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# Chitosan/Starch-based Hydrogels as Drug Delivery Carrier for Controlled Release of Hydroxychloroquine

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**KEYWORDS**
Chitosan, starch, hydrogel, drug delivery systems, controlled release, shape factor, hydroxychloroquine

**ABSTRACT:**
Chitosan/starch cross-linked microspheres were prepared as drug delivery system for controlled release of hydroxychloroquine, the systemic lupus erythematosus drug. Moreover, sodium hexametaphosphate (SHMP) as a physical cross-linker and glutaraldehyde (Glu) as a chemical cross-linker have been used. The size and shape factor(S) of the prepared microspheres was studied using scanning electron microscope technique. The Glu microspheres shows size of their spherical particles (82.5μm) and shape factor (S=0.862) as a high values in comparison with SHMP microspheres with size (39.7μm) and shape factor (S=0.811) means both Glu and SHMP microspheres are rough but Glu microspheres are more roughness. The swelling studies shows Glu microspheres have a higher degree of swelling (DS=130%) in pH= 9 media while SHMP microspheres the more compact microspheres shows higher (DS=160%) in acidic pH= 2 media. The microspheres were loaded with hydroxychloroquine and then sustained the drug in controlled, and because of high electrostatic interactions between drug and system ions, the SHMP microsphere were loaded with higher maximum loading percentage (Lmax=55.8% (w/w)) comparing with Glu microspheres of (Lmax=46.5% (w/w)). Both systems Glu and SHMP microspheres shows controlled drug release behaviors. The Glu microspheres shows (40.0 mg) and (40.5 mg) controlled release in pH = 7.4 and PH= 9.4 media respectively, out of (46.5 mg) maximum loading percentage, while only (30.0 mg) controlled release in pH = 1.3 and only for 18h controlled release time, while 30h and 36h controlled release time in pH= 7.4 and pH= 9.4 respectively. The SHMP microspheres shows controlled drug delivery with more sequence almost in all tested pH of the release media, where they release (45.0 mg), (45.0 mg), and (47.0 mg) under controlled release in pH=1.3, pH= 7.4, and pH=9.4 respectively, out of (55.8 mg) maximum loading percentage and with 30h, 36h and 36h controlled release time, respectively. The prepared microspheres were characterized using FTIR, 1H NMR, (TGA, DTA, and DSC), XRD and FESEM analysis. The prepared systems were found suitable as controlled drug systems for delivery of hydroxychloroquine drug.

## 1. Introduction

Drug delivery systems (DDS) are a technique of carry medication to a patient in such a way that specially increases the drug concentration in its target [1]. The final goal of any delivery system is diffusing, reserve and target the drug in the unsound tissue, thereby maximizing therapeutic efficacy and minimize drug accumulation in the body [2,3]. Last decades, drug delivery systems have been applied effectively in the improvement of health and treatment of diseases due to control of the pharmacological effect of the drug and increase systemic circulation [4]. As a result, it gives rise to the concept of controlled release [5]. The controlled release formulation of a drug shows attracted considerable attention because of its significant advantages over conventional drugs, where the drugs release at a predetermined rate and for a fixed period of

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time. In addition, controlled drug delivery systems are provides spatial control over drug release, include burst and controlled release rate [6,7]. Furthermore, controlled release systems improves dry solubility, efficacy, target site accumulation, pharmacokinetic properties, pharmacological activity, patient acceptance, and compliance, in addition to reduce the drug toxicity [4]. Controlled release systems try to use synthetic polymers to act as carrier for delivering their cargos [8], but most are suffer from weakness or lack in biocompatibility, immunologically, biodegradability and toxicity [9]. Therefore, polysaccharides are natural polymers have biological nature can be use in drug delivery systems and they possess a formulations suitable for drug delivery. Chitosan and starch are polysaccharides have cationic and anionic structures, respectively [Figure 1.1], where both have biomedical properties, including biodegrability, biocompatibility, non toxicity, mucoadhesivity and mechanical strength [10].

**(a)** **(b)**

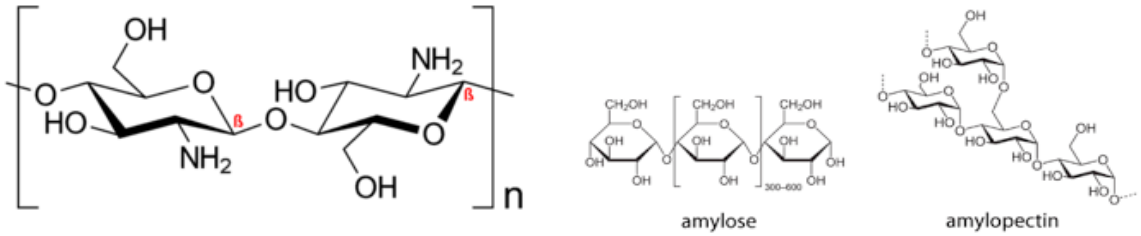


**Figure 1.1: Chemical structure of (a) chitosan, and (b) starch.**

preparations for carry drugs in the form of tablets, films, or gels [19]. The hydrophilic nature of the starch increases its ability for moisture uptake increase its water vapor permeability and tensile strength [20]. Its polymerization with other polysaccharides produces starch-based hydrogel useful as drug delivery systems [21].

Hydroxychloroquine drug is used to treat discoid lupus erythematosus, and it is also used to treat chronic rheumatoid arthritis [22]. It is oral drug and used also to prevent and treat malaria, often used in the form of sulfate [Figure1.2].

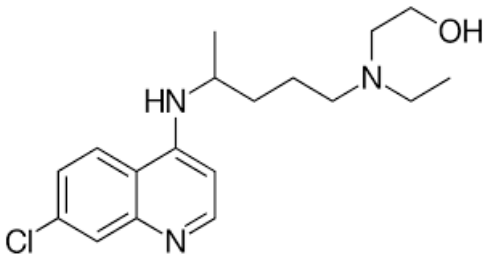

**Figure1.2: Chemical structure of hydroxychloroquine**

Chitosan is a modified polysaccharide, prepared by deacetylation of chitin, and its structure compose of β(1-4) linked residues of 2-amino-2-deoxy-D-glucose [11]. The hydroxyl (-OH) and amine ($NH_2$) groups of chitosan are active groups and they have a significant role in the biochemical and electrostatic interaction in drug delivery systems, and help chitosan to soluble in a slightly acidic solution [12,13]. Starch, is one of the most abundant polysaccharides its structure compose of glucose units are bonded by α-D-[1-4] and α-D-[1-6] linkages [14]. Amylose and amylopectin are the main components present in starch [15]. Starch characterized with hydrophilic property and its strong inter molecular attraction due to its hydrogen bonds formed by the hydroxyl (-OH) groups in starch [16]. Starch, is cheap, has good mechanical properties, besides its biocompatibility and non-toxicity [17]. Starch, and due to its biomedical properties is prefer to blend with other biomedical polymer for increasing such properties [18]. Recently, starch-based drug delivery systems have received a significant attention because starch has high gelling capacity, besides the other biomedical properties which makes it widely used in pharmaceutical

