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Evaluation of *in vitro* drug release kinetics and antibacterial activity of vancomycin HCl-loaded nanogel for topical application

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Abstract

Background: Controlled local release of antibiotics at infected sites is a new strategy to get beneficial treatment of chronic infections. Localized delivery systems, based on biodegradable polymers, are capable of slowing and controlling drug release for a certain period of time. Therefore, the objective of current study was to investigate a new approach for encapsulating vancomycin into carbopol Aqua SF1 using swelling/deswelling mechanism at pH 6.5 which in turn loaded separately with eight different hydrogel formulas containing different concentration of four types gelling agents (Carboxymethylcellulose, Carbopol 974P, xanthan gum, HPMC-K100M).

Methods: The formulation itself was characterized in terms of particle size distribution, morphology, spreadability, rheological properties, in *vitro* drug release behavior and kinetic evaluation of VAC from eight hydrogel formulas.

Results: The results revealed that vancomycin-loaded carbopol nanogel has a spherical shape with average particle diameter and zeta potential which are found to be 400 nm and 36 mV with polydispersity index 0.08 as compared with 100 nm particle size and 32 mV surface charge for only carbopol nanogel. Xanthan gum (2%) provides better viscosity and spreadability in comparison with other formulas. Moreover, the release of VAC from all the prepared formulas was reached to 99% after 24 hours period of application. It is also observed that there was no skin irritation observed on the rabbit after 24 hours and 7 days of hydrogel application. Stability study also revealed that, upon storage for 5 months at room temperature, there were no significant changes in pH or physical appearance of the hydrogel. The antibacterial activities of ten optimized VAC-Aqua SF1 hydrogel formulations were tested towards many of Gram-positive and Gram-negative wound pathogens such as normal *S.aureus* and MRSA. The results proved strong antibacterial activities in comparison with free vancomycin.

Conclusion: This research could be a promising step for enhancing the antibacterial activity for other antibiotics via encapsulating the antibacterial agent into the nanogel which in turn contributes to the development of biomedical applications.

Keywords: Vancomycin Hydrochloride, Carbopol AquaSF-1 nanogel, Hydrogel, HPMC, Xanthan Gum, CMC, Zero-order kinetics.

INTRODUCTION

Topical drug administration depicts the simplest and easiest route of localized drug delivery system that takes place everywhere in the body through skin, rectum, vagina, eyes, ears and nose [1,2] . Skin is the easiest reachable organ on human body for topical administration, thus, it represents main route of topical drug delivery system[1,2]. Most topically applied pharmaceutical preparations probably have some localized actions and are formulated to provide sustained local contact with minimal systemic. Topical delivery system shows valuable properties by bypassing first pass metabolism, avoidance of the risks and unsuitability of intravenous therapy, and of the different conditions of absorption like pH changes, presence of enzymes, gastric emptying time [3,4]. Therefore, topical application of antimicrobial agents is considered an important alternative for oral or parenteral dosage form of various antibacterial chemotherapeutic agents [5].

Controlled local release of antibiotics at infected sites is a new strategy to get benefitial treatment of chronic infections.[6,7]. Localized delivery systems, based on biodegradable polymers, are capable of slowing and controlling drug release for a certain period of time, with initial burst effect to circumvent the infection. Smart nanogels show pH-dependent drug release so antibacterial agents can target and potentially treat polymicrobial infections associated with acidity [8]. Bacterial infection is associated with acidity, because low oxygen fermentation triggers organic acids and natural immune system eliciting inflammatory responses. Therefore, pH-responsive antibiotic delivery systems get much attention owing to their enormous potential and easy applicability in clinical treatment of infections [9]. .

Vancomycin (VAC) is a glycopeptide antibiotic with strong bactericidal activity against many Gram-positive bacterial infections. It is preferred for the bacterial infections in patients allergic to beta-lactam antibiotics and has been used clinically for over 50 years [10]. Vancomycin is not an agent of first choice due to its adverse effects like hypotension, tachycardia, phlebitis, nephrotoxicity, ototoxicity, hypersensitivity reactions, chills, exanthema, immunologic adverse event known as “red man syndrome” and fever. Also, international consensus guidelines for rational use of vancomycin are still controversial and very little is known on safety of systemic use of this drug [11].

Hydrogels, swollen three-dimensional networks of hydrophilic polymers held together by association bonds or cohesive forces, are of special interest in controlled release applications, because of their soft tissue biocompatibility, the ease with which drugs are dispersed in matrix and the high degree of control achieved by selecting the physical and chemical properties of polymer network. They have been investigated extensively for application as carriers in diffusion-controlled release devices. In addition, they represent a common form of topical application. They achieve sustained release by diffusion from a reservoir through microporous membrane into the skin [12]. The present study was conducted to formulate topical hydrogel formulations of VAC. Natural polymer such as Xanthan Gum (XG), synthetic polymer, namely, Carbopol 974 (C), and semisynthetic polymers such as Hydroxypropyl Methylcellulose (HPMC) and Carboxy methyl cellulose (CMC) were used as gelling agents. Hydrogels were evaluated for their physical appearance, rheological behaviour, spreadability and drug release [13].

Carbopol Aqua SF-1 polymer is a lightly cross-linked acrylic polymer dispersion designed to impart suspending, stabilizing and thickening properties to a variety of surfactant-based personal cleansing products. Carbopol Aqua SF-1 polymer is an alkali-swellable acrylic emulsion polymer. As supplied, the majority of polymer’s carboxyl functionality is in the protonated form; the polymer molecules are coiled and impart relatively little suspension and viscosity to the liquid. Upon neutralization, the molecules ionize, expand due to charge repulsion of the anionic carboxylate and provide suspending and thickening properties to the aqueous system in which they reside. This “space-filling” mechanism is distinctly different from the associative thickening

mechanism attributed to hydrophobically-modified polymeric rheology modifiers [14].

In present study we tried to incorporate the optimized vancomycin-loaded nanogel formula into different gelling agents to design localized, controlled release formulations to improve the effectiveness of the drug against infected wound while decreasing its systemic side effects. The main objective of current study was to study the release behaviour and kinetic evaluation of a model drug, vancomycin HCl, from designed pH-sensitive composite hydrogel for smart control release of antibiotics with a focus on bacterial infection with simple preparation process.

