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RESEARCH PAPER

Mechanism of Preparation of Azo Dyes Derived from Cephalosporin by Chromogenic Reagent and Use of Synthesized Nano-Polymers Surface as an Adsorbent model for Future Work of Water Treatment

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ABSTRACT

In this study, preparation of azo dyes as appropriate functional azo groups. Then pointed out best chemical properties of azo dyes by Mechanism preparation of Azo dye derived from Cephalosporin (Ceftazidime and Cefotaxime) and application in Pure Pharmaceutical dosage. Also in this study , synthesized poly (AM-co-AC) hydrogel by free radical copolymerization, was utilized as an initiator for the free radical reaction in the presence of a catalyst, potassium persulfate (KPS), and N,N-methylene-bis-acrylamide (MBA) as crosslinking agent. The overlay nanopolymer was diagnosed utilized techniques, like FESEM, TEM and XRD measurements, this surface have a properties could be applied for future work of water treatment. Precision, selective, rapid, sensitive, inexpensive, and accurate spectrophotometric method has been developed for the study of cefotaxime in pure pharmaceutical dosage. The oxidative coupling reaction of the cefotaxime drug with 2,4-dinitrophenyl hydrazine in potassium periodate as a chromogenic reagent in alkaline medium to preparation of azo dye form a color-stable orang product soluble in water with a maximum λmax of 580 nm for two drug (Ceftazidime and Cefotaxime). The best conditions for the estimation were established, like the effect of volume of the reagent, the order of additions, the effect of volume of sodium hydroxide, the effect of temperature, the effect of solvent, and the effect of oxidation time. That obeyed law lambert beer in linearity of the concentration (1–10 mg/L) of cefotaxime, correlation coefficient of R2 (0.9979), (0.9689) and LOD(1.2×10-4 µg/ml), (1.4×10-3 µg/ml),and LOQ (9.2×10-4 µg/ml), LOQ (8.3×10-3µg/ml), for two drug (Ceftazidime and Cefotaxime) respectively. The value of recovery% was in the range of 99.16–100.7 (n = 3), which indicates the precision of the developed method. This method is useful successfully for the determination of for two drug (Ceftazidime and Cefotaxime) in pharmaceuticals (injection).

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INTRODUCTION

In recent years, the widespread use of pharmaceutical has led to better quality control of medicines. At the same time, the Oxidative Coupling has become one of the most important techniques that contribute significantly to the development of automation in pharmaceutical analysis [1-3]. Oxidative coupled organic reactions appear to be one of the most suitable Oxidative Coupling identifications of drugs like Paracetamol, Methyl dopa, Cefotaxime, Sulphonamide, Folic acid, and phenylephrine, Catechol amines have been determined via visible spectrophotometry after reaction with Chloranil, Fe(III) and o-Phenathroline, Meta periodate, Palladium Chloride Ammonium, and Meta Fandate [1, 4-15].

Cefotaxime and Ceftazidime is from the β-lactam family and a group of cephalosporin antibiotics. It is prescribed mainly because of its widespread antimicrobial use and tissue penetration. The most serious infections occur among children with serious illnesses, including severe sepsis, meningitis, and others. utilized to treat a several of bacterial infections in human, other plant and animals' tissue, like urinary tract infections, pelvic inflammatory disease, pneumonia, meningitis, sepsis, gonorrhea, and cellulitis [16, 17]. Cephalosporin (Cefotaxime and Ceftazidime and) give either via injection in to a muscle or vein. Chemical formula $C_{16}H_{17}N_5O_7S_2$, $C_{22}H_{22}N_6O_7S_2$, molar mass 455.46 g·mol^{-1}, 546.56 g.mol^{-1} and chemical stretcher at the seam order [5], as show in Figure 1.

2,4-Dinitrophenylhydrazine (DNPH) is the solid material a red -orange powder. The chemical formula $C_6H_6N_4O_4$, Linear Formula $(O_2N)_2C_6H_3NHNH_2$, molar mass 198.14 g/mol, Solubility in water Slight. It can be syntheses via the reaction of hydrazine sulfate with 2,4-dinitrochlorobenzene [18-20]. The chemical stretcher shows in Fig. 1. A several techniques have been developed for estimation Cefotaxime which contain fluorimeter, chemiluminescence, voltammetry, infrared spectroscopy, flow injection, spectrofluorometric, spectrofluorometric, LC-MS, HPLC, GC-MS, chromatographic and electrochemical

Hydrogels are characterized by their three-dimensional polymeric networks and hydrophilic properties, and provide a promising solution with their good affordability, non-toxicity, physical and chemical stability, in addition to their exceptional

