Research Paper Medical science

Stimuli-Responsive Hydrogels From Zincoxide Nanocomposite Hydrogels- Synthesis And Characterisation

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ABSTRACT

In this study, we fabricated a series of Poly (N-tert-amylacrylamide –co –N-VinylPyrrolidone) ZincOxidenanocomposite hydrogel (Poly(NTA-NVP)ZnO), Poly (N-tert-butylacrylamide –co –N-VinylPyrrolidone) ZincOxidenanocomposite hydrogel (Poly(NTB-NVP)ZnO), Poly (N-cyclohexylacrylamide –co –N-VinylPyrrolidone) ZincOxidenanocomposite hydrogel (Poly(NCA-NVP)ZnO),by a free-radical polymerization using Azo-bis-iso-butyronitrile(AIBN) and N,N´-methylene-bis-acrylamide (MBA) initiator and cross-linking reagent respectively. The effects of increasing the concentration of crosslinking reagent and pH and the swellablitywere also investigated. These ZincOxideNanocomposite hydrogels were characterised by Fourier transfom infrared Spectroscopy and X-ray diffraction. The results revealed that the copolymers obtained were stimuli-responsive hydrogels.These ZincOxideNanocomposite hydrogels were characterised by Fourier transfom infrared Spectroscopy and X-ray diffraction.Distribution of ZincOxide nanoparticles within the polymer matrix was studied through their characterization using SEM analysis. Protein , Drug loading and the release behaviour were studied using UV Visible spectroscopy.

KEYWORDS copolymerization; gels; swelling; crosslinking; networks.

Introduction

Nanotechnology is emerging as a rapidly growing field with its application in Science and Technology for the purpose of manufacturing new materials at the nanoscale level . Biosynthetic and environment friendly technology for the synthesis of zinc oxide (ZnO) Nanoparticles (NPs) are believed to be nontoxic, biosafe, and biocompatible.

Metal oxide nanoparticles attract great attention in recent years on account of their special electronic and chemical properties. Among the metal oxide, ZnO have been investigated extensively due to their chemical stability and efficient photocatalytic properties. Zinc oxide has achieved applications in various areas such as optical, piezoelectric, magnetic, and gas sensing properties, and apart from this, zinc compounds have been generally regarded as safe. Recently, the use of inorganic antimicrobial agents, such as zinc compounds, has received increased attention.

In this work, we synthesized ZincOxidenanocomposite hydrogel by in situ polymerisation of N-tert- amylacrylamide, N-VinylPyrrolidone(NVP), N-tert- butylacrylamide, N-VinylPyrrolidone(NVP), N-cyclohexylacrylamide, N-VinylPyrrolidone(NVP),using AIBN as free radical initiator and N,N' – methylenebisacrylamide (MBA) as cross linker. These ZincOxideNanocomposite hydrogels were characterised by Fourier transfom infrared Spectroscopy and X-ray diffraction. Swelling behavior and swelling kinetics of the obtained hydrogels were evaluated. Distribution of ZincOxide nanoparticles within the polymer matrix was studied through their characterization using SEM analysis.

EXPERIMENTAL
MATERIAL AND METHODS

The monomer used was Acrylonitrile , N-tert-Amylalcohol ,N-tert-butylalcohol, N-cyclohexanol, N-VinylPyrrolidone, and ZincOxidenanopowder (sigma Aldrich). Azobisbutyronitrile (sigma Aldrich) was used as initiator. N,N'-methylene-bis-acrylamide (Merck, Germany)was used as a cross-linking agent .All other chemicals used were of analytical grade.

Acrylonitrile

Acrylonitrile was first washed with 5% NaOH solution in water to remove the inhibitor and then with 3% Orthophosphoric acid solution in water to remove basic impurities. Then the Acrylonitrile was washed with double distilled water and dried over andhydrous $CaCl_2$. The acrylonitrile was then distilled in an atmosphere of Nitrogen at reduced pressure. It was then collected in a clean dry amber colour bottle and kept in the refrigerator at 5°C.

Preparation of N-tert-amylacrylamide (NTA)

The monomer N-tert-amylacrylamide was prepared by the reaction of t-amyl alcohol with acrylonitrile. N-tert-amylacrylamide was recrystallized in warm dry benzene.

