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RESEARCH ARTICLE

NOVEL SYNTHESIS OF PEG COATED IRON NANOPARTICLES (Fe₃O₄) AND IT'S EVALUATION OF CONTROLLED RELEASE KINETICS IN DRUG DELIVERY SYSTEM

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ABSTRACT:

Magnetic Nanoparticles (MNPs) because of their high magnetic responsiveness, biodegradability, high delivery efficiency and potential targeting function is a possible material as drug delivery system, since drug-loaded MNPs can be directly injected in to solid tumors and are expected to be held in place by an external magnetic field and to release the drug in a controlled manner. In the present research study Synthesis, Characterization and in vitro rate studies of super paramagnetic Iron Nanoparticles coated with PEG has been carried out. The nanoparticle synthesized in the present study had an average particle size of ~ 30 nm. starch-based polysaccharide nanocarrier encapsulated with the drug i.e. THC and MNPs as anticancer drug delivery was constructed. This efficiently reduces tumor growth thus providing a proof of concept for the utilization of this formulation in cannabinoid-based anti-cancer therapies. Evaluation of THC loaded magnetic nanoparticles was successfully carried out and correlation coefficient (R²) value 0.999 and drug released was found to be 97% for 48hrs at pH7.0. The entrapment efficiency and drug loading of THC-MNPs in the starch based nanoparticles was found to be high.

Keywords: Iron Nanoparticles, Magnetic Responsiveness, Tumor Growth**1. INTRODUCTION:**

Nanomaterials are the most challenging areas of current scientific and technological research because of their tremendous possibilities in generating novel shapes, structures and the unusual phenomena associated with materials. The field of nanotechnology is one of the most popular areas for current research and development in basically all technical disciplines. Despite these promising biomedical properties, free curcumin molecules suffered from low water solubility, which in turn have resulted in poor bioavailability and clinical efficacy¹. Hence, researchers have attempted to enhance water solubility and bioavailability of curcumin by loading of curcumin in biodegradable polymeric nanoparticles. For instance, curcumin loaded poly(lactid-coglycolic acid) PLGA nanospheres were formulated for prostate cancer therapy.

Tetrahydrocannabinol (THC) is the active chemical in cannabis and is one of the oldest hallucinogenic drugs. THC comes from the flowering tops and leaves of the hemp plant, Cannabis sativa.² The THC belongs to a class of chemicals called terpenoids. Thus, THC is widely used either as a single agent or in combination with other chemotherapeutics regimens for various kinds of tumors. Intratumoral of chemotherapeutic agents is a potentially more effective modality to overcome the described limitation and this has been extensively evaluated using a number of anticancer drugs. Such targeted delivery may realize drug localization within the tumor tissue and divert the drug from nontarget organs to

improve toxicity and increase efficacy, while decreasing the incidence and the intensity of side effects.³

Magnetic Nanoparticles (MNPs) because of their high magnetic responsiveness, biodegradability, high delivery efficiency and potential targeting function is a possible material as drug delivery system, since drug-loaded MNPs can be directly injected in to solid tumors and are expected to be held in place by an external magnetic field and to release the drug in a controlled manner.⁴

Starch is a well-known, versatile, and inexpensive polysaccharide which has received great attention in drug delivery applications as they are hydrophilic, biodegradable and biocompatible with tissue and cells. Starch has been used as drug delivery system for tumor-targeted drug delivery, trans-dermal drug delivery and brain tumor-targeted drug delivery. A starch microsphere is utilized as drug delivery for tissue engineering. This starch microsphere further loaded with definite growth factors and immobilized, and can be employed for delivery of encapsulate living cells.⁵

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2. MATERIALS:

Fe (SO₄).7H₂O (Sigma Aldrich) Fe₂ (SO₄)₃ (Sigma Aldrich), Ammonium hydroxide (NH₄OH, S.D Fine Chemicals), PEG (Sigma Aldrich), acetone (S.D Fine Chemicals), THC (Sigma Aldrich), Starch. (S.D Fine Chemicals), Pluronic F-127 (Sigma Aldrich), Dichloromethane (FinarChemicals Limited), Triethylamine (Sigma Aldrich), distilled water is also used for preparation of the solution.