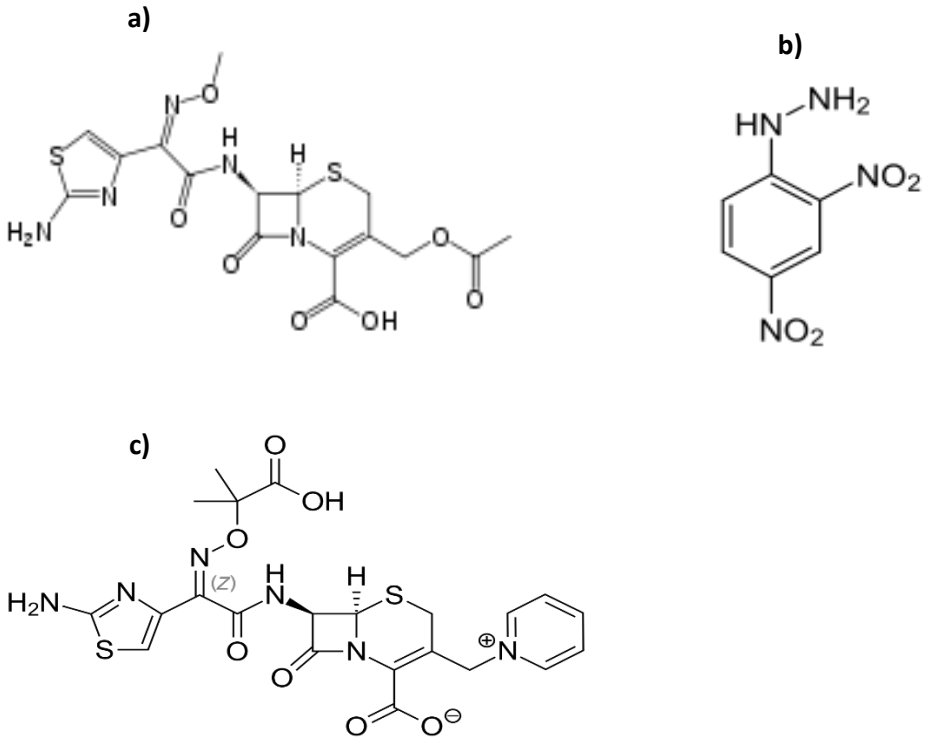

Fig. 1. Chemical stretcher of a) Cefotaxime drug, b) 2,4-Dinitrophenylhydrazine (DNPH) , c) Ceftazidime drug .

Table 1. Analytical factors of spectrophotometric method

Parameter	Value
linearity range mg/L	1-10
Regression equation	Y=0.1113X+0.0185
Linearity R^2	0.9979
Slope	0.1113
intercept	0.0185
LOD mg/L	$1.2*10^{-4}$
LOQ mg/L	$9.2*10^{-4}$
RSD%	0.59
wavelength nm	580

ability to absorb and retain water [21-24]. The gels are prepared from natural polymers such as sodium alginate, starch, chitosan. or from synthetic polymers such as acrylic acid or acrylamide, but it is preferable to prepare it from a mixture of natural and synthetic polymers to increase its efficiency and ability to retain water [25-27].

MATERIALS AND METHODS

Preparation of poly (AM-co-AC) hydrogel

The biopolymer was prepared by free radical copolymerization with a ratio of 5 g acrylic acid in 10 ml distilled water, stirring for 10 minutes. Also, we dissolved 2 g in 5 ml acrylamide (AM) in distilled water, stirring for 10 minutes. We add to the homogeneous mixture the free radical initiator potassium sulfate 0.03 g (KPS), then dissolve 0.08 g in 5 ml of distilled water (MBA) with continuous stirring, then add nitrogen gas (N_2) to the previous total solution with continuous stirring for 15 minutes. The above total solution is transferred to closed tubes and placed in a water bath at a temperature of 60°C. For 3 hr. To complete the reaction.

Preparation of solutions

Nitrite solution

The sodium nitrite solution was prepared via dissolving 0.1 g of the compound in 10 mL distilled water (1% w/v).

Hydrochloric acid

The solution of HCl was prepared via diluting 3 ml of conc. Acid in to 12 ml of distilled water.

Stock solution of cefotaxime drug,1000 mg/L was prepared via dissolving 0.1 g of drug in distilled water and completed by DW in volumetric flask 100 ml. this solution kept in a bulk bottle, where it

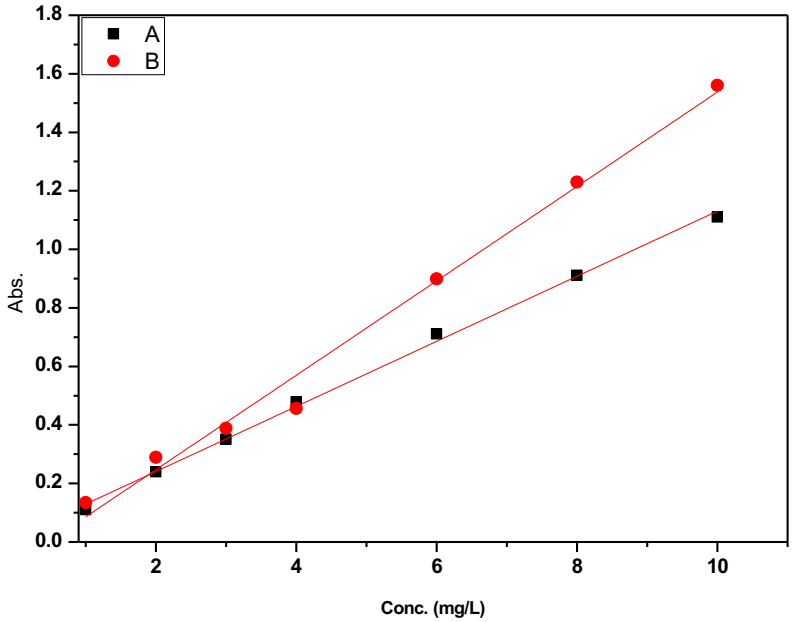

Fig. 2. Calibration curve to color complex azo dye ,A) Cefotaxime, B) Ceftazidime.

is stable at more than month.

Oxidizing agent KIO_3

Preparation solution of KIO3 0.01 M dissolving 0.2301 g from Oxidizing agent by distilled water in volumetric flask 100 ml.

Solution of NaOH

Preparation solution of NaOH 0.1 N by dissolving a 0.4g in distilled water in volumetric flask 100ml.

2,4-dinitrophenylhydrazine DNPH solution:

Preparation solution of DNPH (0.01 M) by dissolving 0.198 g in 1 ml of (6.0 M) H_2SO_4 and completed by distilled water in volumetric flask 100 ml.

