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RESEARCH ARTICLE

SYNTHESIS OF POROUS ZnO SPHERES: CHARACTERIZATION AND *IN VITRO* DRUG RELEASE STUDIES

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Abstract

Zinc Oxide spheres were synthesized using starch as a bio-polymer in an aqueous solution reaction at 85 degree Celsius for 30 minutes. The synthesized spheres were dried at 50 degree Celsius for 24 hours to obtain powdered form of uncalcinated ZnO spheres, which was calcinated at 500 degree celsius for 2 hours to remove the starch present and ZnO hollow spheres were obtained which was ready for the process of drug loading. The uncalcinated and drug loaded spheres were characterized by SEM, TEM, Optical Microscope and FTIR. The spheres were loaded with the drug Captopril in different ratios (1:5, 1:6,1:8) and the amount of free drug and drug entrapped in the spheres were determined. A specific carrier containing a particular amount of drug for all the ratios were taken to study the amount of drug released in a particular pH (1.2pH/7.4pH) for a particular period of time (5hours for 1.2pH and 8hours for 7.4pH). Results was clearly evident that maximum cumulative percentage of drug release was for 1:5 ratio (1.2pH) that is, 51.94% and the least cumulative percentage release was 8.92% for 1:8 (7.4p). The percentage of drug released increased with time and the amount of drug loaded.

Introduction

In the field of health, one of the vital challenges is efficient delivery of drugs in the body using non-toxic nanocarriers. Several recent drug delivery systems (DDSs) are making development to improve the biocompatibility and high drug loading capacity of nanocarriers, reducing in vivo toxicity, and increasing drug selectivity. Drug delivery agents or carriers are increasing in complexity, with improved stability, targeted delivery and controlled release of drug without affecting biological systems (Waterwig *et al*., 2005). Most of the drug carrier materials show poor drug loading and quick release of the proportion of the drug that is simply absorbed at the external/internal surface of the nanocarriers. Among the DDSs presently used, porous nanocarriers (PNCs) are huge challenging as versatile drug delivery carrier. Most of them are responsive to environmental changes such as ionic strength, pH and temperature (Lide *et al*., 2000**).** Nanoparticle characterization is necessary to establish understanding and control of nanoparticle synthesis and applications. Characterization is done by using a variety of different techniques, mainly drawn from materials science. Common techniques are electron microscopy (TEM, SEM), Fourier transform infrared spectroscopy (FTIR).

MATERIALS AND METHODS

Reagents

Potassium phosphate, Sodium hydroxide, Hydrochloric Acid, Potassium Chloride, Zinc nitrate hexahydrate [Zn (NO3)2.6H2O] Ammonium hydroxide, Soluble starch.

Buffer solutions

Preparation of Hydrochloric acid buffer (pH – 1.2)

a) **Preparation of 0.2 (M) Potassium chloride solutions**: About 1.4911 gm potassium chloride was weighed out accurately and taken into a 100 ml volumetric flask and distilled water was added in order to dissolve the potassium chloride and finally the volume was made up to the mark with distilled water.

b) **Preparation of 0.2 (M) Hydrochloric acid solutions**: About 0.617ml of concentrated hydrochloric acid was weighed out accurately and taken in a 100 ml volumetric flask. Finally volume was made up to the mark with distilled water.
c) **Preparation**: Buffer solutions were prepared by mixing 85ml of 0.2M Hcl and 50ml of 0.2M Kcl were made up to 250ml in a volumetric flask.

Preparation of Phosphate buffer (pH – 7.4)

a) **Preparation of 0.2 (M) Sodium Hydroxide**: About 1.6 gm sodium hydroxide was weighed out accurately and taken into a 200 ml volumetric flask. To it distilled water was added in order to dissolve the potassium chloride and finally the volume was made up to the mark with distilled water.
b) **Preparation of 0.2 (M) Potassium phosphate solutions**: About 5.44 gm of hydrochloric acid was weighed out accurately and taken in a 200 ml volumetric flask. Finally volume was made up to the mark with distilled water.
c) **Preparation**: About 39.1ml of 0.2M NaoH and 50ml of 0.2M potassium phosphate was mixed and made up to 200ml in a volumetric flask.

Preparation of standard (1.2pH and 7.4pH)

About 10mg of pure drug was dissolved in 100ml of respective buffer (1.2pH/7.4pH) in 100ml volumetric flask. From the above solution 1ml was taken and made up to 100ml with the respective buffer. From the above solution 1ml, 2ml, 3ml, 4ml and 5ml was taken in separate 10ml volumetric flask respectively and made up to 10ml with the respective buffer. For concentrations 1 to 5 was obtained from this procedure. This procedure was followed to the concentrations of 6 to 25. About 10mg of pure drug was dissolve in 100ml of respective buffer (1.2pH/7.4pH) in 100ml volumetric flask. From the above solution 1ml, 1.5ml, 2ml and 2.5ml was taken in separate 10ml volumetric flasks respectively and made up to 10ml with the respective buffer.

Synthesis of Zinc Oxide Spheres

About10gm of starch was dissolved in 300ml boiling water in a round bottom flask. About 5.9gm of zinc nitrate hexahydrate was added to the starch solution and stirred at 85 degree celsius for 5 minutes.The pH of the solution was adjusted to 8-9 by adding ammonium hydroxide (3 to 5ml) and milk like precipitate was formed. The solution was stirred for an additional 30 minutes. Resulting precipitate was centrifuged at 6000rpm for 10 minutes. The supernatant was discarded and the pellet was washed with distilled water. This process was repeated thrice and the resultant was dried at 50 degree celsius. The obtained powder was calcinated in air atmosphere at 500 degree Celsius to obtain porous ZnO spheres.

Sonication Procedure for Drug Loading

About 100mg of carrier and 20mg of drug was taken and was added 5ml of 1.2pH/1.4pH buffer. It was sonicated for 5 minutes giving 1 minute pulse each. The sonicated samples were poured into ependorff tubes and the samples were centrifuged at 7000rpm for 10 minutes.The supernatant was collected from all the 3 washings and made up to 10ml and measure UV absorbance (Free drug estimation). It was washed for three times with distilled water. The same procedure was repeated for all the ratios in each buffer.

Ratio of Carrier and Drug

Carrier (mg)	Drug (mg)	Ratio
100	20	1:5
120	20	1:6
160	20	1:8

Content analysis of the sample

About 10mg of the prepared ZnO nano particles was taken and added to 10ml of buffer (1.2pH/7.4pH). Sonicated for 15 minutes giving 3min pulse each. One ml of the sample was taken and made up to 10ml with the respective buffer. The UV absorbance was measured. The same procedure was repeated for all the ratios in each buffer.