MATERIALS AND METHODS

The materials utilized in this study for gel preparation were Vancomycin Hydrochloride purchased from Hyper Chem, China**,** Carbopol Aqua SF-1 and Carbopol 974P were supplied from Lubrizol Advanced Materials, Belgium, Carboxymethylcellulose Sodium salt, high viscosity and Propylene glycol purchased from Panreac, Spain, Hydroxy propyl Methylcellulose Methocel K100M supplied from Colorcon Limited, England, Xanthan gum and Hydrochloric acid purchased from Himedia, India, Methyl paraben and Propyl paraben purchased from Interchimiques SA-France, Potassium dihydrogen phosphate and Di-Sodium hydrogen -O-Phosphate purchased from Qualikems Fine Chem Pvt. Ltd., India, Sodium benzoate purchased from Merck, Germany and Triethanolamine purchased from Alpha Chemika, India.

Characterization of Vancomycin HCl

Capillary tube method stated by the USP was used to determine the melting point of vancomycin hydrochloride. A dense line of Vancomycin powder was obtained by insertion of a few amount of the vancomycin powder into a one-side closed tube of capillary glass. Then the capillary tube was positioned in the melting point apparatus where the temperature increased gradually and observed until complete melting of the powder, the temperature interpretation was documented as the melting point [15].

Preparation of VAC-Loaded Nanogel

Carbopol AquaSF1 nanogel suspension 0.3 g was weighed and diluted with 10 mL of deionized water (DW). The prepared suspensions were adjusted up to pH 9 which allows nanogel to swell leading to change the colour of the nanogel from cloudy white to colourless solution. Then, 0.125 g of vancomycin HCl (VAC) was separately weighed and dissolved in a little amount of DW to prepare solution of VAC. The latter was mixed with previously prepared carbopol nanogel suspension. The final VAC-loaded Aqua SF1 formula obtained and pH of the prepared VAC-AquaSF1 formula was adjusted to 6.5 by dropping diluted (0.01M) HCl before completing the volume to 100 mL by DW as shown in Figure 1. Then, particle size, zeta potential and TEM of the prepared formula was calculated to study the precise particle size and stability of VAC-AquaSF1 formula comparing with unloaded Carbopol AquaSF-1 nanogel [16].

Preparation of Vancomycin HCl Hydrogel Formulations

Loading of VAC to hydrogel was accomplished after the mixing of 0.3 wt% of Carbopol Aqua SF1 with 0.125 wt% VAC and controlling pH of this colloidal suspension at 6.5. Hydrogel formulations were prepared using different types and amounts of gelling agents as shown in Table 1. Generally, there are four different classes of excipients usually utilized in the preparation of topical hydrogels these are gelling agents, vehicles, humectants, and preservatives [17]. Natural polymers such as Xanthan gum in range of (1-2)wt%, semisynthetic polymers HPMC in range of (1, 2 and 3) wt%, and Na CMC in (1, 2 and 4) wt% as well as the synthetic polymers C974 in concentration of (1 and 2) wt% were used. Drug-loaded hydrogels were formulated by dispersing the gelling agents slowly in an aqueous media containing previously prepared VAC (0.125) wt%, Aqua SF-1(0.3) wt% nanogel, PG (5 wt%, as a humectant), methyl paraben, ,propyl paraben and Sodium benzoate (0.03 wt%, 0.01 wt% and 0.25 wt% respectively as a preservatives) at room temperature using overhead mechanical stirrer. pH of prepare hydrogel formulas was adjusted using TEA with stirring until desired pH value was approximately reached (6.5-6.6) and the weight completed to 100 g using de ionized water [18]. The prepared hydrogels were packed in wide mouth jar covered with screw capped plastic lid and were kept in dark and cool place after that samples were placed at least 24 h at room temperature prior to performing the characterization [19]. The compositions of different prepared VAC hydrogel formulations are shown in Table 1.

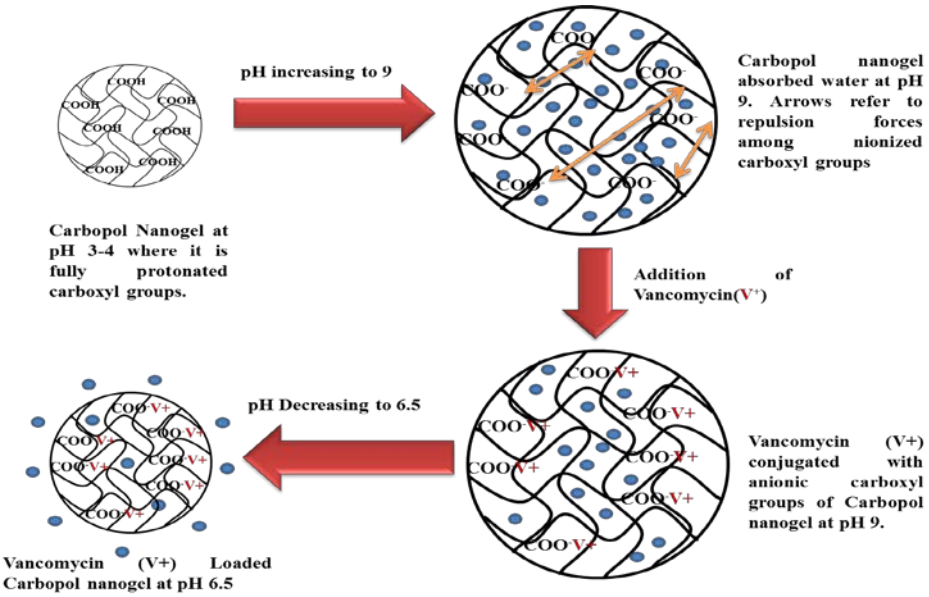


Figure1 : Schematic diagram showing the preparation of vancomycin-loaded carbopol nanogel based on swelling/deswelling mechanism.

Table1 : The preparation of ten formulas using different types and concentrations of gelling agents, Vancomycin Hydrocholride (VAC), Carbopol nanogel Aqua SF1, humectants, preservatives and vehicles.

