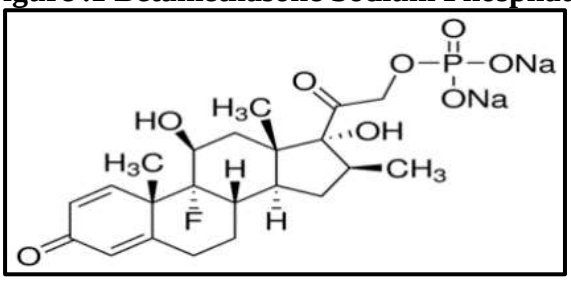
## INTRODUCTION

Betamethasone sodium phosphate (BMSP) is the representative of the synthetic steroids, belonging to the glucocorticoid class(1), Chemically its is known as 9- ( -Fluoro-11β,17-dihydroxy-16 β-methyl-3,20-dioxopregna-1,4-diene-21-yl), White or almost white powder, very hygroscopic molecular formula (C22H28FNa2O8P), The chemical structure of betamethasone sodium phosphate is shown in figure. 1, its is Freely soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride, Natural and synthetic glucocorticoids are known to be highly effective drugs for the treatment of inflammatory diseases. They are widely administrated to relieve joint pain,symptoms of inflammatory skin problems and inflammation due to arthritis, asthma and rhinitis in clinical , It is active in replacement therapy for adrenal insufficiency and as an anti-inflammatory and immunosuppressant, inflammatory bowel disease, reactive airways disease, and respiratory distress syndrome in preterm infants and pruritus in corticosteroid-responsive dermatoses, ulcerative colitis, lupus erythematosus, acute leukemia(14,18), BMSP was estimated in several ways, including the use of UPLC / MS / MS (6, 11) , and was estimated using a voltammetric method (19), and the use of prepared and modified silica compounds (13), Methods were also developed using (RP-HPLC) (12,13), this study was formulation and evaluation of betamethasone sodium phosphate (BMSP) loaded chitosan nanoparticle(CNPs) using cross-linked chitosan malic acid derivative for better therapeutic effect. The prepared BMSP loaded CNPs (16) , A chiral biosensing platform was developed using (BMSP) as chiral recognition element through multilayered electrochemical deposition of BMSP, overoxidized polypyrrole, and nanosheets of graphene (OPPy- BMSP /GR), for enantio-recognition of mandelic acid (MA) enantiomers (9), Were Estimated (BMSP) using Novel magnetic molecularly imprinted polymer nanoparticles (MMIPs) using methacrylic acid as a functional monomer, MAEMA as a cross-linker, and betamethasone as a template The Fe3O4 nanoparticles were encapsulated with a SiO2 shell and functionalized with ACH@CH2 and MMIPs(7), were as Estimated (BMSP) using Novel magnetic molecularly imprinted polymer nanoparticles (MMIPs) using BY precipitation polymerization were prepared MMIPs were prepared by using methacrylic acid as a functional monomer, N,N-p-phenylene bismethacryl amide as a crosslinking agent and betamethasone as template (8) There are a variety of ion selective electrode determined drugs that depended on MIPs as recognition membranes like ibuprofen(18), warfarin (1), phenytoin (3) and metronidazole benzoate (2).

**Figure .1 Betamethasone Sodium Phosphate**




This study aims to development a new method for the estimation of Betamethasone sodium phosphate using a Molecularly Imprinted Polymers method based on solid phase extraction technique and UV-spectrophotometry.

## MATERIALS AND METHODS

**Reagents and Chemicals**: (Acryl amide) (AAM), 2-Acrylamido-2-Methyl-1-Propane Sulphonic Acid (2-AAMMPSA ), Ethylene Glycol Dimethacrylate ethylene glycol(EGDMAC), N, N-Methylene Bisacrylamide (NNMBAAM)) and benzoyl peroxide were purchased from Sigma–Aldrich (St. Louis, MO, USA, www.sigma-aldrich.com), methanol were purchased from Merck (LiChrosolv, Merck KGaA, Darmstadt, Germany, www.merck.com).) Betamethasone Sodium Phosphate (BMSP) was provided from Mahima Life Science PVT.LTD. / India , Sodium hydroxide were purchased from Analar – Germany, nitrogen gas bottle (99.99) from Arab gulf factory Baghdad.

**Instrumentation**

Monitoring of the analyses was performed using UV-Vis (SHIMADZU UV -Visible Spectrophotometer 1800 pc (Japan)) using the (1cm) quartz cells and Scanning Electron Microscopy (SEM) (JSM.6390A) (Tokyo

Japan) and SHIMADZU IRAffinity-1S (FTIR) - 8000 (Japan), heating/ stirring (Germany).During the polymerization process, pure Betamethasone Sodium Phosphate shows absorption band at 238nm, this band can be used to ensure that all Betamethasone Sodium Phosphate was removed after washing, then it measured by using UV-Vis spectrophotometer An Ultrasonic Sensitive Water Bath from (SONERX) (W.GERMANY) was used for stirring the polymer solution.

