Synthesis And Characterization Of Curcumin Loaded Magnesium Oxide Nanoparticles: Ph Dependent Invitro Release Behavior Of Loaded Curcumin And Its Antioxidant Activity

G.Swathi , G.Rajasekhara Reddy, M.Rajesh, M.Venkataswamy, B.Deva Prasad Raju , N.John Sushma

Abstract: Curcumin is an active natural polyphenol component of Curcuma longa using in several different fields, such as food, textile, and the pharmaceutical industry because of its anti-inflammatory and antioxidant properties. To overcome the problem of poor bioavailability, low solubility in aqueous media, we have developed the Curcumin loaded Non-toxic inorganic MgO nanoparticles which can dissolve at slightly acidic conditions can be used as drug delivery systems has advantages such as a long lifetime circulation, ability to improve the drug's aqueous solubility as well the bioavailability, and the capacity to overcome physiological barriers that comprise a very effective and attractive treatment for several diseases. In our present study we have aimed to synthesize Curcumin loaded magnesium oxide nanoparticle as magnesium is main constituent of human body used to load and unload Curcumin molecules by $Mg(OH)_2$ precipitation followed by calcinations technique. Synthesis of Curcumin loaded MgONPs (CuMgONPs) nanoparticles can be primarily confirmed by UV-Visible analysis at 360-380nm and 420-440nm represents the magnesium oxide and the Curcumin. The physicochemical characteristics of CuMgONPs were studied and based on the results of the parameters like particle size, polydispersity index, zeta potential, encapsulation efficiency and drug loading capacity of formulation was 179.2nm, 0.423,-40.8 mV, 83.43 and 17.23 respectively. Fourier transform infrared (FTIR) peaks suggested that magnesium oxide and Curcumin are compatible with each other. X-ray diffraction(XRD) confirmed the reduced crystal formation of Curcumin encapsulated in the metal oxide nanoparticles. SEM analysis has shown the average particle size was found to be in the range of 429-611nm. In vitro release studies of CuMgONPs was observed at different pH conditions (3, 5, 7.4) compared to the Curcumin solution for 5-day period with the maximum release rate of 88% with controlled drug release was observed at acidic pH 3 along with the 82 % of invitro antioxidant activity which is compared with the Ascorbic acid. The studies confirmed that Curcumin loaded MgONPs (CuMgONPs) were prepared using precipitation followed by calcinations technique resulted in promising drug delivery system for control drug release with improved bioavailability with good stability provided by the metal oxide coating.

Index Terms : Curcumin, CuMgONPs (Curcumin loaded Magnesium oxide nanoparticles), antioxidant activity, invitro drug release.

1 INTRODUCTION

Curcumin is an orange-yellow pigment of turmeric which is well known for its natural chemopreventive compound obtained from rhizomes of curcuma longa has broad range of biological and pharmacological activities such as anti-inflammatory, antimicrobial, antioxidant and anticancer[1-5] activities and also helps in treating patients with Alzheimer's by reducing the oxidative damage by suppressing inflammatory factors[6,7]. Beside the beneficial prospective of Curcumin its low aqueous solubility along with rapid metabolism are the major hurdles for the beneficial use of Curcumin[8]. various strategies have been tried to rectify the problem of Curcumin solubility and bioavailability innovations in nanotechnology provided a way for hydrophobic drugs through nanocarriers mediated drug delivery.