Journal of Chemical Health Risks
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JCHR (2023) 13(3), 1457-1471 | ISSN:2251-6727

water and added gently with stirring at room temperature. The formed viscous solution of chitosan-starch was blows gently through a fine nozzle into a beaker containing 100mL of (2.5 wt%) glutaraldehyde (Glu) the covalent cross-linker (GLU solution was prepared by mixing 10mL of (25wt%) Glu stock solution with (90mL) distilled water), and the cross-linker solution was heated at 60°C with moderate stirring. A fine microspheres were formed, which kept under stirring for extra 1h, separated and washed several times with hot and cold water and dried in vacuum oven at 30°C. A similar procedure was done in 100mL sodium hexametaphosphate (SHMP) as ionic cross-linker prepared with (6% w/w) concentration and their formed microspheres were also separated, washed and dried.

### 2.3 Measurement of Size and Morphology of Prepared Particles

Studying the size and morphology of the prepared particles will give a good idea about their loading and release behaviors. However, the size and morphology of the microspheres shows significant effects on their physical character [23]. FESEM technique was used for measuring the shape factor (S), where FESEM-Imaging-EDS-Mapping-Line-EBSD/ Germany instrument was used, and the size and morphology of the particles will change as the type of the hydrogel cross-linker change [24]. The shape factor (S) of the hydrogel particles was calculated depends on size parameters for the surface characteristics of the particles and the following equation (1) was depended [25].

$$S = \frac{L^2}{4\pi A} \quad \text{.....(1)}$$

where L is the perimeter, A is the surface area of the particles. The out finding results from literatures indicate that a value of (S) above 0.80 shows surface roughness increases progressively [24].

### 2.4. Swelling Measurements of Hydrogel Particles

The degree of swelling (DS) of the particles of both prepared hydrogels as (CH-co-ST)/Glu and (CH-co-ST)/SHMP was measured.

Where 100 mg of dry microspheres were kept in 20 mL phosphate buffered solutions of pH=2, pH=7,

The main purpose of this work is preparing a hydrogel in the form of microspheres from chitosan and starch. The hydrogel is cross-linked physically by sodium hexametaphosphate and chemically by gluteraldehyde. The prepared hydrogels were tested for their degree of swelling and their structures by FTIR, and $^{1}$H NMR, and crystalline structure by XRD, and their thermal stability by TGA, DTA and DSC, beside their surface morphology by FESEM analysis. Maximum leading and efficiency of loading percentages were measured, and finally the loaded microspheres were allowed to release in different pH-buffered solutions and their burst, controlled, and cumulative release percentages were measured.

## 2. Experimental

### 2.1. Materials and Methods

chitosan (CH) sample (75 wt% DDA) was obtained from HiMedia Lab. Pvt. Ltd. India. Chitosan powder was dissolved in 2% (w/w) acetic acid and then filtered use vacuum for purification to remove undissolved particles of chitosan. The filtrate was subsequently precipitated with 1M NaOH solution, then filtered and dried inside vacuum oven at 25°c. Starch (ST), ammonium persulfate (APS), and sodium hexametaphosphate (SHMP) were obtained from BDH, U.K. Glutaraldehyde (Glu) 25% (w/w) solution was supplied by Thomas Baker (chemicals) Pvt. Ltd.; Mumbai-India. Acetic acid, methanol and buffered solutions (different pHs) were analytical grade chemicals supplied by (Fluka, Swiss, Buchs, Switzerland).

### 2.2. Preparation of Chitosan-Starch Blend Copolymer

Chitosan (CH) solution was prepared by dissolving 1g of pure chitosan in 100mL of 2% (w/w) acetic acid. The formed viscous solution was stirred for 3h at room temperature starch (St) solution was prepared by dissolving 1g powder starch in 100ml distilled water heated at 60°C because the starch is insoluble in cold water and the heat is necessary to break the strong hydrogen bonds in its semi-crystalline granules [22]. The chitosan-starch blend copolymer was prepared by mixing 100mL of (1%w/v) chitosan with 100 ml of (1% w/v) starch solutions for preparation of (1 : 1M) in a 250mL beaker were mixed. Then, 5 mL (10% w/w) of APS initiator was prepared in distilled

Journal of Chemical Health Risks
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The releasing behavior of the chitosan/starch microspheres of both cross-linkers hydrogels were studied by immersing 100mg of loaded microspheres in 20mL buffered solutions of pH=1.3 representing simulated pH of gastric fluid, pH=7.4, the plasma blood fluid, and PH=9.4 the intestinal fluid in the human body and the released solutions were kept at body temperature of 37°C [29]. The absorbance of the released hydroxychloroquine in the different media were measured by UV-Visible spectrophotometer at $\lambda_{max}$=331nm and for a fixed time interval of 3h. The calibration curve was used for calculation the concentrations of released hydroxychloroquine using their measured absorption (A) values. The release measurement process was continued until no hydroxychloroquine was released. The controlled release ($CR_{max}$%) percentage was calculated using equation (5) [28];

$$\text{Controlled release } (CR_{max})\ \% = \Sigma \left(\frac{W_t}{W_o} \times 100\right)_{constant} \quad \ldots\ldots(5)$$

Further, the burst release ($BR_{max}$%) percentage was calculated using equation (6) [28];

$$\text{Burst release } (BR_{max})\ \% = \Sigma \left(\frac{W_t}{W_o} \times 100\right)_{variable} \quad \ldots\ldots(6)$$

The overall release of hydroxychloroquine was calculated in the form of cumulative release ($R_{cum}$%) percentage using equation (7) [28];

$$\text{Cumulative release } (R_{cum})\ \% = \left(\frac{W_t}{W_o} \times 100\right) \quad \ldots\ldots(7)$$

Where $\left(\frac{W_t}{W_o}\times 100\right)_t$ is represent a variable amount for burst release and a constant amount for controlled release for a fixed time interval of 6h and at 37°C. Whereas, the $W_t$ is the cumulative amount of hydroxychloroquine released at time (t) and $W_o$ is the total amount of the drug released [27].