Ingredients	A	B	C	D	E	F	G	H	I	J
VAC %w/w	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
Aqua-SF1 %w/w	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
XG	1	2	--	--						
CMC			1	2	4					
HPMC						1	2	3		
COP									1	2
PG	5	5	5	5	5	5	5	5	5	5
Sodium benzoate	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methyl paraben	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Propyl paraben	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Deionized water to	100	100	100	100	100	100	100	100	100	100

Characterization of vancomycin hydrogel formulations

VAC hydrogels were inspected visually for their colour, homogeneity (appearance and presence of any aggregates), grittiness (presence of particles or grits) and syneresis (phase separation). The pH of all hydrogel formulations was also determined using pH-meter by putting the tip of the electrode into the hydrogel without dilution and after 2 minutes the result was recorded. The spreadability of VAC-AquaSF1 hydrogel formulas was determined via sandwiching approximately 0.5 gm of each prepared hydrogel formula at the center of two glass plates (20 cm × 20 cm). Then, 0.5 Kg weight was gently employed on the upper side of the plate; as a result the hydrogel spread out in between the plates. After one minute the weight segregates and the diameter of the spread area (cm) was calculated. The diameters of spread rings were calculated in cm and compared with the initial circle diameter. The spreadability was calculated using the following equation (diameter of the spread circle - initial circle diameter) [20]. Further, viscosity of the prepared VAC hydrogels was determined using Brookfield viscometer (Brookfield LV, spindle no. 64) by filling the glass bowl with hydrogel sample and then immerse it in a beaker (fill with water) which positioned on heat source to maintain the temperature at 37^{o}C. Then the spindle was allowed to rotate at a particular speed inside gel formulas and the viscosity of the formulation was measured after 30 seconds between two successful measurements. All measurements were performed in triplicate [21]. The extrudability of VAC-AquaSF1 optimum hydrogels formula was determined by measuring the amount of hydrogel that extruded from tube which was filled with the formula and having a tip of 5 mm opening when the finger pressure was applied on the tube [22]. The stability of prepared formulations were also studied through packaging into air tight plastic container, stored at room temperature (25^{o}C) and in the refrigerator (4^{o}C) over a period of three months and then subjected to stability study via visual examination.

Antibacterial Activity

In vitro antibacterial activity of all prepared hydrogel formulas of VAC-AquaSF1 (A-J) and optimum drug-loaded nanogel (L3) were carried out by using Muller Hinton agar plate. The agar plate was prepared by dispersing 28 g of powder in 1 liter of deionized water, swirled to mix and sterilized using autoclave at pressure of 15 lbs (121°C) for about 15 minutes then reducing the temperature to 47°C in order to transfer culture media into disinfected plates under aseptic conditions. The poured media was allowed to solidify at room temperature. Accurately 0.1 ml bacterial suspension having a uniform turbidity (10^6 CFU/mL) was distributed gently over the surface of the medium with a sterile glass spreader. The wells were made aseptically with cork borer having 6 mm diameter [23]. In each of these plates, sufficient quantity of 10 hydrogel formulas and vancomycin loaded nanogel (L3) as well as 0.3 wt% AquaSF1 polymer at pH 6.5 and phosphate buffer at pH6.5 (both as a negative control) were placed in the wells with the help of syringe , then the plates were incubated at 37^{o}C for 24 hrs. The diameters of the inhibition zones were measured in millimeters. All tests were performed in triplicate. Vancomycin (Va) standard disk used as positive control.

***In Vitro* Drug Release of Formulated Hydrogel**

In vitro drug release study was achieved with some modifications. Dialysis tubing (MWCO of 12000 Da) placed in the release medium under constant stirring using dissolution apparatus. The membrane was soaked in phosphate buffer solution for 24 hours and opened from both sides and then a quantity of 5 g of eight hydrogel formulations were individually packed into dialysis tube with the ends being tightly fastened. The membrane was fixed around the paddle of the USP dissolution test apparatus and submerged in the dissolution jar previously filled with 500 mL. The medium was maintained at 37^{o}C±0.5 and stirred continuously at 50 rpm. Aliquots of 5 mL of the release medium were withdrawn at predetermined time intervals (10 min, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 20 and 24 h) and replaced by fresh phosphate buffer to provide sink condition. Each withdrawn sample was measured using UV-visible spectrophotometer at a λ_{max} of 280 nm. Absorbance was converted to drug concentration using a linear equation of calibration curve and then the cumulative percentage of VAC released was calculated taking into consideration the dilution factor. All measurements were performed in triplicate (n = 3).

Release kinetics study

To study the release kinetics of VAC from the hydrogels, the data obtained from the *in vitro* release study were analysed using various kinetic models to describe the mechanism of drug release from the hydrogels. Four kinetic models, (1) Zero-order, (2) First-order, (3) Higuchi square root models and (4) Korsmeyer-Peppas semi-empirical model, were applied on the release data as follows:

I. Zero order equation [24]:

$Q_t = k_o t$ …….. (1)

Where Q_t stands for the percentage of drug released at time t and k_o is the release rate constant;

II. First order equation [24]:

$\text{Log } Q = \text{Log } Q_0 - kt/2.303$…….. (2)

Where, Q_0= is the initial concentration of drug, k= is the first order rate constant, t = release time;

III. Higuchi's equation [24]:

$Qt = k_H t^{1/2}$ …….. (3)

Where k_H represents the Higuchi release rate constant;

IV. Korsmeyer-Peppas semi empirical model was applied [24]:

$Qt/Qe = Kt^n$ …… (4)

Where Qt/Qe is the fractional drug release from the gel into the receptor media, K is a constant corresponding to the structural and geometric characteristics of the device and n is the release exponent which is indicative of the mechanism of the drug release. The (n) value of 0.5 that indicates Quasi-Fickian diffusion mechanism, while if (n>0.5) then anomalous or non-Fickian diffusion mechanism exists and if it is (=1) then the Zero order release one exists [20].

RESULTS AND DISCUSSION

Preparation and Evaluation of Vancomycin HCl (VAC)-loaded nanogel

Carbopol Aqua SF1 considered as pH responsive nanogel thereby at low pH the nanogel is cloudy due to shrink of the polymer while by increasing the pH, nanogel is converted to colourless solution indicating the swelling of the nanogel polymer at high pH. Colour change of the nanogel from white cloudy to colourless solution is an indication of swelling of the nanogel with pH increase [16]. Particle size distribution has proved the collapsed state of unloaded nanogel particles at a pH below 6.5 as they showed an average diameter of about 100 nm. VAC cations bounded into the core of the nanogel particles through increasing the pH of the nanogel suspension up to 9.0. At this pH, the nanogel particles are swollen and the carboxyl groups of the poly acrylic acid are fully deprotonated which allows vancomycin cations to interact. After drug loading, pH was dropped to 6.5 which thus results in shrinkage of the VAC-nanogel with average diameter is bigger than that of unloaded nanogel which may refers to the loading of vancomycin into carbopol nanogel polymer (Figure 2A&C). The electrostatic potential of this aqueous pH sensitive smart nanogel is called Zeta Potential and is related to the surface charge of the dispersed nanoparticles. Nanomaterials with Zeta Potential values greater than +30 mV or less than -30 mV typically have high degrees of stability for cationic and anionic, respectively [25]. So that the drug-loaded nanogel was more stable at pH 6.5 as well as the unloaded nanogel at the same pH of medium (Figure 1B&D). Additionally, the TEM study of formulated nanogel was conducted in order to provide more insight into the morphology and size of the drug-nanogel formula and unloaded nanogel. The particle size of examined unloaded AquaSF1 nanogel and VAC-AquaSF1 (Figure 2E&F) revealed that the prepared drug loaded nanogel particles were almost spherical with smooth morphology, appeared as black dots with bright surroundings, well dispersed and separated on the surface. The average particle size was found to be less than 115 nm and larger than unloaded nanogel which was about lower than 100 nm. The difference in the particle size may be due to the entrapment of the drug into nanogel polymer. The sizes derived from TEM might be considerably smaller than that obtained by Zeta sizer and are considered that the real diameters of the drug loaded nanogel [16,26].