3. EXPERIMENTAL PROCEDURE:

(a) Preparation of MNPs

3.2 gm of PEG taken in 25 ml of D.H₂O and stirred. 3.3 gm of Fe (SO₄).7H₂O in 10 ml of D.H₂O, 9.6 gm of Fe₂ (SO₄)₃ in 20 ml of D.H₂O are stirred separately. Now both Fe (SO₄).7H₂O and Fe₂ (SO₄)₃ Solutions are added drop by drop into the PEG solution. Then the solution is stirred for 30mins, Ammonia solution is added for maintain pH-10. The mixture was further stirred for 4hrs and filtered, washed with D.I. water and finally rinsed with acetone, dried in hot air oven at 60°C/ 8h. The dried compound was calcined at temperatures at 400°C for 4hrs to get Fe₃O₄ nanoparticles.

(b) Preparation of THC-MNPs

MNPs containing PEG coated Fe₃O₄ nanoparticles and THC were prepared by the conventional oil-in-water single emulsion evaporation method. Three equivalents of triethylamine were added to an aqueous solution of (5mg/mL), and the drug was then extracted to prepare a dichloromethane solution THC. Initially, synthesized nanoparticles were dispersed in dichloromethane, and the resulting dispersion was mixed with the dichloromethane solution of THC. Starch (20mg) was added to the solution of PEG –coated Fe₃O₄ nanoparticles THC (1ML). This mixture was emulsified in aqueous solution (100mL) of Pluronic F-127(7wt %) by use of a probe – type sonicator at 600W for 10 minutes in an ice bath. The resulting suspension was stirred for 12 hours at room temperature to evaporate the organic solvent and was then centrifuged at 10,000 rpm for 10 minutes. The precipitate was washed twice in water to remove the remaining Pluronic F-127and starch. The supernatant was used for analysis of THC loading efficiency.

4. EVALUATION STUDIES

For evaluation of drug contents, drug loading efficiency and percentage yield , THC MNPs(5mg) were dissolved in dimethyl sulfoxide (10mL).The THC concentration was evaluated using an ultraviolet and visible spectrophotometer at 238nm. Empty nanoparticles of PLGA were used as a blank test.

Percentage yield

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical Yield}} \times 100 \text{-----(1)}$$

Drug loading

$$\% \text{ drug loading} = \frac{\text{Weight of drug in nanoparticles}}{\text{Weight of nanoparticles taken}} \times 100 \text{-----(2)}$$

Encapsulation efficiency

$$\text{EE}\% = \frac{\text{Total amount of drug} - \text{Free drug}}{\text{Total amount of drug}} \times 100 \text{-----(3)}$$

Drug content

$$\% \text{ drug content} = \frac{\text{Weight of the total drug} - \text{weight of free drug}}{\text{Weight of nanoparticles}} \times 100 \text{---(4)}$$

4.1 Invitro Studies.

The Invitro release studies of THC nanoparticles were carried out at 37±2°C in phosphate buffer saline (PBS) pH7.0, buffer media for a period of 48hrs. A horizontal water bath shaker was to conduct invitro release studies. The 10mg of drug (THC) was suspended in 30ml of buffered solution .the platform was allowed to vibrated horizontally at an average speed of 100rpm to induce mixing in the release medium. At periodic intervals of every 2hrs.5.0ml of the released medium was sampled and replaced with fresh 5.0ml of release medium to

provide the necessary sink condition. The sample was diluted with methanol ,and finally analyzed by double beam U.V spectrophotometer for the amount of drug released from nanoparticles .the cumulative percentage drug released was calculated to establish the drug release profile of the drug(THC) loaded nanoparticles.