In this regard the use of organic based nanocarriers have hindered their successful utilization in nanomedicine because of its low drug loading and instability enhanced the attention towards inorganic nanoparticles for better stability and biocompatibility with high drug encapsulation with minimum drug loss during circulation are the desirable characteristic features of drug carrier systems with clinical efficacy. $CaCO_3$ and MgO are the non-toxic inorganic nanoparticles which can dissolve slightly in acidic conditions used for the transport of the drugs which cannot dissolve at the physiological pH of blood (7.2). In this study we have made use of Magnesium Oxide nanoparticles which are odorless and nontoxic white powder for hydrophobic Curcumin delivery because of its biocompatibility, biodegradability and relatively low cost. When compared with other inorganic nanocarriers magnesium-based carriers are the fourth most abundant essential mineral in the body which is proved as more biocompatible than FDA approved magnetite for MRI. In medical applications it is used to reduce heartburn and sour stomach, improves bone regeneration and also as antitumor agent [9]. The activity of the MgONPs increases with decreased particle size [10]. To our knowledge we report for the first-time using Magnesium Oxide nanoparticles (MgONPs) as pH responsive carrier system for Curcumin delivery which involves the loading of Curcumin into Magnesium Oxide nanoparticles that forms the coordination bond between divalent magnesium and ketonic groups of Curcumin [11,12]. In our previous report [13] we have already optimized the concentrations and conditions for the synthesis of MgONPs and with all the physicochemical investigations. In this study we are making use of standardized

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MgONPs for Curcumin delivery and also going to investigate the properties of Curcumin loaded Magnesium Oxide nanoparticles(CuMgONPs) using zeta potential(ZP), X-ray diffraction (XRD), Fourier transform infrared spectroscopy(FTIR), Particle size analysis, Scanning electron microscope (SEM) along with invitro antioxidant Assay and pH dependent invitro release of Curcumin at pH (3, 5 and 7.4) from CuMgONPs.

2 Materials and methods:

2.1 Materials: $MgCl_2$-$6H_2O$, NaOH, Hcl and C_2H_5OH were of analytical grade chemicals (Merck and Himedia), Curcumin with melting point of 175.11ºC was purchased from SRL Scientifics.

2.2 Synthesis of Curcumin loaded Magnesium Oxide Nanoparticles:

For Synthesis of CuMgONPs the optimized concentrations are taken as per our previous report mentioned in ref 13. Dissolve the 2.5mg of optimized concentration of Curcumin in ethanol as it is insoluble in water and add Curcumin solution to the aqueous 25 mM MgCl2 hexahydrate solution by adding NaOH drop wise under continuous stirring, at room temperature stopped adding at pH 12 for reduced particle size according to our previous reports. The complex formation was depicted by colour change from yellow to reddish brown/orange colour based on the pH of the solution. The solution generates $CuMg(OH)_2$ which acts as precursor of CuMgO was centrifuged at 10,000 rpm for 10 min. The collected precipitation was washed with ethanol to remove impurities and formed pellet was calcinated at 100–300^{O}C, which gives the reddish brown powder form of CuMgONPs. The preliminary confirmation of nanoparticles were carried out by Thermo scientific (GENESYS 10S UV-vis spectrophotometer) at a wavelength of 200-800nm. The absorbance peak of pure MgO was observed at range between 260-280nm which was compared with Curcumin loaded MgONPs two absorbance peaks were observed at 350nm and at 420nm shown in Fig.1. Increase in absorbance peak in CuMgONPs leads to highly dispersed small sized nanoparticles with reduced aggregation due to presence of hydroxyl ions at high concentrations on the surface of nanoparticles.

