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Synthesis optimization and characterization of Magnetic nanoparticles in gastric cancer for drug delivery

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ABSTRACT

Magnetic nanoparticles (MNPs) of targeted drug delivery were employed for some drugs including omeprazole, ranitidine and metronidazole in peptic ulcer disease and gastric cancer.This study emphasizes on the potential of silica-coated magnetic nanoparticles (SiO2-MNPs) as a kind of nano carrier gastric cancer and peptic ulcer therapy. The synthesis of magnetic nanoparticles was performed on two types of nanoparticles MgNiFe2O4 and PdNiFe2O4. SiO2-MNPs used in the targeted therapeutic delivery of omeprazole, ranitidine and metronidazole were prepared by a combination of two techniques consisting of controlled precipitation and hydrothermal methods. Response surface methodology (RSM) was carried out in order to evaluate the efficiency of the SiO2-MNPs. Silica and amine groups in the coated nanoparticles were loaded to target drugs. The three drugs were encapsulated by means of a silanizing agent with some surface rich in 3-aminopropyltrimethoxysilane layered around the SiO2-MNPs the optimum maximized conditions for producing MNPs were found to be at pH = 9 and at a temperature of 85 °C by rated 1500 (rpm). The silica-coated magnetic nanocarriers enhanced in this study show potential for promoted clinical Treatment of gastrointestinal diseases. Scanning electron microscope image shows the morphology of fine particle size.

Keywords: *Magnetic nanoparticles; Drug delivery; Omeprazole; Ranitidine and Metronidazole*.

1. INTRODUCTION

Gastric cancer remains the third cause of cancer death in the world, although the incidence has declined over the last decade [1]. A large amount of patients has advanced or metastatic disease at the time of diag nosis because of lack of symptoms specific to this disease. Cancer cells that are exfoliated from the preliminary tumor can be widespread in the peritoneal cavity once cancer invades the serosa, and peritoneal carcinomatosis as a result is an ordinary type of metastasis that makes the disease incurable [2]. Effective means of treating patients with peritoneal disease by systemic administration of anticancer medicines has not been reported.

Peptic ulcer illness has been found to be a main cause of morbidity and fatalities for more than a century. The pathophysiology of peptic ulcer disease lies in the imbalance existing between aggressive and protective elements in the stomach. Twenty years ago when Marshall and Warren discovered the connection between a bacterium called "Campylobacter pylori" and the peptic ulcer disease [3]. There is, however, much evidence now showing that "Helicobacter pylori" infection is a prerequisite for duodenal and gastric ulcers [4, 5].

Omeprazole belongs to group of medicines by the name of proton pump inhibitors OMZ enters into the gastric parietal cell through the blood system and [6]. Omeprazole is used for the treatment of gastro esophageal reflux disease (GERD), ulcers, erosive esophagitis and Zollinger-Ellisonsyndrome. Omeprazole may also be used along with antibiotics to treat gastric ulcer caused by infection with

helicobacter pylori [7, 8]. Ranitidine was introduced to the pharmaceutical market in 1981. It is a histamine H2 receptor antagonist and has been used for the short-term treatment of active duodenal ulcer [9–14]. Metronidazole is a nitroimidazole derivative [15] to cure trichomonas vaginalis infections but currently widely used for treatment of, and prophylaxis against, anaerobic bacterial infections such as those due to Clostridium difficile .as long as it has antibacterial and anti-inflammatory activities, metronidazole has been enormously used for the treatment of protozoal diseases, including trichomoniasis and giardiasis [16]. However, the long-term abuse of this medicine may cause serious harm to the human health for its genotoxic, carcinogenic and mutagenic type side effect [17].

Advances in nanotechnology and molecular biology may lead to the bring about development of in nanoparticles with specific functional properties, therefore compensating for the lackage of traditional disease diagnostic and therapeutic agents [18-20]. Amongst the wide variety of various nano scale substances employed for biomedical uses, MNPs are of great owing to their intrinsic magnetic properties [21].