Drug release kinetic analysis by using different release model of extended release in nanoparticles

Drug release kinetics can be analyzed by various mathematical models. Following equations presents the models tested. Depending on these estimations, suitable

mathematical models to describe the invitro profiles were determined. The following plots were made:

- cumulative % drug release versus time (zero-order kinetic model);
- log cumulative % drug remaining versus time (first-order kinetic model);
- cumulative % drug release versus square root of time (Higuchi model);
- cube root of drug % remaining in matrix versus time (Hixson–Crowell cube root law)

Zero order kinetic:

Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly (assuming that area does not change and no equilibrium conditions. are obtained) can be represented by the following equation:

$$Q_1 = Q_0 + K_0t \text{ -----(5)}$$

Where Q is the amount of drug dissolved in time t,

Q is the initial amount of drug in the solution (most times) and

K is the zero order release constant.

First order kinetics:

The application of this model to drug dissolution studies was first proposed by Gibaldi and Feldman (1967) and later by Wagner (1969). This model has been also used to describe absorption and/or elimination of some drugs, although it is difficult to conceptualise this mechanism in a theoretical basis. The following relation can also express this model:

$$\ln Q_t = \ln Q_0 - k_1t \text{(6)}$$

Where Q_t is the amount of drug released in time t,

Q_0 is the initial amount of drug in the solution and

K is the first order release constant.

In this way a graphic of the decimal logarithm of the released amount of drug versus time will be linear. The pharmaceutical dosage forms following this invitro profile, such as those containing water-soluble drugs in porous matrices (Mulye and Turco, 1995), release the drug in a way that is proportional to the amount of drug remaining in its interior, in such way, that the amount of drug released by unit of time diminish.

Higuchi model:

Higuchi (1961, 1963) developed several theoretical models to study the release of, water soluble and low soluble drugs incorporated in semi-solid and/or solid. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media. In a general way it is possible to resume the Higuchi model to the following expression.

$$Q_t = K_H t^{1/2} \text{(7)}$$

Where Q_t is amount of drug released in time t and

K_H is release rate constants.

Higuchi describes drug release as a diffusion process based in the Fick's law, square root time dependent. This relation can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems and matrix tablets with water soluble drugs.

Hixson Crowell model:

Hixson and Crowell (1931) recognizing that the particle regular area is proportional to the cubic root of its volume derived an equation that can be described in the following manner:

$$W_0^{1/3} - W_t^{1/3} = K_s t \text{(8)}$$

Where W is the initial amount of drug in the pharmaceutical dosage form,

W is the remaining amount of drug in the pharmaceutical dosage form at time t and

K is a constant incorporating the surface– volume relation.

This expression applies to pharmaceutical dosage form such as tablets, where the Invitro occurs in planes that are parallel to the drug surface if the tablet dimensions diminish proportionally, in such a manner that the initial geometrical form keeps constant all the time. This model has been used to describe the release profile keeping in mind the diminishing surface of the drug particles during the dissolution.

Korsmeyer–Peppas model:

Korsmeyer et al. (1983) developed a simple, semiempirical model, relating exponentially the drug release to the elapsed time (t). An equation that can be described in the following manner:

$$M_t / M_\infty = a t^n$$

Where a is a constant incorporating structural and geometric characteristics of the drug dosage form, n is the release exponent, indicative of the drug release mechanism, and the function of t is M_t / M_∞ (fractional release of drug). Peppas (1985) used this n value in order to characterize different release mechanisms, concluding for values for a slab, of $n = 0.5$ for Fick diffusion and higher values of n , between 0.5 and 1.0, or $n = 1.0$, for mass transfer following a non-Fickian model.