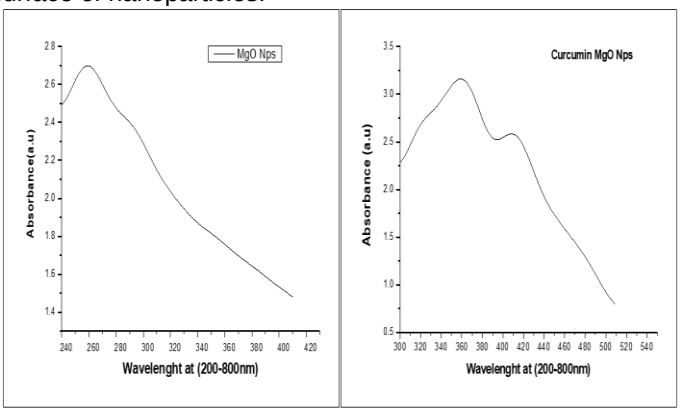

Fig .1 *UV-visible spectra of pure MgO and CuMgONPs*

2.3 Physicochemical characterizations of CuMgONPs:

The synthesized CuMgONPs were characterized for physi-cochemical parameters such as particle size, polydispersity index and Electrophoretic mobility using a Zetasizer Nano based on scattering light intensity, Chemical composition of CuMgONPs formulation can be detected by FTIR (Shimadzu), Crystalline nature or complete dissolution of the compounds can be determined by X-ray diffraction analysis (XRD) using PANalytical X'pert PRO X-ray diffractometer and also the Scanning electron microscope (SEM) of OUCT-Hyderabad set at 15.0 kV was used to know the morphology.

2.4 Invitro studies:

Entrapment efficiency (%EE) and drug loading capacity (%DLC), in vitro antioxidant activity by DPPH and reducing power assay and also invitro release behavior of Curcumin at pH (3.5,7.4).

3 Results and discussion:

3.1 Particle size, stability and morphology analysis of CuMgONPs:

The size of Curcumin loaded MgONPs measured by DLS technique which analyzes particle size distribution in the solution phase. The histograms for synthesized CuMgONPs clearly exhibit that mean hydrodynamic diameter of size distribution for CuMgONPs was 95.6nm with polydispersity index of 0.289 shown in fig 2a. The magnitude of the zeta potential (−30 mV to +30 mV) gives indication of the potential stability of the colloidal solution which indicates it requires more electrostatic repulsions between the surface charges of the particles but many experiments have proved that stability of the nanoparticle suspension not only depends on the repulsion but also depends on the use of stearic stabilizers [14, 15]. Curcumin loading was also confirmed by zeta potential analysis. In our present study loading of negatively charged oxygen rich Curcumin resulted in the zeta potential value of −40.8 mV shown in fig.2b which indicates the synthesized CuMgONPs are absolutely stable for long period of time.