Preparation N-tert-butyl acrylamide (NTB)

The monomer N-tert-butylacrylamide was prepared by the reaction N-Tertiary-butyl alcohol with acrylonitrile. N-tert-butylacrylamide was recrystallized in warm dry benzene.

Preparation N-Cyclohexyl acrylamide (NCA)

Themonomer N-Cyclohexylacrylamide was prepared by the reaction N-Cyclohexanol with acrylonitrile. N-Cyclohexylacrylamide was recrystallized in warm dry benzene.

Preparation of ZincOxideNanocomposite Hydrogels–(Poly(NTA-NVP)ZnO), (Poly(NTB-NVP)ZnO),(Poly(NCA-NVP)ZnO).

The ZnONanocomposite hydrogel were prepared by free radical copolymerization of NTA, NVP in the presence of MBA as crosslinker and AIBN for initiating the polymerisation system. Aqueous solution containing a weighed amount of NTA, NVP, MBA, AIBN were dissolved in methanol – water (3:1) mixture and final volume was made 10mLin a polymerization tube. A solution containing 10mg of ZincOxide nanoparticle was added with constant stirring. After bubbling nitrogen for 15 min, the contents were placed in thermostatic water bath at 65^0C and the polymerization was conducted for 1 day. The prepared hydrogels were air-dried followed by vacuum drying. Similarly Poly (N-Tert-butylacrylamide-co-N-VinylPyrrolidone) ZIncOxidenanocomposite hydrogels andPoly(N-Cyclohexylacrylamide-co-N-VinylPyrrolidone)ZincOxidenanocomposite hydrogels were prepared.

Characterization FTIR Spectra Poly (NTA-NVP) ZnONanocomposite Hydrogel

The vibrational spectrum of a molecule is considered to be a unique physical property and is characteristic of the molecule .The following characteristic bands were identified. The peak at 3478 cm^{-1} is due N-H stretching and a peak at 1592 cm^{-1} corresponds to C=O. We can see two bands at 1359, 1118 cm^{-1} represent the C-N and C-H bonding respectively. There was only new band at 485 cm-1 specific for stretching vibration of ZnO group.

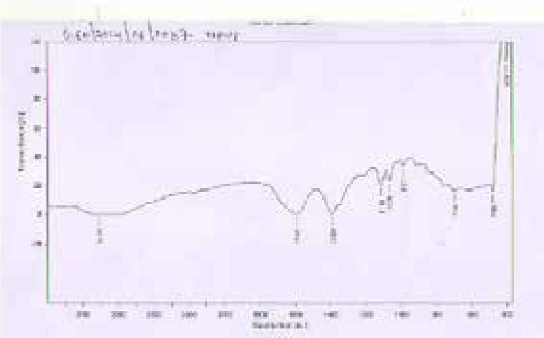

Fig.1 FT-IR Spectra of Poly (NTA-NVP) ZincOxideNanocomposite Hydrogel

Characterization FTIR Spectra Poly (NTB-NVP) ZnONanocomposite Hydrogel

The Peaks at 1217 and 1389 cm^{-1} are corresponding to [-C(CH3)3] groups of NTB and at 1594 cm^{-1} C=O group in acrylamide A broad spectrum peak corresponding to NH stretching of acrylamide was observed at 3393 cm-1. There was only new band at 486 cm-1 specific for stretching vibration of ZnO group.

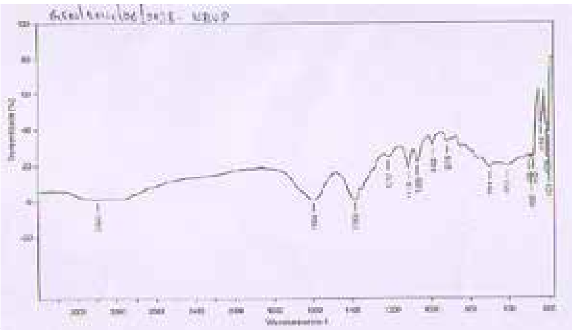

Fig 2.FT-IR Spectra of Poly (NTB-NVP) ZnONanocomposite Hydrogel

.Characterization FTIR Spectra Poly (NCA-NVP) ZnONanocomposite Hydrogel

The IR analysis of the hydrogels showed that the presence of peaks corresponding to the functional groups of monomeric units present in the copolymeric hydrogel chain. A broad peak corresponding to NH stretching of acrylamide was observed around 3461cm^{-1} , the peaks were also observed at 1592 cm^{-1} corresponding to C=O , We can see two bands at 1389, 1123 cm^{-1} represent the C-N and C-H bonding respectively and there was only new band at 500 cm^{-1} specific for stretching vibration of ZnO group.