**Preparing of Standard solutions**

preparing of standard solution (100 $\mu g.ml^{-1)}$ Betamethasone Sodium Phosphate by dissolving (0.01 gm )of standard Betamethasone Sodium Phosphate in the methanol and completed to(100 mL) in the volumetric flask .The other solutions were prepared in100 mL at the ranged from (10-100 $\mu g.ml^{-1}$) in the same procedure.

**Synthesis of the Imprinted Polymer BMSP-($MIP_1$-AAM)**

Unbreakable glass tube (25 ml) was utilized, and 0.42 mmol from the mold material BMSP was added to the tube. BMSP was dissolved in 7 ml of methanol. Furthermore. An amount of 4.6 mmol of Acrylamide (AAM) was added to the mixture. Further, the combination was stirred via the ultrasonic waves for 5 minutes. Later, cross linkers of Ethylene Glycol Dimethacrylate (EGDMAC) (9.9 mmol) and Benzoyl Peroxide (0.165 mmol) (BPO), which acts as a starting point for polymerization, were added to the glass tube. Bubbles in liquid were moved out by using high-purified Nitrogen for 30 minutes. Immediately thereafter, a rubber cap tightly locked the tube orifice, and the resulting liquid was placed in a water bath at 60 $C^o$ for two days without moving. After polymerization finishes, the mold was removed by frequent washing of the polymer using a combination of (10%) (v/v) of Acidic acid/Methanol utilizing the extractor (Soxhlet) for 24 hours. Following mold removal, it was necessary to guarantee that there were no reactive materials by checking it, following the process of frequent washing and drying at 40 $C^o$ for one hour. After drying, the material was smashed into powder using a grinder of Granit and a steel sieve whose porosity is 125µm. For evaluating the extracted material, a plastic syringe (3 ml) was exploited by filling it with a polymer material. Furthermore, a standard liquid, which lies within the calibration curve, was prepared and permitted to pass through the plastic syringe. Finally, the liquid was removed from the plastic syringe by a washing solution and under a pressure of 5 pa.

**Synthesis of the Imprinted Polymer BMSP - ($MIP_2$-2-AAMMPSA)**

Unbreakable glass tube (25 ml) was utilized, and 0.6 mmol from the mold material BMSP was added to it. BMSP was dissolved in 7 ml of methanol. In addition. An amount of 3.5 mmol of 2-Acrylamido-2-Methyl-1-Propane Sulphonic Acid (2-AAMMPSA) was added to the blend. Further, the combination was stirred via the ultrasonic waves for 5 minutes. Later, cross linkers of N, N-Methylene Bisacrylamide (NNMBAAM) (25 mmol) and Benzoyl Peroxide (0.32 mmol) (BPO), which represents a beginning point for polymerization, were added to the glass tube. Bubbles in the liquid were moved out using high-purified Nitrogen for 30 minutes. Directly thereafter, a rubber lid tightly locked the tube outlet, and the resulting liquid was placed in a water bath at 60 $C^o$ for two days without moving. After polymerization finishes, the mold was removed by frequent washing of the polymer using a combination of (10%) (v/v) of Acidic acid/Methanol and utilizing the extractor (Soxhlet) for 24 hours. Succeeding mold removal, It was necessary to be certain that there were no reactive ingredients by checking it following the process of frequent washing and drying at 40 $C^o$ for one hour. After drying, the material was smashed into powder using a grinder of Granit and a steel sieve whose porosity is 125µm. For evaluating the extracted material, a plastic syringe (3 ml) was exploited through filling it with the polymer material. Furthermore, a standard liquid, which lies within the calibration curve, was prepared and permitted to pass through the plastic syringe. Finally, the liquid was removed from the plastic syringe by a washing solution and under a pressure of 5 pa.

**Preparation of pharmaceutical BMSP solutions**

The pharmaceutical form, which is available in local markets and contains BMSP, has tablets shape and is produced by the company "The