## 3. Results and Discussion

Polysaccharides are one of the main branches of natural polymers, are biocompatible materials, which are suitable to use in drug delivery systems. Polysaccharides have the ability to encapsulate drug and keep it for a long time and then release in a controlled manner [6]. The physico-chemical properties of the blend polymer system can play a significant role in the controlled release process. Blending chitosan of cationic and pH=9 and the solution was measured at room temperature. Then, after 6h the swollen microspheres were taken out of the swelling solution and filtered through fine sieve of (100 mesh), and after drain for 10 min, the microsphere weighted and returned to the same solution. The process was repeated every 6h until no change in the weight of the microspheres, and the DS of different hydrogels were calculated using equation (2) [26];

$$\text{Degree of swelling } (\%) = (W_t - W_o / W_o) \times 100 \quad \ldots\ldots\ldots..(2)$$

Where $W_t$ is the weight of the wet microspheres at a time (t), and $W_o$ is the weight of microspheres at zero time

### 2.5. Loading of Hydroxychloroquine on Microspheres

The loading of hydroxychloroquine drug on microspheres was carried out by immersing 100mg microspheres of (CH-co-ST) hydrogel cross-linked with Glu and SHMP in 100ml phosphate buffered solution of pH=7, containing different concentrations of hydroxychloroquine starting from 50mg (0.05 g/L); 100mg (0.1 g/L), and 150mg (0.15 g/L). The temperature of the loading solution was fixed at 25°C and the microspheres were immersed inside the loading solutions under gentle stirring for the following periods of time: 10h, 30h, and 60h. Different drug concentrations and time of loading are done for comparison to select the suitable loading conditions. A calibration curve of known hydroxyhydrequine concentrations was done at its λmax = 331 nm, using UV-1800 Shimadzu Spectrophotometer, Kyoto, Japan. The loading of hydroxychloroquine on microspheres were calculated as maximum loading ($L_{max}$%) according to equation (3) [23],

$$L_{max}(\%) = \frac{\text{weight of hydroxychloroquine loading (mg)}}{\text{weight of microspheres taken for loading (100mg)}} \times 100 \quad \ldots\ldots(3)$$

In addition, the loading of hydroxychloroquine was calculated as efficiency of loading EL (%) using the following equation [23];

$$EL\ (\%) = \frac{\text{weight of hydroxychloroquine loaded (mg)}}{\text{weight of hydroxychloroquine taken for loading}} \times 100 \quad \ldots\ldots(4)$$

### 2.6. Release of Hydroxychloroquine from Loaded Microspheres

Journal of Chemical Health Risks
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JCHR (2023) 13(3), 1457-1471 | ISSN:2251-6727

Glu hydrogel and (CH-co-ST)/SHMP hydrogel were studied using FTIR/ATR/ Far-IR/Near-IR Thermo Fisher Scientific, USA, instrument, where the absorption frequency of the hydrogel (Figure 3.1a and Table 3.1) for (CH-co-ST) / Glu appears at 3443 $cm^{-1}$ is belongs to $\nu$ $(O\text{-}H)_{str}$ groups present in both polymers. The bands at 1556 $cm^{-1}$ and 1622 $cm^{-1}$ are represent the amide-II and amide-I of chitosan. Whereas, the (CH-co-ST)/SHMP hydrogel shows (Figure 3.1b and Table 3.1) bands almost similar to these of (CH-co-ST)/Glu hydrogel in addition to the bands of (p-o-p) which are belongs to SHMP cross-linker which appears at 740 $cm^{-1}$, 1109 $cm^{-1}$ and 1251 $cm^{-1}$.

structure with starch of anionic nature will produce hydrogels having functional groups enhance the hydrogel for swelling to a high extent in different pH media. The hydrogel with high degree of swelling easily loaded with chronic drugs, especially those of harmful effects on the digestive system and shows long-term controlled release [23]. The prepared hydrogels from blending of chitosan (CH) with starch (ST) and cross-linked chemically by glutaraldehyde (Glu) and physically using sodium hexametaphosphate (SHMP) are characterized and their chemical structure, thermal behavior, crystalline composite and surface morphology, were studied. The FTIR of (CH-co-ST)/

**Table 3.1 FTIR main functional groups with their absorption frequencies of studied hydrogels**

| FTIR Characteristic Functional Groups | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Sample** | **$\nu$(O-H) str** | **$\nu$(C-H) str** | **$\nu(N\text{-}H)_{str}$ Amid - II** | **$\nu(C{=}O)_{str}$ Amid - I** | **$\nu$ (C=O) Asym/ Sym** | **(p-o-p) SHMP** | **$\nu$ $(C\text{-}O)_{str}$** | **$\nu$ (C-O-H)** |
| | Wave Number $\nu$ /$cm^{-1}$ | | | | | | | |
| (CH-co-ST)/ Glu | 3443 | 2922 | 1556 | 1622 | 1390 | - | 1088 | 852 |
| (CH-co-ST)/ SHMP | 3449 | 2926 | 1559 | 1623 | 1373 | 740<br>1109<br>1251 | 1109 | 868 |

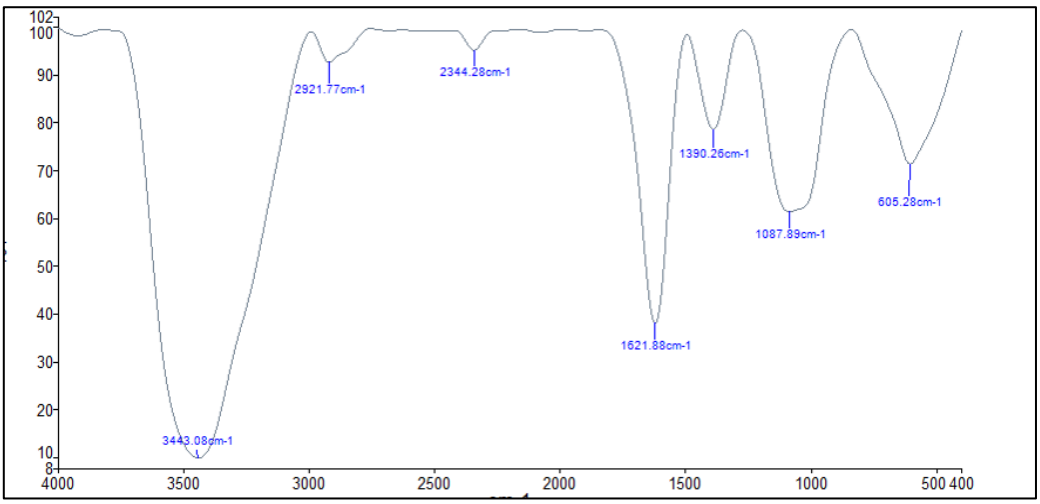


**Figure 3.1a: FTIR spectrum of (CH-co-ST)/Glu hydrogel**

Journal of Chemical Health Risks
www.jchr.org
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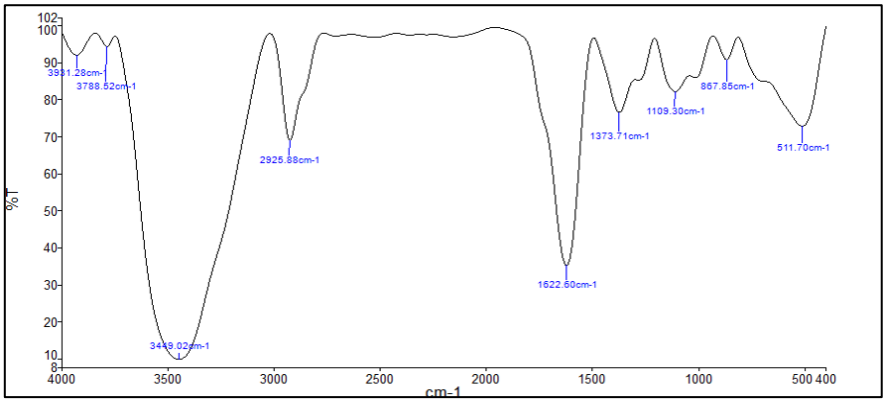