Preparation of Azo dye of drug

(0.01 mole, 6.51 g, 7.54 g) from the drug compounds (cefotaxime, ceftazidime) was dissolved respectively in the beaker having 5ml

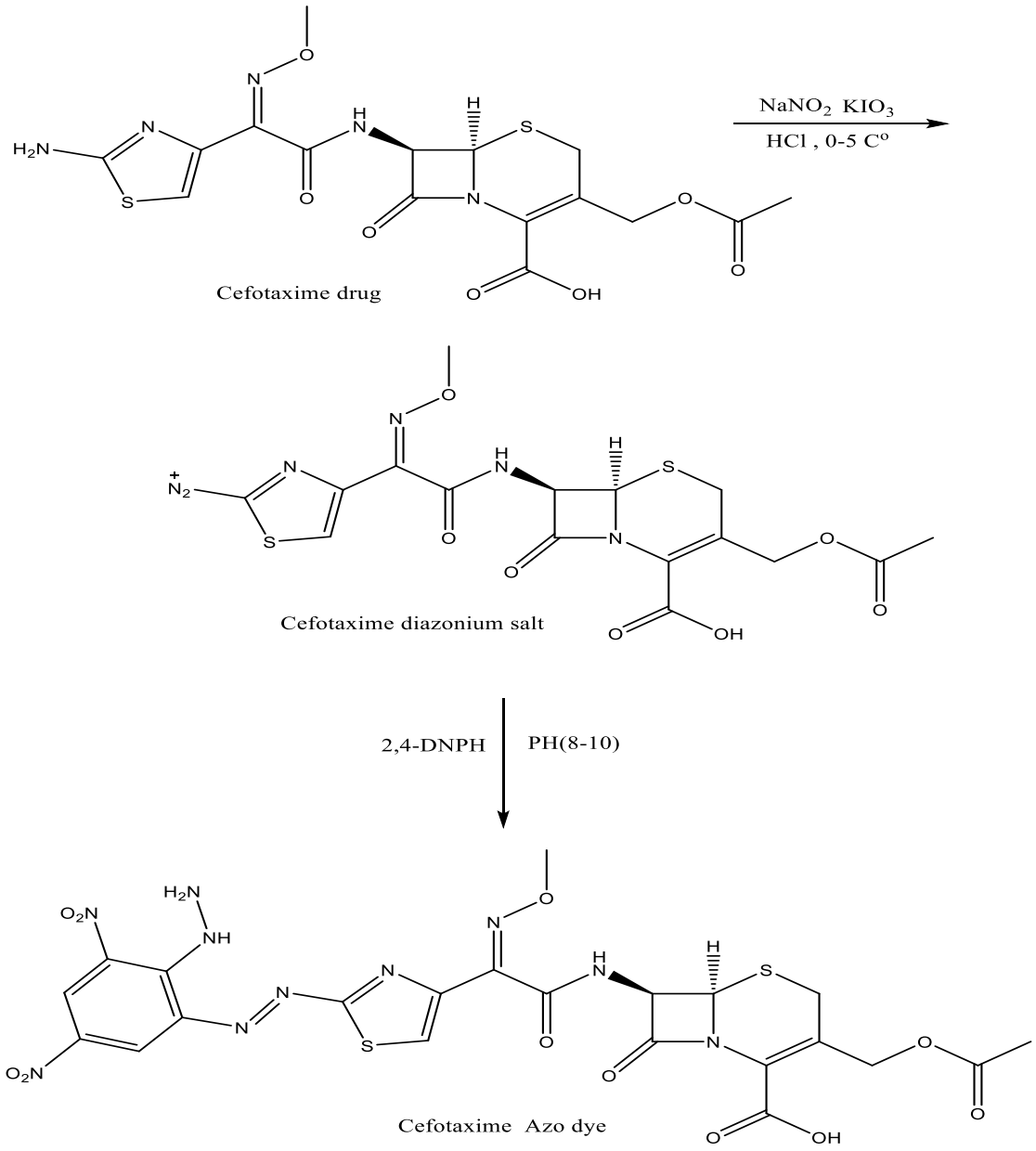

Fig. 3. Synthesis of Cefotaxime Azo dye.

of prepared HCl and then the solution cooled in ice water bath at (0-5˚C). In another beaker, the solution sodium nitrate was taken about 5 from prepared solution and also cooled at (0-5˚C) and then in the same solution added slowly to at the same temperature with stirring via magnetic stirrer. The formed the solution Diaz onium salt was kept at (0-5˚C) and added drop by drop to (0.01mol, 1.98 g) 2,4-DNPH solution prepared in solution sodium hydroxide 10% , the PH was maintained among (8-10) at (0-5˚C) and then the mixture was stirred for 30 min. The final product was precipitated, filter and washing several time by distilled water and re-crystallized by ethanol, Rf = 0.7 (benzene : ethanol, 2: 1).

Calibration curve

The calibration curve was prepared via addition 2ml of Cefotaxime drug, 3 ml of solution KIO3 and 2 ml of 2,4-dinitrophenylhydrazine DNPH solution to volumetric flasks of 10 ml, in basic medium 0.5 ml NaOH, then the absorbance was measured against