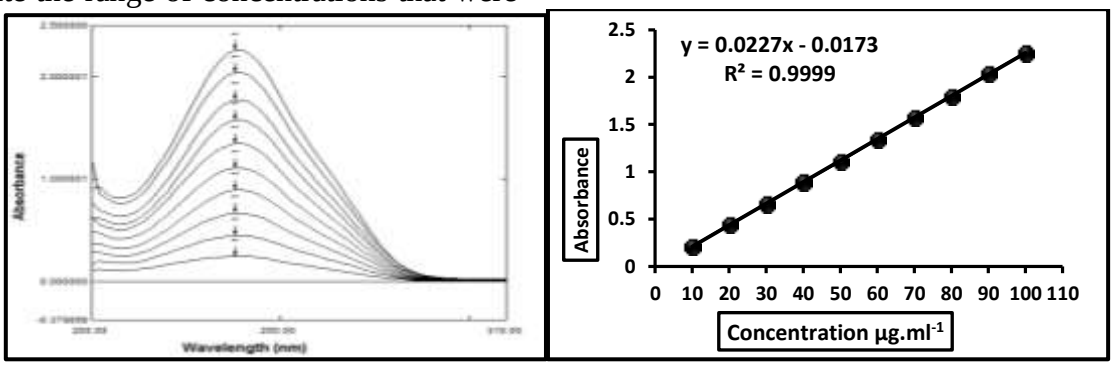
Gulf Jilfar for medical industry" in UAE. Ten tablets of pharmaceutical form, which have 0.5 mg of the effective material, were weighed to get an average weight of 1.905 g. The collection was smashed and well mixed using a ceramic grinder. Then, an average of one tablet weight (0. 10905 g) was considered and dissolved in a volumetric vial (100 ml) using Methanol as a solvent. Following the process of placing in a water bath to dissolve by ultrasonic waves, the liquid was filtered through an infiltration paper (Whatman No. 42) to get rid of any undissolved materials. Additionally, the leachate, containing 50 $\mu g.ml^{-1}$ of the effective material BMSP, was obtained and applied in tests.

**Procedure of BMSP standard solution**

Different quantities of (1 – 10) ml of the standard liquid BMSP, whose concentration is 100 $\mu g.ml^{-1}$, were moved to a collection of volumetric bottles having 10 ml each, and were slaked up to the mark of this solvent. Then, the UV ray device scanned the wavelength (190 nm– 400 nm) of the combination to plot the zero spectrum and the absorption spectrum record (for each bottle) to calculate the range of concentrations that were consistent with Pier – Lambert law. The study showed that the maximum absorption was at 238 nm.

**RESULTS AND DISCUSSION**

**Absorption spectra**: Absorption of Betamethasone sodium phosphate versus its photo liquid was measured. Consequently, BMSP showed a maximum absorption at 238 nm, as in figure. 2.a. Then, a calibration curve for BMSP drug was organized by plotting absorption versus concentration, as in figure. 2.b. The linearity of BMSP drug was in the range (10 – 100) $\mu g.ml^{-1}$, the gradient coefficient of BMSP ($R^2$) was 0.9999, the molar absorption coefficient with Sandal indication of BMSP were 11722.28 $L.mol^{-1}.cm^{-1}$ and $0.044053^2$ $\mu g.cm^{-1}$ respectively, and the identification limit with the estimation limit of BMSP were 0.002985 $\mu g.ml^{-1}$ and 0.009949 $\mu g.ml^{-1}$ respectively. This method depicted satisfying accuracy and harmony, where the reconstruction percentage (Rec%) value of BMSP drug took the range (99.058149 % - 101.887004 %), and the relative standard deviation (RSD%) value took the range (0.224149 % - 0.743651 %).




(b) (a )

**Figure 2. (a) zero-order spectra of (BMSP) at 238 nm and (b) calibration curve of (BMSP) with concentrations (10 – 100) $\mu g.ml^{-1}$**

**Accuracy and precision**

Accuracy and consistency of the method were computed through Rec% and RSD% for two concentrations within the calibration curve, where Table 1 Shows the obtained results. Rec% value took a range of (99.058149 % - 101.887 %), and RSD% took the range (0.464235 % - 0.688368 %) for BMSP drug.

**Table 1. Accuracy and Consistency of (BMSP) drug**

| Sample | Drug conc (µg/ml) | | Rec % | RSD % |
|---|---|---|---|---|
| | Taken | Found | | |
| PMSP | 20 | 20.3774 | 101.8870 | 0.6884 |
| | 50 | 49.5291 | 99.0582 | 0.4642 |

**Synthesis of MIPs for Betamethasone Sodium Phosphate (BMSP)**: Two MIPs of Betamethasone sodium phosphate were prepared via polymerization. In addition, polymerization method requires the drug as a mold, and requires choosing monomers that

have a great role in reacting with mold and forming molecular printed polymers. Two types of monomers were utilized, which were Acrylamide (AAM) and 2-Acrylamido-2-methyl-1-propane Sulphonic Acid (2-AAMMPSA) that supports checking of the printing process. The molecular printed polymers needed appropriate type and quantity of cross linkers to complete polymerization to become a hard and a high selective polymer. Many attempts to prepare molecular printed polymers were conducted, and they included finding the perfect ratios of (monomer: cross: linker drug) to prepare NIPs and MIPs, The prepared NIPs and MIPs included convenient properties regarding their performance, as shown in Table 2.