MNPs, which are made of iron, cobalt or nickel, are not usually employed for biological applications owing to their chemical instability [22]. It has been found that Nano-based drug delivery systems improve pharmacotherapy to a great extent as because they can change the pharmacokinetics of conventional medicines and new drugs ones, so prolonging drug retention time, reducing toxicity and increasing the half-life of drugs [23].

MNPs are normally coated by organic or inorganic molecules during the precipitation process; this is carried out to prevent the possible oxidation which is caused by air and agglomeration [24]. Silica surfaces are biocompatible; therefore, they can be readily functionalized for bio conjugation intentions [25]. Silica-coated NPs are negatively charged at the pH of blood, thereby involving electrostatic repulsion, which, in turn, leads to avoiding aggregate formation [26].

In this research attempted to develop, synthesize and characterize a carrier system based on SiO2-MNPs. Drug carriers were generated by changing crystalline MNP parameters in order to reach an optimal size, therefore making it possible to transport omeprazole, ranitidine and metronidazole to the selective sites for local targeting and sustained drug release. As a result, Central composite design (CCD), which is known as the most popular RSM employed for the experimental designs.

2. EXPERIMENTAL

2.1 Reagents and apparatus

Iron (III) nitrate 9 hydrates; nickel nitrate 6 hydrates, magnesium nitrate 6 hydrates, Palladium chloride and all chemicals reagents used in this work were of analytical level and were obtained from Merck Company. Deionized distilled water (DDW) was used to prepare all the solutions.

X-ray diffraction (XRD) (X'Pert Pro MPD, PANalytical, 2009) was used in order to examine the PS and the crystal structure. The PS and morphology of the MNPs were investigated by employing scanning electron microscopy (SEM) (KYKY-EM3200, Madell Technology, USA) at 15 kV. Furthermore, the Fourier transform infrared spectroscopy (FT-IR) was hired to explore the absorption spectra of the SiO2-MNPs from 400 to 4500 cm_1 (Nicolet Magna 550 series II FTIR, USA). The NP powder was blended with KBr and the spectra were obtained through the analyzer. The magnetic properties of the samples were then studied using vibrating sample magnetometer (VSM) (Lakeshore 7400). Ultrasonic (Bandelin SONOREX RK 514H, capacity 18.7 L, 600 W) was employed for belended phase synthesis.

2.2 Synthesis of MgNiFe2O4, PdNiFe2O4 and SiO2-MNP- omeprazole - ranitidine -metronidazole

2.2.1 Optimization of MNPs

RSM is, in fact, a combination of mathematical and statistical methods used to define the relationship between response and independent variables. RSM is defined as "the effect of the independent variables, alone or in combination, on the processes". It ought to be noted that although the possible effects of the independent variables can be analyzed, this methodology can also yield a mathematical model. The graphical illustrated outlook of the mathematical model has led to the term Response Surface Methodology. The design involveds low, medium and high levels (–1, 0, and +1); a total of twenty runs were carried out by takeing Rate (X1), temperature (X2), and pH (X3) as the independent variables and particle size (Y) as the response. All experiments were repeated three times in the model. The levels used in the experiments can be observed in Table 1. The set of twenty experiments employed in the present study has been displayed in Table 2.

Table 1. Factors and levels used in the factorial design

Factor / Level	-1	+1
Reaction rate(rpm) (x_1)	500	1500
Temperature (_C) (X_2)	60	85
pH (X3)	9	13

Table 2. Particle size obtained with RSM design.