**Figure 3.1b: FTIR spectrum of (CH-co-ST)/SHMP hydrogel**

(Figure 3.2 and Table 3.2) representing the (O-H) group of chitosan and starch. In addition, the resonance of (IH, w) protons at $\sigma$ = 4.85ppm and $\sigma$ =5.41 ppm are belongs to hydrogen atoms of the starch backbone [29].

The $^1$H NMR spectroscopy of (CH-co-ST)/Glu hydrogel using Agilent Technologies of 499.35 MHz spectrometer freq. Germany with deut. DMSO solvent, where it shows resonance of (1H, w) at $\sigma$=3.66 ppm

**Table (3.2): $^1$H NMR chemical shifts with their protons descriptions of (CH-co-ST)/SHMP hydrogel.**

| Sample | Chemical shift $\sigma$ /ppm | Description of proton |
|---|---|---|
| **(CH-co-ST)/Glu** | 1.25 | ($CH_2$) groups of Glu |
| | 3.3 | Cross-linking of glucosamine group with Glu |
| | 3.66 | (O-H) groups of (ST) and (CH) |
| | 4.85 and 5.41 | H atoms of (ST) backbone |

The resonance of (2H,m) protons at $\sigma$ = 1.25 ppm representing the $CH_2$ group of the cross-linker, beside the resonance of (3H,w) at $\sigma$ = 3.3 ppm which ascribed to the $CH_3$-$N^+$ group (cross-linking of glucosamine group with Glu).

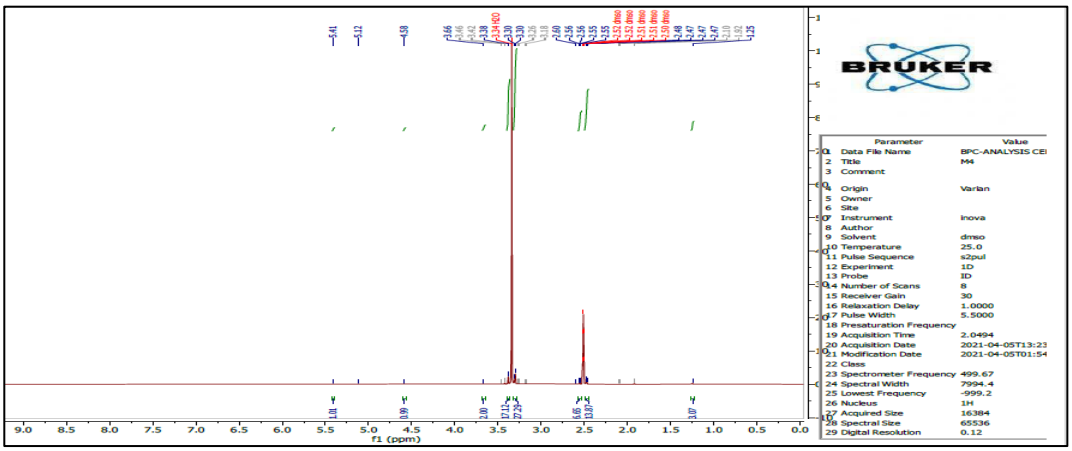


**Figure 3.2: $^1$H NMR spectrum of (CH-co-ST)/Glu hydrogel**

Journal of Chemical Health Risks
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JCHR (2023) 13(3), 1457-1471 | ISSN:2251-6727

The XRD analysis of (CH-co-ST)/SHMP hydrogel was done by Panalytical X Pert Pr, UK, where the hydrogel pattern (Figure 3.3) shows the hydrogel has two maxima peaks at 18.38° and 24.5° along the 2θ axis, where both are broad and they shows the hydrogel is semi-crystalline, and its physical cross-link is contributed to raising its crystallinity [30].

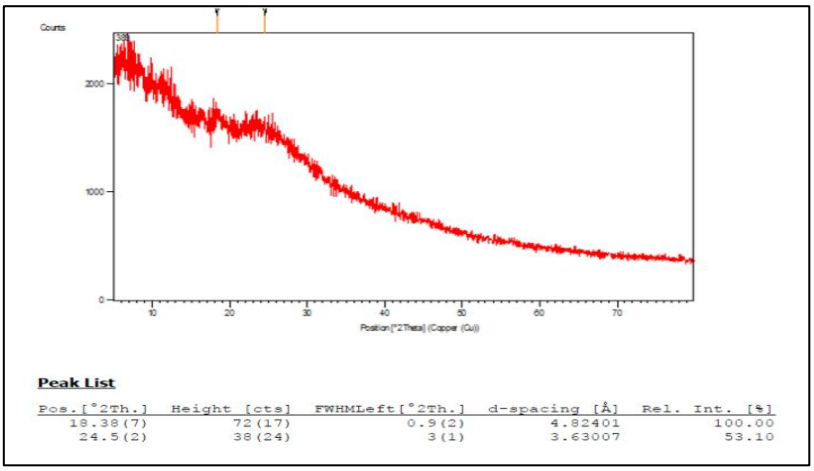


**Figure 3.3: XRD pattern of (CH-co-ST)/ SHMP hydrogel**

The thermal analysis of (CH-co-ST)/SHMP hydrogel was studied using Mettler Toledo,USA and its TGA, DTA, and DSC thermograms (Figure 3.4a and Table 3.3) shows the weight loss (%) in TGA thermogram with initial decomposition temperature (IDT) is (0.9%) at 50°C, and at final decomposition temperature (FDT) is (25.5%) at 400°C means the hydrogel is thermally stable. In addition, the (18.5%) weight loss at 255°C which representing the maximum decomposition temperature ($T_{max}$) with the crystalline decomposition temperature ($T_{cr}$) 410°C with (28.0%) weight less emphasizes the thermal stability of the hydrogel.