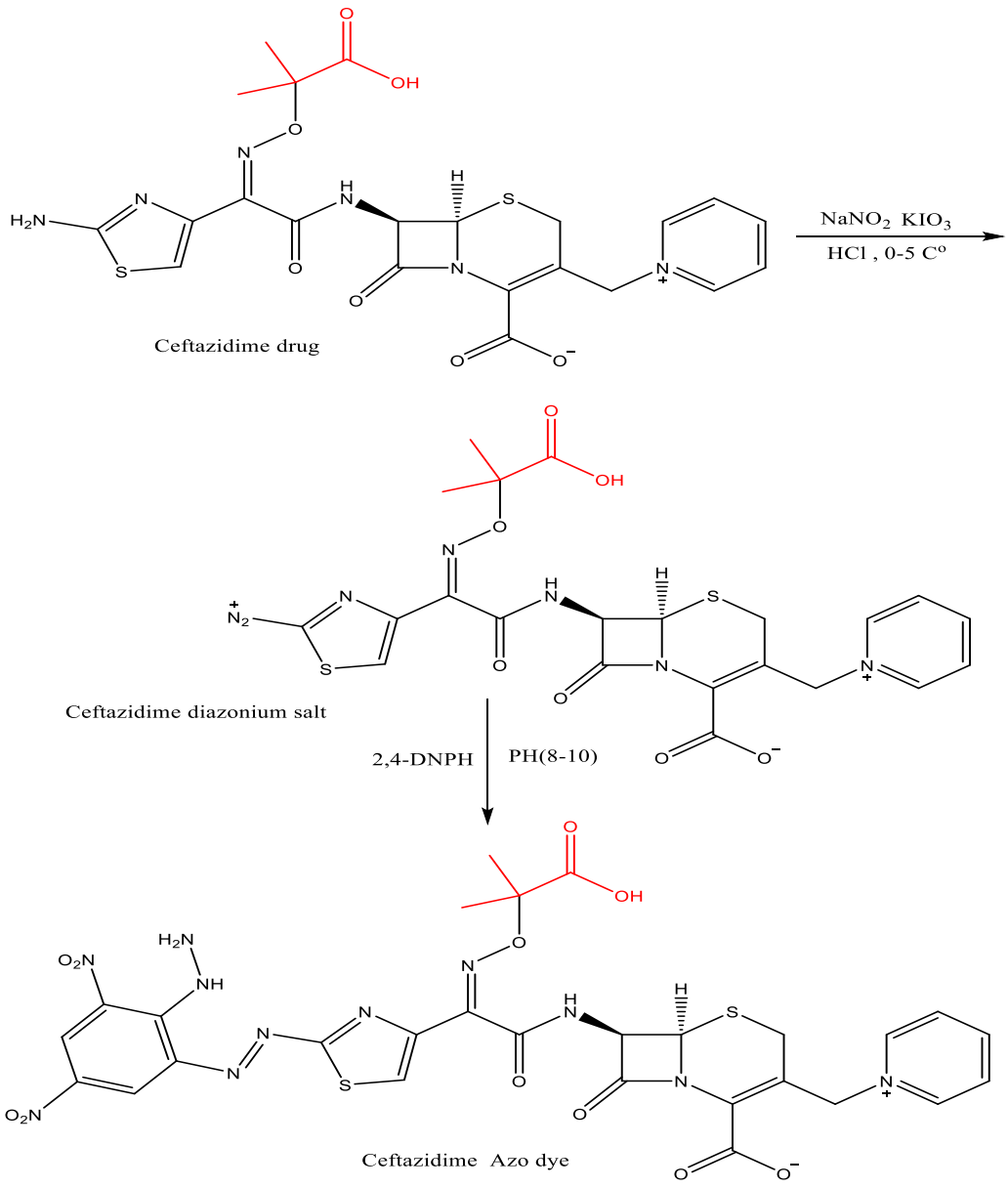

Fig. 4. Synthesis of Ceftazidime Azo dye

reagent blank after dilution with distilled water at the wavelength 580 nm Fig. 2 show linearity range (1-10 mg/L) of estimation of drug. The value of coefficient R2 0.9979, which statistical data shows that it has excellent linear characteristics as show in Table 1. LOD (1.2×10^{-4} µg/ml), LOQ (9.2×10^{-4} µg/ml) respectively.

Mechanism of the Reaction method of Azo dye of Cefotaxime

Cefotaxime drug was reacted with a solution of $NaNO_2$ to form a diazonium salt to form a complex color with reagent 2,4-dinitrophenylhydrazine DNPH in basic medium in the presence of potassium iodate (KIO_3). Under the best optimum conditions of the reaction, 2,4-dinitrophenylhydrazine DNPH oxidation with potassium iodate which is Oxidative Coupling Reaction method species. The intermediate undergoes substitution electrophilic with the phenolic moieties of Cefotaxime drug to result a complex color azo dye .as shown in Fig. 3.

Mechanism of the Reaction method of Azo dye of Ceftazidime

Ceftazidime drug was reacted with a solution of $NaNO_2$ to form a diazonium salt to form a complex color with reagent 2,4-dinitrophenylhydrazine DNPH in basic medium with presence potassium iodate (KIO_3). Under the best optimum conditions of the reaction, 2,4-dinitrophenylhydrazine DNPH oxidation with potassium iodate which is Oxidative Coupling Reaction method species. The intermediate undergoes substitution electrophilic with the phenolic moieties of Ceftazidime drug to result a complex color azo dye .as shown in Fig. 4.

RESULT AND DISCUSSION

Characterization of Adsorbent

The Field Emission Scanning Electron Microscopy (FE-SEM) technique is utilized to study the surface characteristics of the grafted hydrogel. This technique provides insights into the particle shape, their aggregation nature, crystalline structure, and surface area[26, 28]. It also helps in determining the surface's porosity or smoothness and the homogeneity of the composite components' distribution on the surface. The FE-SEM images, as shown in Fig. 5 reveal that the surface of the hydrogel is smooth, clear, and flaky. It also possesses a porous structure resembling a sponge and a network of tightly packed layers due to the cross-linking agent between the polymeric chains [25].

X-ray diffraction (XRD) spectra were used to study the structural properties, represented by composition, crystalline size, and spacing between crystalline planes [24], of the prepared Poly(AM-co-AC) Hydrogel in its solid state using single light of wavelength 1.5104 A^0 from a (Cu-Kα) source within the angular range 2θ (5-80) degree, the XRD patterns for the Poly(AM-co-AC) were observed, the broad peak at 20.109° indicates that the hydrogel composites are semi crystalline, with a significant proportion of amorphous material