Std order	Run order	X_1:reaction rate	X_2:tempeature	X_3:pH	Y_{EXP}:MNPsize(nm)
8	1	1500	85	13	43.46
7	2	1500	85	9	40.37
4	3	1500	60	13	51.35
12	4	1840	72	11	72.75
2	5	500	60	13	94.15
10	6	1000	72	14	72.75
20	7	1000	72	11	52.59
1	8	500	60	9	57.09
11	9	159	72	11	62.82
14	10	1000	93	11	57.09
18	11	1000	72	11	49.36
6	12	500	85	13	47.09
16	13	1000	72	11	54.25
13	14	1000	51	11	72.43
5	15	500	85	9	93.50
15	16	1000	72	11	50.34
3	17	1500	60	9	43.47
9	18	1000	72	7	47.41
17	19	1000	72	11	51.36
19	20	1000	72	11	47.25

2.2.2. The effect of Reaction Rate

To study the effect of various Reaction Rates on the properties of MNPs, following the preparation of a mixture of salts of Mg, Pd, Ni and Fe, it was permitted to be stirred in an oil bath under a nitrogen atmosphere at 500 rpm, 1000 rpm and 1500 rpm.

2.2.3. The effect of Temperature

For the determination of the optimum temperature for the oil bath, the mixture was permitted to be stirred in the bath under a nitrogen atmosphere at 60 °C, 72 °C and 85 °C; then the resulting PS was measured for each increment.

2.2.4. The effect of pH

The pH of the mixture was adjusted to 9, 11 and 13 using a pH meter that had been calibrated prior to each measurement; this was done by dripping a 1.2 M NaOH aqueous solution, under magnetic stirring, into the mixture. The PS obtained in this way was measured for each pH.

2.2.5. Synthesis of Mg0.5Ni0.5Fe2O4 and Pd0.5Ni0.5Fe2O4 NPs.

Mg0.5Ni0.5Fe2O4 and Pd0.5Ni0.5Fe2O4 were obtained by a combination of controlled precipitation and hydrothermal methods [27]. FeN3O9_9H2O, MgN2O6 _6H2O, and NiN2O6_6H2O (Mg: Ni: 2Fe) and FeN3O9_9H2O, Pdcl2, and NiN2O6_6H2O (Pd: Ni: 2Fe) were then blended in water at room temperature under magnetic stirring at 1500 rpm for 30 min. Following that, the mixture was placed in an oil bath heated to 85 °C under a nitrogen atmosphere with constant stirring at 1500 rpm for 40 min. then, 1.2 M NaOH solution was added in a drop-wise manner to the solution. Following this procedure, the mixture was transported to a Teflon-lined autoclave and heated at 250 °C for 12 h. The product obtained in this way was rinsed three times in water; then it was magnetically decanted to separate the particles and dried at 90 °C for 15h in a vacuum oven. at last, the MNPs were heated at 800 °C for 2h in an oven.

2.2.6. Synthesis of SiO2-MNP- omeprazole - ranitidine - metronidazole

For the synthesis of SiO2-MNPs (Mg0.5Ni0.5Fe2O4 and Pd0.5Ni0.5Fe2O4), ethanol, water and tetraethyl orth silicate were mixed in a two-neck flask and allowed to be stirred magnetically for 30 min while being warmed at 40 °C in a water bath. NH4OH was stirred into the solution to allow it to react for 30 min. The MNPs were added to the solution and stirred for further 6h. The SiO2-MNPs obtained in these experiments were separated by magnetic decantation, washed with ethanol three and dried at 90 °C in a vacuum oven. The amine-modified SiO2-MNPs became ready by washing the SiO2-MNPs in ethanol and water for 30 min, using an ultrasonic processor. (3- Aminopropyl) triethoxysilane and dimethylformamide were added to the solution; then the solution was then shaken in an incubator for 2 h. The SiO2-MNPs were rinsed with phosphate-buffered saline (PBS) three times, isolated with a magnet and stored in PBS., finally the SiO2-MNP suspension was mixed with glutaraldehyde (GA) in PBS and shaken for 2h. For the preparation of the activated SiO2- MNPs, the suspension was washed three times with PBS. SiO2- MNPs were then blended with omeprazole, ranitidine and metronidazole for 3 h at 25 °C using a rotor at 7 rpm; after that, they were rinsed with PBS and isolated magnetically.

