Formulation and Evaluation of Nystatin Microparticles as a Sustained Release System

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Abstract

Nystatin is the drug of choice for treatment of cutaneous fungal infections with main disadvantage that is the need for multiple applications to achieve complete eradication which may reduce patient compliance. Microparticles offer a solution for such issue as they are one of sustained release preparations that achieve slow release of drug over an extended period of time. The objectives of this study were to fabricate nystatin-loaded chitosan microparticles with the ultimate goal of prolonging drug release and to analyze the influence of polymer concentration on various properties of microparticles. Microparticles were prepared by chemical cross-linking method using glutaraldehyde as cross-linking agent. Five formulas, namely N1C1, N1C2, N1C3, N1C4 and N1C5, were prepared and the effect of drug to polymer ratio was studied with respect to drug loading, encapsulation efficiency, particle size and morphology. Furthermore the prepared microparticles were subjected to various physico-chemical studies, such as drug- polymer compatibility by Fourier Transform Infrared Spectroscopy (FTIR) and *in-vitro* drug release characteristics. Microparticles obtained from N1C1, N1C2 and N1C3 formulas were regular in shape with mean particle size ranging between 1µm and 10µm. N1C5 formula was resulted in particles with irregular shape while N1C4 showed a blend of microparticles and deformed particles. The effect of chitosan concentration on drug loading and entrapment efficiency was studied. The results showed increment in these parameters that was directly proportional to the increment in polymer concentration. Percentage yield showed a significant increment which was related to the increment in the ratio of chitosan used during the study. FTIR results showed no interactions between nystatin and chitosan. DSC studies proved the crystalline nature of nystatin and chitosan. On other hand, the thermogram of loaded microparticles showed the absence of endothermic peak corresponding to nystatin which may indicate the loss of the crystalline nature of the drug presented inside the microparticles. *In-vitro* release studies resulted in 95.6% release of nystatin for N1C1 after 15 hours. N1C1 appeared to be promising in formulating microparticles that provide nearly complete release of the drug within15 hours. This formula can be selected in future work to be formulated as topical gel that prolongs the release of nystatin.

Keywords: Nystatin, Chitosan, Glutaraldehyde, Chemical cross-linking.

التصييغ و التقييم المختبري للجزيئات المايكروية للنستاتين كنظام تحرير دوائي طويل الأمد

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الخلاصة

يعتبر النستاتين العلاج المثالي لإصابات الجلد الفطرية مع تضمنه عيب رئيسي وهو الحاجة إلى الاستعمال المتعدد لغرض الوصول إلى الشفاء التام مما يؤدي إلى تقليل التزام المريض. تقدم الجزيئات المايكروية حلا للمشاكل المشابهة وذلك لكونها إحدى تحضيرات التحرير طويلة الأمد والتي تحقق تحريرا بطيئا للدواء يستمر لفترات ممتدة من الزمن. الغرض الأول من هذه الدراسة هو تصنيع جزيئات مايكروية من الكايتوسان محملة بالنستاتين هدفها النهائي إطالة تحرير الدواء, أما الغرض الثاني فهو دراسة تأثير تركيز الكايتوسان على مختلف خصائص الجزيئات المايكروية.حضرت الجزيئات المايكروية بطريقة الربط الكيميائي وباستخدام كلوترالديهايد كعامل ربط.تم تحضير خمس صيغ (N1C1,N1C2, N1C3, N1C4 and N1C5) لدراسة تأثير نسبة البوليمر على تحميل الدواء, كفاءة التغليف و الخصائص الشكلية وحجم الجسيمات. خضعت الجزيئات المايكروية المحضرة لدراسات فيزيوكيميائية متعددة مثل دراسة التوافق بين الدواء و البوليمر بواسطة جهاز FTIR و كذلك دراسة خصائص تحرير الدواء. كانت الجزيئات المايكروية الناتجة من الصيغ N1C1, N1C2 و N1C3 منتظمة الشكل مع متوسط حجم يتراوح بين ١ مكم و ١٠ مكم. أنتجت الصيغة N1C5 جسيمات غير منتظمة الشكل بينما أظهرت الصيغة N1C4 خليط من الجزيئات المايكروية التقليدية و جزيئات مشوهة. تمت دراسة تأثير تركيز الكايتوسان على تحميل الدواء و كفاءة التغليف. أظهرت النتائج زيادة في هذين العاملين وكانت هذه الزيادة ذات علاقة طردية بالزيادة في تركيز البوليمر. أظهرت النسبة المئوية للمحصول زيادة معتبرة والتي عزيت إلى الزيادة في نسبة الكايتوسان المستخدم خلال الدراسة. أظهرت نتائج FTIR عدم وجود أي تفاعل بين النستاتين و الكايتوسان. أثبتت دراسة DSC الطبيعة البلورية للنستاتين و الكايتوسان. من جهة أخرى, أظهر الرسم الحراري للجزيئات المايكروية المحملة بالدواء غياب قمة