Preparation and characterization of formulated vancomycin HCl hydrogel

Figure 3 shows all the prepared hydrogel formulas. It can be seen in the formulations that xanthan gum based formulations were generally white appearance and have very soft and light consistency. Carbopol based hydrogel formulations gave transparent appearance with smooth and excellent homogenous consistency. CMC formulations at 4% concentration was very thick than 2% and 1% formulas that were homogenous in consistency and transparency. HPMC formulas were thicker, more transparent and quite sticky than other formulations. The pH values of all prepared hydrogel formulas were controlled at 6.5-6.6 to match the skin requirements for topical application preparations to avoid skin irritation [27].

The spreadability is important feature for topical formulation, because the patient prefers the application of the formulation to the inflamed skin if it is easily spread and uniformly applied [22]. Figure 4 shows the histogram of spreadability of the prepared formulas. The spreadability of formulations also plays an essential role in the topical formulations efficacy which indicates that the formulations are easily spread by small application of shear and it showed the behavior of hydrogel when it comes out from the container. Spreadability of the hydrogel decreases with the increase in the concentration of the gelling polymer as it was found with (xanthan1%), (xanthan 2%), (CMC1%) and (CMC2%) due to an increase in the viscosity of the formulas with increasing its concentration [27]. As a general view on all formulations the spreadability of xanthan gum and Carboxymethylcellulose based hydrogel formulations have higher spreadability than other formulas due to the excellent homogeneity and lower viscosity which easily spread with a little application of shear. Formulas with carbopol and HPMC based gel had lower spreadability owing to these formulas have higher viscosity causing inability to spread easily.

The viscosity was performed to assess the effect of the type and concentration of the gelling agent on the physical properties of the final hydrogel products and their viscosity [28]. Figure 5 shows the viscosities of vancomycin loaded hydrogel formulations at low and high shear rates. It can be seen in the figure that the effect of the types of gelling agents on the viscosity. Generally, the formulations of carbopol 974 have higher viscosity than other formulations, because carbopol 974 is a cross-linked polymer of acrylic acid with high molecular weight that has the ability to absorb and retain water upon neutralization, resulting in a viscous gel. In the current study, when carbopol 974 is being under hydration condition, it can form a physically bounded structure that is crucial for providing the proper mechanical strength to the hydrogel. The consistency depends on the ratio of solid fraction which produces structure to liquid fraction. The differences in the type of the gelling agents produce changes in structure consistency. The viscosity of the hydrogel formulations generally reflects its consistency. Methylcellulose-based formulations have lower viscosity than carbopol 974-based hydrogel formulations. This finding may be attributed to the higher hygroscopicity of cellulose derivatives as compared to carbopol [28]. In general, increasing the concentration of the polymer from (1% xanthan gum) to (2%xanthan gum), (HPMC2%) to (HPMC3%) and (CMC 1%) to (CMC 4%) led to increased viscosity of hydrogel [27]. So that, the type and the concentration of the base used play an important role in the topical preparation design since it affects the viscosity of the hydrogel.