3. RESULTS

3.1 Analysis of Particle Size

Scanning electron microscopy (SEM) is known as a common technique to determine the morphology and size distribution of prepared particles in different measures from micro to nano. The SEM picture in Fig. 1 shows the structure and surface morphology of MNPs. a good amount of less than 50 nm can be seen in both nanoparticles.

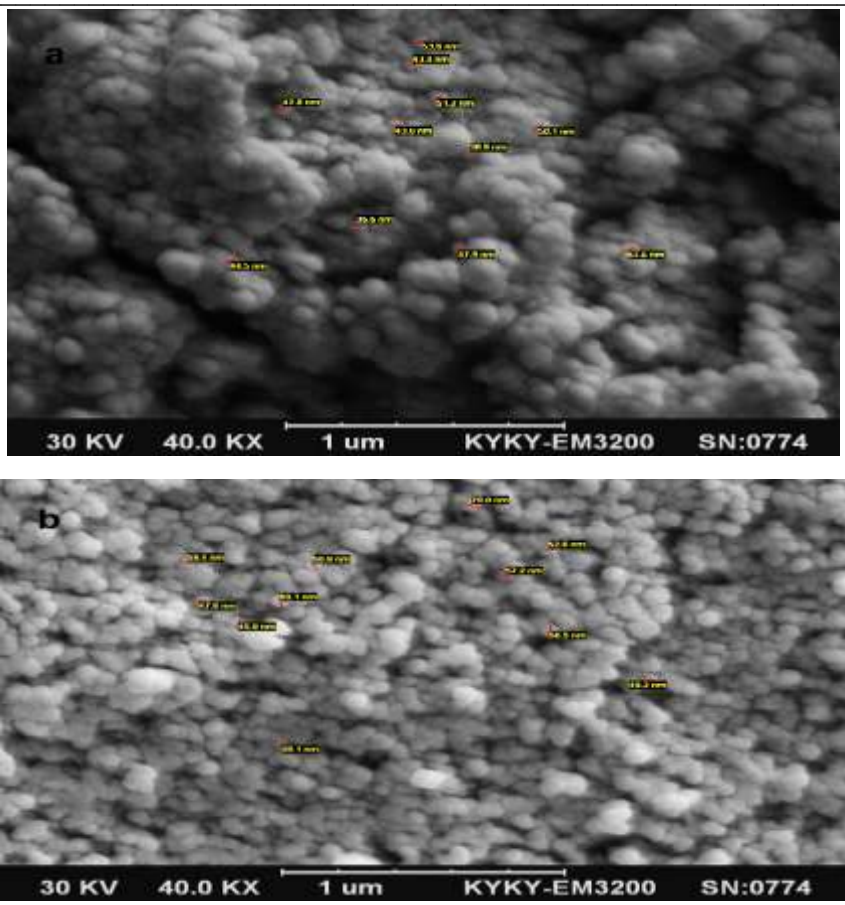


Fig1. SEM image of nanoparticles (a) MgNiFe2O4 and (b) PdNiFe2O4.

FT-IR spectroscopy is recognized as a useful tool used to show the functional group of any organic molecule. FT-IR has been widely employed to confirm the attachment of different functional groups to each step of functionalization. In the FT-IR spectra (Fig. 2 and Fig 3), magnetic nanoparticles are shown in the lower peaks.