امتصاصية مقابلة لنقطة انصهار النستاتين مما يمكن أن يدل على على فقدان الشكل البلوري للدواء الموجود داخل الجزيئات. أسفرت دراسة التحرير الدوائي في المختبر عن تحرير ٩٥٫٦% بالنسبة للصيغة N1C1 و ذلك بعد مرور ١٥ ساعة. أظهرت الصيغة N1C1 نتائج واعدة في تصييغ الجزيئات المايكروية وذلك بتوفيرها تحرير شبه كامل للدواء بعد ١٥ ساعة. هذه الصيغة يمكن أن تختار في عمل مستقبلي لتصييغ شكل دوائي موضعي هلامي طويل الأمد من النستاتين.

الكلمات المفتاحية : نستاتين, كايتوسان, كلوترالديهايد, ربط كيمائي.

Introduction

The development of new delivery systems for the controlled release of drugs is one of the most interesting fields of research in pharmaceutical sciences. Microparticles are one of sustained release preparations that achieve slow release of drug over an extended period of time. Microparticles are micrometric matrix systems essentially spherical in shape having size ranging between 1μm - 1000μm [1]. Microparticles can be used for the controlled release of drugs, vaccines, antibiotics and hormones. Microparticles have many advantages such as providing a larger surface area and possessing an easier estimation of diffusion and mass transfer behavior. Besides these, the encapsulated small molecules could diffuse out of the barrier with precise kinetics modeling and control-release of drugs to the body fluid [2]. Candida albicans infection is a problem of growing clinical importance worldwide. Literature data point out that about 96% of all opportunistic mycoses are caused by Candida species [3]. Nystatin is a polyene antifungal antibiotic characterized by a potent broad-spectrum antifungal action against a variety of fungal pathogens including Candida, Aspergillus, Histoplasma, and Coccidioides [4]. Successful eradication of cutaneous fungal infections requires 2-4 applications of all available topical dosage forms of nystatin [5]. Consequently, the aim of this study was to fabricate nystatin-loaded, sustained-release formulation that would prolong the release of the drug at the site of application, reduce the frequency of application, reduce the amount of drug administered and improve patient compliance and acceptance. Chitosan was selected as a release-retardant polymer and the chemical cross- linking was selected as the technique. To find the best formula (the one with the highest drug content, best encapsulation efficiency, optimum particle size and morphology), different formulas were prepared using different ratios of chitosan.

Materials and methods

Materials

Nystatin was obtained as a gift from Ninavah Drug Industry (NDI). Chitosan was obtained from Johnson CAO, US. Span 80 is purchased from BDH® (UK). Liquid paraffin, methanol and glutaraldehyde were obtained from TEDIA® (USA). Glacial acetic acid was obtained from THOMAS BAKER (UK) while hexane obtained from POCH® (Poland).

Preparation of microparticles

Glutaraldehyde cross- linking method was employed for preparation of microparticles [6]. Accurately weighed amounts of polymer and drug (see Table 1 for the different amounts of drug and polymer) were added step by step into 2% acetic acid solution. The resultant nystatin- chitosan dispersion was added drop wise by syringe with 23G needle into a continuous phase consisting of 125 mL of light liquid paraffin containing 0.5% of Span 80 as an emulsifying agent. The mixture was stirred for 30 minutes at 1500 rpm using a 4-blade mechanical stirrer to form a w/o emulsion. A drop-by-drop solution of a measured quantity of aqueous glutaraldehyde (25% v/v) was added to the prepared emulsion at 15, 30, 45, and 60 minutes. Stirring was continued for 2.5 hours to obtain microparticles which were washed first with hexane and then with distilled water to remove the adhered liquid paraffin and glutaraldehyde. Finally the resultant microparticles were filtered, dried at room temperature and stored in a desiccator for further evaluation.