**Table 3.3: TGA, DTA, and DSC thermal data of (CH-co-ST)/ SHMP hydrogel**

| Sample | TGA weight loss (%) | | | | DTA 1/°C | | | DSC (w/g) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | IDT °C | FDT °C | Tmax °C | Tcr °C | 1st decomp 1/°C | 2nd decomp 1/°C | 3rd decomp 1/°C | Tg °C | Δ $H_f$/ (J/g) | | | |
| (CH-co-ST)/ SHMP | 0.9 | 25.5 | 18.5 | 28 | $-1.3x10^{-3}$ | $-2.6x10^{-3}$ | $-1.67x10^{-3}$ | 53 | -231.8 | -3.87 | 27.0 | -194.32 |
| | 50 °C | 400 °C | 255 °C | 410 °C | 65 °C | 207 °C | 300 °C | | 97.3 °C | 173.2 °C | 205.7 °C | 231.9 °C |

The DTA thermograms (Figure 3.4b, and Table 3.3) shows the (CH-co-ST)/SHMP hydrogel has three decomposition rates at different temperatures.

The hydrogel was decomposed with three stages where it loses its free water first, and then its bonded water, and finally, some of its functional groups because only (28.91%) of the total used weight was decomposed and (71.09%) of the hydrogel remain undecomposed on the examined temperature scale (600°C). The DSC thermogram of (CH-co-ST)/SHMP hydrogel (Figure 3.4c, and Table 3.3) shows the hydrogel has (Tg=53.0°C), and the hydrogel has four physical

Journal of Chemical Health Risks
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JCHR (2023) 13(3), 1457-1471 | ISSN:2251-6727

transformations as heat of fusion ($\Delta H_f$), first two are exothermic, representing loss of free and bonded water. The third one is endothermic which belongs to the physical interaction between polymer chains due to cross-linking by SHMP. Finally, the fourth heat of fusion is exothermic resulting from partial decomposition of chitosan and starch functional groups.

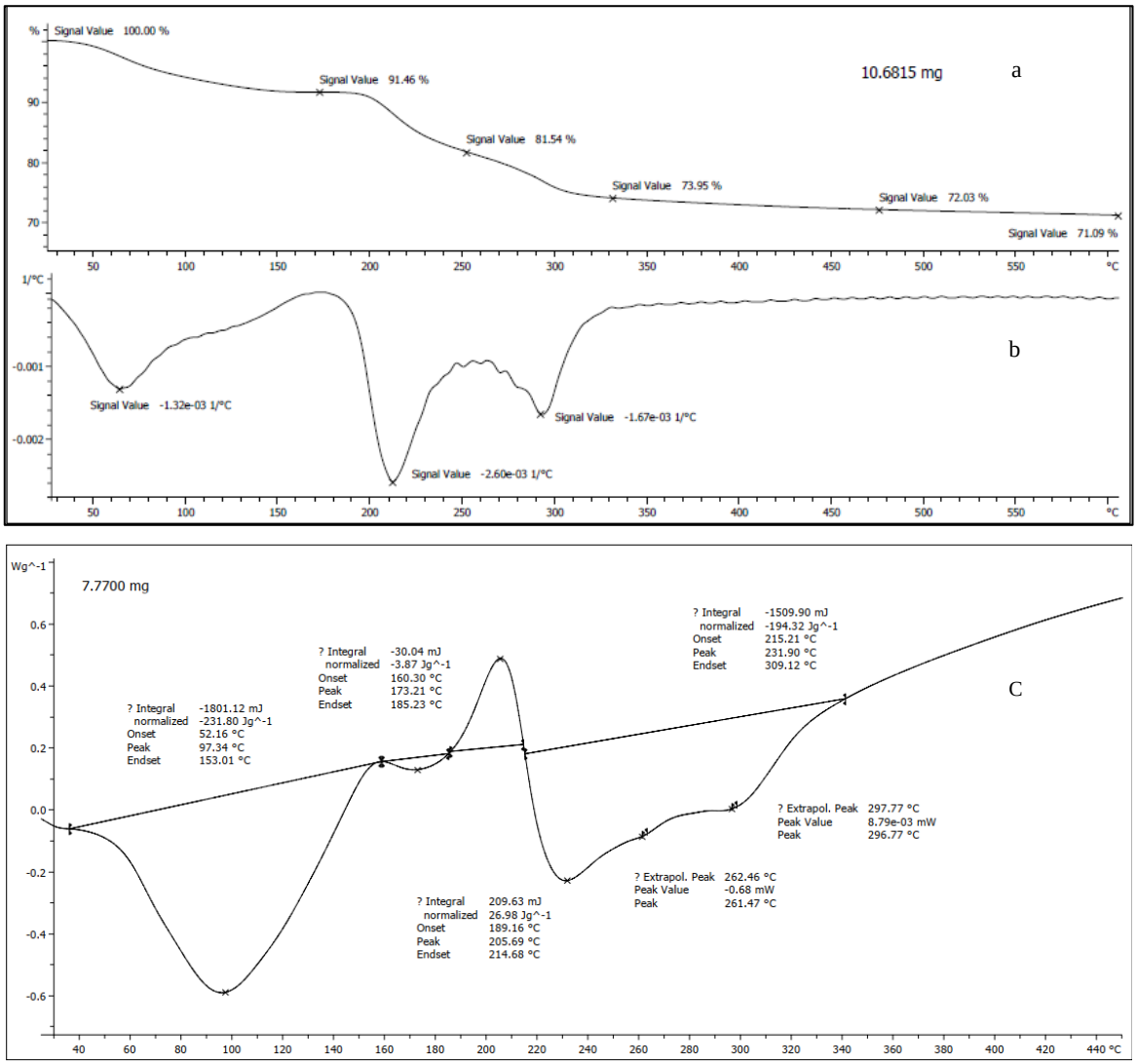
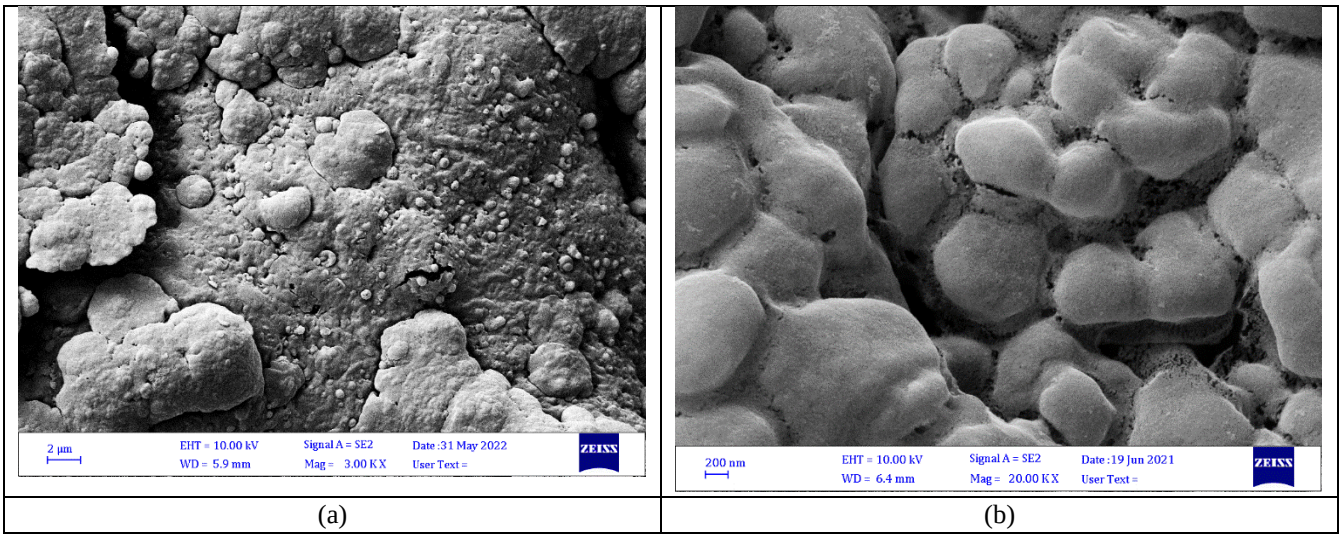