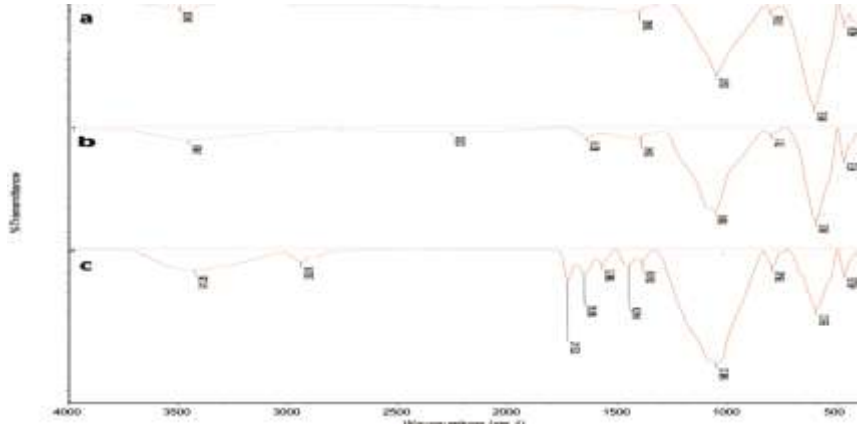


Fig2. FTIR spectra of magnetic nanoparticles of(a) MgNiFe2O4 , (b) SiO2 – MNP of MgNiFe2O4 and (c) SiO2 – MNP-omeprazole-ranitidine-metronidazole of MgNiFe2O4.

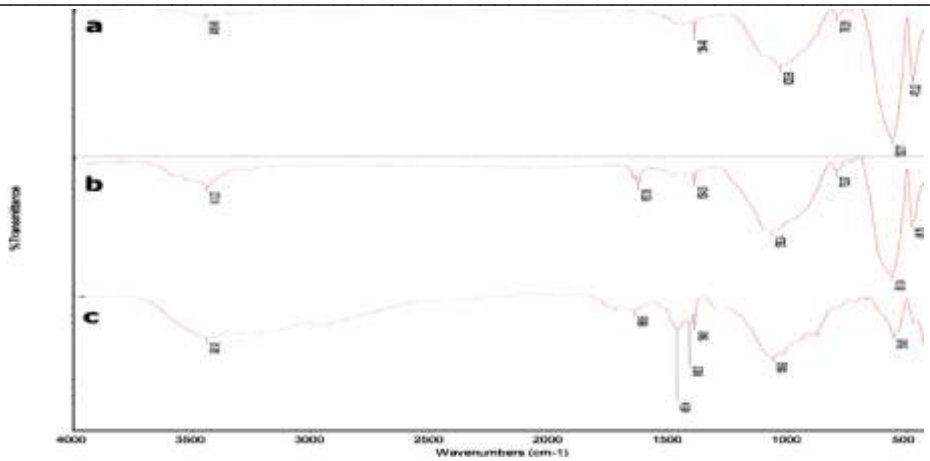
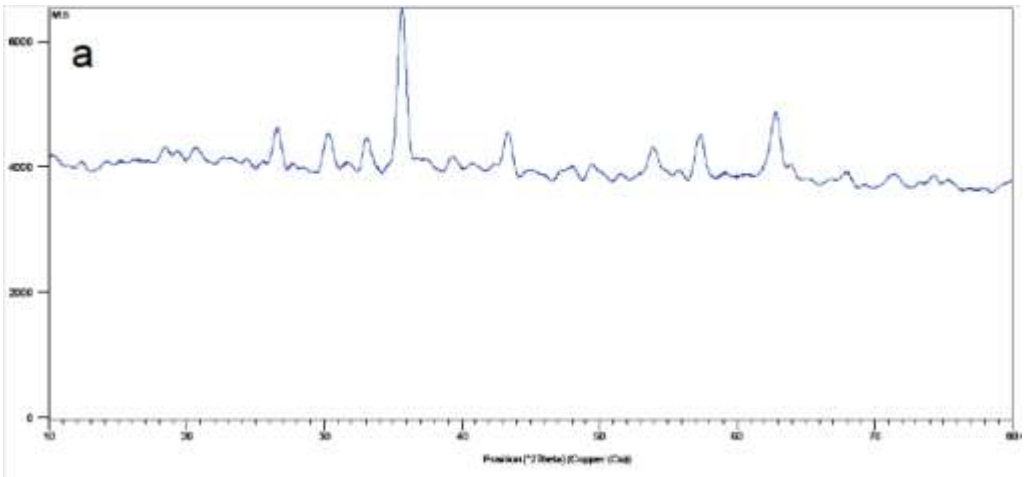