Physicochemical characterization

Morphological characteristics and particle size analysis:

A microscopical image analysis technique was applied for determination of particle size. The morphology and particle sizes were determined with Motic® digital microscope equipped with imaging accessory. The particle diameters of 200 microparticles were measured randomly by supplied software and the average particle size was determined [7]. In order to be able to define a size distribution or compare the characteristics of particles with many different diameters, the size distribution can be broken down into different size ranges, which can be presented in the form of a histogram.

Determination of drug loading and entrapment efficiency

A known quantity of microparticles (10 mg) was triturated by mortar and pestle and extracted with 10 ml methanol (containing 5% acetic acid). The resultant solution was centrifuged and the obtained supernatant was diluted appropriately with respective solvent

system [8]. Absorbance was measured at 305 nm using LABOMED® UV-VIS spectrophotometer and concentration of nystatin in microparticles was calculated according to the standard regression. Each determination was made in triplicate. The drug loading of prepared microsphere was determined by applying the following equation[9]:

Drug loading (%) =

$$\frac{\textbf{calculated drug content}}{\textbf{total amount of microparticles}} \; \textit{x } \textbf{100}$$

Entrapment efficiency represents the proportion of the initial amount of drug incorporated/associated into/with the microparticles[10]. This parameter was determined by applying the following equation[11]:

Entrapment efficiency (%) =

$$\frac{\textbf{actual drug content}}{\textbf{Theoretical drug content}} \; \textit{x } \textbf{100}$$

Determination of percentage yield

The relative yield was calculated based on the amount of microparticles of each formulation obtained relative to the amount of the total weight of the polymer and drug used in the dispersed phase. The percentage yield was calculated according to the following equation[12].

Yield (%) =

$$\frac{\textbf{Total weight of microparticles obtained}}{\textbf{Total weight of initial raw materials}} \; \textit{x } \textbf{100}$$

Fourier transforms infrared spectroscopy (FTIR)

Drug-polymer interactions were studied by FTIR spectroscopy. The spectrum was recorded for chitosan, pure nystatin, blank chitosan microparticles, physical mixture of blank chitosan microparticles and nystatin and for nystatin loaded chitosan microparticles using BRUKER® (Germany) infrared spectrophotometer [13].

Differential Scanning Calorimetry (DSC)

Thermal analysis was performed using DSC (Shimadzu DSC plus, Japan). Approximately 10 mg of each sample (chitosan, pure nystatin, blank chitosan microparticles, physical mixture of blank chitosan microparticles and nystatin in the ratio of (1:1) and nystatin loaded chitosan microparticles) were used. Each sample was placed in a sealed aluminum pan and heated at a scanning rate of 10ºC/min from 30ºC to 250ºC [14].

In- vitro drug release studies

Nystatin- loaded chitosan microparticles equivalent to (10) mg of nystatin were subjected to in-vitro drug release studies to ascertain the drug release pattern of nystatin from the prepared microparticles formulations.

Microparticles were introduced in a beaker containing (300) mL of phosphate buffer solution (pH 5.5), used as the release medium. The beaker was rotated at 100 rpm and maintained at 32±0.5°Cin a thermostat shaking water bath [15, 16]. An aliquot of (5) mL was withdrawn periodically at 1, 2, 3, 4, 5, 6, 9, 12 and 15 hours and replaced with a same volume of fresh medium each time. The withdrawn samples were filtered, then diluted appropriately and analyzed with LABOMED® UV–VIS spectrophotometer at 305 nm to determine the amount of drug released.

Drug release kinetics

To examine the drug release kinetics, the cumulative release data were fitted to models representing zero-order (Q v/s t), first-order (log (Q_0-Q) v/s t) and Higuchi's square root of time (Q v/s $t^{1/2}$), respectively, Where Q is the cumulative percentage of drug released at time t and (Q_0-Q) is the cumulative percentage of drug remaining after time t [17]. To determine the mode of drug release, the initial 60% drug release values were fitted to the Korsmeyer–Peppas model:

$M_t/M\infty = Kt^n$, where $M_t/M\infty$ is the fraction($\leq 60\%$) of drug released at time t, K is the drug release rate constant, and n is the release exponent. The n value is employed for the characterization of the mechanism of solute release from formulation [18].

Statistical analysis

All experiments were repeated at least three times. Results are expressed as means ± standard deviation (SD). Statistical analysis was carried out employing one-way ANOVA test. A p-value ≤ 0.05 was considered statistically significant.