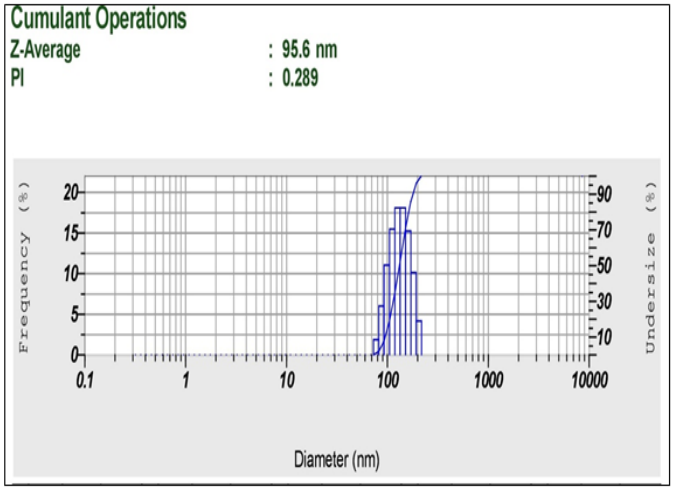

Fig.2a *representing the particle size distribution of CuMgONPs*

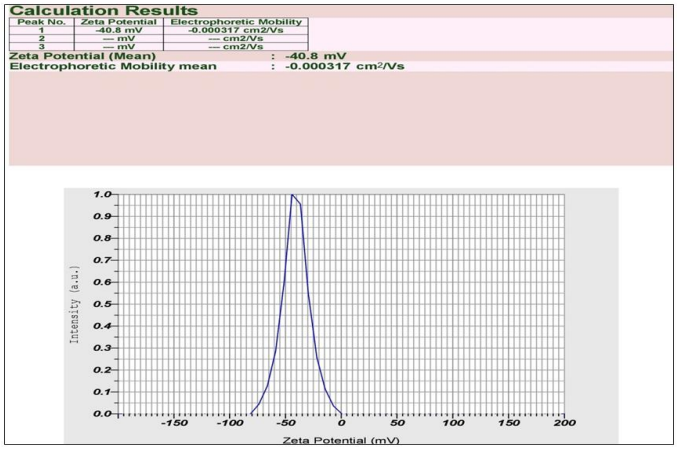

Fig.2b *showing the stability of CuMgONPs by zeta potential analysis*

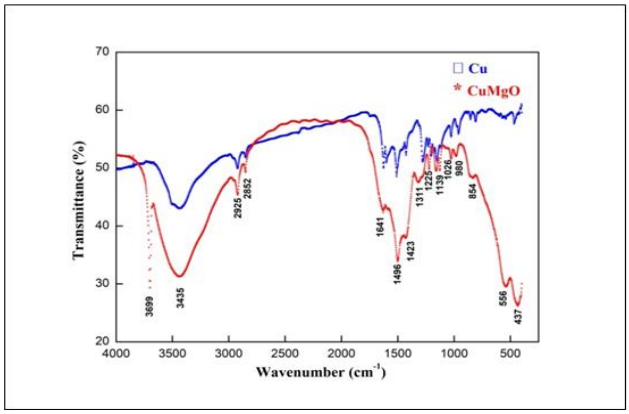

Fig.3 *FTIR spectrum of Curcumin and synthesized CuMgO NPs*

3.2 Morphological analysis of the nanoparticles by SEM:

SEM analysis reveals the polydispersity and rod-shaped nanoparticles after Curcumin loading at a optimized pH of 12 with a magnification of 3700 X resulted in the particle size in the range of below 500nm and particle size distribution measured from DLS in the range of 95.6nm. Agglomeration of particles can be observed from the figure due to high pH gives reduced particle size.

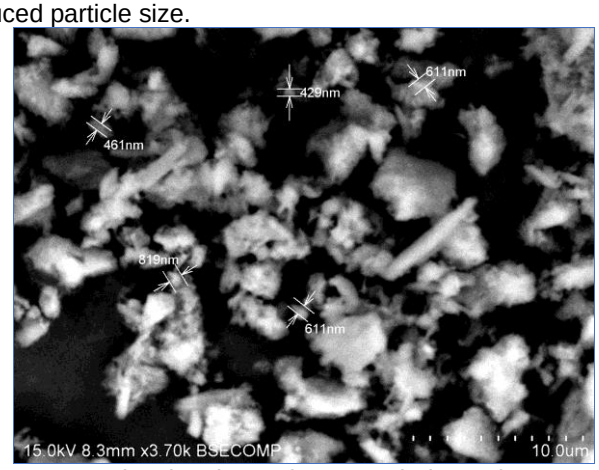

Fig.2c *SEM showing the surface morphology of CuMgONPs*

3.3 Functional group studies using spectroscopic studies (FTIR):

The functional molecules present in the Curcumin and the synthesized MgO NPs were examined by FTIR spectrum shown in fig.3 revealing the similarity in functional groups both in pure Curcumin and CuMgONPs. Peak at 3435 cm^{-1} indicates stretching vibration of phenolic (O-H) stretching, peaks at 2852 and 2925 cm^{-1} indicating stretching vibration of (C-H) bond of alkanes, peaks from bands at wavelength ranging from 1000 to 1700 cm^{-1} indicates the presence of loaded Curcumin in MgONPs which reveals the formation of Curcumin and metal ion complex. 1026 cm^{-1} indicates the C-O stretching, the band at 1496 cm^{-1} indicates Mg-O bond vibration [16] and peak at 1641 cm^{-1} indicates the presence of carbonyl groups (C=O) of Curcumin which are responsible for chelating divalent magnesium ions. The band at 854 cm^{-1} indicates bending vibration of intercalated metal oxide species [17]. Broad absorption at 556 cm^{-1} indicate lattice vibrations of MgO nanoparticles. The peak at 437 cm^{-1} indicates Mg-O stretching of nanoparticles.