**Figure 3.4: a) TGA, b) DTA, and c) DSC thermograms of (CH-co-ST)/ SHMP hydrogel**

The FESEM images of (CH-co-ST) hydrogels were studied using FESEM-Imaging-EDS-Mapping Line-EBSD/Germany of both Glu and SHMP cross-linking (Figure 3.5) shows the hydrogels are present in spherical-particles have wide surface area. The (CH-co-ST)/Glu hydrogel (Figure 3.5a) shows a cluster of microspheres are connected with each others have whiten spots indicate the hydrogel have crystalline regions. While the (CH-co-ST)/SHMP hydrogel (Figure 3.5b) shows a folded surface with huge numbers of spherical beads seems cumulating in compacting form due to their physical interactions by SHMP cross-linker.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(3), 1457-1471 | ISSN:2251-6727




(a) (b)

**Figure 3.5: FESEM images of (a) (CH-co-ST)/Glu , and (b) (CH-co-ST)/SHMP hydrogels.**

### 3.1 Studying the Surface Shape of the Hydrogel Microspheres

Hydrogels have three-dimensional network structures and the type of their cross-linking has a significant effect on the morphology of the hydrogel microspheres [23]. The particle size of (CH-co-ST) hydrogels of both Glu and SHMP cross-linking were studied using the FESEM images (Figure 3.5) which shows spherical particles having average size according to their image scale are (82.5µm) for (CH-co-ST)/Glu hydrogel particles (Figure 3.5a), and (39.7µm) for (CH-co-ST)/SHMP hydrogel particles (Figure 3.5b), means physical cross-linker attracted the polymer chains in three-dimensional network and produce small size particles due to the high ionic interactions between cross-linker and hydrogel functional groups. The other studying factor which has significant effects on surface roughness of the hydrogel microspheres as changes occur in the type of cross-linker is the shape factor (S) of the microspheres, depending on equation (1) for calculation. The value of shape factor (S) greater than (0.80), the microsphere surface tends to be rough, while it becomes smooth if (S) is equal to or lower than (0.80). The shape factor of (CH-co-ST)/Glu hydrogel have shown a high value (S=0.862), means its particles have high degree of roughness (Figure 3.5a), whereas the (CH-co-ST)/SHMP hydrogel have shown a low value (S=0.811) but still it is higher than (0.80), means the particles are rough but less than Glu cross-linked hydrogel.

### 3.2. Degree of Swelling

Estimation of degree of swelling of hydrogel microspheres could control their loading with drug and their release behavior, hence, the swelling behaviors of Glu and SHMP cross-linked chitosan/starch hydrogels were studied. The swelling curve of (CH-co-ST)/Glu hydrogel (Figure 3.6a) shows its microspheres in pH=2 having lowest but stable DS% and are stable for four days, means the microspheres are highly compact in acidic medium under the effects of Glu cross-linker. Similarly, the Glu microspheres in neutral medium (pH=7) (Figure 3.6a) are also stable and for four days with higher DS%, means the polymer chains are less compact and the neutral medium help the polymer functional groups to be relax. In basic medium (pH = 9) (Figure 3.6a) the microspheres are swell and reach their highest DS% comparing to those in pH=2 and PH=7, and may be because of the repulsive force between hydrogel functional groups and swelling media ions and also the microspheres in pH=9 shows stable swelling state for three days. Furthermore, the (CH-co-ST)/SHMP hydrogel microspheres shows (Figure 3.6b) highly DS% in acidic medium (PH=2) where reach more than (DS = 160%) after four days with stable structure, which is due to the physical interactions

Journal of Chemical Health Risks
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JCHR (2023) 13(3), 1457-1471 | ISSN:2251-6727

between both ions of swelling media and SHMP cross-linker [31]. Wherein, the attraction force between media ions and SHMP ions, which opens the blockade of polymer chains and take their freedom to swell [28]. Where the SHMP cress-linked microspheres in neutral (pH= 7) and basic (pH = 9) medium (Figure 3.6b) are swell and reach DS% less than that in acidic medium, but their swelling behavior and DS% are almost same and remain stable and compact inside the swelling solution for more than four days.

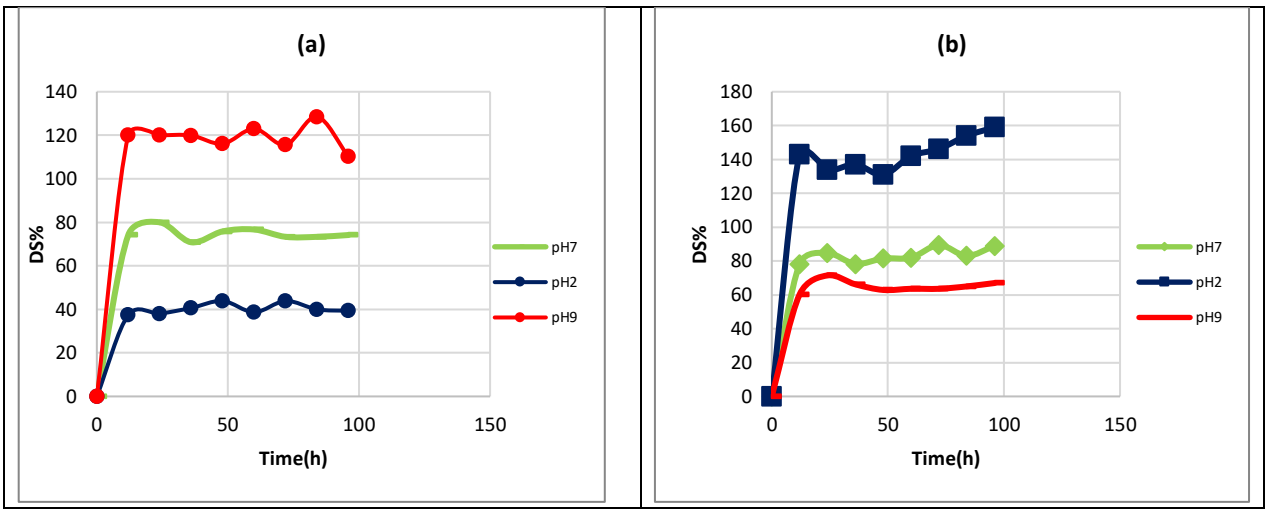


**Figure 3.6: Degree of swelling versus time (h) of (a) (CH-co-ST)/Glu, and (b) (CH-co-ST)/SHMP microspheres in different pH media, T=25$^0$C.**