Amount of drug =

Present in the sample = sample absorbance/ Standard Absorbance * standard concentration

Procedure for Drug Release

Ratio	Amount of Carrier
1:6 (1.2pH)	56.64mg
1:6 (7.4pH)	55.4mg
1:8 (1.2ph)	55mg
1:8 (7.4pH)	30mg

The amount of carrier for the specific ratios was taken. The water jacket was maintained at 37 degree Celsius and poured into 200ml of the respective buffer (1.2pH/ 7.4pH) in the water jacketed circulatory beaker. The sample was poured in a dialysis bag after tying one end with a twine and was added 4ml of the respective buffer and tie the other end. The bag in the water jacketed circulatory beaker and magnetic stir at low speed (100rpm). After 15min 5ml of the buffer was taken from the water jacket for analysis in a test tube and replace 5ml of the respective fresh buffer in the beaker. This process was repeated at 15min interval for 5hours for 1.2pH ratios and 30min interval for 7.4pH for 8 hours.

RESULT

Zinc Oxide spheres were synthesized using starch as a bio-polymer in an aqueous solution reaction at 85 degree Celsius for 30 minutes. The synthesized spheres were dried at 50 degree Celsius for 24 hours to obtain powdered form of uncalcinated ZnO spheres, which was calcinated at 500 degree Celsius for 2 hours to remove the starch present in the spheres and ZnO hollow spheres were obtained which was ready for the process of drug loading. The uncalcinated and drug loaded spheres were characterized by SEM, TEM, Optical Microscope and FTIR. The spheres were loaded with the drug Captopril in different ratios (1:5, 1:6,& 1:8) and the amount of free drug and the amount of drug entrapped in the spheres were determined. A specific amount of carrier containing a particular amount of drug for all the ratios were taken to study the amount of drug released in a particular pH (1.2pH/7.4pH) for a particular period of time (5hours for 1.2pH and 8hours for 7.4pH). From the results obtained is was clearly evident that maximum cumulative percentage of drug release was for 1:5 ratio (1.2pH) that is, 51.94% and the least cumulative percentage release was 8.92% for 1:8 (7.4pH). From all the graphs obtained it was clear that the percentage of drug released increased with time and the amount of drug loaded.

Characterization

Scanning Electron microscope (SEM) was used to characterize the morphologies of the products. The morphologies and microstructures of the prepared samples were further examined using Transmission Electron Microscope (TEM). Fourier Transform Infrared Spectroscopy was done to establish that the characteristic properties of both the carrier as well the drug were maintained even after the drug was loaded in the carrier. This proves that there were no reactions between the two. Microscopic view of both uncalcinated and calcinated ZnO spheres were studied to confirm the complete removal of soluble starch after the process of calcination and also to study the morphology of the ZnO mesosphere.

Mechanism of starch aggregation with Zn^{2+}:

The soluble starch played an important role in the formation of sphere like particles. The starch becomes soluble in boiling water. The granules swell, burst and the semi crystalline structure is lost as the smaller amylose molecules start leeching out of the granules. The smaller amylose molecules could complex with Zn^{2+} because of the higher number of coordinating functional group. Majority of Zn ions were associated with the starch molecules, so nucleation and crystal growth might preferentially occur within regions of both high starch concentration and high Zn^{2+} concentration.

Mechanism of drug loading into the mesoporous ZnO spheres

In Most cases, the wanderwalls interactions between the ZnO surface molecules of the nanocrystallites, that form the driving forces of drug molecules, were attracted over the pores partially, and then ZnO nanocrystallites pores were adsorbed partially

Fourier Transform Infrared Spectroscopy (FTIR)

From the graph 1 obtained, the maximum peak for pure drug (1591 cm-1), uncalcinated ZnO (3429 cm-1), calcinated ZnO (2920cm-1) and drug loaded ZnO (1632cm-1) were determined. This characterization was done to check whether the characteristic properties of the respected compound (ZnO and Captopril) were maintained after the process of drug loading (Tomasik *et al.*, 1989*)*. From the result we can conclude that there were no reactions between the carrier and the drug as peaks of both the carrier and drug were remained even after the drug was loaded into the carrier.

Standard Absorbance at Different Concentrations

The pure drug was taken (10mg) and dissolved in 100ml of the respective buffer (1.2ph/7.4ph), from this solution 1ml, 2ml, 3ml, 4ml etc was taken and diluted in 10ml volumetric flask with the corresponding buffer (Shan. G. et al.,2000). The slope values obtained from graph 2 were used to determine free drug estimation and in vitro drug release study.

Free drug estimation

From Table 1, the least amount of drug loss was for in 1:5 ratios (1.2pH) and the maximum amount of drug loss was for 1:6 ratios. This proves that maximum drug was entrapped in the carrier for 1:5 ratios. The maximum drug loss was for 1:8 ratios (7.4ph) and the minimum amount of drug loss was for 1:5 ratios (7.4pH). This indicates that 1:5

ratios have the maximum amount of drug entrapped in them than the other ratios. The amount of drug present in the carrier was estimated by subtracting the amount of drug loaded (20mg) from the amount of free drug. We can conclude that only minimal amount of drug was gone as wastage. This procedure shows that maximum drug was present in 1:5 ratios.

Sample Content Analysis

The drug loading capacity of the carrier was determined by taking a small amount of the drug loaded carrier (10mg) and diluting it with the respective buffer and UV absorbance was taken. The drug loading capacity was relatively high for 1:5 ratio and low for 1:8 ratios. This proves that more drug is entrapped in 1:5 ratio than the other 2 ratios. The drug loading capacity plays a very important role in the percentage of drug released. More the amount of drug entrapped more there are chances for increase in percentage of release.

In Vitro Drug Release Studies

A specific amount of carrier was taken containing a particular amount of drug and was dissolved with 4ml of the respected buffer. The ratios 1:5, 1:6 and 1:8 with different concentration of the drug (captopril) was taken for in vitro drug release studies. The results obtained show that the 1:5 (1.2pH) has maximum percentage of cumulative drug release 51.94% (table7) and the least was obtained from 1:8 (7.4pH), 8.92% (table12). This proves that the release is higher in acidic medium than alkaline medium and the amount of drug present in the carrier also plays a vital role. The drug loading capacity also plays an important role and 1:5 ratios had the maximum drug loading capacity which indicates a high amount of drug was entrapped in the carrier. For all the ratios the drug release for a particular time (5hrs for 1.2pH and 8hrs for 7.4pH) was in increasing order and this can be studied from the graphs obtained, which proves that as time increases there was an increase in drug release as well as continuous release of drug.