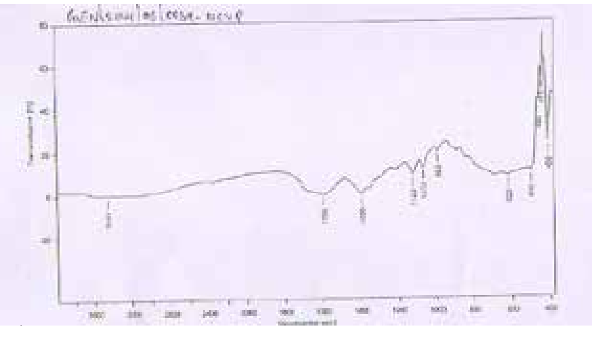

Fig 3.FT-IR Spectra of Poly (NCA-NVP) ZnONanocomposite Hydrogel

Scanning Electron microscope (SEM)

Images for the ZincOxide Nanoparticle and ZincOxideNanocomposite hydrogels were recorded using Hitach, model-JSM-5000 imaging mode at 30 kV with varying levels of magnification. SEM/ EDAX was used to study the internal or cross morphology of the nanocomposite hydrogel. The image indicates the ZNPs are spherical in shapeZincOxideNanocomposite hydrogels distributed uniformly throughout the polymer matrix.