Fig3. FTIR spectra of magnetic nanoparticles of (a) PdNiFe2O4 ,(b SiO2 – MNP of PdNiFe2O4 and (c) SiO2 – MNP-omeprazole-ranitidine-metronidazole of PdNiFe2O4.

Peak broadening in the area around 1700 and 3400 could be related to the silica coating. Since FT-IR spectrum could be more relevant to the operating groups, the functional groups on the drug structure were as follows: Area of NH was in the area between 2500- 4000, C = C was in the area between 1550 - 1650, CH3 was between1375-1450, and S = O was placed in the area between1050-1375. The C-N was in the area between 1000 -1350, C = N was in the area between 1640- 1690, and N = O was in the region between 1350 -1550. Also, the carbonyl peak in the area was about 1700. Regarding the spectrum FT-IR taken from nanoparticles, it can be seen that MgNiFe2O4 peaks are better than PdNiFe2O4.

The XRD spectra were utilized to determine the crystallographic identity of the produced material, and phase purity known for calculating the mean particle size; it must be clarified that this was done according to the broadening of the most prominent peak observed in the XRD profile. The diffraction peaks of the XRD patterns of the prepared MNP examples can be seen in Fig. 4, as determined by Mini tab software and the Scherrer equation. The MNPs showed minor strong reflection peaks at low 2θ values that originated from the amorphous silica matrix. In the MgNiFe2O4 spectrum, better separation of phases and higher purity can be observed.



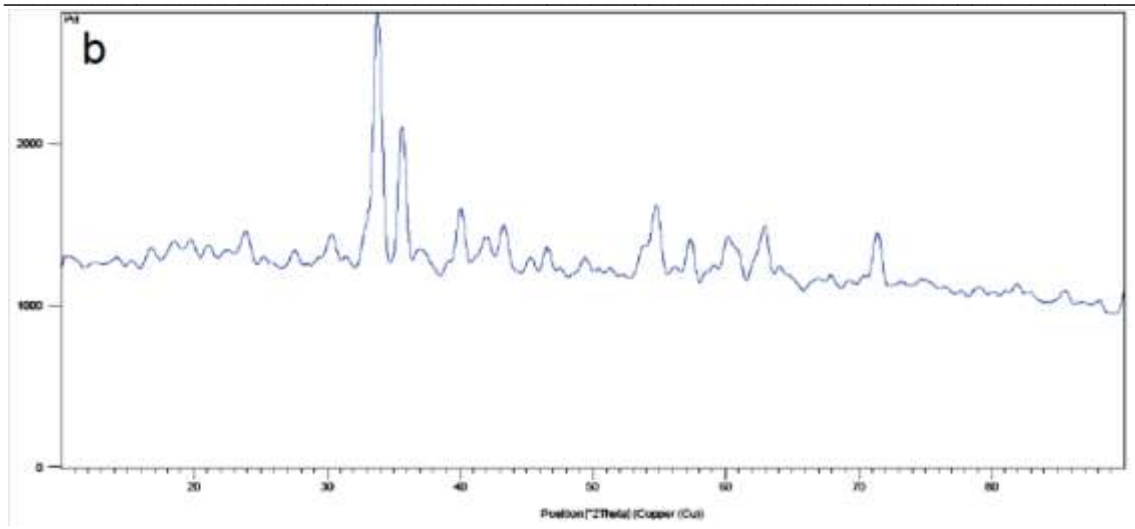


Fig4. X-Ray diffraction patterns showing magnetic nanoparticles of (a) MgNiFe2O4 and (b) PdNiFe2O4 .