The amount of drug released through the pores of ZnO is due to diffusion process and drug release depends on the amount of drug perfused into the pores. Sample content analyses for the 3 ratios were done and the amount of drug present in the carrier was determined. From the results obtained we can conclude that 1:5 ratios entrapped maximum drug and percentage of release was more compared to all the other ratios.

Fig-1 Scanning Electron Microscope (SEM) ,Uncalcinated ZnO

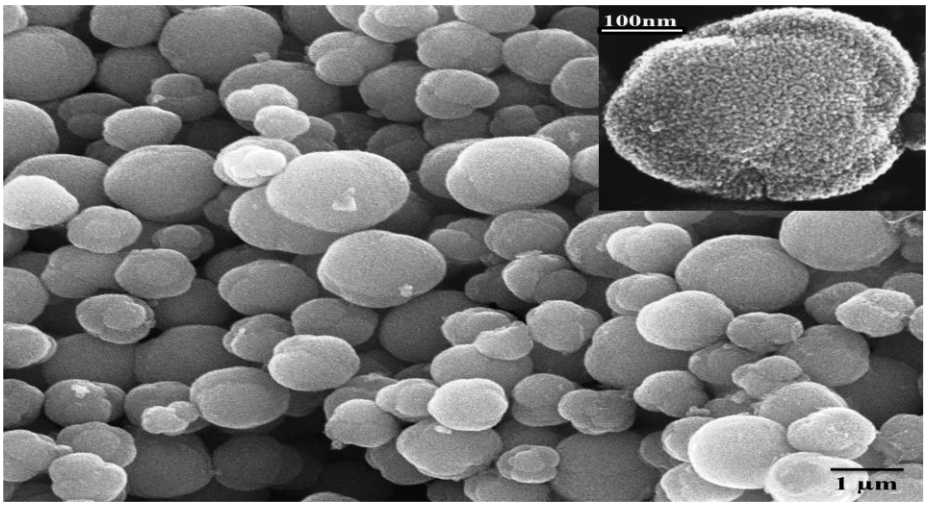


Fig-2Transmission Electron Microscope (TEM) Drug Loaded ZnO

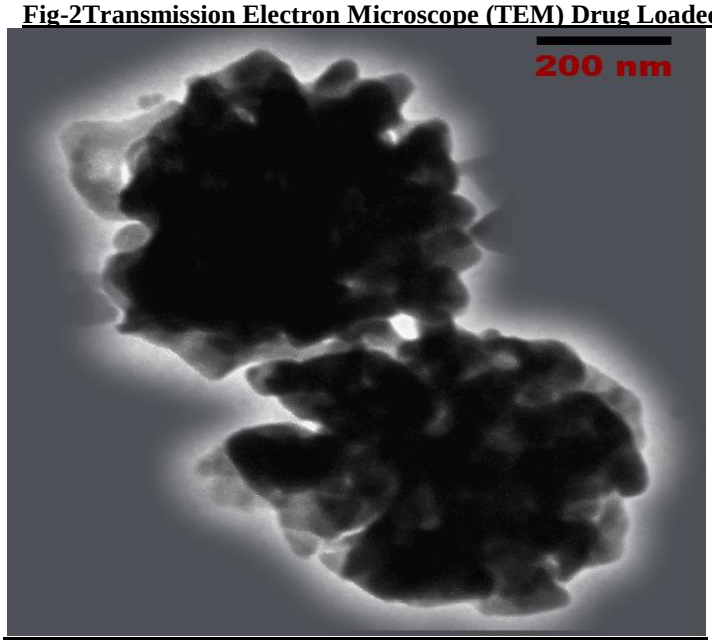


Figure 1 and 2 displayed SEM and TEM images of uncalcinated powder and calcinated (at 500^0C for 2 hrs) drug loaded, from the Fig.1, it could be seen as monodisperse sphere-shaped particles with diameter 100nm. Fig 2 shows that the surface of single particle consists of many particles with diameter 200nm. A single porous ZnO sphere results from the agglomeration of crystallite particles and diameter ranges from 20-40nm.The diameters of ZnO spheres decrease after calcination as a result of the removal of soluble starch and aggregation of the particles.