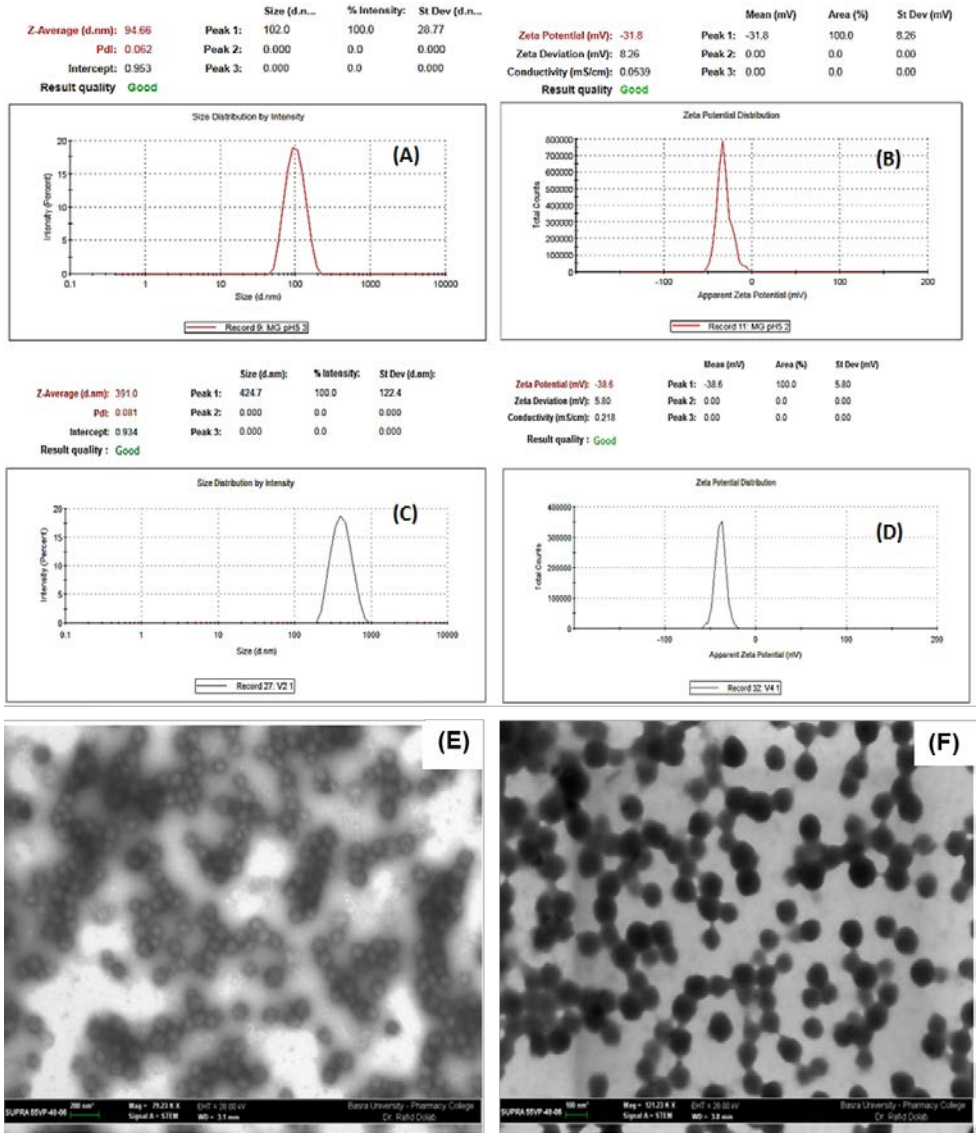


Figure 2: Particle size distribution (A) and zeta potential (B) of bare carbopol nanogel at pH 6.5 while (C) and (D) are representing particle size distribution and zeta potential of vancomycin-loaded carbopol nanogel at pH 6.5, respectively. The morphology of microstructure of bare carbopol nanogel and vancomycin-loaded carbopol nanogel are demonstrated in the TEM images (E) and (F), respectively.

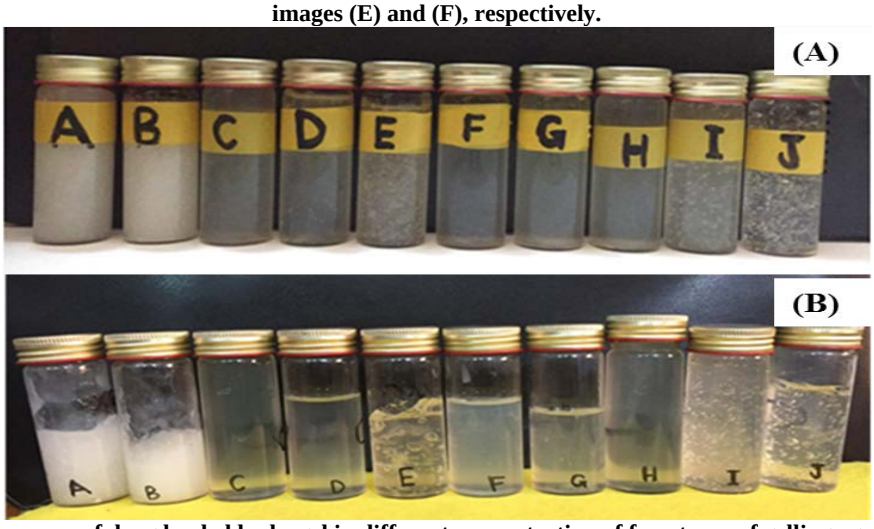


Figure 3: Visual appearance of drug loaded hydrogel in different concentration of four types of gelling agents Xanthan Gum (A & B), Carboxymethylcellulose (C, D, E), HPMC (F, G, H), Carbopol 974P (I, J) to prepare dermal delivered hydrogel dosage form. (A) Freshly prepared formulations. (B) After three months.

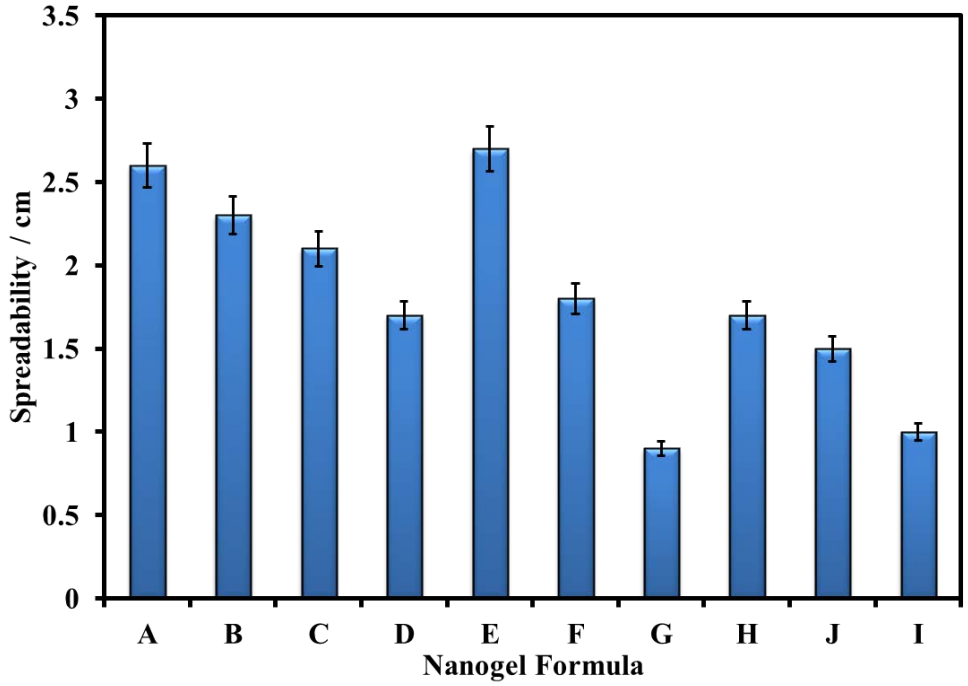
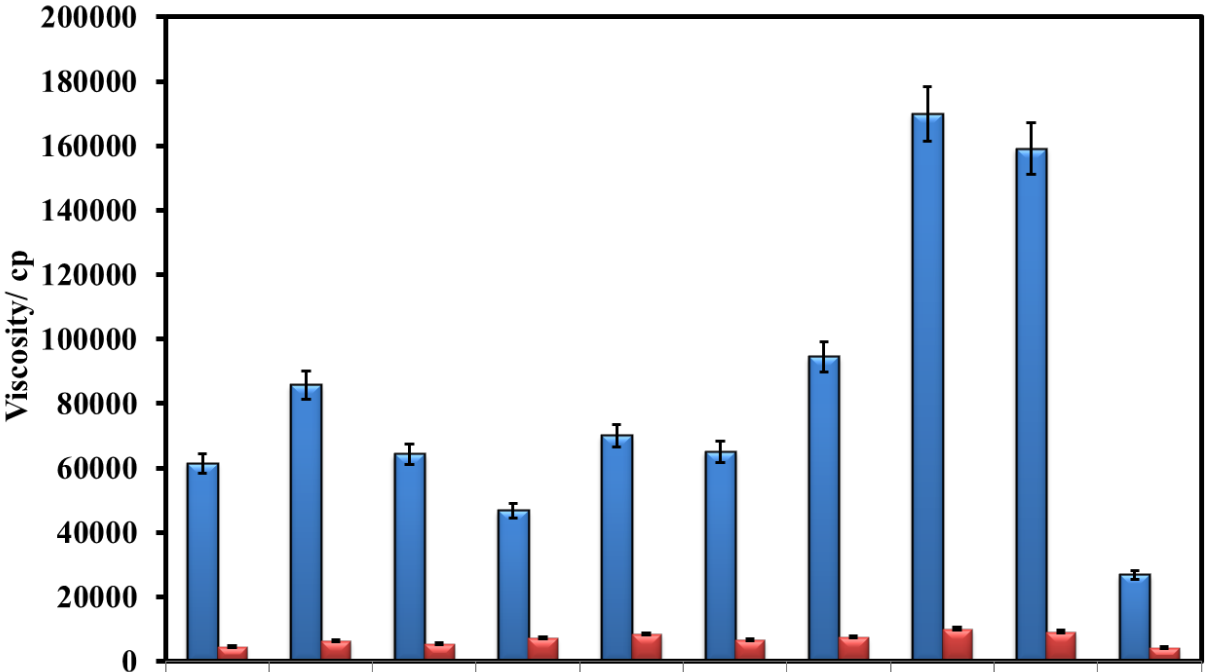