**Table 2. The various ratios (D: M: C) that were used to prepare NIPs and MIPs for (BMSP).**

| No.MIP | Ratio | Drug BMSP | Monomer AAM | Cross linker EGDMAC | Initiator BPO | Solvent | Result |
|---|---|---|---|---|---|---|---|
| MIP1 | % | 2.26 | 37.596 | 60.156 | 0.2 | 7ml $CH_3OH$ | White suspensions |
| | mmol | 0.3 | 5 | 8 | 0.165 | | |
| MIP1 | % | 3.04 | 36.36 | 60.61 | 0.2 | 7ml $CH_3OH$ | White suspensions |
| | mmol | 0.5 | 6 | 10 | 0.165 | | |
| MIP1 | % | 2.82 | 30.83 | 66.35 | 0.2 | 7ml $CH_3OH$ | White hard powder |
| | mmol | 0.42 | 4.6 | 9.9 | 0.165 | | |
| NIP1 | % | | 30.83 | 66.35 | 0.2 | 7ml $CH_3OH$ | White hard powder |
| | mmol | | 4.6 | 9.9 | 0.165 | | |
| No.MIP | | Drug BMSP | Monomer 2-AAMMPSA | Cross linker NNMBAAM | Initiator BPO | Solvent | Result |
| MIP2 | % | 1.92 | 11.54 | 86.54 | 0.3 | 7ml $CH_3OH$ | White suspensions |
| | mmol | 0.5 | 3 | 22.5 | 0.32 | | |
| MIP2 | % | 1.96 | 14 | 84.03 | 0.3 | 7ml $CH_3OH$ | White suspensions |
| | mmol | 0.7 | 5 | 30 | 0.32 | | |
| MIP2 | % | 2.06 | 12.03 | 85.91 | 0.3 | 7ml $CH_3OH$ | White hard powder |
| | mmol | 0.6 | 3.5 | 25 | 0.32 | | |
| NIP2 | % | | 12.03 | 85.91 | 0.3 | 7ml $CH_3OH$ | White hard powder |
| | mmol | | 3.5 | 25 | 0.32 | | |

**All ratios of MIPs and NIPs were prepared employing a water bath at (60 – 70) $C^o$.**

## FTIR analysis

FTIR spectra of BMSP drug appear at forming MIPs that stand on the monomer Acrylamide and 2-Acrylamido-2-methyl-1-propane Sulphonic acid. Before and after drug removing, basic functional groups perform, as shown in figure. (3 – 7).

**Table 3. Demonstrates the most recognized peaks in FTIR spectra of the molecular printed polymer of (BMSP) using AAM as a functional monomer**

| No. | Functional Group | BMSP | BMSP-($MIP_1$-AAM) before template removal | BMSP-($MIP_1$-AAM) after template removal |
|---|---|---|---|---|
| 1 | N-H str. | --- | 3444 | 3448 |
| 2 | O-H str. | 3406 | 3367 | --- |
| 3 | C-H aliphatic. | 2987, 2945 | 2956 , 2866 | 2995 , 2958 |
| 4 | C=O str.ester. | --- | 1670 | 1728 |
| 5 | C=O str.Carbonyl | 1722 | --- | --- |
| 6 | C=O str.α.β.unsaturated | 1662 | 1728 | --- |
| 7 | C=O str.amid | --- | 1631 | 1676 |
| 8 | C=C str.exocyclic | 1606 | --- | --- |
| 9 | C-H bending | 1454 | 1454 | 1456 |
| 10 | C-O str. asymm. | 1174 | 1149 | 1145 |
| 11 | C-O str. symm. | 1099 | 1047 | 1049 |

FTIR spectra of pure Betamethasone sodium phosphate were measured. The same operation occurred to the molecular printed polymers (before and after removing the mold) through scanning within the range (400 – 4000) $cm^{-1}$ utilizing the solid tablets method (KBr).Through FTIR spectra, a wide band of OH group was observed. The frequency band of this group became less than its previous value, because of the linkage between OH of BMSP drug with atoms existing within the monomer (AAM) via hydrogen bonds. Consequently, the hydrogen bonds drag the (O-H) bond and change the dynamics of this bond. Furthermore, we can observe that Carbonyl group (C=O) disappeared after the process of removing the mold molecule finished. In addition, groups (C=O amid) and (N-H) that belong to monomer AAM appeared. In spite of conducting the process of removing the mold molecule, the groups did not disappear. This verifies that washing and removing actions were effective.

**Table 4. Shows the most recognized peaks within FTIR spectra of the molecular printed polymer of (BMSP) using 2-AAMMPSA as a functional monomer**

| No. | Functional Group | BMSP | BMSP-($MIP_2$-2-AAMMPSA) before template removal | BMSP-($MIP_2$-2-AAMMPSA) after template removal |
|---|---|---|---|---|
| 1 | O-H str. | 3406 | 3523, 3409 | 3438 |
| 2 | C-H aromatic. | --- | 3068 | 3076 |
| 3 | C-H aliphatic. | 2987, 2945 | 2945 | 2933 |
| 4 | C=O str.Carbonyl | 1722 | --- | --- |
| 5 | C=O str.α.β.unsaturated | 1662 | --- | --- |
| 6 | C=O str.amid | --- | 1656 | 1654 |
| 7 | C=C str.exocyclic | 1606 | --- | --- |
| 8 | C-H bending | 1454 | 1452 | 1452 |
| 9 | C-O str. asymm. | 1174 | 1114 | 1114 |
| 10 | C-O str. symm. | 1099 | 1064 | 1039 |

FTIR referred to the existing of a wideband of OH group having frequencies that became higher than its preceding value, because the new band represents a summation of OH frequencies of BMSP drug and the frequencies existing in 2-AAMMPSA monomer. Moreover, we observed that the Carbonyl groups (C=O) disappeared after the operation of removing the mold molecule. In addition, the groups (C=O amid), which belongs to the monomer, appeared during the formation of MIPs and did not disappear after removing the mold molecule. The operation proves that the frequent washing using a combination of 10 % (v/v) of Acetic acid/Methanol and mold molecule removal was effective.