Results and Discussion

Preparation of microparticles

Different formulas of nystatin microparticles were fabricated successfully using chemical cross-linking method (Table 1). The formula N1C5 has shown very high viscous dispersion that was difficult to be extruded from a 23G needle, so it was applied by a disposable syringe without needle. Chitosan has 1 primary amino and 2 free hydroxyl groups for each C6 building unit. Due to the availability of free amino groups in chitosan, it carries a positive charge and thus it reacts with many negatively charged compounds [6]. Chitosan could be covalently cross-linked with glutaraldehyde through its amino groups. The aldehyde groups of the glutaraldehyde formed covalent imine bonds with the amino groups of chitosan, due to the resonance established with the adjacent double ethylenic bonds via a Schiff reaction [19].

Table (1): Composition of nystatin- loaded chitosan microparticles formulas

Formula code	Drug: polymer ratio (w/w)	Nystatin (mg)	Chitosan (mg)	Aq. Glutar- -aldehyde solution (ml)	aqueous acetic acid solution (ml)
N1C1	1:1	100	100	15	10
N1C2	1:2	100	200	15	10
N1C3	1:3	100	300	15	10
N1C4	1:4	100	400	15	10
N1C5	1:5	100	500	15	10

Particle size analysis and morphological characteristics

Optical microscope images show that N1C1, N1C2 and N1C3 formulas have uniform spherical shaped microparticles while N1C5 microparticles were irregularly shaped and highly aggregated in nature and practically difficult to be distinguished as individual microparticles. In other hand, N1C4 showed a blend of microparticles and deformed particles (Figure. 1). It was noted that as the concentration of chitosan increased, the color of microparticles changed from light yellow to dark brown.

The size distribution profiles of the microparticles are shown in Figure 2. The particle size of all formulas was ranged from 1µm to 10 µm. The results showed that particle size was affected significantly ($p < 0.05$) by the polymer concentration, as the chitosan concentration increased a larger mean size of microparticles was obtained (Table. 2). This effect may due to the increase in the viscosity of the droplets (due to the increase in concentration of polymer solution) [11]. The increase in solution viscosity led to a decrease in stirring efficiency hence result in difficulty in dispersion and subdivision of droplets and thus increased the resultant microsphere size [20].
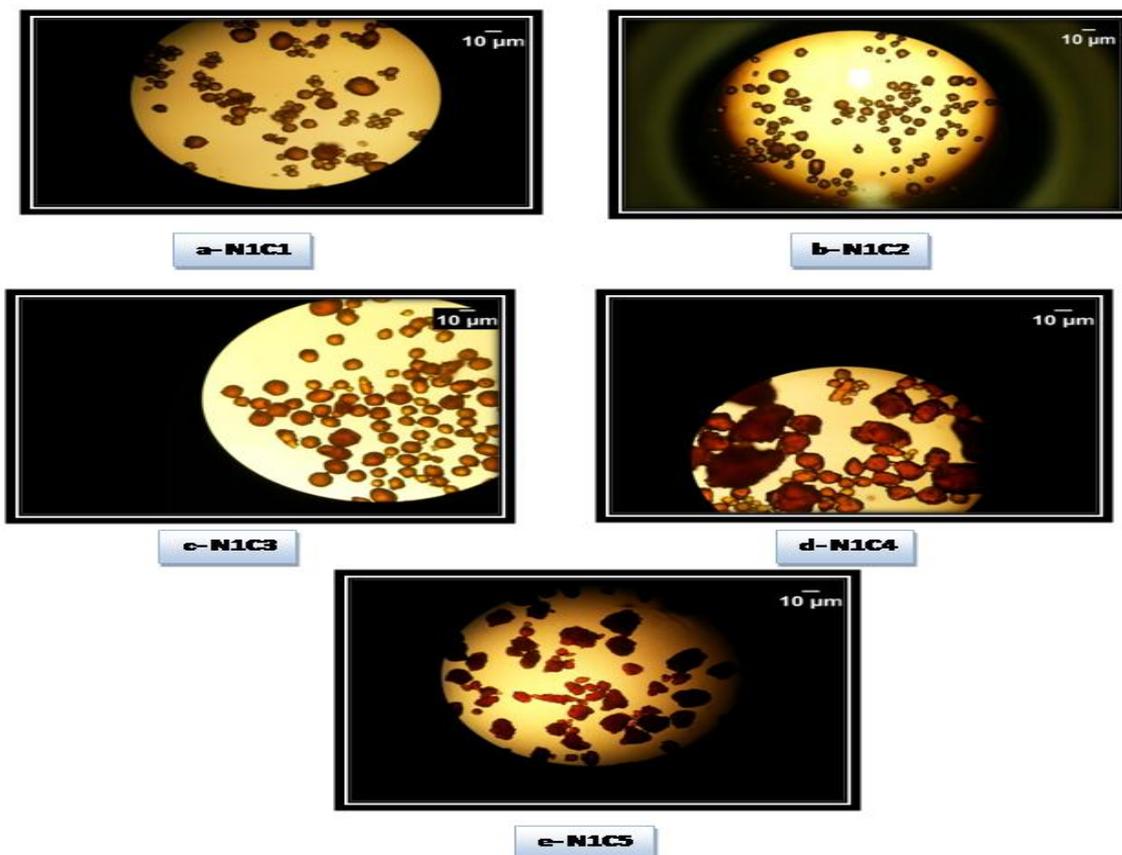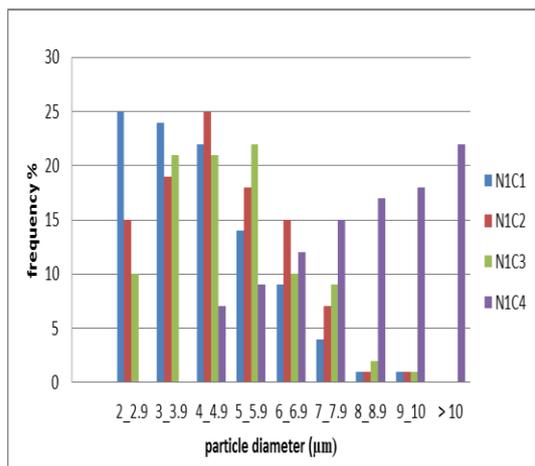