Fig-3 Optical Microscopic View,Uncalcinated ZnO

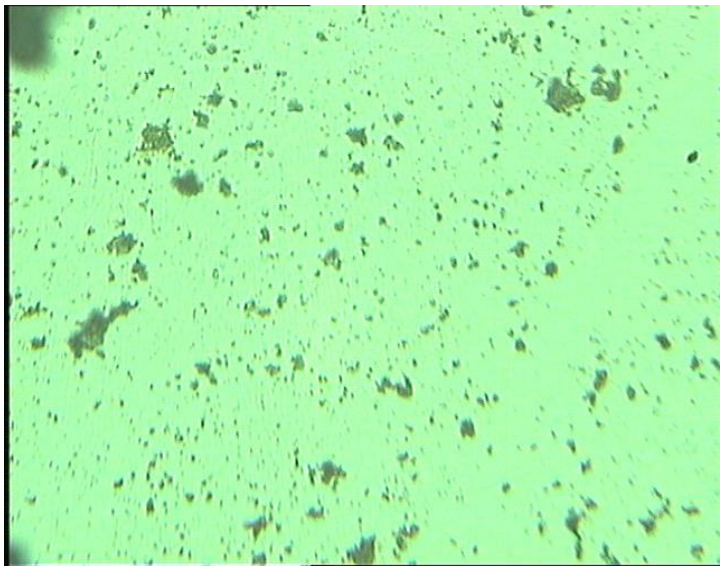
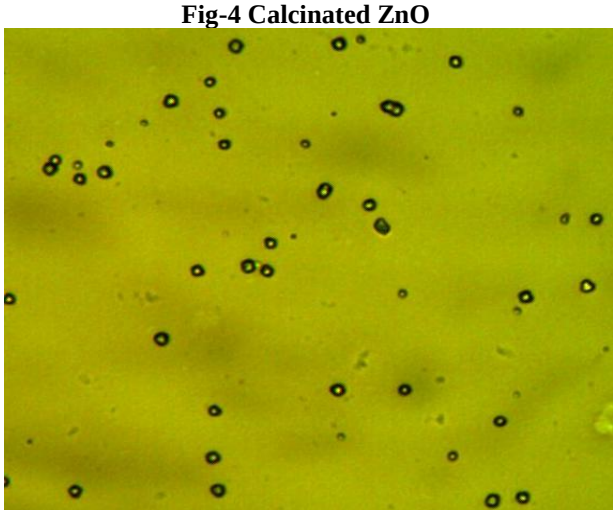
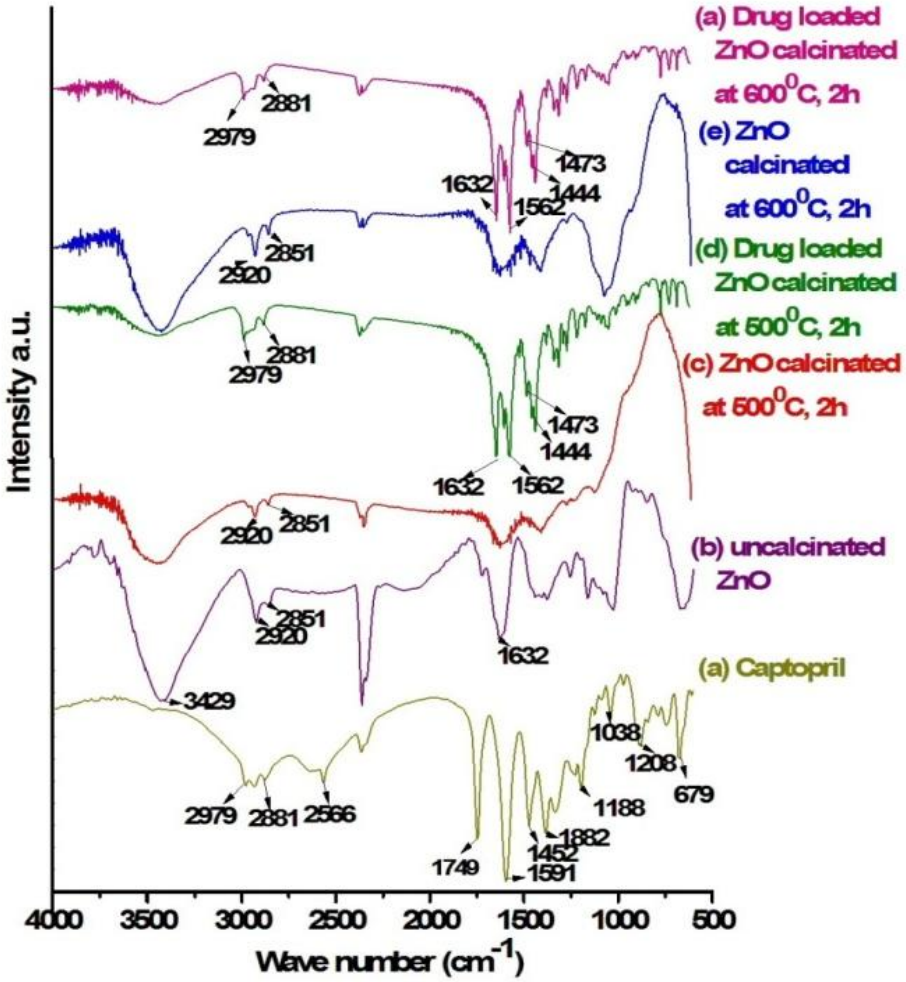


Fig-4 Calcinated ZnO

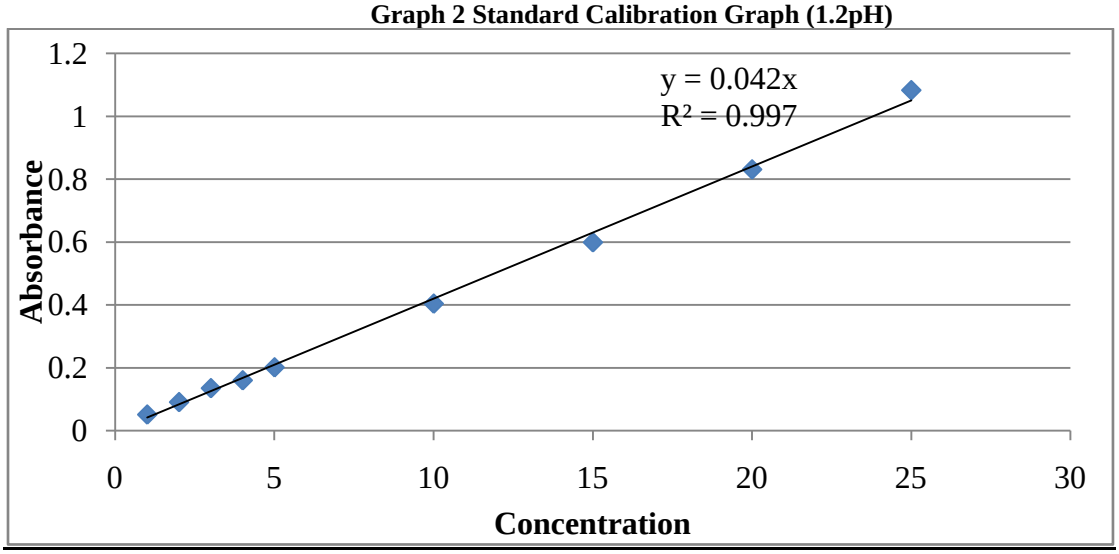


The microscopic view was taken with the help of an optical microscope with a magnification of 40X. At first the powder was examined before calcination (after sonicating 10mg of the drug with distilled water for about 2min) and thus sphere shaped structures had larger diameter as it was filled with starch, which acted as a biopolymer for the synthesis of ZnO nanoparticles (figure 3). After calcination at 500^0C for 2 hrs the spherical structures shrunk as the starch was completely removed from the hollow spheres. This resulted in the decrease in the diameter of the nanoparticles and making a hollow sphere for the entrapment of the drug (figure 4).