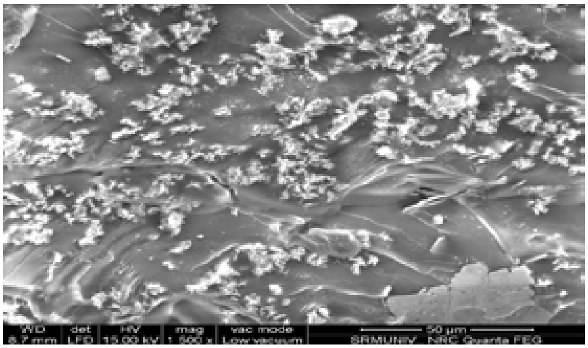

Fig 4.SEM image ofPoly (NTA-NVP) ZincOxideNanocomposite Hydrogel

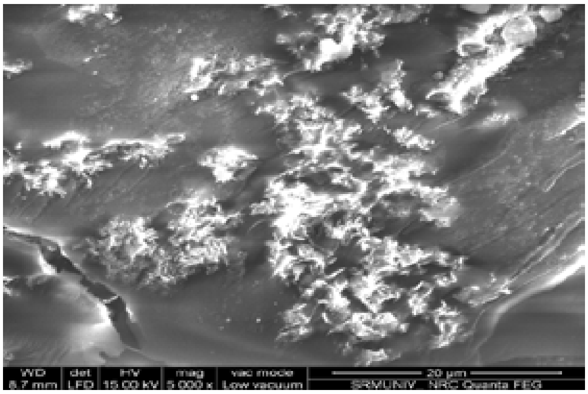

Fig 5.SEM image ofPoly (NTB-NVP) ZincOxideNanocomposite Hydrogel

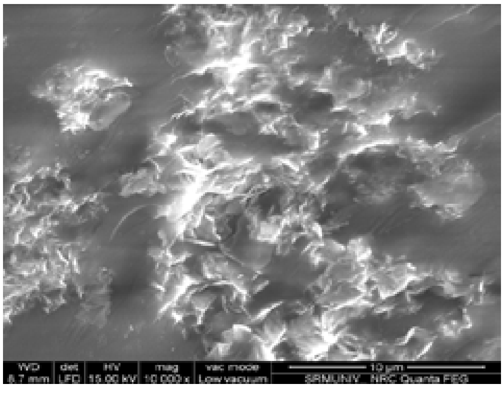

Fig 6.SEM image ofPoly (NCA-NVP) ZincOxideNanocomposite Hydrogel

EDAX spectrum of ZNP Hydrogel

SEM/EDAX micro analysis was employed to determine the constitution of the ZincOxide nanoparticles dispersed in the hydrogel matrix. The surface/cross sectional micrographs of the ZincOxidenanocomposite hydrogel Energy dispersive analysis X-ray confirms the presence of ZincOxide nanoparticles in the hydrogel polymer matrix.The representative EDAX spectrum showing well-resolved peaks for Zinc, carbon, oxygen which are the elements present in the ZincOxidenanocomposite hydrogel.

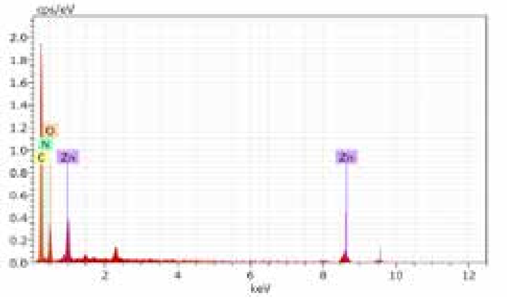

FIG 7.EDAX image ofPoly (NTA-NVP) ZincOxideNanocomposite Hydrogel

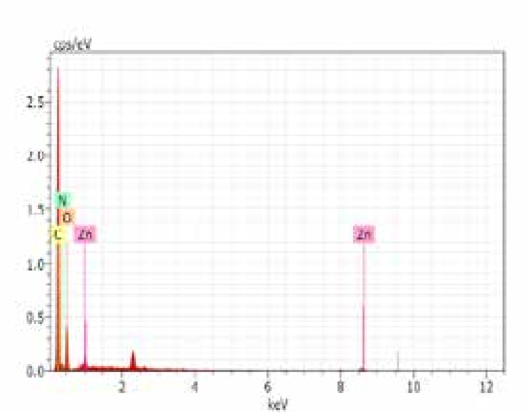

Fig 8.EDAX image ofPoly (NTB-NVP) ZincOxideNanocomposite Hydrogel

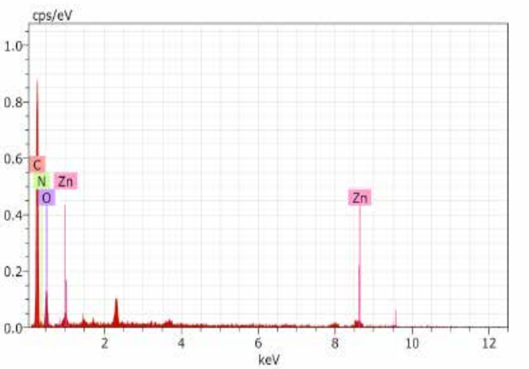

Fig 9 EDAX image ofPoly (NCA-NVP) ZincOxideNanocomposite Hydrogel

X-ray Diffraction (XRD)

XRD patterns of ZincOxidenanocomposite hydrogel was measured using Riga-ku DMAX-2000 X-ray diffractometer with the Cu Ka radiation at a scanning rate of 2s-1 in 2 ranging from 10 to 80. The sample for XRD was supported on glass substrates.