Figure (1): Optical microscope images of nystatin loaded microparticles

Figure(2): Histogram showing the effect of increasing chitosan concentration on frequency distribution analysis of nystatin-loaded microparticles formulas

Determination of drug loading and entrapment efficiency

The concentration of chitosan had significant ($p < 0.05$) effect on the drug loading and entrapment efficiency of nystatin- loaded microparticles which is reflected by the variations in the percentage of drug loading and entrapment efficiency that observed in all formulations as shown in Table 2. Better entrapment efficiency were achieved by increasing the ratio of chitosan which complies with Patel *et al.* who obtained the same results with Tramadol-loaded chitosan microspheres

[21]. Senthil *et al.* revealed that entrapment efficiency was also increased with the higher polymer concentration [22]. Another study conducted by Palanisamy *et al.* also achieved highest drug entrapment with drug: polymer ratio of 1:3 indicating that polymer concentration has a great impact on this parameter [23]. Increase in the concentration of chitosan increases the yield of the prepared microparticles and thereby resulting in higher drug entrapment levels [24]. It was further observed that the drug entrapment was proportional to the size of the microparticles [21]. The contribution of a high polymer concentration to drug loading can be interpreted in two ways. First, the high viscosity of the polymer solution would be expected to decrease the diffusion of the drug into the external phase which would result in high entrapment efficiency [8, 25]. Second, the higher polymer concentration produce large size microparticles in which the loss of drug from surface during washing process is lesser in comparing to small microparticles. Thus size of microparticles is also affecting the drug loading [26, 27].

Determination of percentage yield

It was observed that as the polymer ratio increased, the product yield percentage also significantly ($p < 0.05$) increased (Table 2). The low percentage yield in N1C1 in relative to other formulas may be due to microparticles loss during washing process.

Table (2): Drug Loading (%), Entrapment Efficiency (%), Yield (%) and Particle Diameter of Nystatin- Loaded Microparticles Formulas.