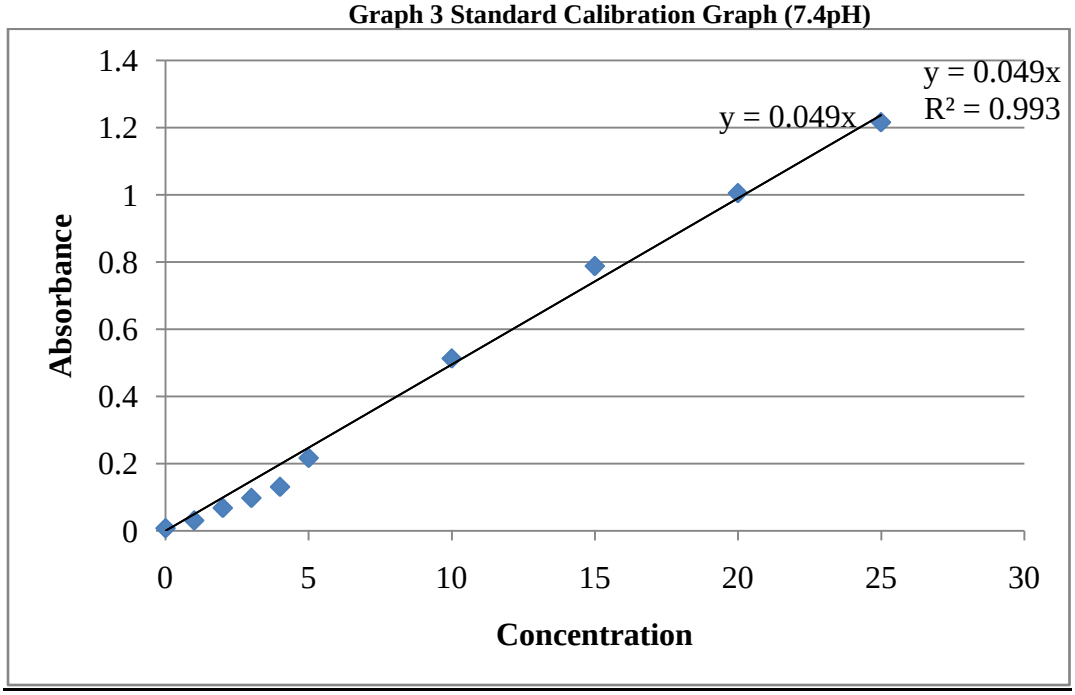
Graph 1 - Fourier Transform Infrared Spectroscopy (FTIR)



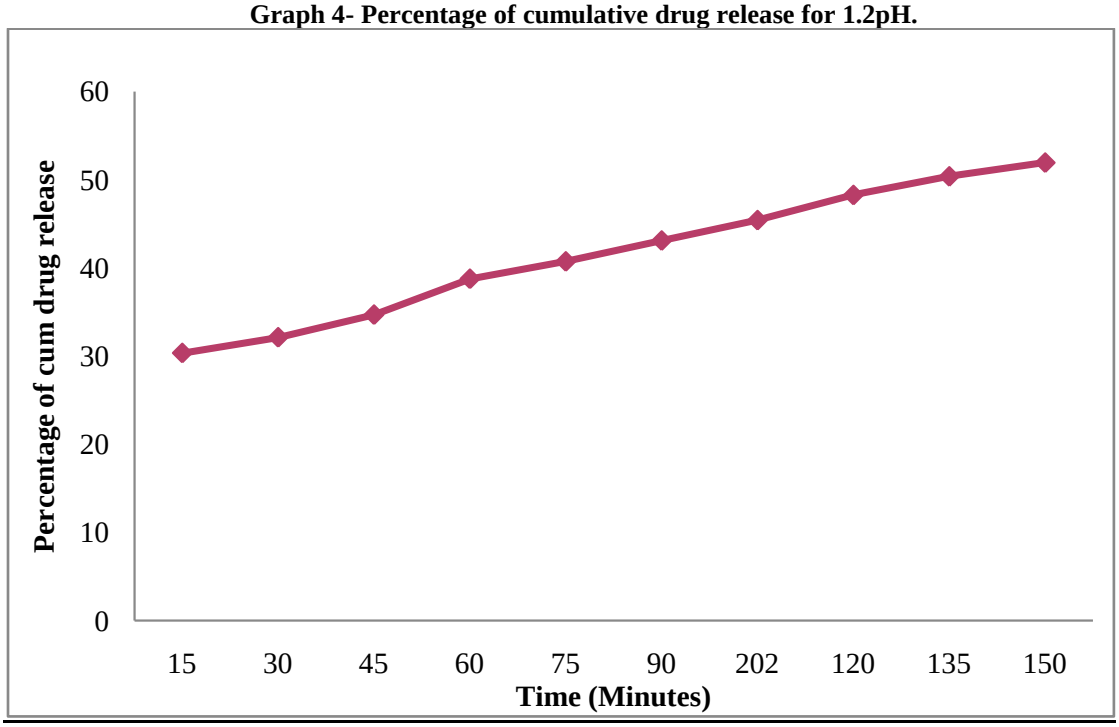
Graph 2 Standard Calibration Graph (1.2pH)



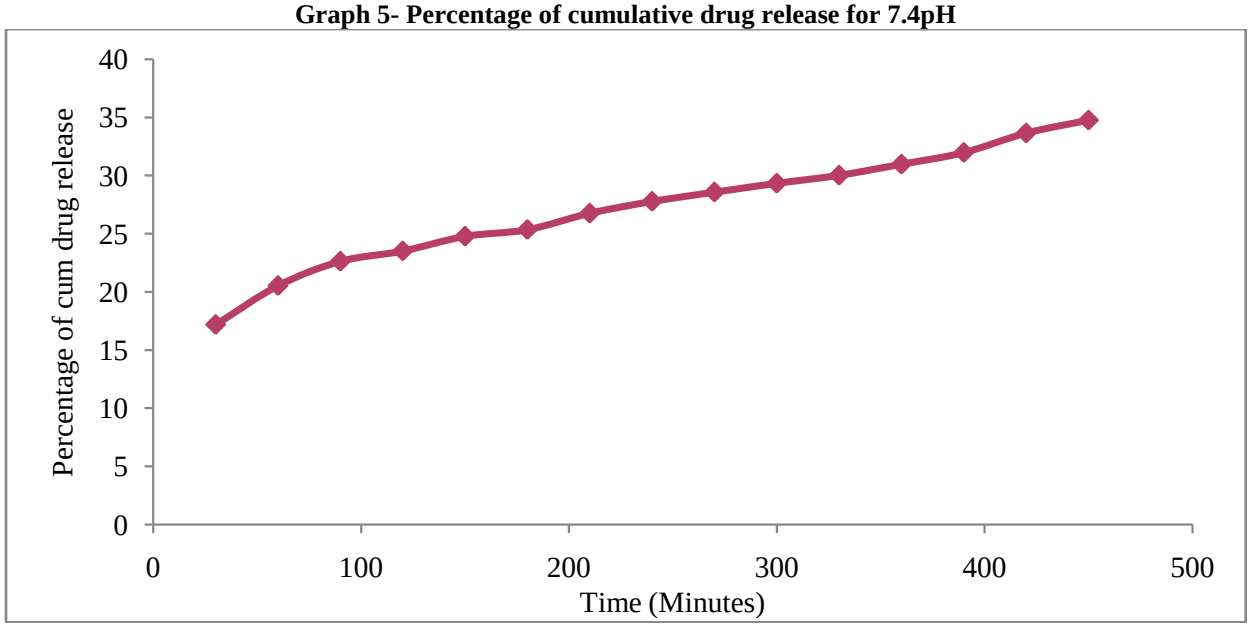
Graph 3 Standard Calibration Graph (7.4pH)