5. CHARACTERIZATIONS:

X-ray powder diffraction data were recorded on a Siemens (D5000) diffractometer using $\text{Cu K}\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$) in the range of $2\theta = 2-65^\circ$. EDAX and Scanning Electron microscope was performed by Hitachi S3000N operating at 10kv. Particle size was recorded by particle size analysis Horiba SZ100.

6. RESULTS AND DISCUSSIONS:

6.1 X-ray Diffraction (XRD).

XRD patterns of Fe_3O_4 nanoparticles as-synthesized and calcined at temperatures at 400°C in figure 6.1. From the patterns of samples, it was found that all the different peaks at (311), (400), (422), (511), (440) and (533) corresponds to 36° , 42° , 52° , 55° and 62° . For calcined

sample we observed peaks (220), (311), (400), (422), (511), (440) and (533) are well indexed at 30°, 36°, 42°, 52°, 55° and 62° to the inverse cubic spinel structure of Fe₃O₄. In the above spectra phase identification is one of the most important uses in XRD. As shown in fig XRD

pattern of Fe₃O₄ nanoparticles after annealing the XRD patterns well indexed to the cubical spinel phase of magnetite and no other peaks observed in as synthesized material.

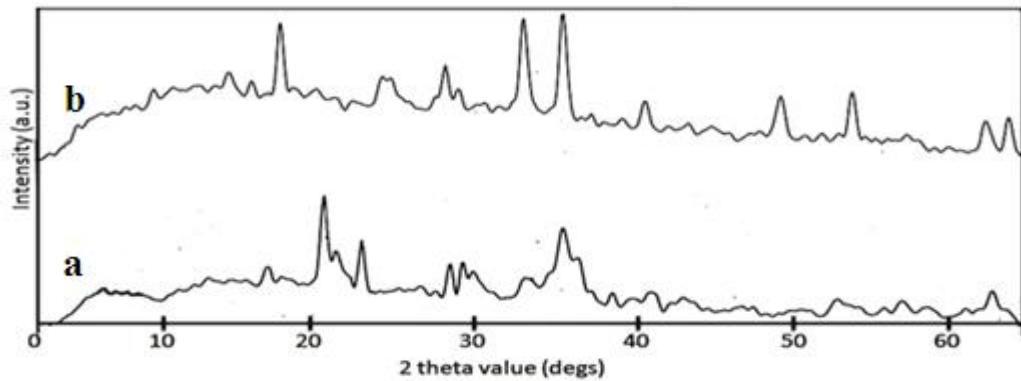


Figure 6.1: X-Ray Diffraction (XRD) of iron nanoparticles at (a) as-synthesized (b) 400 ° C.

6.2 Energy-Dispersive Spectroscopy (EDAX)

The energy-dispersive spectroscopy (EDX) of the nanoparticles dispersion confirmed the presence of Fe, and O composition in the iron oxide nanoparticles (Fe₃O₄) in table 6.2. It is clearly displayed that as-synthesized materials contained only iron and oxygen

elements. The composition of iron and oxygen are 54.11%, and 45.84% respectively. No other peak related with any impurity has been detected in EDX, which confirms that the synthesized nanoparticles are composed only with iron, and oxygen which confirmed the purity of synthesized sample.

Table 6.2: Energy-Dispersive Spectroscopy of iron nanoparticles

Element	Spect.	Element%
O K	ED	45.84
Fe K	ED	54.11
TOTAL		100

6.3 Raman Spectra.

Figure 6.3: Illustrates the Raman spectra of Fe₃O₄ magnetite nanoparticles for as-synthesized material and calcined at 400°C temperature, peak observed at 220cm⁻¹, 290cm⁻¹, 400cm⁻¹, 490,cm⁻¹, 610cm⁻¹, 650cm⁻¹ and 720cm⁻¹ correspond to Fe₃O₄ vibration mode for as synthesized material and calcined at 400°C temperature, low intense peak at 610cm⁻¹, 720cm⁻¹ correspond to Fe₃O₄. These results well match with our XRD results.