Figure 4: The spreadability of the prepared hydrogel formulas values (n=3). These measurement were obtained through sandwiching two glass plates and the formulations are put in between which then obeyed to external pressing on top of the glass plates with 0.5 kg weight.



	A	B	C	D	E	F	G	H	I	J
RPM2.5	61400	85700	64300	46730	70100	65000	94500	169900	159100	26900
RPM50	4620	6480	5410	7300	8500	6700	7554	10100	9150	4310

Figure 5: Histogram of viscosities (in poise) of VAC-AquaSF1 hydrogel formulations at low and high shear rates.

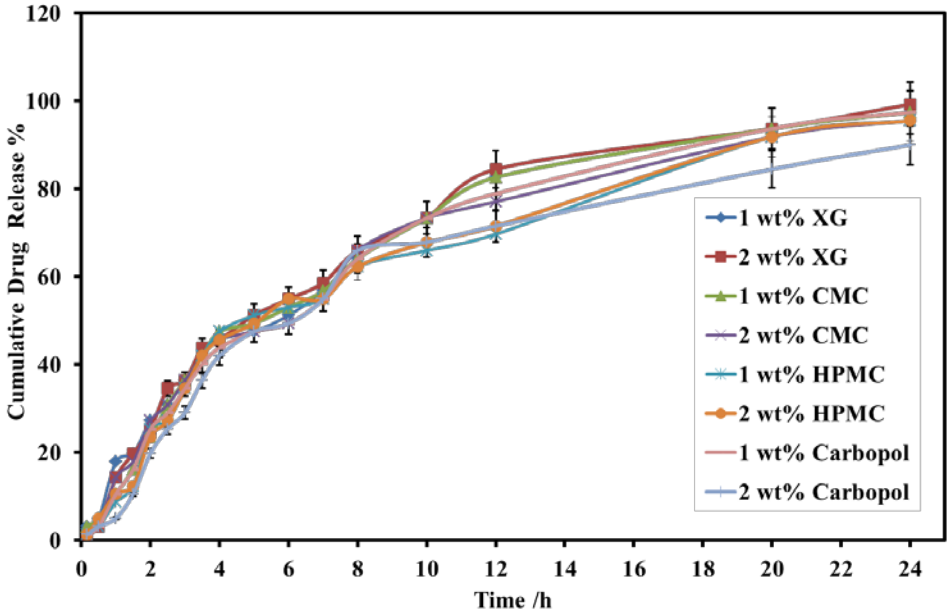


Figure 6: Effect of concentrations of gelling agents on the release profile of Vancomycin-loaded carbopol nanogel incorporated with hydrogel formulations in phosphate buffer solution (pH 5.5) at 37°C. This experiment was conducted through mixing 0.125% vancomycin loaded 0.3 wt% carbopol nanogel with 1 wt% and 2 wt% Xanthan gum, 1% and 2 wt% carboxymethylcellulose, 1 wt% and 2 wt% HPMC and 1 wt% and 2 wt% carbopol 994.

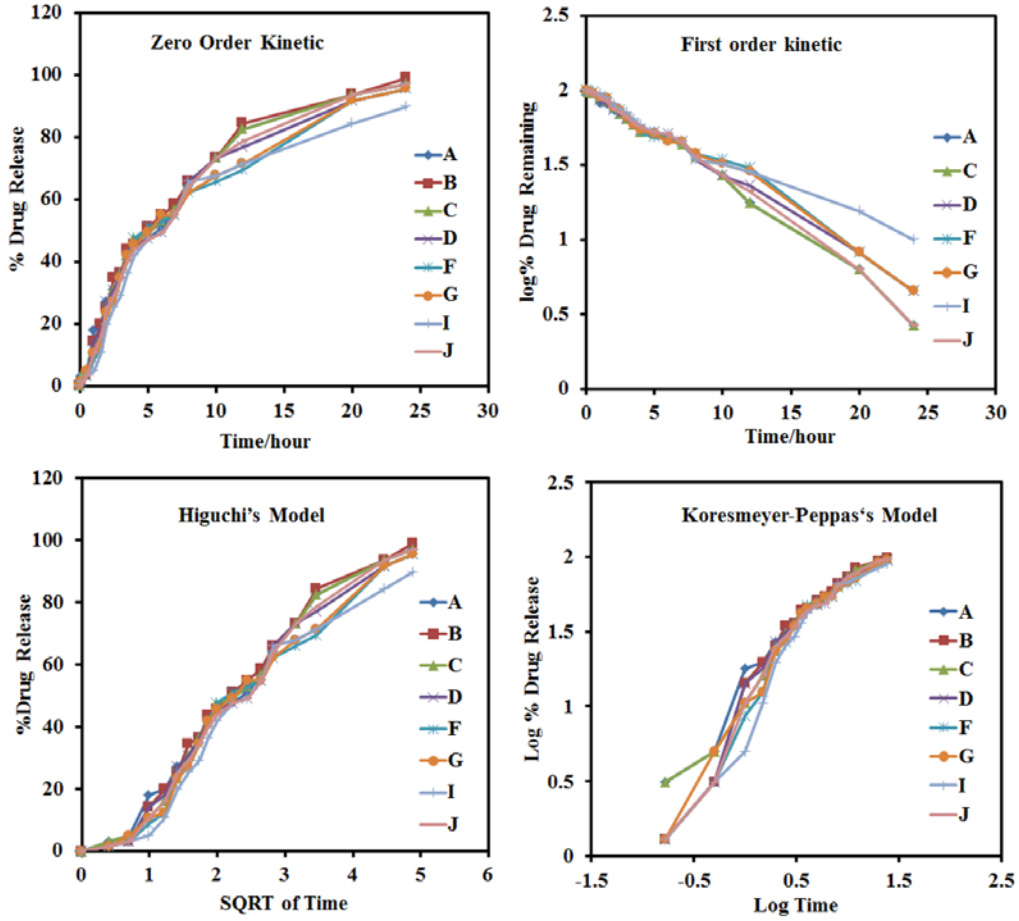


Figure 7: Mathematical models of release profiles of prepared VAC hydrogel to prove the slow and sustained drug release using zero-order, first-rder kinetics and Higuchi model according to the curve linearity of % cumulative drug release as a function of time while Korsmeyer-Peppas model represents drug release depending on diffusion through membrane.