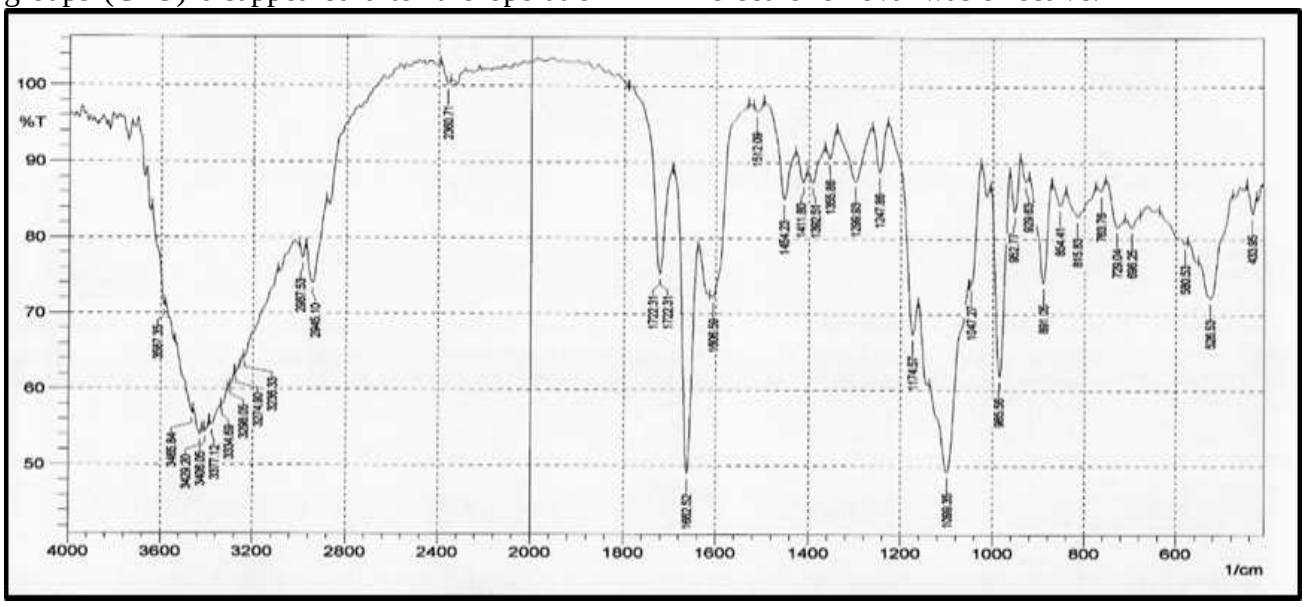



**Figure 3. FTIR of (BMSP) drug**

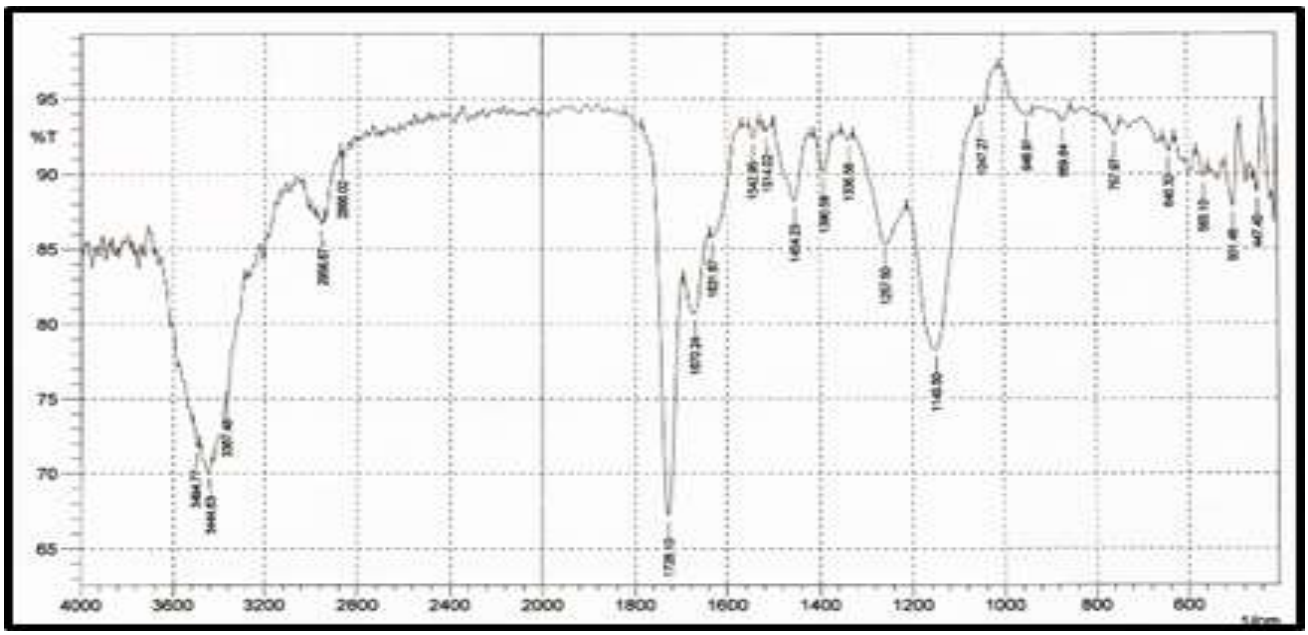



**Figure 4. FTIR BMSP-MIP1-AAM before the removal of (BMSP) .