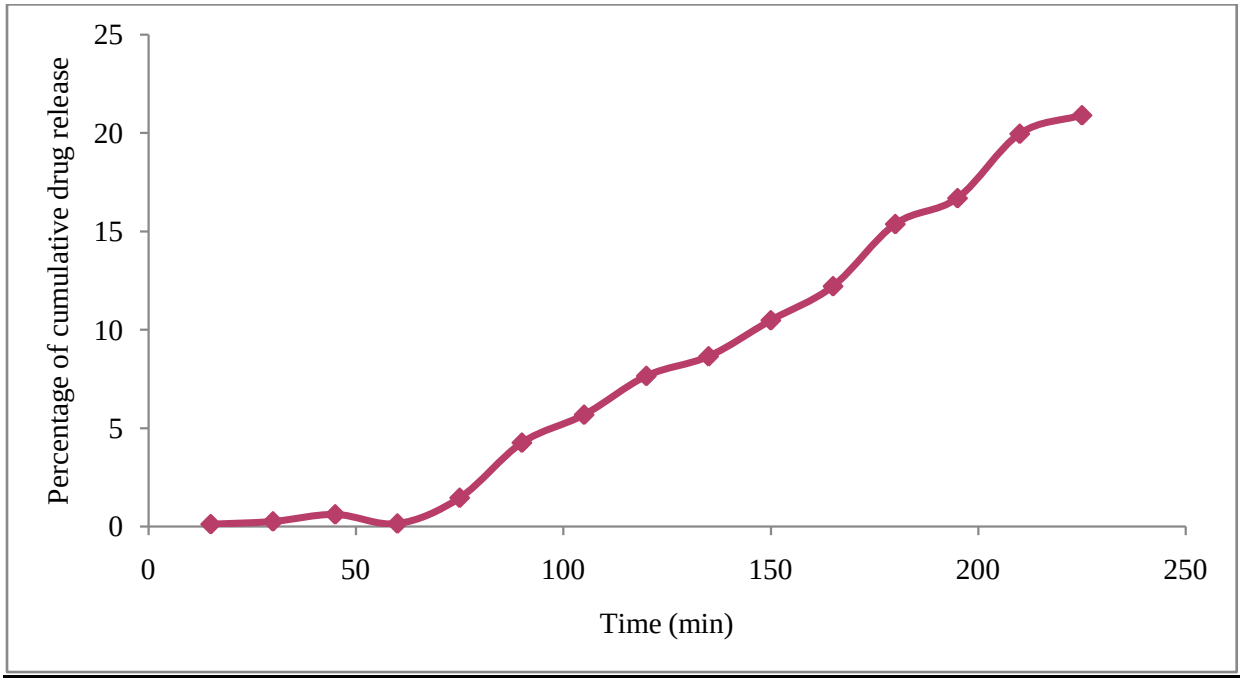
Graph 4- Percentage of cumulative drug release for 1.2pH.



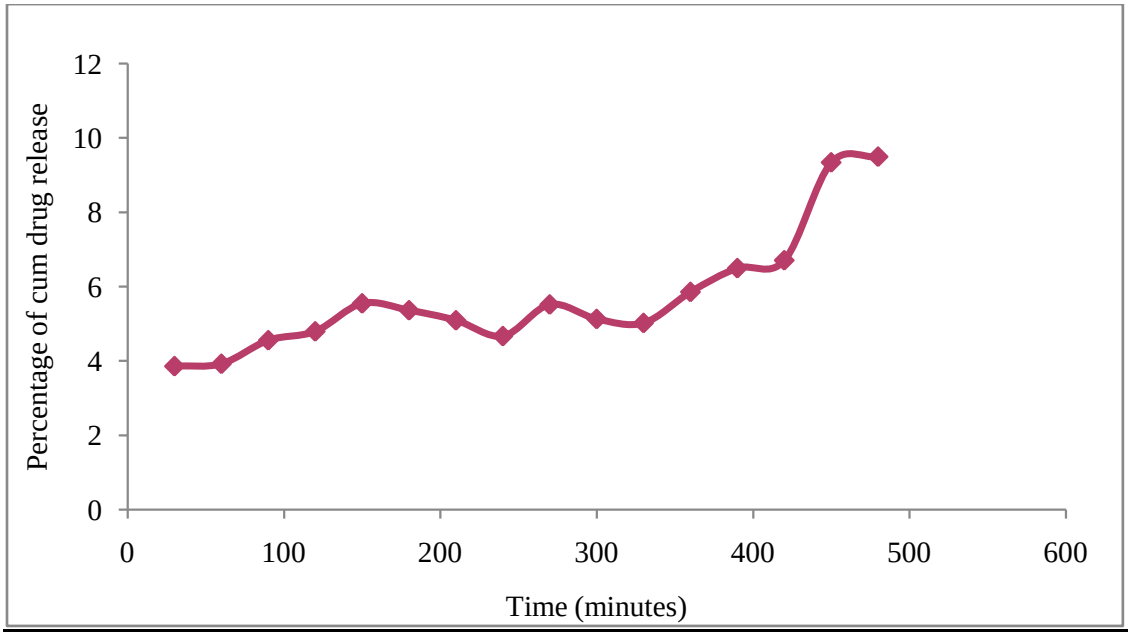
Graph 5- Percentage of cumulative drug release for 7.4pH