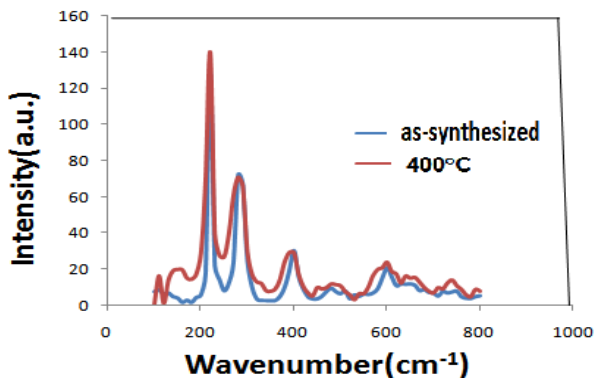


Figure 6.3: Raman Spectra of iron nanoparticles at (a) as-synthesized (b) 400 ° C.

6.4 U.V DRS Spectra.

The diffuse reflectance spectroscopy (UV-DRS) of the synthesized oleic acid coated Fe₃O₄ nanoparticles and calcined at temperatures (400°C) are shown in figure 6.4. From UV results obtained, it is evident that the Fe₃O₄ nanoparticles were formed & this was confirmed by the surface Plasmon resonance exhibited at 250nm & 550nm. The U.V –DRS patterns indicate existence of the magnetic core during the both as-synthesized and calcined nanoparticles.

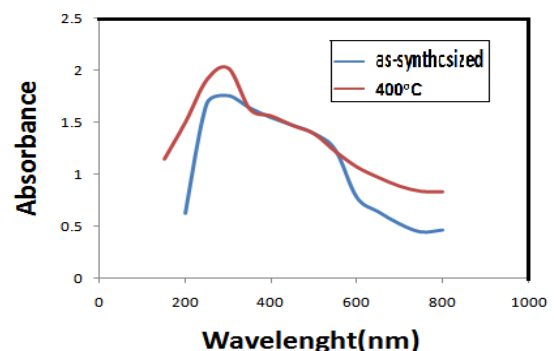
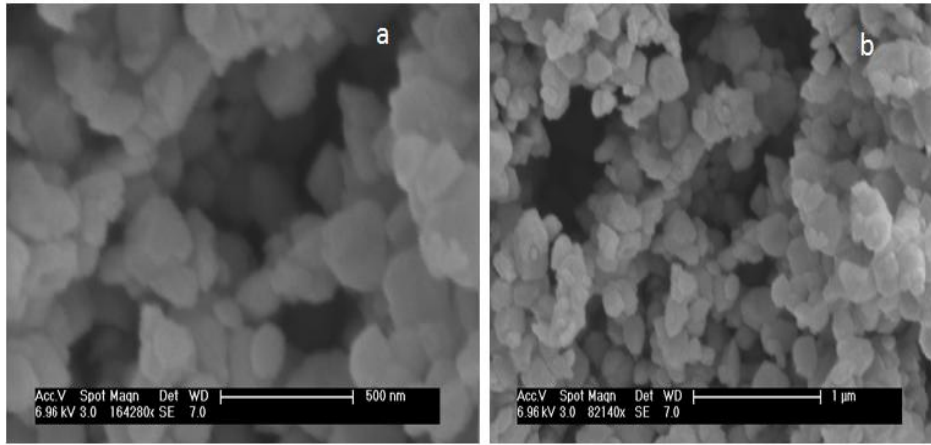


Figure 6.4: (UV-DRS) of iron nanoparticles at (a) as-synthesized (b) 400 ° C



6.5 SEM and Particle Size Analysis

Particle size distribution of Fe_3O_4 nanoparticles are shown in the figure 6.5 (A), (a) as-synthesized and calcined at 400°C . It is observed that as-synthesized sample as per average particle size of $\sim 30\text{nm}$ and calcined at 400°C as average particles size of 40nm . It is also seen that particle size increases with increase in calcinations temperature.