Formula Code	Drug: Polymer Ratio w/w	Drug Loading* (%)	Entrapment Efficiency* (%)	Percentage Yield* (%)	Particle diameter (μm)*
N1C1	1:1	43.9±1.6	88.9±0.4	82.15±3	4.19±1.6
N1C2	1:2	28.6±0.8	91.2±0.4	86.67±4	4.65±1.7
N1C3	1:3	21.6±0.5	93.5±0.6	93.57±2.2	4.81±1.6
N1C4	1:4	17.8±0.4	96.2±0.5	95.33±2.1	7.91±2.9
N1C5	1:5	15.9±0.1	97.2±0.6	97.12±1.1	ND
*: mean± standard deviation, ND: not determined					

Fourier transforms infrared spectroscopy (FTIR)

Owing to that each specific chemical bond often has unique energy absorption; FTIR spectroscopy has been extensively applied to identify the presence of certain functional groups or chemical bonds in a molecule. Spectral data could confirm the stability of the drug during microparticles

preparation and the absence of any drug-polymer interaction. FTIR spectra of pure nystatin, chitosan, blank chitosan microparticles, physical mixture of blank chitosan microparticles and nystatin and the spectrum of nystatin loaded chitosan microparticles are shown in Figure 3. Nystatin displays characteristic absorption bands at about 1702 cm^{-1} due to the stretching vibration of carbonyl group [28]. On the other hand, the characteristic absorption of the chitosan

situated at 1647 cm^{-1} due to C=O stretching in amide group (amide I vibration) and at 1576 cm^{-1} due to N–H bending in amide group (amide II vibration) [29]. Some changes can be observed after chitosan cross-linking with glutaraldehyde. The characteristic peak at 1642 cm^{-1} is due to Schiff base (C=N) formed by a cross linking reaction between the amino group and the aldehydic group of glutaraldehyde. Moreover, no absorption is observed at ~1715 cm^{-1}, related to the free aldehyde group which further confirms the cross-linking reaction [18]. FTIR spectra of nystatin loaded microparticles and blank polymer microparticles showed the same characteristic absorption peaks with the exception of characteristic band value at ~1700 cm^{-1} for loaded microparticles which can be assigned to the C=O group of the drug. This result clearly indicated the stability of the drug during the microparticles preparation and revealed the absence of any drug- polymer interaction.

Figure(3): FTIR spectra of pure nystatin (a), chitosan (b), blank chitosan microparticles (c), physical mixture of nystatin and blank microparticles (d) and nystatin loaded microparticles (e)

Differential scanning calorimetry (DSC)