3.4 Evaluation of Crystal nature of CuMgONPs by XRD analysis:

The diffraction peaks shown in fig.4 indicates the hexagonal structure of the nanoparticles. The strong diffraction peak of Curcumin at 2θ value of 24.48^{o} represents the crystal structure of Curcumin was absent in synthesized CuMgONPs which indicates the loaded Curcumin was in highly dispersed in nature when combined with $Mg(OH)_2$ during the synthesis process . The diffraction peaks of synthesized CuMgONPs observed at 2θ value of 19^{o} (001), 38.25^{o} (101), 50.93^{o} (102), 58.89^{o} (110), 62.25^{o} (220), 68.32^{o} (103), 72.22^{o}(111) respectively. The sharpness of the diffraction peaks indicates the crystalline nature of the nanoparticles. The pattern shows strong diffraction peak at 38.25^{o} corresponds to the diffraction from the (101) plane. Applying the Scherer equation to this peak gives the average crystalline size of 40nm [18] . The data obtained was matched with reported JCPDS data card no: 002-1207.

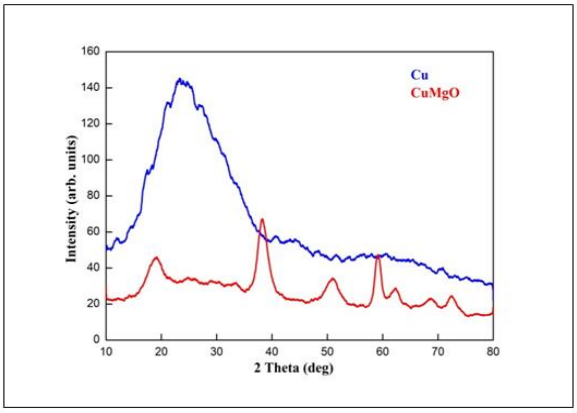

Fig.4 *XRD pattern of Curcumin and CuMgONPs*

3.5 Entrapment efficiency and drug loading capacity of CuMgONps:

In order to evaluate the Curcumin entrapment efficiency of the CuMgONPs, firstly the free content or unentrapped Curcumin present in the CuMgONPs was measured by centrifugation at a high speed of 10,000 rpm for 20 minutes and the supernatant was assayed for unbound Curcumin concentration using Thermo scientific (GENESYS 10S UV-vis spectrophotometer) at a wavelength of 423nm. Entrapment

efficiency (EE) and drug loading (DL) was calculated with formula[19].

$$EE\% = \frac{\text{Total amount of drug added– Unbound / Unentrapped drug}}{\text{Total amount of drug added}} \times 100$$

$$DL\% = \frac{\text{Wt. of the loaded/ bound drug}}{\text{Wt. of the nanoparticles}} \times 100$$

Different concentrations of Curcumin in the range of 0.1-0.3mg/ml to that 1mg/ml of MgO nanoparticles were added. The mixture was homogenized/sonicated for uniform dispersion followed by centrifugation performed at 10,000 rpm for 20 minutes to remove un bound Curcumin present in the supernatant and measured using UV-visible spectroscopy at 423nm to know the absorbance of un entrapped drug.

S.No	Curcumin concentration (mg)	% Entrapment efficiency of MgONPs	% Drug loading capacity of MgONPs
1.	2.5	68.40±0.65	12.82±0.28
2.	5	80.38±0.85	15.76±0.34
3.	7.5	85.66±0.43	19.25±0.56

Table.*1 showing the entrapment and drug loading capacity of CuMgONPs*

Entrapment of Curcumin is based on the presence of ketonic, phenolic, one active methylene group on the Curcumin acts as a brilliant ligand for any chelation which uses the β-diketone moiety as the binding site present in the divalent ions maximum Entrapment efficiency 85.66±0.43% and drug loading of 19.25±0.56 % was observed at Curcumin concentration of 5mg which is optimized for further studies