The surface morphology and particles size of THC loaded Fe_3O_4 nanoparticles were examined by Scanning Electron Microscopy (SEM). Nanoparticles prepared by this procedure were spherical, showed in figure 6.5(B) as smooth surface and had an average size of $10\ \mu\text{m}$.

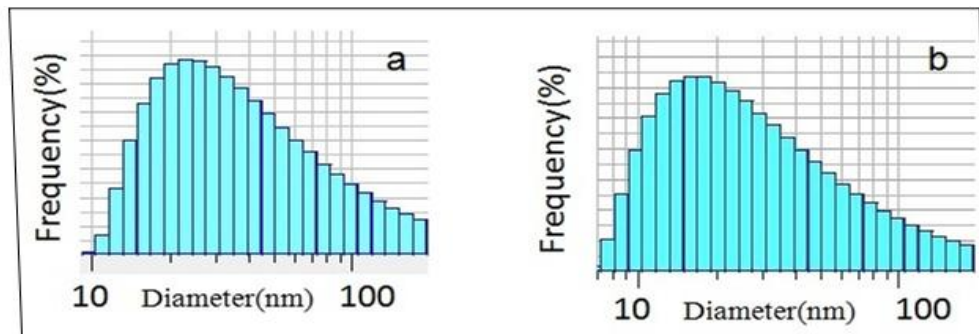


Figure 6.5(A): a-b corresponds as –synthesized, $400^\circ\ \text{C}$, of Fe_3O_4 nanoparticles.

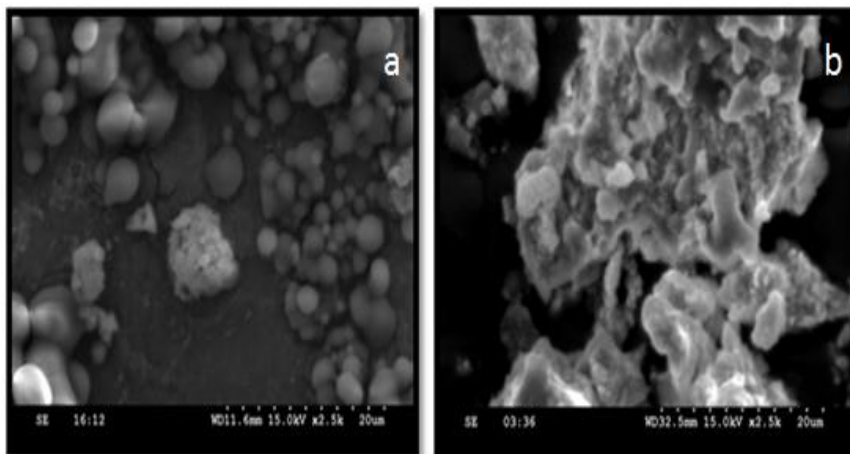


Figure 6.5(B): SEM pictures of THC loaded Fe_3O_4 Nanoparticles (a) as-Synthesized and calcined at (B) 400°C .

6.6 Invitro Studies.

The encapsulation efficiency is calculated and demonstrated in Table 6.6(A). Where the values are range from 99.4-99.05%. Previously, it was reported that THC encapsulation efficiency of iron nanoparticles

decreased by elevation of THC amounts during formation of nanoparticles. In this study, results are in good correlation with the reported study.