**

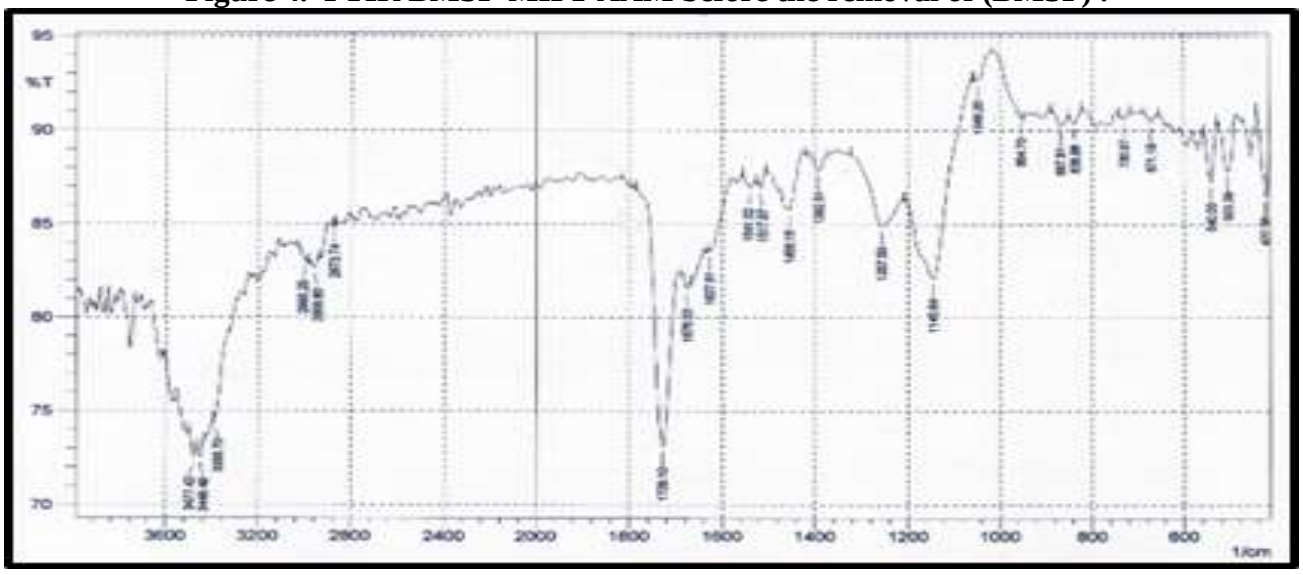



**Figure 5. FTIR BMSP-MIP1-AAM after the removal of (BMSP) .**

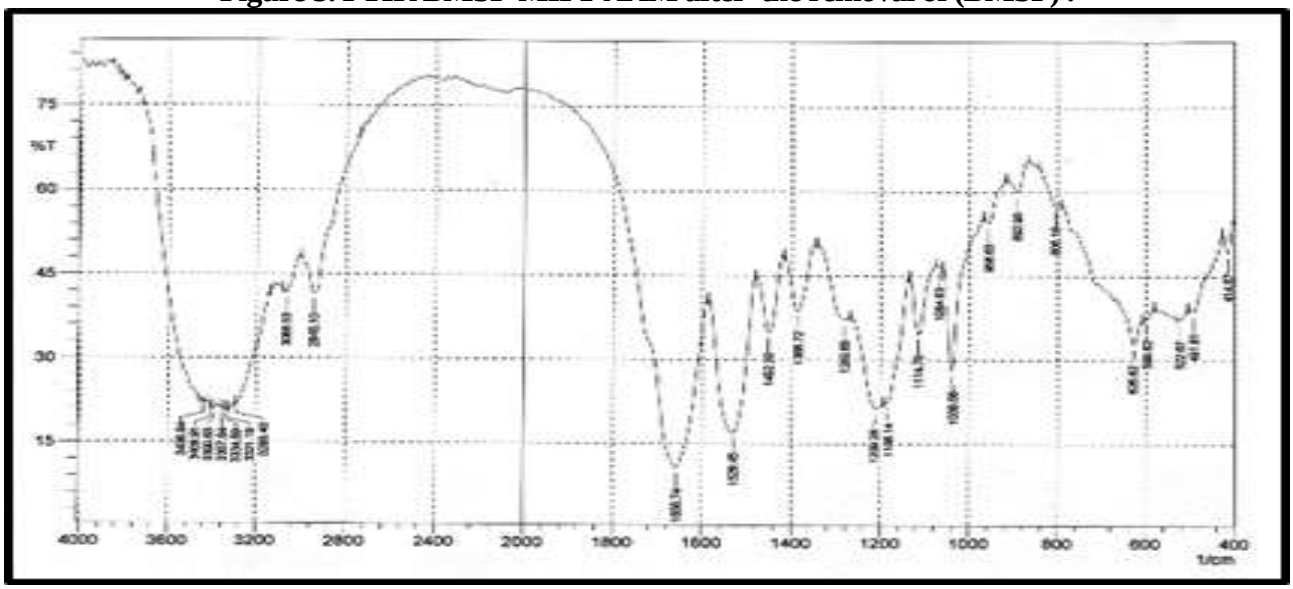