The thermograms of nystatin (a), chitosan (b), physical mixture of chitosan and nystatin (c), plain microparticles (d) and loaded microparticles (e) are shown in figure 4. Nystatin presented a single, well defined sharp endothermic peak at 165.5°C which is corresponded to the melting point of nystatin. Chitosan showed initial endothermic peak at 75.15°C which can be correlated with loss of water associated to hydrophilic groups of polymer [30, 31]. Physical mixture thermogram showed an endothermic peak at 164°C due to the presence of nystatin, which reveals the absence of interactions between the nystatin and chitosan. Thermogram of drug-loaded chitosan microparticles showed the same peaks observed in blank microparticles, but there was no peak corresponding to nystatin. This might be an indication of loss of crystallinity of nystatin presented within the prepared micoparticles [21, 24, 29].
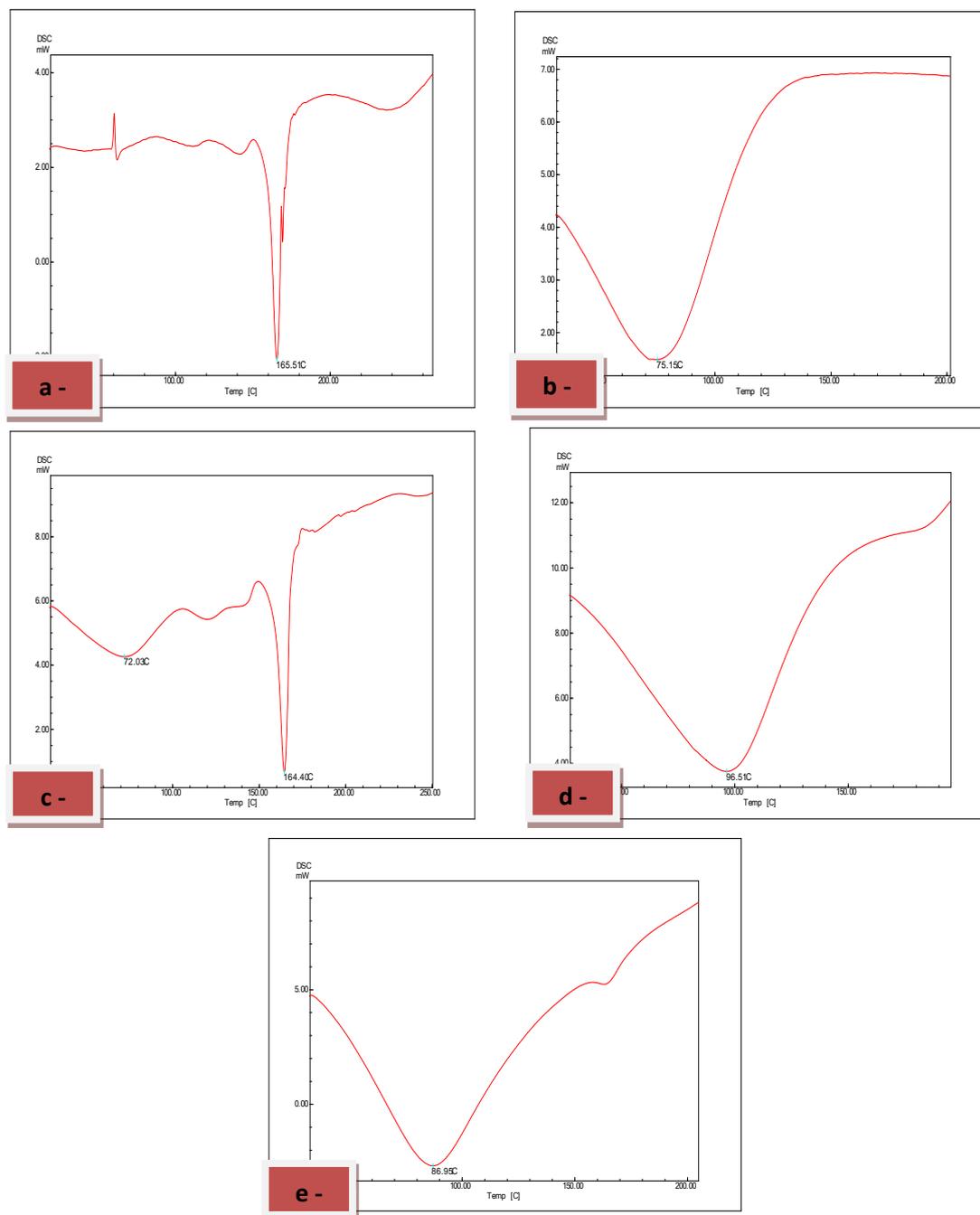

Figure 4: DSC thermograms of pure nystatin (a), chitosan (b), physical mixture of chitosan and nystatin (c), plain microparticles (d) and loaded microparticles(e).

In- vitro drug release studies

The cumulative percentage of nystatin release from chitosan microparticles versus time was drawn and represented graphically (Figure 5).
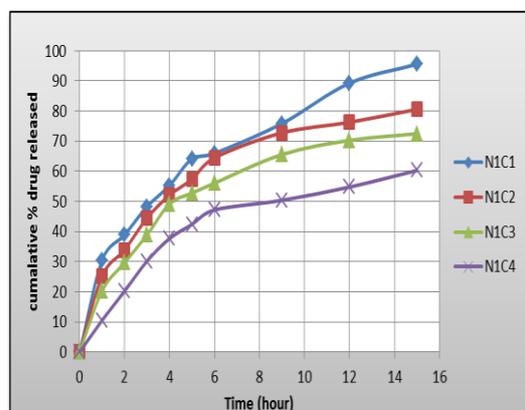

Figure (5): Cumulative % of nystatin released versus time.

The *in- vitro* drug release plot exhibited a biphasic mode with an initial 'burst' release for the different plots. Thereafter, a slow release phase was started. The initial rapid release of drug might due to fast dissolution of drug molecules attached to the surface of the microparticles while subsequent slow but steady release was related to diffusion of drug molecules present in the core of the microparticles [20]. This is in agreement with Kumar *et al.* who found that increasing the concentration of chitosan leads to decrease the release of Indomethacin from chitosan microparticles [29]. Khalandar *et al.* who prepared nasal microspheres of Sumatriptan using different concentrations of chitosan also concluded that the drug release is retarded by increasing polymer ratio [32]. The results show that an increase in polymer ratio reduced the first phase (burst phase) significantly (p< 0.05) as is evident from $T_{50\%}$ value (time required to release 50% of the loaded dose) as seen in table 3. Nystatin was released from microparticles in a controlled manner for about 15 hours.