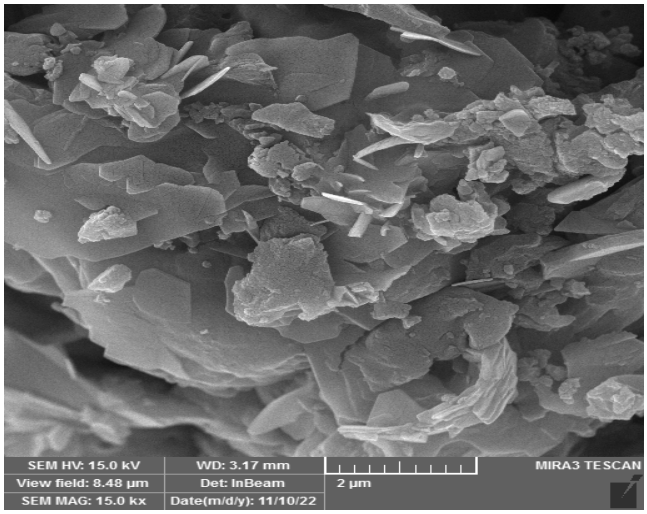

Fig. 5. FESEM image of Poly(AM-co-AC) Hydrogel

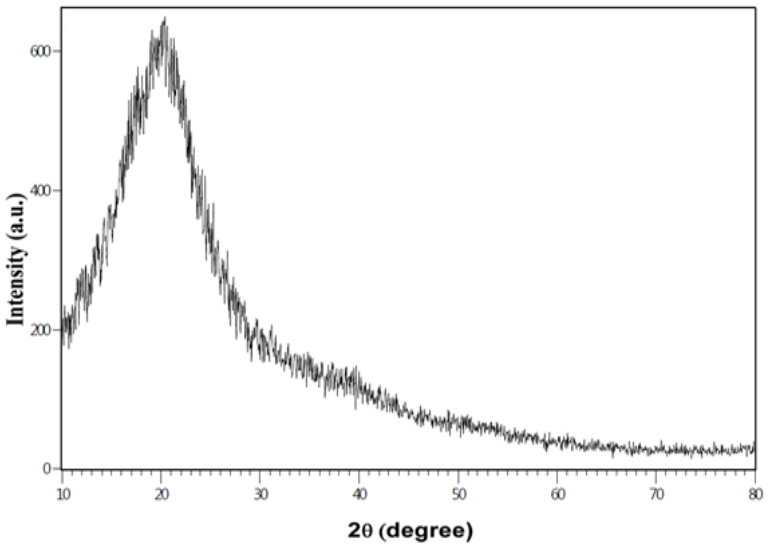

Fig. 6. XRD of of Poly(AM-co-AC) Hydrogel

[29, 30], as show in Fig. 6.

Characterization of azo dye

The FT-IR spectrum of the prepared azo dye was recorded as KBr disk using shimadzu apparatus in rang (4000-400 cm^{-1}). The spectral data of the product was gathered in Figs. 7 and 8: The of cefotaxime azo dye exhibited intense IR absorptions at 3371 cm^{-1} [ν(-O-H)], 3236 cm^{-1} [ν(-NH_2)], 3101 cm^{-1} [ν(C-H aromatic)] , 2928 cm^{-1} [ν(C-H aliphatic)], 1716 cm^{-1} [ν(C=O) lactam] ,1624 cm^{-1} [ν(C=C)], 1593 cm-^{1} [ν(-N=N-)] .The IR spectrum of ceftazidime azo dye which is shown in Fig. 7 exhibited intense IR absorptions at 3360 cm^{-1}