Table2 : Kinetics study of the *in vitro* drug release data of prepared vancomycin-loaded carbopol nanogel incorporated into hydrogel formulations[27]

Formulation	Zero Order		First Order		Higuchi		Korsmeyer-Peppas		
	$K_0(min^{-1})$	R^2	K_1	R^2	K_H	R^2	K_{KP}	n	R^2
A	4.084	0.8455	0.0626	0.9919	22.431	0.9776	1.1411	0.7229	0.9508
B	4.1801	0.8282	0.0751	0.9498	23.120	0.9711	1.0245	0.8759	0.9189
C	4.1669	0.8350	0.0630	0.9929	22.931	0.9691	1.1012	0.7595	0.9582
D	4.0136	0.8337	0.0547	0.9953	22.238	0.9721	1.0150	0.8663	0.9195
F	4.0228	0.8339	0.0536	0.9842	22.111	0.9655	0.9673	0.8976	0.9331
G	4.0378	0.8399	0.0540	0.9896	22.168	0.9703	1.0041	0.8583	0.9436
I	3.93	0.8171	0.0418	0.9783	21.683	0.9533	0.9081	0.9282	0.9404
J	4.1903	0.8506	0.0627	0.9913	22.919	0.9752	0.9856	0.8896	0.9386

Formulation	Staphylococcus aureus Zone inhibition /mm.	MRSA Zone inhibition /mm.
A	18±0.6	20±0.9
B	19 ±0.4	20±0.6
C	17±0.7	19±0.5
D	17±0.5	19±0.4
E	19±0.5	19±0.7
F	21±0.9	20±0.5
G	20±0.7	20±0.9
H	19±0.4	19±0.7
I	19±1.2	19±0.4
J	18±0.5	20±0.3
Vancomycin Loaded Nanogel (L)	20±0.5	20±0.4
Control (K)	---	---
Carbopol Nanogel Polymer (P)	---	---
Vancomycin Disk	11	11

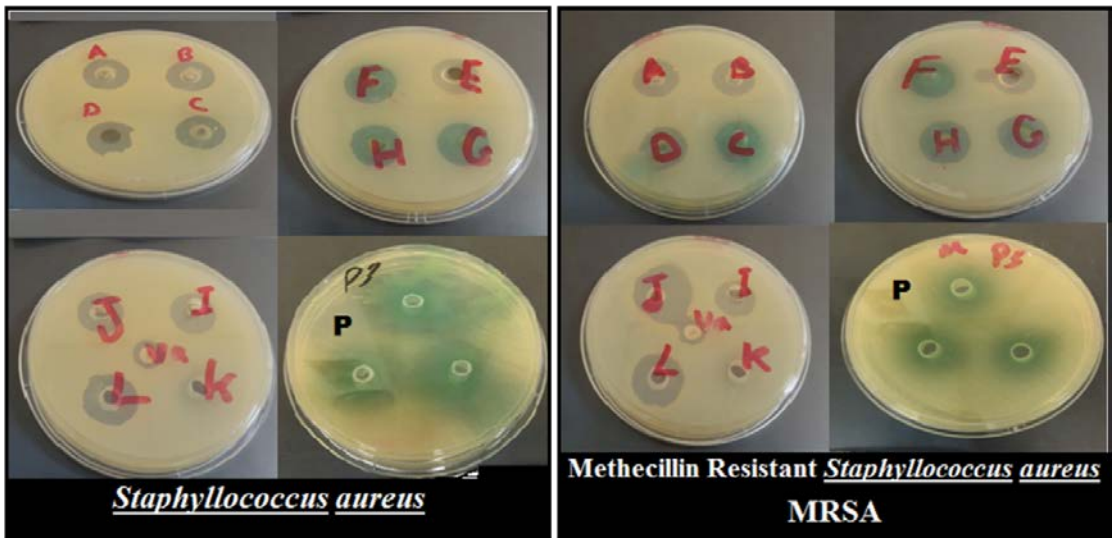


Figure 8: Antimicrobial zone inhibition diameter of vancomycin disk as positive control (Va), Carbopol Aqua-SF1 0.3wt% (P) and phosphate buffer 6.5 as negative control at different drug-loaded Aqua-SF1 hydrogel formula (A- J). (Values are mean±SD) (n=3). Images represent Minimum Inhibitory Concentration of carbopol nanogel formulations (A to J formulations) against Staphylococcus aureus and Methecillin Resistant Staphylococcus aureus bacteria.

As the shear stress is increased, the normally disarranged molecules of the gelling material are caused to align their long axes in the direction of flow. Such orientation reduces the internal resistance of the material and hence decreases the viscosity. Figure 5 also showed that VAC-hydrogel formulations possessed pseudoplastic flow with thixotropic behaviour. Thixotropy, or time-dependent flow, occurs because the gel requires a finite time to rebuild its original structure that breaks down during continuous shear measurements. It is noteworthy that thixotropy is a desirable characteristic in pharmaceutical preparations in order to deliver an initially thick product as a thinner and easily spreadable material. These findings are in agreement with all the prepared hydrogel formulations [28,29]. All the prepared VAC hydrogel formulations exhibited a shear thinning behaviour since the viscosity decreased with increasing the shear rate which means that all developed formulations exhibited pseudoplastic flow. Such pseudoplasticity results from a colloidal network structure that aligns itself in the direction of shear, thereby decreasing viscosity as the shear rate increases [27,28].