**Figure 6. FTIR BMSP-MIP2-2-AAMMPSA. before the removal of (BMSP)**

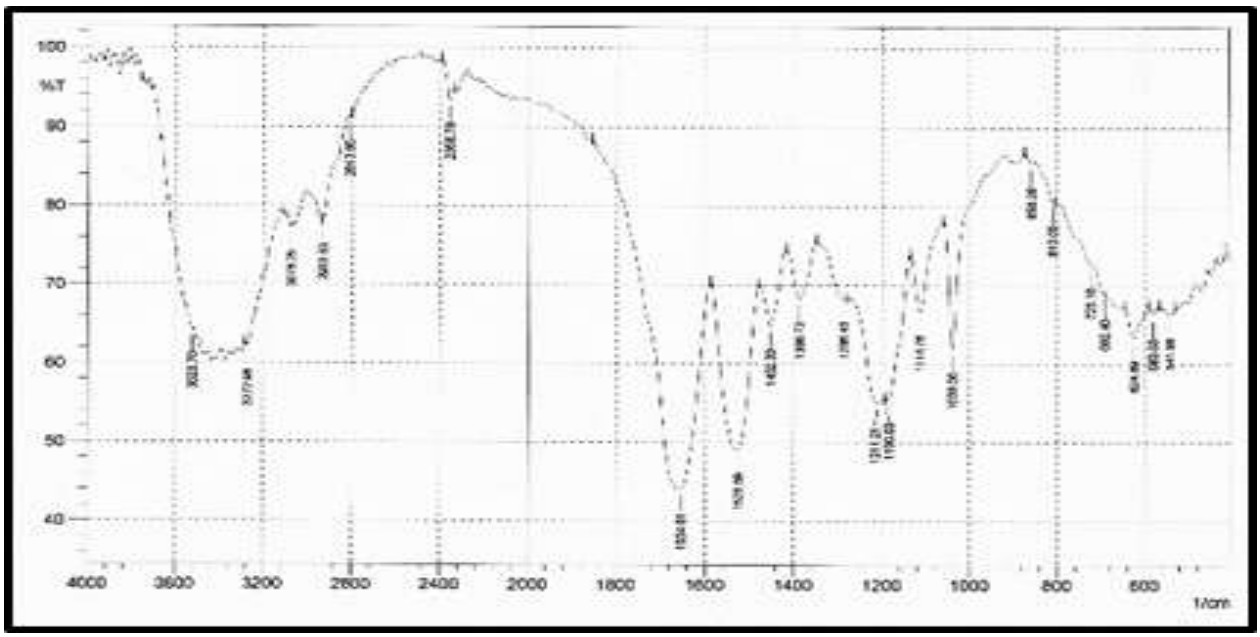
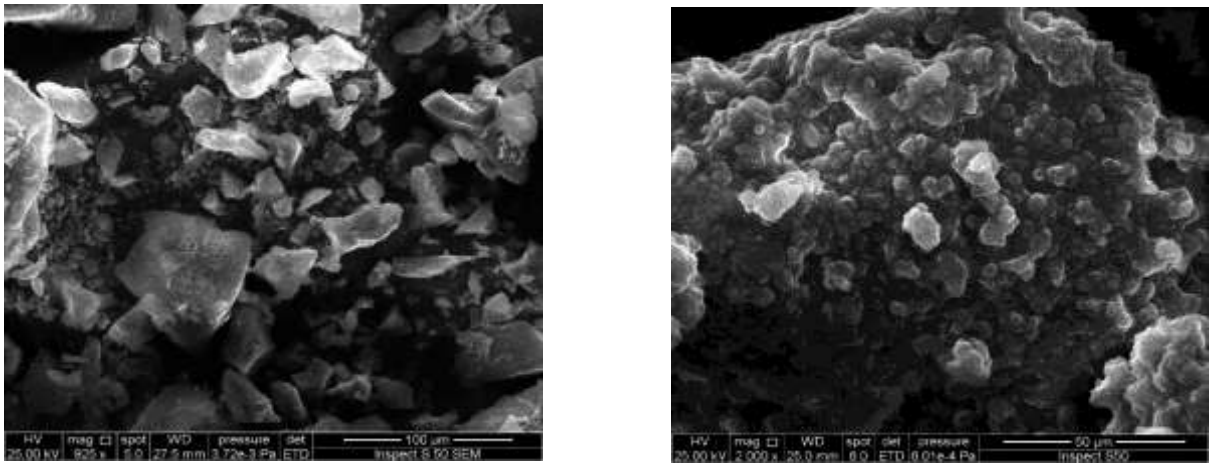