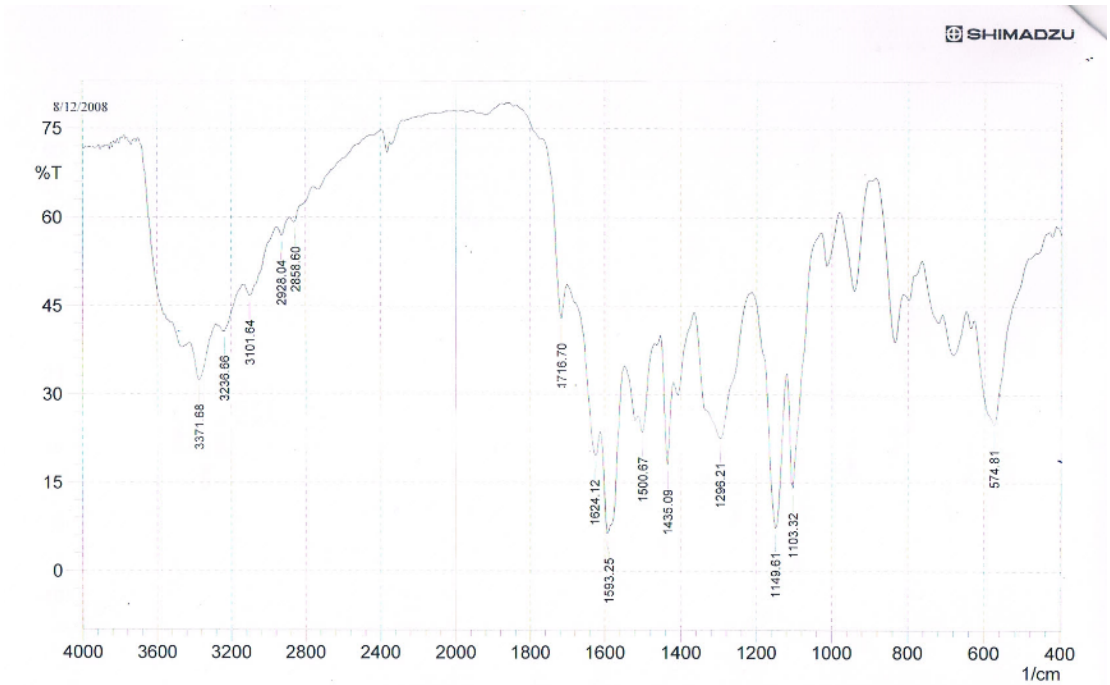

Fig. 7. IR spectrum of cefotaxime azo dye

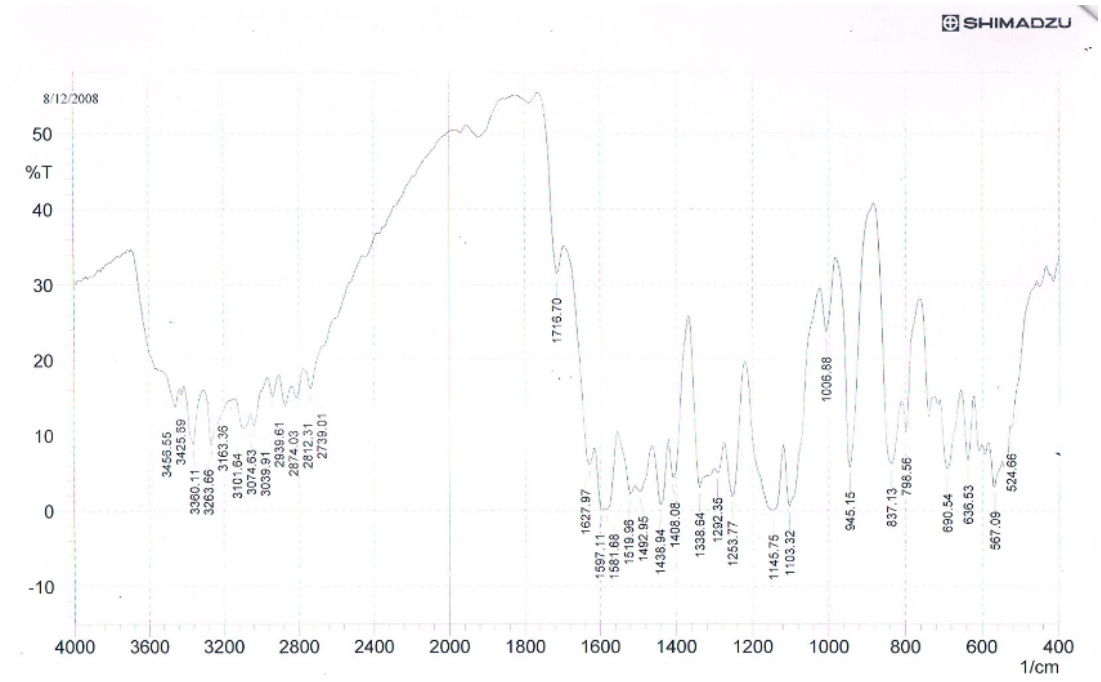

Fig. 8. IR spectrum of ceftazidime azo dye

[ν(-O-H)], 3263 cm^{-1} [ν(-NH_2)], 3101 cm^{-1} [ν(C-H aromatic)], 2939 cm^{-1} [ν(C-H aliphatic)], 1716 cm^{-1} [ν(C=O) lactam], 1627 cm^{-1} [ν(C=C)], 1597 cm^{-1} [ν(-N=N-)].

Type of oxidizing agent

Several kinds of oxidizing agents were utilized to select the better oxidizing to give the maximum color intensity as show in (Table 2). The data showed in Table 2 oxidizing agent potassium Periodate give the higher absorbent, and maximum color intensity of compound. The influence of several volumes of potassium Periodate (0.5-4 ml) on the stability of intensity color has been studied, it was experiential potassium Periodate about 3ml is the utmost suitable quantity, since it gives the maximum color intensity of the formed compound thus it is chosen for further studies as show in Fig. 9.

Effect of 2,4-dinitrophenylhydrazine DNPH solution

The reagent has a fundamental and important role in the Oxidative Coupling Reaction, forming the colored complex and increasing sensitivity and selectivity. Different volumes of 2,4-dinitrophenylhydrazine DNPH solution (1-5 ml) were study, the data indicated that utilizing about 2ml DNPH solution gives best color intensity and absorbance of the compound at 580 nm and the volume was considered as a best value [3, 31] as show in Fig. 10.

Effect of type and volume base

The preliminary experiments have appeared Cefotaxime drug give best color intensity with DNPH in the found of KIO_4 in base medium, so that several kind types of bases as show in (Table 3).

It is noted from the results shown in Table 3

Table 2. Effect of different oxidizing agent to formation color complex

Type of oxidizing agent	Abs. Cefotaxime	Abs. Ceftazidime
$K_2Cr_2O_7$	0.0678	0.117
KIO_3	0.351	0.454
K_2CrO_4	0.0987	0.232
KIO_4	0.311	0.411

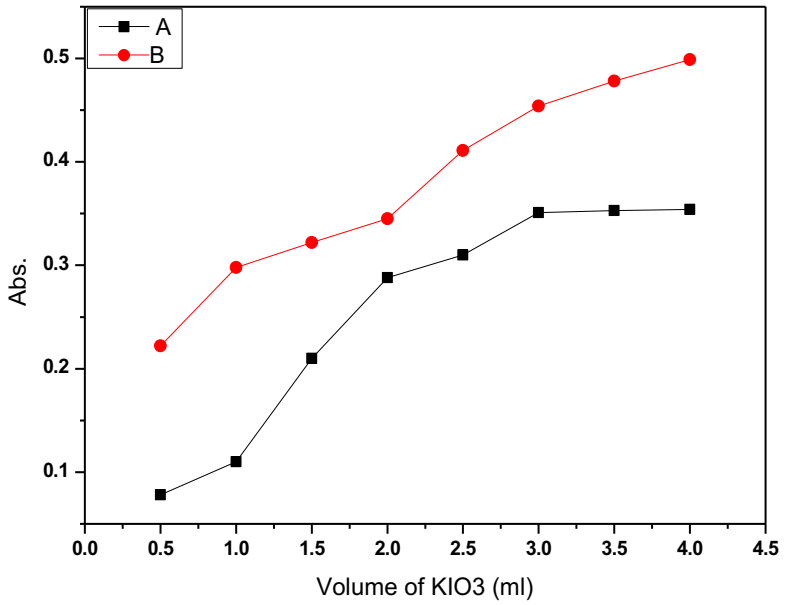

Fig. 9. Effect of volume of oxidizing agent (potassium Periodate) ,A) Cefotaxime, B) Ceftazidime.