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3.6 Free radical scavenging activity by DPPH assay:

The free radical scavenging activity of Curcumin loaded MgONPs was evaluated by 2,2-diphenyl-2-picryl-hydrazil (DPPH) scavenging assay according to the method reported by Blois[20]. Powder form of CuMgONPs were dissolved in distilled water and added to the 1 ml of 1 mM DPPH. The mixture was shaken well and incubated at room temperature for 30 min and absorbance was measured at 517 nm in a spectrophotometer. The same process was carried out using unloaded MgONPs compared with free Curcumin and Ascorbic acid as shown in fig.5. The reaction of DPPH with the donor of antioxidant changes the solution from deep violet to pale yellow colour based on the antioxidant activity of the CuMgONPs.

$$DPPH + H\text{-}A \longrightarrow DPPH\ –H + A$$

$$\% \text{ Scavenging activity} = \frac{\text{Absorbance of control – absorbance of sample} * 100}{\text{Absorbance of control}}$$

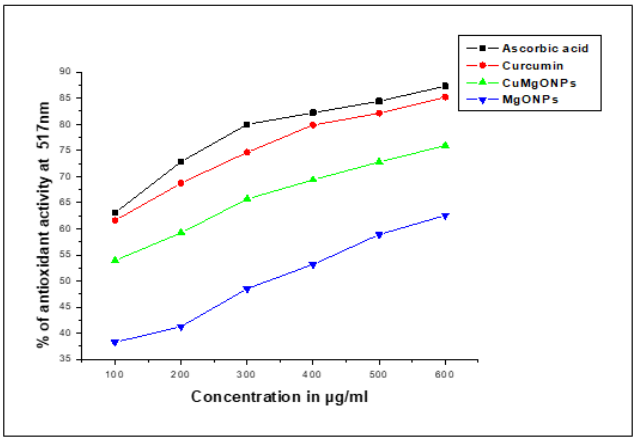

Fig.5 *Antioxidant activity of CuMgONPs by DPPH Assay*

The maximum inhibition effect of CuMgONPs showed 75.97% and Curcumin alone has the inhibition of 85.21%.

3.7 Reducing power assay:

The ability of the antioxidants presents in the sample to reduce Fe^{+3} to Fe^{+2} (reducing effect) according to the method given by Oyaizu et al [21]. The reduced activity of the compound is a indicator of potent antioxidant activity of the sample which was compared with the standard Ascorbic acid [22]. The compounds with reducing power acts as a electron donors with electron transfer property of polyphenols and flavonoids help in the reduction of the oxidized intermediates which are generated during lipid peroxidation process acts as primary and secondary antioxidants. Briefly 100-600 µl concentrations of loaded and unloaded Curcumin MgONPs and pure Curcumin solutions along with standard ascorbic acid was added to the 2.5ml of 0.2 M Phosphate buffer to which 2.5 ml of 1% of potassium ferricyanide was added and kept in water bath at 50^{o}C for 20 minutes. After cooling the solution 2.5ml of 10% TCA was added and the mixture was centrifuged at 3000 rpm for 10minutes. After centrifugation 2.5ml of distilled water and 0.5ml of 0.1% ferric chloride was added to the reactant mixture resulted in the different shades of bluish green colour formation depending on the reducing ability of the sample which was measured at a absorbance of 700nm. The inhibition effect of CuMgONPs and Curcumin with 82.22 % and 87.57%.