### 3.3. Loading of Hydroxychloroquine Microspheres

Maximum loading percentage ($L_{max}$%) of the prepared microspheres with model drug shows effects with many factors. The type of cross-linking, degree of swelling, pH of loading media, time of loading and concentration of loaded drug where all have a significant effects on $L_{max}$% of drug on microspheres. The $L_{max}$% and EL% of the loaded microspheres are calculated using equations (3 and 4) respectively, and the collected data were given in (Table 3.4) which shows an increase in loading trend as the concentration of loaded drug in the solution is increase.

**Table (3.4): $L_{max}$% and EL% of Glu and SHMP cross-linked microspheres leaded with different concentrations of hydroxychloroquine.**

| Sample/drug | Type of cross-link | $L_{max}$% (period) | EL% (period) | $L_{max}$% (period) | EL% (period) | $L_{max}$% (period) | EL% (period) |
|---|---|---|---|---|---|---|---|
| | | 50 mg/dL drug | 50 mg/dL drug | 100 mg/dL drug | 100 mg/dL drug | 150 mg/dL drug | 150 mg/dL drug |
| (CH-co-ST) hydroxychloroquine | 2.5 wt% Glu | 18 (30h) | 36 (30h) | 41 (60h) | 41 (60h) | 46.5 (60h) | 31 (60h) |
| | 6.0 wt% SHMP | 20.2 (60h) | 40.4 (60h) | 45 (30h) | 45 (30h) | 55.8 (60h) | 37.2 (60h) |

100 mg microspheres in 100ml loading solution of pH=7, T=25°c

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The time of loading also shows a significant effect on $L_{max}$% and 10h loading time was not sufficient, whereas 60h was more common in most drug concentrations. Finally, the SHMP hydrogel cross-linking has shown higher $L_{max}$% and even higher EL% of drug on microspheres, which was due to the highly electrostatic interactions between the functional groups of Hydroxychloroquine and the highly ionic salt sodium hexametaphosphate in the loading solution at pH=7. In addition to the dispersal holes and folds on the microspheres surfaces which increase the retention capacity of SHMP cross-linked microspheres with higher $L_{max}$% and EL% of drug.

**3.4 Release of Hydroxychloroquine from Microspheres**

The release profile of hydroxychloroquine from drug loaded microspheres were studied in three pH media representing simulated fluids in the human body and at 37°C. The highest loaded microspheres were selected for drug release with $L_{max}$% = 46.5 for (CH-co-ST)/Glu hydrogel and $L_{max}$% = 55.8 for (CH-co-ST) / SHMP hydrogel. The controlled ($CR_{max}$%) and burst ($BR_{max}$%) release percentages (Table 3.5) were calculated using equations (5 and 6), and the cumulative ($R_{cum}$%) release percentage (Figure 3.7) was calculated using equation (7).

**Table (3.5): Loading and release characteristic of Glu and SHMP cross-linked (CH-co-ST) microspheres.**

| Microsphere sample | $L_{max}$% | Release Solution pH | Burst Release (mg) | Controlled Release (mg) | Controlled Release Time (h) |
|---|---|---|---|---|---|
| (CH-CO-ST)/ Glu | 46.5 | 1.3 | 9.3 | 30.0 | 18 |
| | | 7.4 | 5.5 | 40.0 | 30 |
| | | 9.4 | 4.0 | 40.5 | 36 |
| (CH-CO-ST)/ SHMP | 55.8 | 1.3 | 10.5 | 45.0 | 30 |
| | | 7.4 | 7.5 | 45.0 | 36 |
| | | 9.4 | 8.8 | 47.0 | 36 |

The release character of Glu cross-linked microspheres (Figure 3.7a) shows the microspheres were released hydroxychloroquine in the release media of pH=1.3, pH=7.4 and pH=9.4 in controlled manner, only a few differences are shown in controlled time (Table 3.5) occurred in different pH media. In basic solution pH=9.4 the loaded microspheres shows the best trend of controlled release comparing with other solutions, where Glu microspheres reach their high structural relaxation in an acidic release solution of pH=1.3 and high $R_{cum}$% were released for a short controlled time (Figure 3.7, and Table 3.5). Whereas in an basic release solution of pH = 9.4, the microspheres are more compact and the drug was interact with hydrogel, therefore, the hydrogel gives a controlled release trend with (40.5mg) and for 36h controlled time. The SHMP cross-linked (CH-co-ST) hydrogel were studied and their data (Figure 3.7b, and Table 3.5) shows release of hydroxychloroquine with highly controlled manner in all studied cases. The (CH-co-ST)/SHMP hydrogel loaded hydroxychloroquine shows about 80% of the loaded drug was release in controlled way in all pH media (pH=1.3,pH=7.4and pH=9.4). the controlled trend of drug release in all pH media was due to the electrostatic atrraction between the drug functional groups and those of the hydrogel , which hinders the release of hydroxychloroquine [23].