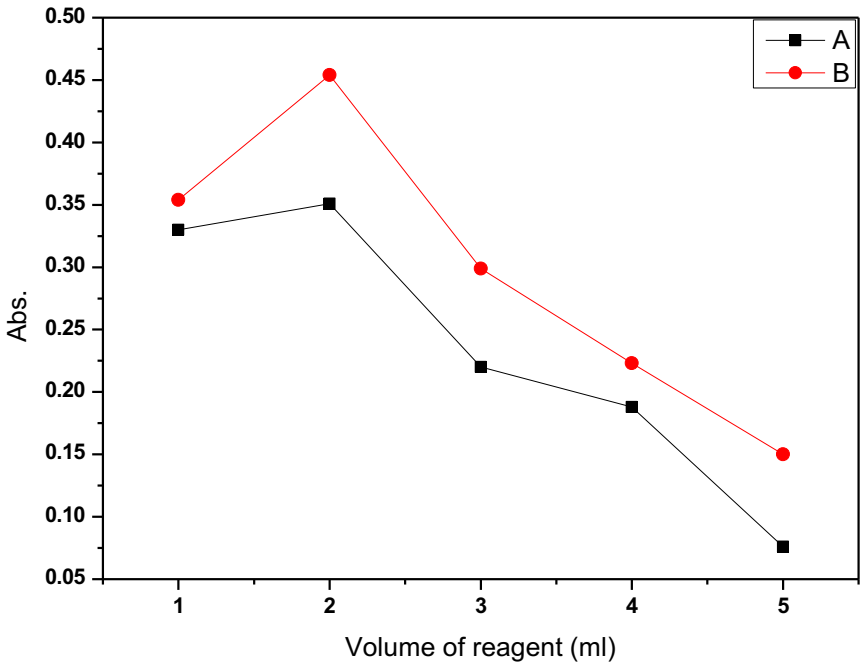

Fig. 10. Effect of reagent 2,4-dinitrophenylhydrazine DNPH solution. ,A) Cefotaxime, B) Ceftazidime .

that the use of sodium hydroxide base increases the absorbance and also increases the intensity of the colored product and makes the reaction stable [32, 33]. This indicates that the reaction is affected in the basic medium, and the absorbance increases with the increase in the volume of the base until the reaction stabilizes over time as show in Fig. 11.

Table 3. Several kind types of bases to stability of color complex

Type of base	Abs Cefotaxime	Abs Ceftazidime
KOH	0.267	0.411
NaOH	0.351	0.454
$NaHCO_3$	0.111	0.322
Na_2CO_3	0.178	0.222

Effect of time

The best maximum intensity color and higher absorbance, after drug was reaction with DNPH and potassium parodied after 10 min in alkane medium. Therefore, 10 min. development time was selected as best in the general reaction and sufficient for complete the reaction and adopted

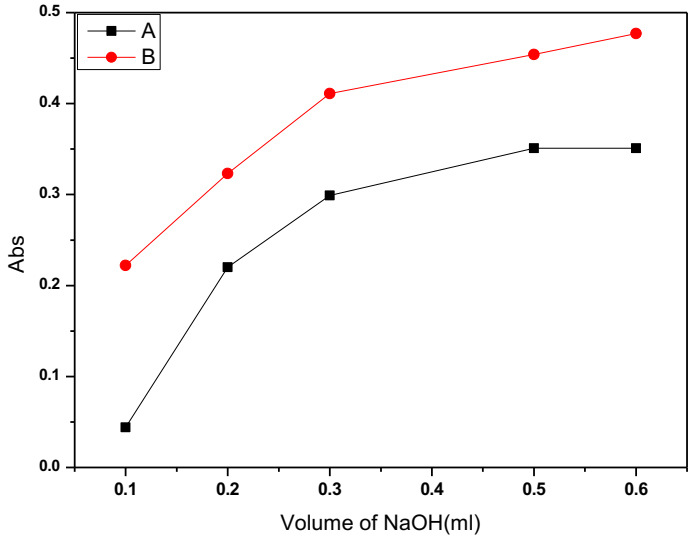

Fig. 11. Effect of volume of sodium hydroxide. ,A) Cefotaxime, B) Ceftazidime .

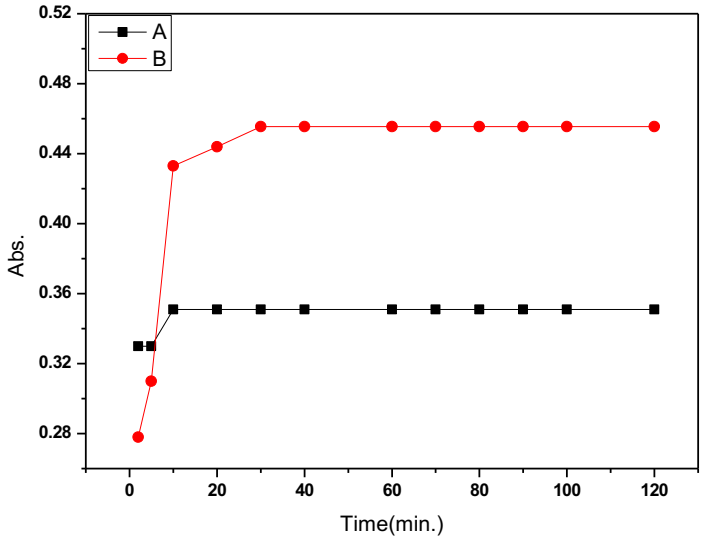

Fig. 12. Effect of equilibrium time onto stability of complex formation. ,A) Cefotaxime, B) Ceftazidime .