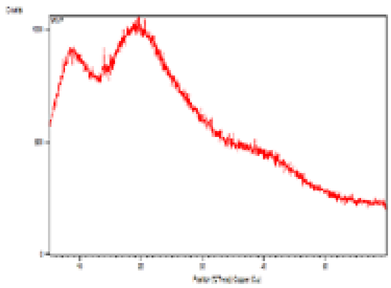

Fig 10. X-ray diffraction pattern of ZnOnanocomposite (NTA-NVP) hydrogel

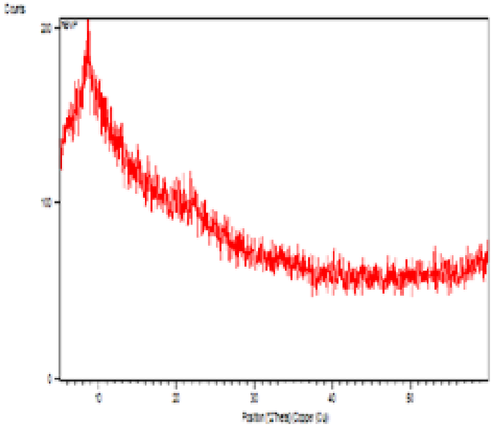

Fig 11. X-ray diffraction pattern of ZincOxidenanocomposite (NTB-NVP) hydrogel

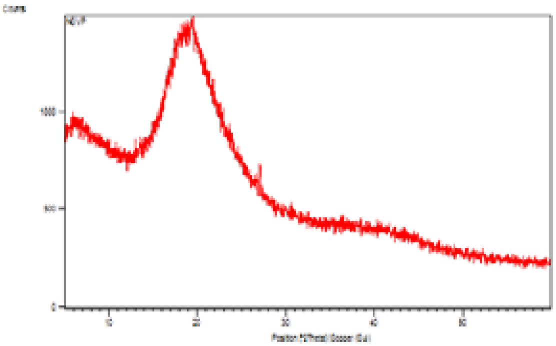

Fig 12.X-ray diffraction pattern of ZincOxidenanocomposite (NCA-NVP) hydrogel

Swelling Behaviour

Swelling experiments were carried out with a view of evaluation of the swelling capacity of the hydrogels under investigation in distilled water. Results of these experiments indicated that the increase in weight of the swollen hydrogels is directly related to the duration of swelling. The swelling behaviour observed could be associated with absorption mechanism, which, in turn, is determined by the diffusion process.

Swelling measurements

A fundamental relationship exists between the swelling of a polymer in a solvent and the natures of the polymer and the solvent. The percentage swelling (or mass swelling) is the most important parameter about swelling studies. The percentage swelling (%S) was calculated from the following equation

$$\%S = (M_t - M_0)/M_0 \qquad \text{-------}(1)$$

Where M_t is the mass of the swollen gel at time t, and M_0 is the mass of dry gel at time 0.

Porosity measurement

The solvent replacement method was adopted to determine porosity. Dried hydrogels were immersed in absolute ethanol overnight and weighed after blotting excess ethanol from the surface. The porosity was calculated by following equation

$$\text{Porosity} = (M_2 - M_1) \times 100$$

$$\rho V$$

where M1 and M2 are the masses of hydrogel before and after immersion in ethanol, respectively, ρ is the density of absolute ethanol and V is the volume of hydrogel.

Swelling behavior of ZincOxidenanocomposite hydrogels in different pH

Dynamic and equilibrium swelling coefficients of cross-linked hydrogels were determined in 0.05 M USP phosphate buffer solutions of pH 2.7,3.5,4.2,5.1,6.8 while ionic strength was adjusted to 0.11 by adding the calculated amount of NaCl. Diffferent buffer solutions of pH 2.7, 3.5, 4.2, 5.1, 6.8 were prepared. Dried hydrogels were left to swell in a solution of desired pH at a temperature of 27°C. For dynamic swelling, swollen gels removed from the solution, blotted with filter paper, weighed, at regular intervals for 8 h and placed in the same flask. The swelling ratio of each hdrogel was calculated .

Table 1.Swelling behavior of Poly (NTA-NVP) ZincOxidenanocomposite hydrogels in different pH

pH	2.7	3.5	4.2	5.1	6.8
Dyanamic swelling Coefficient	0.87	1.0	1.1	1.46	1.74
Equlibrium swelling coefficient	0.46	0.5	0.52	0.59	0.63
Diffusional Exponent n	0.551	0.551	0.552	0.552	0.551
Porosity %	14.58	15.07	15.23	15.39	15.50

Table 2.Effect of increasing the concentration of Cross-linker MBA in Poly (NTA-NVP) ZincOxidenanocomposite hydrogels in different pH

pH	Concentration of crosslinker In g	Dyanamic Swelling coefficient	Equilibrium swelling coefficient
2.7	0.025	0.87	0.46
	0.05	0.75	0.42
	0.075	0.5	0.33
3.5	0.025	1	0.5
	0.05	0.8	0.44
	0.075	0.63	0.42
4.2	0.025	1.1	0.52
	0.05	0.9	0.47
	0.075	0.75	0.42
5.1	0.025	1.46	0.59
	0.05	1	0.5
	0.075	0.8	0.46
6.8	0.025	1.74	0.63
	0.05	1.1	0.52
	0.075	0.87	0.46