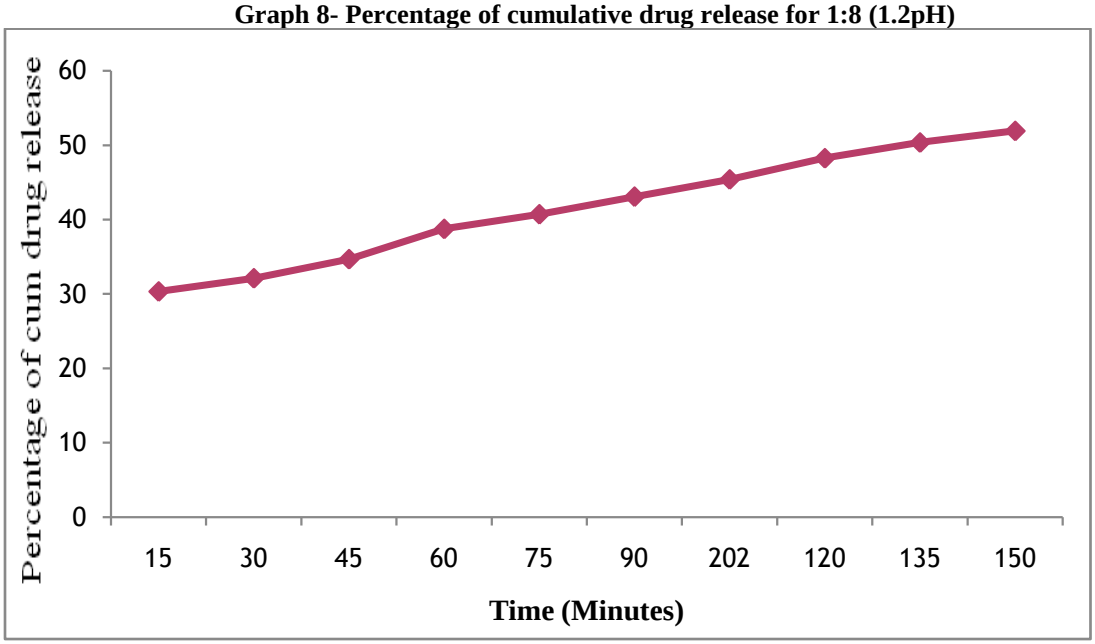
Graph 6- Percentage of cumulative drug release for 1:6 (1.2pH)



Graph 7- Percentage of cumulative drug release for 1:6 (7.4pH)



Graph 8- Percentage of cumulative drug release for 1:8 (1.2pH)



Graph 9- Percentage of cumulative drug release for 1:8 (7.4pH)

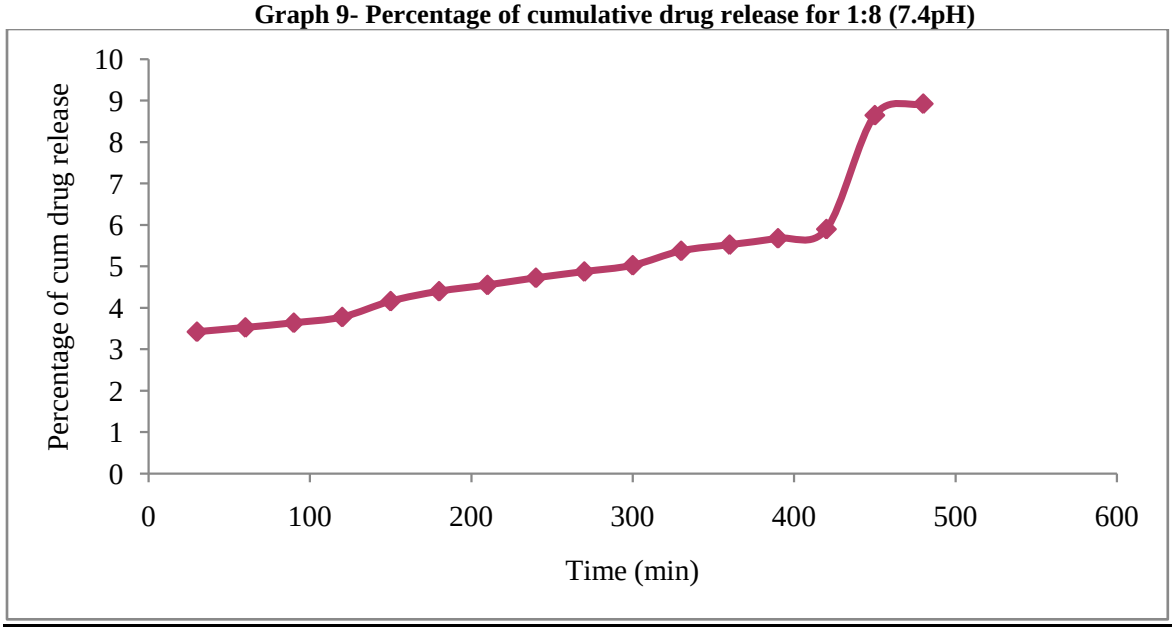


Table 1 Free drug estimation for 1.2pH and 7.4pH

pH	Ratio of the drug/ carrier	Absorbance	Microgram/ml	For 6ml	mg/6ml
1.2	1:5	0.731	17.404	104.4	0.104
	1:6	0.1979	4.711	28.27	0.028
	1:8	0.1637	8.897	23.38	0.023
7.4	1:5	2.3745	47.969	287.81	0.2878
	1:6	2.7020	54.585	327.51	0.3275
	1:8	2.7898	56.359	338.15	0.3381