***In vitro* Drug release of formulated vancomycin HCl**

The formulations contain four types of gelling agents to study the effect of the type and concentration of gelling agents on the release profile of the drug from the nanogel incorporated into hydrogel formulations. The release profiles for vancomycin HCl from different hydrogel formulas using phosphate buffer saline, pH 5.5, A, B, C, D, F, G, I and J are shown in Figures 5. The results revealed that there is no considerable delay on drug release thereby as 2% Carbopol was used; about 90% of drug was released after 24 hours with more delay in release profile comparing with the other hydrogel formulas. It is also more sustained release than that upon addition of 1% Carbopol which had no marked effect on the drug release. This result may be due to the effect of lower pH medium that causes more collapsing in the acrylic cross-linking of the carbopol gel which caused more entrapment of the drug particles within the used hydrogel. This effect was also observed with the formula J which contains lower carbopol weight percentage as the drug release was delayed at the first six hours when the same 49% was reached in both formulas [30]. Xanthan gum also showed no effect on the drug release as well as HPMC and CMC at different concentrations with noticeable increase with xanthan 2% formula B that reached 99% of vancomycin release after 24 hours which is still more than CMC and HPMC. These observations could be attributed to the low viscosity of xanthan gum-based formulas in comparison with that for both of methylcellulose-, and HPMC-, based formulations and higher viscosity of carbopol-based formulations.

First-order and Higuchi release models for different formulations are represented in Table 2. Data obtained from the *in vitro* release study were analysed using linear regression method. In case of zero order kinetics observation, the graphical representation of cumulative % of drug release against time describes that VAC release from the matrix does not follow perfectly the principle of zero order release kinetics, based on the low R^2 values obtained as compared to those of the first order models of the drug release (R^2=0.8171- 0.850) [32]. Whereas, the observation of first-order kinetics describes the dependency on the drug concentration i.e. the greater the concentration, the faster the release which means it follows linear kinetics. Hence, to study the drug release kinetics data obtained from *in-vitro* dissolution study is plotted against time i.e. log % of drug remaining *vs.* time and the slope of the plot gives the first order rate constant. The correlation coefficient of the above plot gives information whether the drug release follows first order kinetics or not. Thus, this model was applied in the release profile of VAC (log Cumulative % drug remaining vs. time; Table 2) and evaluation was achieved in the graphical presentation as shown in Figure 7. The graphical representation of cumulative % of drug release against time describes that drug release of VAC from the polymeric matrix follows the principle of first-order release kinetics as there is high value of coefficient of correlation (R^2=0.949-0.995) [33]. Previous studies showed that drug release was decreased with an increase in gelling agent concentration, because the viscosity is increasing as polymer concentration increases [31].

***In Vitro* drug release kinetics**

Various kinetic models were used to describe the release kinetics of VAC hydrogels as shown in Figure 7. With respect to Higuchi kinetics, the plots were found to be fairly linear as indicated by their highest regression values. Higuchi equation is considered one of the widely used and the most well-known controlled-release equation. Thus, this model was applied in the release profile of VAC (Cumulative % drug release *vs.* Square root time; Table 2) and evaluation was done in the graphical presentation (Figure 7) .It can be observed in the figure that graphical representation of cumulative % of drug release against time represents that drug release of VAC from the hydrogel is perfectly following Higuchi drug release model as the drug release profile is very closest to trend line or regression line and the highest value of coefficient of correlation values (R^2) was in the range of (0.953–0.977) for all hydrogel formulations [30].

In Korsmeyer-Peppas release kinetics model which is considered the way of drug release from the gel across the membrane into the receptor media. To study release kinetics according to Korsmeyer-Peppas model, a graph is plotted between log cumulative % drug release *vs.* log time. In general, it can be perceived that the mechanism of drug release of all formulations except formula (I) was anomalous diffusion. As mentioned above, an (n) value in the model were ($0.5 < n < 1$) revealing that two mechanisms for the drug release from the hydrogels were involved. These area non-Fickian (anomalous) and super case II transport (table 2) [35].. Formulations A, B, C, D, F, G, I and J showed a release exponents *n-values* 0.7229, 0.8759, 0.7595, 0.8663, 0.8976, 0.8583, 0.9282 and 0.8896, respectively, indicating anomalous diffusion coupled with erosion.

Antibacterial activity

In vitro antimicrobial activity of vancomycin loaded carbopol nanogel hydrogel formulations was assessed by well diffusion agar method for screening the antibacterial potential against clinical strains of bacteria: *Staphylococcus aureus* and MRSA. Phosphate buffer 6.5 (K) and 0.3 wt% nanogel polymer (P) at pH 6.5 were used as a negative controls and both had no inhibition zone on the tested microorganism while vancomycin disk (Va) used as positive control to compare its inhibition zone values with the values of VAC-AquaSF1(L) and hyydrogels that contain VAC-Aqua SF1(A-J). The antibacterial activity was expressed as diameter of the inhibition zones in millimeter (mm) as shown in Figure 8. It can be illustrated in the figure that at 24 hour incubation time, the diameters of inhibition zones values were between 20 and 23 mm. It clearly explains that the drug-loaded nanogel showed relatively good inhibitory activities against the tested bacteria when compared with the positive control of vancomycin HCl disk as seen in Figure 8. The images also clearly demonstrated that there was no significant difference in zone of inhibition among the hydrogel formulations. The diameter of inhibition zones of hydrogel formulations were approximately similar to that of VAC-AquaSF1 against both of the strains tested which are in agreement with the fact that incorporation of the drug into the gel base does not decrease its antibacterial activity [27]. Samples C and D have showed the least antibacterial activity against *S. aureus* though it was non-significant. Although MRSA

is found to be resistant to many routinely used antibiotics, in this study it showed susceptibility to vancomycin [36].

CONCLUSIONS

Vancomycin hydrochloride was successfully incorporated into the different topical gel formulations. Formula B showed good pH value, viscosity and highest *in vitro* release profile after 24 hours among all the prepared different formulations. Mathematical models played a vital role in the interpretation of mechanism of drug release from a dosage form to understand the drug release kinetics of a dosage form. The Higuchi kinetic was found to be fairly linear as indicated by their highest regression values. The correlation coefficients values (R^2) were in the range of (0.953–0.977) for all hydrogel formulations. Also, the model Korsmeyer-Peppas states the type of diffusion which was evaluated by value, *n* (release exponent) which is higher than 0.72 which implies that the drug release from the system follows Super case II transport. In general, it was observed that the mechanism of drug release of all formulations was either anomalous diffusion or non-Fickian super case II. Therefore, it was concluded that our formula could be very promising topical alternative for the treatment of bacterial wound infection. However, further preclinical, clinical and long-term stability studies should be performed.

Ethical Clearance was obtained from the Research Ethics Committees in the College of Pharmacy/ Al-Mustansiriyah University and the College of Veterinary Medicine/ The Green University of AL-Qasim.

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