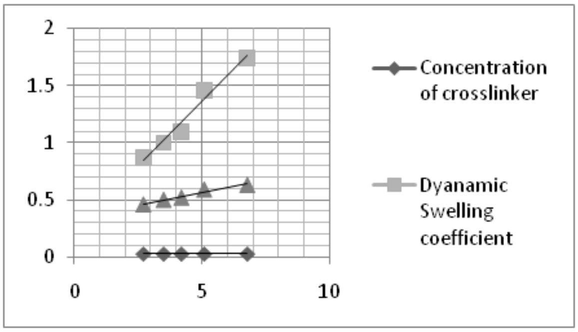

Fig 13.Effect of increasing the concentration of Cross-linker MBA in Poly (NTA-NVP) ZincOxidenanocomposite hydrogels in different pH

Table 3.Swelling behavior of Poly (NTB-NVP) ZincOxidenanocomposite hydrogels in different pH

pH	2.7	3.5	4.2	5.1	6.8
Dyanamic swelling Coefficient	0.90	1.05	1.85	2.05	2.65
Equlibrium swelling coefficient	0.47	0.51	0.64	0.67	0.72
Diffusional Exponent n	0.61	0.60	0.58	0.59	0.62
Porosity %	18.17	18.25	18.39	18.48	18.53

Table 4.Effect of increasing the concentration of Cross-linker MBA in Poly (NTB-NVP) ZincOxidenanocomposite hydrogels in different pH

pH	Concentration of crosslinker	Dyanamic Swelling coefficient	Equilibrium swelling coefficient
2.7	0.025	0.9	0.47
	0.05	1	0.5
	0.075	1.1	0.52
3.5	0.025	1.05	0.51
	0.05	1.2	0.54
	0.075	1.38	0.58
4.2	0.025	1.85	0.64
	0.05	1.46	0.59
	0.075	1.53	0.61
5.1	0.025	2.05	0.67
	0.05	1.66	0.62
	0.075	1.6	0.61
6.8	0.025	2.65	0.72
	0.05	1.74	0.63
	0.075	1.63	0.65

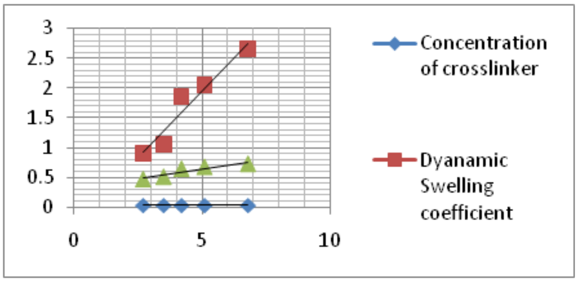

Fig 14.Effect of increasing the concentration of Cross-linker MBA in Poly (NTB-NVP) ZincOxidenanocomposite hydrogels in different pH

Tabe 5.Swelling behavior of Poly (NCA-NVP) ZincOxidenanocomposite hydrogels in different pH

pH	2.7	3.5	4.2	5.1	6.8
Dyanamic swelling Coefficient	0.50	0.93	0.9	1.0	1.2
Equlibrium swelling coefficient	0.33	0.47	0.48	0.5	0.54

Diffusional Exponent n	0.53	0.55	0.55	0.54	0.55
Porosity %	16.13	16.5	16.5	16.3	16.3

Table 6.Effect of increasing the concentration of Cross-linker MBA in Poly (NCA-NVP) ZincOxidenanocomposite hydrogels in different pH

pH	Concentration of crosslinker	Dyanamic Swelling coefficient	Equilibrium swelling coefficient
2.7	0.025	0.55	0.33
	0.05	0.66	0.4
	0.075	0.75	0.42
3.5	0.025	0.8	0.44
	0.05	0.87	0.46
	0.075	1	0.5
4.2	0.025	1	0.48
	0.05	1.1	0.52
	0.075	1.2	0.54
5.1	0.025	1.1	0.52
	0.05	1.2	0.54
	0.075	1.3	0.57
6.8	0.025	1.2	0.54
	0.05	1.38	0.58
	0.075	1.46	0.59