The in vitro release of THC from the THC-MNPs showed a sustained release pattern in all the sample

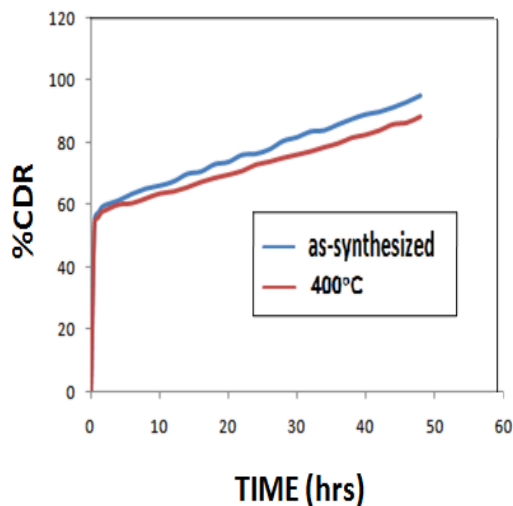
under natural (pH7.0). After 48hrs of incubation as-synthesized released was 40% release rate. 400°C released rate is 88% and at (pH9.2) after 48hrs of incubation as-synthesized released was 8.0% release rate. 400°C released rate is 15%.

Release Kinetics and Mechanisms of Drug Release. Understanding mechanism for release of drugs from nanoparticles has been well described in the literature, suggesting the mechanism to be desorption, diffusion, and matrices degradation. However, recent studies focus on biopolymers responsive to physiological changes such as pH, temperature, and external stimuli that can trigger a control release of the therapeutic agent. In this study, the

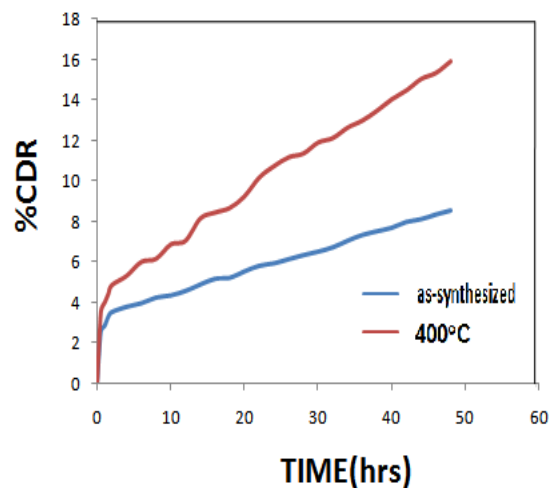
release kinetics and mechanisms of THC release from iron nanoparticles were evaluated by several mathematical models (zero order, first order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas.). Table 6.6(C) demonstrates correlation values (R^2) and release parameters determined from the results of model fitting of the release profiles. As seen from Table, according to correlation values, release data well fitted to the Hixson-Crowell model and zero order on all pH values evaluated which indicates that THC is released by diffusion. Moreover, the Korsmeyer-Peppas release model (high correlation values) exponent, n , is about 0.6, which confirms that the Fickian diffusion is the controlling factor in drug release.

Table 6.6(A): Encapsulation Efficiency of THC loaded Fe_3O_4 Nanoparticles

S.no	Formulation code	Encapsulation Efficiency	Drug Loading	Drug content	Percentage yield.
1.	A synthesized	99.4%	63.68%	6.71%	95%
2.	400°C	99.05%	62.8%	6.63%	89.6%



(a)



(b)

Figure 6.6(B): In vitro drug release profile of THC loaded Fe_3O_4 nanoparticle (a) at (pH7.0) and (b) at (pH9.2) as-Synthesized and calcined between 400°C.

Table 6.6(C) Kinetics Release Rate Data of THC loaded Fe_3O_4 nanoparticles

Formulation code	Zero order		First order		Higuchi model		Korsmeyer Peppas model		Hixson crowell		Best fit model
	(R^2)	K	(R^2)	K	(R^2)	K	(R^2)	n	(R^2)	K	
Fe_3O_4 normal	0.9965	0.331	0.998	0.0021	0.9697	2.6067	0.8651	0.1199	0.9977	0.0066	First order
400°C	0.9962	0.6614	0.9708	0.011	0.9653	5.197	0.8576	0.1058	0.9869	0.0247	Zero order

Further, bad fitting release data of the first order suggests that there is no change in surface area as a function of time and also may confirm that the change depends on the stimuli-induced release systems.

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