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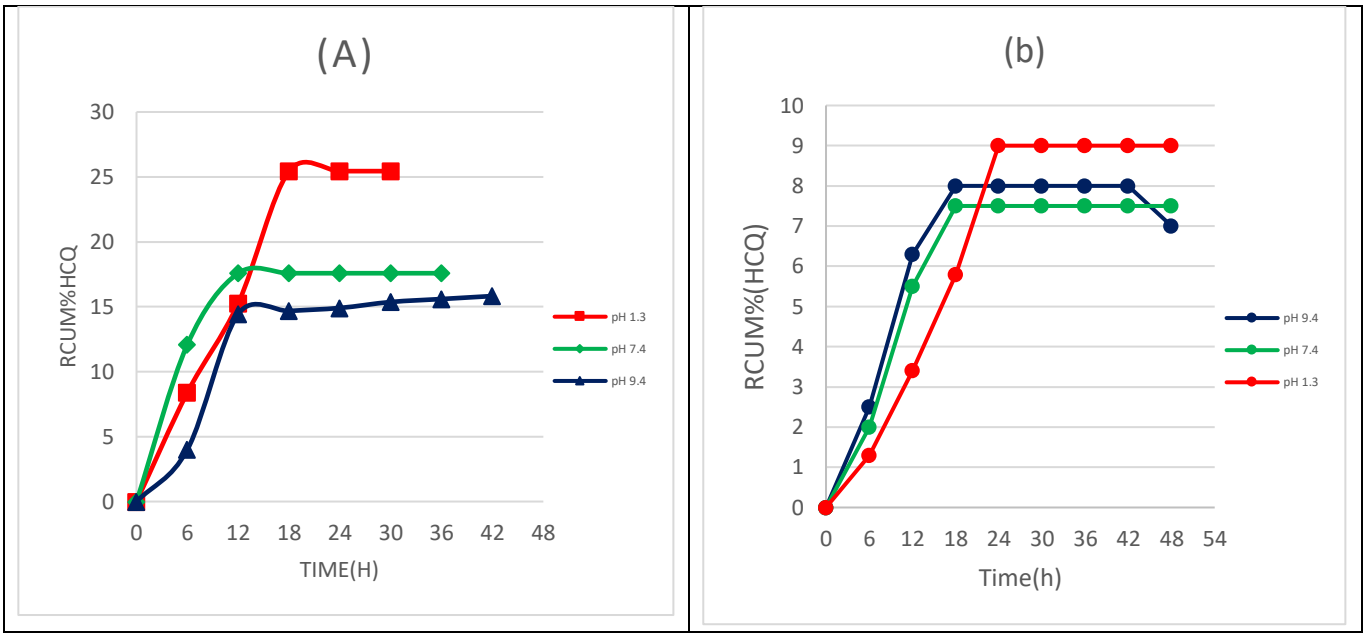
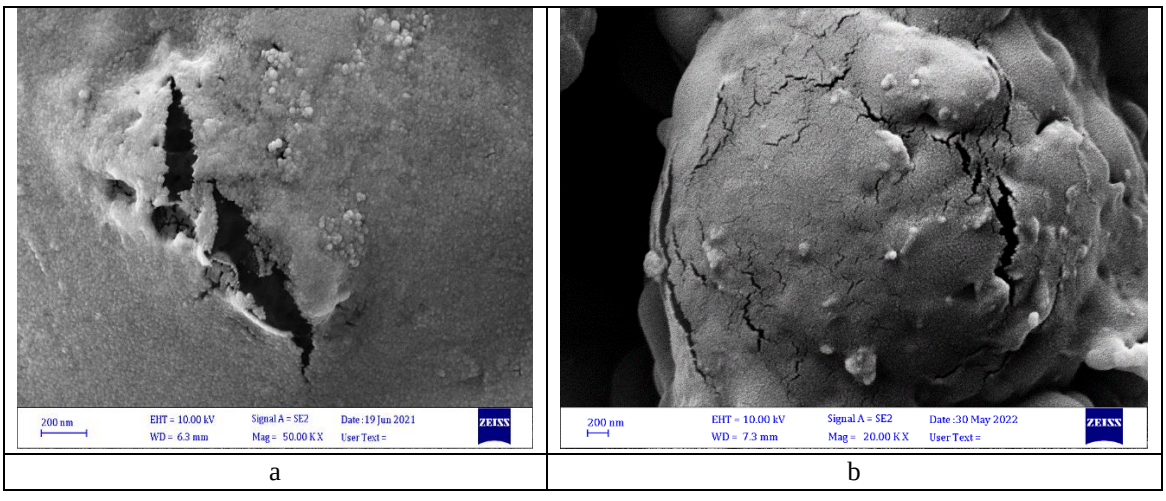



**Figure 3.7: Effect of solution pH on the release of hydroxychloroquine from (a) Glu and (b) SHMP cross-linked microspheres. Release media =100mg microspheres in 20 ml buffered solution (pH =1.3, pH = 7.4, and pH = 9.4 ) at T=37$^0$C .**

Some FESEM images of chitosan /starch microspheres of both cross –linked after release hydroxychloroquine (figure 3.8) shows the clear large open cracks permeated the microsphere surface with some holes which allowed drug molecules to exit from (CH-co-ST)/Glu microspheres (figure 3.8a). Similarly, the (CH-co-ST)/SHMP microspheres shows FESEM images ( figure 3.8b) with brittle surface interspersed with cracks help the drug to release.




a b

**Figure 3.8: FESEM images of (a) (CH-co-ST)/Glu , and (b) (CH-co-ST)/SHMP microspheres after release hydroxychloroquine .**

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## 4. Conclusions

Blend copolymerization of chitosan with starch polysaccharide will produce hydrogel with cationic and anionic character with high water absorption properties, need cross-linking for microspheres production. Therefore, the microspheres were cross-linked with glutaraldehyde and sodium hexametaphosphate representing chemical and physical cross-linking respectively. The formed microspheres are characterized using different analysis and their shape factor calculations shows the prepared microspheres, have rough surfaces, where Glu microspheres are more roughness than SHMP particles. The XRD pattern shows the SHMP cross-linked microspheres are semi crystalline material due to the electrostatic interactions between polymer chains. The degree of swelling of microspheres which is the controlled key of their drug loading and release shows for Glu cross-linked microspheres the (DS=120%), (DS=80%), and (DS=35%) in pH=9, pH=4, and PH=2 swelling media respectively, where the microspheres are highly compact in acid medium, whilst relaxed are in neutral and basic media. The SHMP cross-linked microspheres have higher degree of swelling in acidic media (pH=2) with (DS=150%) due to the ionic interactions between SHMP and swelling solution ions. While its (DS=80%), and (DS=75%) in pH=7 and pH=9 swelling media respectively, due to the attraction forces formed between the polymer chains in neutral and basic swelling media. The prepared hydrogels shows after 60h in (100 mg/dL) hydroxychloroquine solution a maximum loading percentage $L_{max}$= 46.5% (w/w) for Glu microspheres and $L_{max}$=55.8% (w/w) for SHMP microspheres inside a pH=7 buffered solution and at 25°C. The release behaviors of loaded microspheres shows differences when varying the pH of the release media from 1.3 to 7.4 and then to 9.4. In both systems, Glu and SHMP microspheres, the release manner was in controlled action due to their roughness nature as the shape factor (S) measurement proved, and also their good swelling behaviors. Nevertheless, SHMP microspheres have controlled release character more sequence especially in pH=7.4 and pH=9.4 release media, where they release hydroxychloroquine of 45.0 mg(w/w) and 47.0 mg (w/w) respectively in controlled manner from total $L_{max}$= 55.8 mg (w/w) and only few milligrams of drug was released in burst way, where ions in the release media could help for more interactions.

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