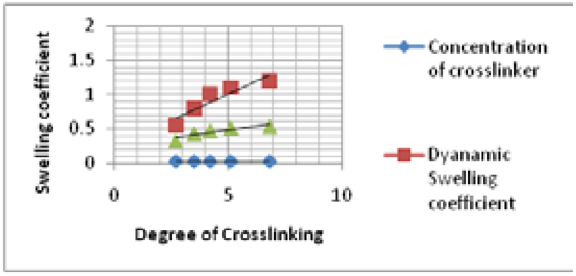

Fig 15.Effect of increasing the concentration of Cross-linker MBA in Poly (NTB-NVP) ZincOxidenanocomposite hydrogels in different pH

Analysis of drug release pattern

Peppas model $M_t/M_\infty = Kt^n$(12) where M_t/M_∞ is the fraction of drug released at time "t", K is a constant incorporating the structural and geometric characteristics of the gels and "n" is the release exponent or diffusional exponent. When n = 0.5, order of release is Fickian, n = 1 responds to a case II transport, while 0.5<n<1 corresponds to a diffusion mechanism that is non-Fickian .

This indicates that the solvent transport mechanism becomes non-Fickian as gel ionization becomes prominent. The dynamic swelling behavior of crosslinked polymers is dependent upon the relative magnitude of the solvent diffusion and polymer relaxation diffusion and relaxation times. Thus, non-Fickian (anomalous) transport is observed as the pH of the surrounding fluid increases above $pK_{a.}$

Drug Loading

50ml of 100 ppm (drug) Chloramphenicol solution was used for loading the Nanocomposite hydrogels. Prepared gels were extracted, dried and weighed. Drug solution was added to the nanocomposite hydrogels then it was closed and stored away from direct sunlight. The nanocomposite hydrogels were swollen. The gel was removed from the drug and wiped. It was added with the required amount of solvent and stored, then the absorbance was measured during different time intervals using UV-Vis spectrometer

Drug release

Dissolution studies were performed in 500 mL of freshly prepared 0.05 M phosphate buffer solutions having pH of 4.2. Temperature of dissolution medium was maintained at 37 °C. For each sample, 5 mL of media was withdrawn at a predetermined time and replaced with the same volume of fresh medium to maintain constant volume, , then the absorbance was measured during different time intervals using UV-Vis spectrometer to determine the percentage of drug release.

Table7. PERCENTAGEOF SWELLINGOF ZincOxide NANOCOMPOSITE HYDROGELS IN CHLORAMPHENICOL

Samples	Dyanamic swelling Coefficient	Equlibrium swelling coefficient	% drug Loading
Poly (NTA-NVP) ZnO hydrogel	1.48	0.324	148
Poly (NTB-NVP) ZnO hydrogel	5.84	0.828	584
Poly (NCA-NVP) ZnO hydrogel	1.96	0.490	196

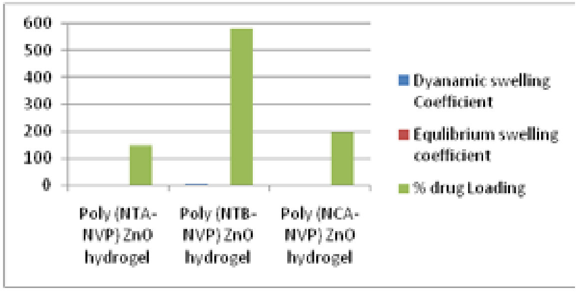

Fig 16. PERCENTAGE OF SWELLING OF ZincOxide NANOCOMPOSITE HYDROGELS IN CHLORAMPHENICOL

Table 8.DRUG LOADED AND RELEASED UV ABSORBANCE IN ZincOxide NANOCOMPOSITE HYDROGELS

SAMPLES	% of UV absorbance of drug loaded samples	% of UV absorbance of drug released samples
Poly (NTA-NVP) ZnO hydrogel	74	104
Poly (NTB-NVP) ZnO hydrogel	83	194
Poly (NCA-NVP) ZnO hydrogel	46	53