**Figure 7. FTIR BMSP-MIP2-2-AAMMPSA after the removal of (BMSP)**

**Characterization**

Morphological analysis is very important for clarifying the particles design and their volumes before and after the mold (BMSP) molecule removal of the polymer occurs. Structural analysis of molecules shows an existence of very small particles, which are polymeric spherical particles having tiny volumes BMSP-$MIP_2$-2-AAMMPSA (0.315 µm - 0.4082 µm) before the mold (BMSP) molecule removal happens. The other set of volumes (0.2392 µm - 0.2944 µm) of BMSP-$MIP_2$-2-AAMMPS comes after the mold (BMSP) molecule removal, where the wholes becomes obvious.



**(b)** **(a)**

**Figure 8. SEM photograph of the surface of BMSP-MIP2 - 2-AAMMPSA, (a) before (BMSP) removal (b) after (BMSP) removal**

**Application of Method**

The aforementioned method was applied utilizing Solid Phase Extraction and was conducted for two concentrations (within the calibration curve) that are (25 and 50) µg.$ml^{-1}$ for two materials. The materials are BMSP (the standard material) and Betasone pharmaceutical and have the same concentrations for three repetitions for every measurement process. Then, a scan with wavelengths of (200 – 400) nm for the prepared combinations was carried out; hence, the results exhibited efficient accuracy and consistency. Moreover, Rec% took values of (98.400035 - 99.404218) %, and RSD% took values of (0.572589 - 1.012777) % of BMSP

drug for the Betazone pharmaceutical, as depicted in Tables (5) and Tables (6).

**Table 5. Results of applying the method on BMSP-MIP$_1$-AAM and BMSP-MIP$_2$-2-AAMMPSA that were prepared using Solid Phase Extraction for the concentrations (25 and 50) µg.ml$^{-1}$ in their pure form**

| Sample | Method | conc (µg/mL) | | Rec % | RSD % |
|---|---|---|---|---|---|
| | | Taken | Found | | |
| Standard solutions (BMSP) | BMSP-MIP$_1$-AAM. | 25 | 24.6983 | 98.7932 | 0.5726 |
| | | 50 | 49.5869 | 99.1738 | 0.7994 |
| Standard solutions (BMSP) | BMSP- MIP$_2$-2-AAMMPSA. | 25 | 24.8511 | 99.4042 | 1.0128 |
| | | 50 | 49.2000 | 98.4000 | 0.8882 |

**Table 6. Results of applying the method on BMSP-MIP$_1$-AAM and BMSP-MIP$_2$-2-AAMMPSA that were prepared using Solid Phase Extraction for the concentrations (25 and 50) µg.ml$^{-1}$ for Betasone pharmaceutical**

| Sample | Method | conc (µg/mL) | | Rec % | RSD % |
|---|---|---|---|---|---|
| | | Taken | Found | | |
| Betasone Tablet 0.5mg | BMSP-MIP$_1$-AAM. | 25 | 25.3054 | 101.2215 | 0.5969 |
| | | 50 | 50.4165 | 100.8331 | 0.7770 |
| Betasone Tablet 0.5mg | BMSP- MIP$_2$-2-AAMMPSA. | 25 | 25.1498 | 100.5994 | 0.7287 |
| | | 50 | 50.8129 | 101.6259 | 0.6726 |

**Method comparison**

The proposed method was compared with a reference method, which is the Constitution of British Medicine, through the test F-test at a confidence level of 95 % confidence level and at the rate of three replicates. The results showed significant differences as compared to F (Table 19). The calculated values of F were 15.2 and 14.7 for the polymers BMSP-MIP1-AAM and BMSP- MIP2-2-AAMMPSA respectively. The results signifies the successful method of the printed molecule polymer in estimating Betamethasone sodium phosphate in pharmaceuticals.

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