VSM is meant to evaluate the magnetization of the MNPs as a function of an applied external magnetic. The results related to the magnetization of the samples at room temperature (Fig. 5) revealed that the saturation magnetization value of SiO2-MNP- omeprazole, ranitidine and metronidazole was lower than that of MNPs, owing the diamagnetic contribution of the silica shell surrounding the magnetite. The magnetic property is visible well before and after coating.

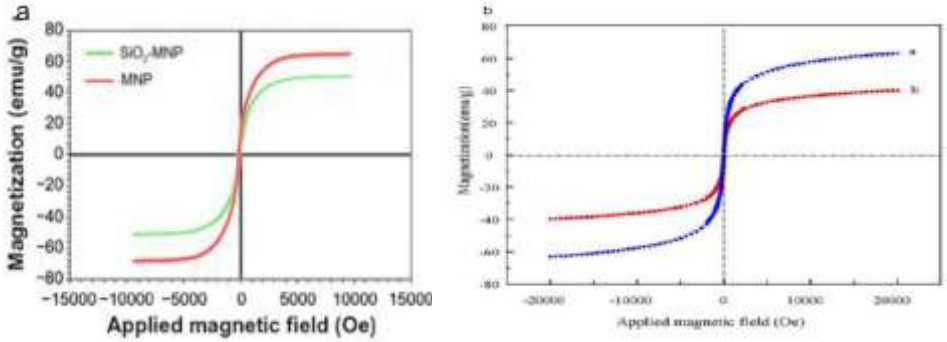


Fig5. Superconducting quantum interference device magnetization curves of (a) MgNiFe2O4 and (b PdNiFe2O4.

3.2. Preparation & Properties of MNPs and SiO2-MNP-Omeprazole-Ranitidine-Metronidazole

To determine the optimal parameters required for the preparation of MNPs, CCD was employed exploited as a reliable methodology to evaluate the interactions between the independent variables. Accordingly, the influence of pH, temperature and rate of reaction was studied. The MNP PS obtained under each synthesis condition and the relationship between the reaction conditions and PS can be seen in Table 2. By comparison the information obtained in this study with those already found in the previous studies [28], it was clear that the PS obtained here was smaller; in fact, it was the smallest, with the run 2 MNP PS of 40.37 nm.

3.3. Characterization of Three-Dimensional Response Surface Plot

Based on the experiments conducted, three-dimensional response surface plots shown in order to examine the main interactional effects of the factors (Fig. 6).

3.4. The effect of Different Factors on the Synthesis of MNPs

3.4.1. Reaction Rate

The reaction rate for MNPs was found to be important to achieve complete reduction. As shown in Table 2, the optimal PS of 40.37 nm was achieved by following a reaction rate of +1 (1500 rpm) at an oil bath temperature. The results obtained indicated that variation in the reaction rate could affect PS.The rate-temperature interaction showed that at 85 °C, for 1500 rpm of the reaction rate, both values minimized the PS (Fig. 6a). The effect of varying the pH-to-rate relationship indicated that pH had a considerably significant ($p < 0.005$) impact on PS (Fig. 6b).

3.4.2. Temperature

The effect of temperatures between 60 °C and 85 °C on PS was studied. The smallest PS was obtained at higher temperatures. So, 85 °C was chosen as the best condition for the formation of the optimal PS since it could result in lead to a higher yield of MNPs with a uniform size. The best condition for the formation of optimal PS was found at +1 (85 °C).