DISCUSSION

Mucoadhesive is a topic of current interest in the design of drug delivery systems to prolong the residence time of the dosage form at the site of application or absorption and to facilitate intimate contact of the dosage form with the underlying absorption surface to improve and enhance the bioavailability of drug (Mathiowitz et al., 1999). Microparticulate delivery system includes many pellets, beads, microcapsules, microspheres, liposomes etc. Generally these micro particulate delivery systems are intended for oral and topical use (Benita S. 1996). Present study describes the formulation of monodisperse ZnO hollow spheres which act as carriers to study continuous in vitro drug release study in both acidic as well as alkaline medium. Different types of coated particles can be obtained depending on the coating process used. The particles can be embedded within a polymeric or proteinic matrix network in either a solid aggregated state or a molecular dispersion resulting in the formation of microspheres (Abubakr et al., 2003). The ultimate objective of microparticulate delivery system is to control and extend the release of the active ingredient from the nano particles without attempting to modify the normal bio fate of the active molecules in the body after administration and absorption. ZnO is nontoxic and is compatible with human skin, it has large surface area, synthesis of ZnO hollow spheres is an easy process and does not require many contributing ingredients, and it has inexpensive manufacture compared to other lipid based or biological nanoparticles making it a suitable carrier for drug delivery. Captopril is an orally active inhibitor of angiotensin converting enzyme and it is widely used in the treatment of hypertension and congestive cardiac failure. The bioavailability of captopril is approximately 65 % have relatively short half-life of 3 hours and requires frequent administration of dose 25 – 50 mg, 2-3 times daily (Oates 1996). Hence it is necessary to develop sustained release formulation to overcome this draw back. Gastro retentive dosage forms are interesting extended release delivery systems for drugs with a narrow window of absorption in the upper intestine (Rouge *et al*., 1996), for drugs with pH-dependent solubility, for drugs degraded by higher pH intestinal fluids (Badve *et al*., 2007) or for drugs with local action in the proximal part of the GI tract, such as antibiotic administration for *Helicobacter pylori* in the treatment of peptic ulcer (Yang *et al*., 1999). Several approaches to prolong gastric retention have been investigated: Magnetic systems, high density systems, mucoadhesive systems, swelling and expanding systems and floating systems (Streubel *et al*., 2002). Mohammed G Ahmed *et al*., (2010) reported that, prolonged inhibition of ACE (Angiotensin converting enzyme) activity of captopril could be achieved by control release dosage form, using oily matrix formulation filled in gelatin capsules. Because of oily vehicles gastric emptying time was delayed and decreased GI motility, thereby retaining the drug for longer period of time at the site of absorption. The challenges of using matrix is that the remaining matrix should be removed after the drug has been completely released to avoid accumulation, following multiple administrations, release rate continuously diminishes due to an increase in diffusional resistance and/ or a decrease in effective area at the diffusion front (Qiu and Zhang, 2000). Thus this technique did not attain potential development. ZnO get dissolved in the body after the complete release of the drug. As the body requires an adequate amount of Zinc it does not cause any harm to the biological system, making it a convenient method for drug delivery compared to the use of matrices. Sameer Singh et al., (2011) studied on the formulation and evaluation of floating tablet of captopril and he reported that the release profiles showed tri-phasic with initial burst effect (less than 30 min) followed by a polymer-controlled slower release in the second phase. The difference in burst effect was the result of difference in the viscosity of the polymers. The polymers with increased viscosity showed slower burst effect. The use of metal based nanoparticles need not worry about the viscosity but only the porosity of the hollow spheres which is achieved by the process of chemical etching. One of the greatest advances offered by ZnO nanoparticles is their ease of use compared long shaking process required to hydrate surfactants in the conventional dry film method (Ankur *et al*., 2007).

The synthesis and evolution of zinc oxide hollow microspheres using zinc powder precursor was studied and found out that high surface area hollow spherical structures may find potential applications in sustained and continuous drug release studies (Yu Tian *et al*., 2009).

Lun Hsiao *et al*., (2011) effects of various physiochemical characteristics on the toxicities of zinc oxide and titanium oxide nanoparticles towards human lung epithelial cells was studied and found out that nano zinc oxide has greater potential to enter cells than nano titanium oxide therefore, concluded that the chemical composition of a nanoparticle is a major influence on its cytotoxicity. Zinc oxide spheres and pseudosphereical structures were synthesized using a surfactant free wet chemical method. This low cost and high yield synthesis method makes the products promising materials for drug delivery (Zijie Yan *et al*., 2008). From all these we can state that, besides providing the controlled systemic delivery of captopril, ZnO nanospheres provides an effective means of delivering the drug through route and it can be further processed to make tablets and capsules to increase patient compliance. (Sunil *et al*., 2007).

ZnO as bio-nanocarriers, they have a wide range of biomedical applications for drug delivery carriers, diagnostic agents, bio sensing probes and adsorption and separation. In addition, the process of selective chemical itching on

ZnO porous nanoparticles was achieved (Zeng *et al*., 2005) Smigelkas *et al*., (2007) demonstrated that, at elevated temperature, the difference between the diffusion rates of two metals leads to the formation of pores in the species. Hollow/ porous structure nanocarriers have been advantageous to achieve high drug load because of their large storage volume. Inorganic drug delivery agents of hollow cores in their interior have the ability of loading and releasing as well as transferring therapeutic agents and would enhance the therapeutic value. Organic hollow particles such as colloidal-templated spheres, macroporous capsules, micelles, liposome and dendrimers have been the focus of this line of studies.

In vitro release studies were carried out in acid medium (pH 1.2) and alkaline medium (7.4pH) and there was a slow and controlled release of drug for all the formulations in 1.2pH as captopril is a mucoadhesion drug and most of the release took place in the stomach than the intestine (Yuan *et al*., 2009). From the work carried out and from the results obtained we can confirm that ZnO nanoparticles or any other metal-based nanoparticles are the most effective and convenient carriers for the delivery of drugs and sustained and continuous drug release was obtained at the required site in the time period given.

CONCLUSION

Studies were undertaken on the development and characterization of captopril loaded ZnO nanospheres with a view to develop control release formulation. The Captopril-ZnO Nanospheres were synthesized and confirmed by the characterization studies such as SEM, TEM and FTIR. The ZnO Nanospheres act as a drug delivery carrier for the drug captopril. The drug release from different proportions of the drug-carrier ratio depends on the pore size of the carrier and ability of drug to perfuse into the pores. Thus drug loading capacity depends on the pore size. In the preparation of nanospheres, carrier zinc oxide was used with different concentrations of captopril and different formulations are made. Interaction studies like UV and IR spectra showed that there were no interaction between drug and carrier used. In the preparation of nanospheres, captopril was used with different concentration with carrier zinc oxide and formulations were made using two different buffer solutions pH 1.2 (acid buffer) and pH 7.4 (alkaline buffer) and out of which formulation 1:5 shows optimum release in pH 1.2. Results of dissolution studies shows that formulation (1:5) of pH 1.2 gives good release profile compared to other formulations.

The objectives of the present work was achieved i.e., formulation, evaluation and usefulness of Zinc Oxide hollow spheres which acted as the carrier for mucoadhesive drug Captopril. Certainly these findings can be applied for sustained and continuous delivery of drugs with mucoadhesion. Further findings will help the industry to scale up the commercial production.

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