Table 4. Effect of several order addition

NO	TYPE OF ORDER ADDITION	ABS CEFOTAXIME	ABS CEFTAZIDIME
I	Drug+KIO4+ DNPH+ NaOH	0.351	0.454
II	Drug+ DNPH +KIO4 +NaOH	0.333	0.433
III	Drug + DNPH+ NaOH+ KIO4	0.311	0.411
IIII	DNPH +Drug+ NaOH+ KIO4	0.1111	0.0932
IIIII	DNPH + KIO4+ Drug+ NaOH	No color	0.022

Table 5. Effect of different temperature to improve color of complex

Temperature (°C)	Abs Cefotaxime	Abs Ceftazidime
10	0.011	0.029
15	0.09	0.301
20	0.23	0.433
25	0.35	0.454
40	0.278	0.111

Table 6. Effect of several solvents to improve color of complex

Type of Solvent	Abs.	Abs Ceftazidime
Water	0.351	0.454
Acetone	0.121	0.093
Ethanol	0.098	0.033
Methanol	0.065	0.025

Table 7. determination of Cefotaxime drug in pharmaceutical formulation

Pharmaceutical preparation	Conc. Of drug (mg L^{-1})		E %	Rec%
	present	Found		
Cefotaxime injection 250 mg, Iran	8	7.96	-0.5	99.497
	6	6.11	1.8	101.8
	4	4.12	2.9	102.9
Cefotaxime injection 500 mg India	8	8.20	2.4	102.4
	6	5.98	-0.33	99.6
	4	4.11	2.67	102.6
Cefotaxime injection 10 mg Iran	8	7.89	-0.01	99.98
	6	6.2	3.2	103.2
	4	3.98	-0.5	99.49

in the all experiments. thus, the color stable obtained for 2 h [34], as show in Fig. 12.

Effect of order addition

The effect of different subsequent addition reaction component on the absorption of the intensity colored dye was studied and data appear in Table 4, indicate that subsequent (I) was best order for reaction components, so it was chosen for next experiments [35].

Effect of temperature

The influence of solution temperature on the formation of color dye was studied at several temperatures and data appear in the Table (5), confirm that when the reaction is carried out at 25

Table 8. Optimum preparation of Nano polymers and application to removal azo dye

Sorbent	Azo dye	Initial	pH	t (hr.)	Cross-linked	Monomer	Ref.
SA-g-Poly(AM-co-AC)/TiO_2	BB	KPS	7	3	MBA	AM	[40]
Poly(AM-co-GO)	CR	KPS	6	3	MBA	AM	[41]
Poly Nano Chitosan/ ZnO	CR	KPS	6	2	MBA	CS	[42]
Poly Nano Chitosan/ ZnO	MB	KPS	6	3	MBA	CS	[42]
SA/acid activated bentonite beads (A-AAB)	CV	-	9	4	CaCl2	SA	[43]
Poly (SA/bentonite beads)	CV	-	9	4	CaCl2	SA	[43]
Poly (AM/SH/clay)	MB	KPS	5	3	MBA	AM	[44]
Poly (SA-co-PAA)	MB	KPS	3	2	MBA	SA	[45]
Poly (SA-g-PAA/TiO2)	Methyl violet	KPS	4	2	MBA	SA	[45]
poly (AM-co-AC)	New azo dyes	KPS	4.2	3	MBA	AM	In this work

^{o}C . The effect of temperature solution on the color intensity of the product was studied. Through practical experiments, the best absorption and highest color intensity were obtained at a temperature (25 ^{o}C), however, when the temperature was increased by placing the solution in a water bath (40 ^{o}C) or the temperature was decreased by placing the solution in an ice bath (10 ^{o}C). It was observed that the absorbance, color intensity and stability decreased, and therefore the reaction must be carried out at a temperature of (25 ^{o}C) [36, 37].

Effect of organic solvents

The effect of the organic solvents on the absorbance of formed best color dye was study via using several solvents instead of water the data appear in Table 6 and Fig. 8 reveal that using water as solvent give the maximum absorbance of color dye formed, and water best solvent, safety in use, extensive, eco-friendly comparing with other solvent[38].

Application

The proposed method was applied in indirect determination of Cefotaxime drug (in pharmaceutical formulation) like injection formulations using several concentrations about 4,6,8 mg/L were transferred volumetric flasks 10 ml and treated as in construction as method in the calibration curve [39]. The absorbance was measured at 486 nm for three times. and appear the capacity and successfully of the developed way to estimation of drug in its pharmaceutical formulation, the recovery was 99.4-103.2 %. as show in Table 7.

Literature survey for the preparation of Nano polymers

Table 8 represents the preparation of environmentally friendly, biodegradable polymers that can be prepared from natural polymers and synthetic polymers, as they improve their ability to increase active hydrophilic groups. Previous research can be compared with the current work in terms of efficiency and ease of preparation, also about the high ability of Nano polymers to remove different types of azo dyes. In this work, a polymer was efficiently prepared and it is proposed to be applied as a future work in removing azo dyes, as

shown in the Table 8.

CONCLUSION

in this study studied preparing the poly (AM-co-AC) hydrogel by free radical copolymerization, was utilized as an initiator for the free radical reaction in the presence of a catalyst, potassium persulfate 0.03 g (KPS), and N,N-methylene-bis-acrylamide (MBA)0.08g as crosslinking agent . The method of the reaction Cefotaxime drug with reagent 2,4-dinitrophenyl hydrazine in basic medium proses to produce orange dye product to estimate the for two drug (Ceftazidime and Cefotaxime) pure and in pharmaceutical. The concentration obeys Beer's law at range 1 and 10 mg/L. Beer's law is met by the product in terms. That obeyed law lambert beer in linearity of the concentration (1–10 mg/L) of cefotaxime, correlation coefficient of R^2 (0.9979), (0.9689) and LOD (1.2×10^{-4} µg/ml), (1.4×10^{-3} µg/ml), and LOQ (9.2×10^{-4} µg/ml), LOQ(8.3×10^{-3} µg/ml), Recovery between 99.6%-103.3% at wave length 580 nm were obtained at a temperature (25 °C).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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