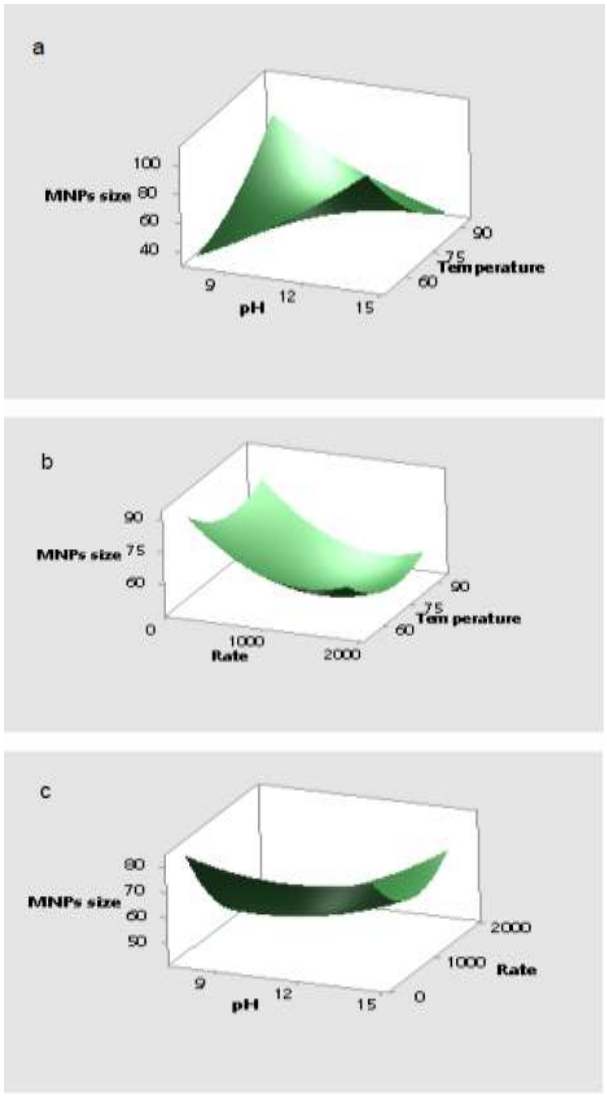


Fig.6. Response surface plots representing the effect of three variables: reaction (a) rate to temperature. (b) reaction rate to pH and (c) pH to temperature.

3.4.3. pH

The results showed that pH played a very important role in determining the PS of MNPs. The pH values of all the solutions were measured using a digital pH meter and then recorded with respect to their NaOH concentration. These interactions between pH and reaction or rate or temperature can be seen in Fig. 6b–c. The results of other studies suggest that the optimal pH for producing the smallest PS was within the range of 9.7–10.6. pH -1 (9 rpm) was, therefore, chosen as the best condition for the formation of the optimal PS. Accordingly, the run 2 was selected as the optimal condition, and the MNPs produced in that run were employed for further experiment.

4. CONCLUSION

In this study, omeprazole, ranitidine and metronidazole were immobilized in SiO2-MNPs using GA as a crosslinking agent and their effectiveness in targeted therapy was studied .The results obtained in this study showed that SiO2-MNP omeprazole - ranitidine – metronidazole could be employed for the immobilization of omeprazole, ranitidine and metronidazole, therefore ensuring high protein-loading efficiency and activity retention; this, in turn, could prevent the hemorrhagic side effects of the drug and enzyme. On the other hand, by examining the spectra, we found that the magnesium-bearing nanoparticle was better than the palladium nanoparticle. It was found that the immobilization improved enzyme stability; also, it could be stored in PBS, therefore including amino acids required for the catalytic activity. Our observations confirmed that all of the enzyme activity of omeprazole, ranitidine and metronidazole was preserved by following immobilization.In another research, GA was introduced as an effective crosslinking agent contributing to the formation of imide bonds between the amine groups of SiO2-MNPs or omeprazole, ranitidine and metronidazole.In one previous research with magnetic nanoparticles targeted drug delivery was investigated [28]. In other research, GA was introduced as an effective crosslinking agent in formation of imide bonds between the amine groups of SiO2-MNPs or the aldehyde groups of GA [29].

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