The particle size would be expected to influence the rate of drug release [29]. This can explain the higher initial burst release of drug from N1C1 which has a smaller particle size. The overall release of nystatin from chitosan microparticles (from N1C1 to N1C4) decreased as chitosan concentration increased, suggesting that drug release could be controlled by varying chitosan concentration. N1C1 released the highest cumulative percentage of nystatin as 95.6% was released after 15hr. N1C2 did not show much variation. The remaining formulas (N1C3 and N1C4) exhibited less drug release. At the end of dissolution test , the release of nystatin was found to be incomplete in all formulas; this could be due to the relatively slow erosion of the polymeric substance under dissolution test conditions, with a consequential slow release of entrapped drug from the matrices [33].

Drug release kinetics

The *in- vitro* release data were plotted according to different models, and these curves were used to draw some conclusions regarding the mode of drug release from the microparticles. The curve fitting and plotting was performed in Excel (Microsoft Software Inc., USA). Correlation coefficient (R^2) values were calculated for the curves obtained by the regression analysis of the plots. The model with the highest correlation coefficient was considered to be the best fitted model. As Table 3 shows, the kinetic model fits more appropriately with the Higuchi matrix model (linear nature of curve). This was confirmed by high values of regression coefficients obtained in all formulas. All other models produced curvilinear plots with lower values of regression coefficient. To find out the mechanism of drug release, the obtained drug release data were fitted in Korsmeyer-Peppas model. This equation was used to calculate the value of release point (n) which gave an additional evidence for the diffusion controlled mechanism. The values of (n) for all formulas suggested a non-Fickian (anomalous) diffusion mode of nystatin release from chitosan microparticles, for spheres, when n= 0.85 it indicates case-II (swelling − controlled drug release). Case-II is the relaxational release drug transport mechanism that is associated with stresses and state-transition in hydrophilic glassy polymers which swell in water or biological fluids. This term also includes polymer disentanglement and erosion [33]. When n value is between 0.43 and 0.85 (0.43 < n < 0.85) it corresponds to non-Fickian (anomalous) model while if n≤ 0.43, it indicates Fickian diffusion mechanism[34]. Non-Fickian refers to a combination of both diffusion and erosion to obtain controlled dug release [10]. Typically, the diffusion process consists of an initial "burst" release of drug at or near the surface of the microsphere followed by the additional release of drug from the pores of the microsphere. Erosion occurs by hydrolysis of the polymer matrix that generate pores which expose interior pockets of Nystatin to the bathing liquid. For continuous release, the diffusion and erosion process must balance each other to allow the drug to diffuse out of the microsphere at a constant rate [35].

Table (3): Release mechanism and kinetic model of nystatin release from chitosan Microparticles in PBS (pH 7.4) (n=3). (R^2= correlation coefficient, n = diffusion exponent from eq. $Mt/M\infty= Kt^n$).

Formula code	n	Release mechanism	R^2				Release kinetic	$T_{50\%}$ (hour)
			First-order	Higuchi model	Zero-order	Korsmeyer–Peppas		
N1C1	0.45	Anomalous transport	0.96	0.98	0.85	0.99	Higuchi	4.5
N1C2	0.52	Anomalous transport	0.93	0.96	0.79	0.99	Higuchi	5.54
N1C3	0.58	Anomalous transport	0.90	0.95	0.79	0.99	Higuchi	6.75
N1C4	0.61	Anomalous transport	0.89	0.95	0.80	0.92	Higuchi	10.18

Conclusion

In an attempt to formulate nystatin as sustained release microparticles by chemical cross-linking method, five different formulas were prepared and evaluated. Chitosan concentration had a substantial effect on the main parameters of microparticles (particle size, drug loading, entrapment efficiency and yield) as any increase in polymer ratio was accompanied by a similar increase in the aforementioned parameters. *In vitro* release studies of microparticles showed that N1C1 formula has resulted in higher release of nystatin from the loaded microparticles after 15 hours of study (95.6% release). The kinetic study demonstrated that nystatin release from the prepared microparticles is likely to follow Higuchi matrix model. From the aforementioned results, chitosan appears to be a good choice as a polymer for producing a modified release microparticles. The produced microparticles have been found to have a good potential for prolonged nystatin release and therefore can be beneficial for use in treatment of fungal infections. In the future, the optimized nystatin microspheres formula will be incorporated in a topical gel that will be evaluated for its possible utilization in the prolongation of nystatin release.

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