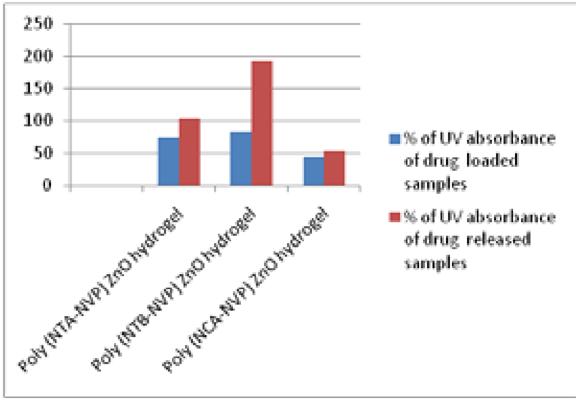

Fig17. DRUG LOADED AND RELEASED UV ABSORBANCE IN ZincOxide NANOCOMPOSITE HYDROGELS

Protein Loading and Release

50ml of 100 ppm (BSA) Bovine –Serum-Albumin solution was used for loading the Nanocomposite hydrogels. Prepared gels were extracted, dried and weighed. Protein solution was added to the nanocomposite hydrogels then it was closed and stored away from direct sunlight. The nanocomposite hydrogels were swollen. The gel was removed from the drug and wiped. It was added with the required amount of solvent and stored, then the absorbance was measured during different time intervals using UV-Vis spectrometer to determine the loading and release behavior of the hydrogel.

Table 9.UV ABSORBANCE OF PROTEIN LOADED IN ZincOxide NANOCOMPOSITE HYDROGELS

SAMPLES	% of UV absorbance of Protein loaded samples	% of UV absorbance of Protein released samples
Poly (NTA-NVP) ZnO hydrogel	65	147
Poly (NTB-NVP) ZnO hydrogel	29	113
Poly (NCA-NVP) ZnO hydrogel	43	87

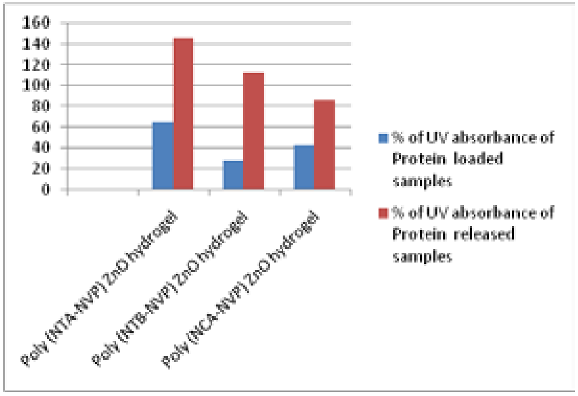

Fig 18.UV ABSORBANCE OF PROTEIN LOADED IN ZincOxide NANOCOMPOSITE HYDROGELS

Conclusion

The swelling , drug loading capacity decreases with increase in bulky groups in structure as Poly (NTB-NVP)ZnO has better swellablity than Poly (NTA-NVP)ZnO and Poly (NCA-NVP)ZnO. In Protein loading and release behavior is dominant in Poly (NTA-NVP)ZnO.X-ray diffraction pattern shows the ZincOxidenanocomposite hydrogel is micro crystalline in nature.The swelling capacity of the hydrogel depends on pH of the solution.Swelling behaviour has increased as pH increases.Thus, non-Fickian (anomalous) transport is observed as the pH of the surrounding fluid increases above pK_a. Porosity is decreased by increasing the concentration of MBA because it augments cross-linking density and reduces mesh size of hydrogels. The percentage of equilibrium swelling is decreased with increasing cross-linking agent concentration . The increasing cross-link density restricts the swelling of the hydrogels.Increasingcrosslinker concentration also increased the number of polymer chain entanglements within a network allowing the network to bear more load giving a higher modulus.

Significance of the study :

ZincOxidenanocomposites hydrogels with the ability to absorb many hundred times their dry weight of water have received considerable attention. Such materials are widely used as absorbents in medical, chemical, and agricultural applications.

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