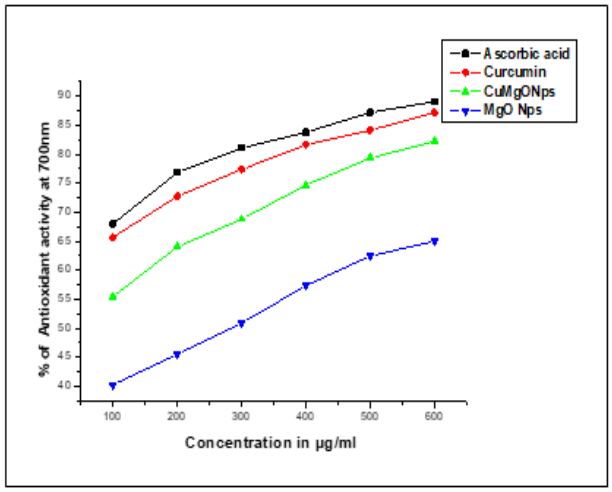

Fig.6 *showing the reducing power activity of CuMgONPs*

3.8 In vitro release profile of Curcumin from Curcumin loaded MgONPs:

In-vitro release studies were performed on prepared nanoparticles at room temperature in three different buffer solutions i.e., (a) KHP buffer at pH 3.0 (b) acetate buffer at pH 5.0 (c) phosphate buffer at pH 7.4. The CuMgONPs of 5mg dissolved in appropriate buffers was transferred to egg membranes and kept in beakers containing 100ml of corresponding buffer solutions of pH (3,5 and 7.4) and kept in room temperature and samples are withdrawn at regular intervals of time and same volume of fresh buffer was transferred to the beakers. Invitro release behaviour of Curcumin form loaded magnesium oxide nanoparticles was observed at 3 different pH buffer solutions. The burst Curcumin release in acidic pH 3 as MgO dissolves readily by releasing 46% of Curcumin within 1hr of time indicates pH responsive degradation of magnesium Oxide nanoparticles and also due to the drug-carrier bond is simultaneously broken due to protonation of Curcumin ligand where as in other buffer solutions of pH 5 where MgO dissolves very slow releasing of Curcumin with 38% and at pH 7.4 MgO releasing Curcumin of 3% within 1hr. The percentage of Curcumin released from MgONPs at end of 120hrs is 88.03 %,64.95% and 20.03% respectively at pH 3,5 and 7.4 respectively which is compared with unloaded or free Curcumin has shown the maximum release rate of 91.28% at pH 3, slow release of 64.95% at pH 5 and 20.25% at pH 7.4 at the end of 120hrs of release data. The pH mediated drug release rate was in the order of pH 3.0 > 5.0 > 7.4. In our present study we have made use of biocompatible nanocarrier that can release the drug by disintegrate with response to mildly acidic conditions which makes our system more efficient for site specific drug delivery.

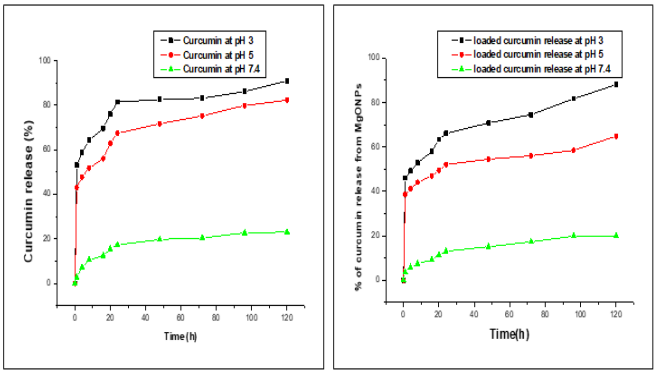

Fig.7 *Invitro Curcumin release form MgONPs at different pH compared with unloaded Curcumin for 120hours.*

4 CONCLUSION:

In this study we reported CuMgONPs for acidic pH responsive nanocarriers as drug delivery systems which is because of the chelating components present in the Curcumin for effective binding or entrapment with the biocompatible magnesium ions and insolubility problem of Curcumin was solved by loaded on to Magnesium Oxide nanoparticles through coordination chemistry and invitro release has confirmed the effective release at acidic pH and also showed the potential invitro antioxidant activity. CuMgONPs can also be used as safe oral route for therapeutic applications of Curcumin.

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