Preparation and In vitro Characterization of Aceclofenac Nanosuspension (ACNS) for Enhancement of Percutaneous Absorption using Hydrogel Dosage Form

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Abstract

Aceclofenac (AC) is an orally active phenylacetic acid derivative, non-steroidal anti-inflammatory drug with exceptional anti-inflammatory, analgesic and antipyretic properties. It has low aqueous solubility, leading to slow dissolution and inadequate bioavailability (15%). The aim of the current study was to prepare and characterize AC-NS-based gel to enhance the dissolution rate and then percutaneous permeability. NS.s were prepared using solvent/antisolvent precipitation method at different drug to polymer ratios (1:1, 1:2, and 1:3) using poly vinyl pyrrolidone (PVP-K25), hydroxy propyl methyl cellulose (HPMC-E5) and poloxamer® (388) as stabilizers alone in combinations of two polymers (1:2 and 1:4 drug: polymer ratio). Fifteen formulas of AC-NS.s were prepared and characterized for loading efficiency, particle size, polydispersity index and physical stability. The best formulas of NS were F11 (PVP K25, poloxamer® 338 and AC), and F15 (HPMC E5, poloxamer® 338 and AC) that gave the best results of physical stability and entrapment efficiency which were lyophilized to be characterized by FTIR, DSC, P-XRD and SEM. After that, the best prepared formula of AC-NS regarding the involved characterization methods was incorporated in gel dosage forms using (1% W/V carbopol®9840). From this study, we conclude that the solubility and dissolution rate of AC were improved when the particle size was reduced to Nano-scale as compared with pure drug.

Keywords: Aceclofenac, Nanosuspension, Solvent/antisolvent method, Hydrogel.

Introduction

The word ‘solubility’ is defined as a maximum quantity of solute that can be dissolved in a given amount of solvent. There are various techniques for solubility enhancement like particle size reduction, solid dispersion, use of surfactants, pH modification, complexation, hydrotopy, use of co-solvent and others (1). When the particle becomes smaller, the surface area with volume ratio will be increases.

The large surface area enables greater contact with the solvent which lead to elevate solubility. Nanoparticles are particulate dispersions or solid particles with a size between 10-1000nm (2).

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Nanoparticles stabilization is crucial to ensure their effectiveness. The massive rise in surface area causes excessive surface energy, which is undesirable thermodynamically. The increase in surface energy accelerates particle agglomeration to reduce over-energy on the surface. Agglomeration affects over all stability of the formulation of Nanoparticles. Polymers like Poloxamers® (338), HPMC (E5), PVP (K25) and others provide stabilization for nanoparticles by surface binding as well as through the steric bulk of their three-dimensional structure.

AC is a non-steroidal anti-inflammatory drug, it is a phenyl acetic acid derivative (figure-1), a white crystalline solid, almost insoluble in water and soluble in ethanol (96%). After oral administration AC is absorbed and undergoes first pass hepatic metabolism. It is recommended for the short and long term therapy of the signs and symptoms of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis.

Figure 1. Chemical structure of Aceclofenac

Hydrogel is a water-swollen, cross-linked polymeric network formed by the simple reaction of one or more monomers.

The main objective of this study is to modify AC particles to be prepared as NS using solvent/anti-solvent precipitation method and then incorporated in gel forming NS-based hydrogel type with improved dissolution rate and dermatological permeation.

Materials and Methods

Materials

Aceclofenac powder, poloxamer® (338) were purchased from Lishui Nanning Chemical Co., Ltd (China). PVP®-K25, HPMC-E5 and carbopol® 940 are gifts from Sama Alfayhaa for Pharmaceutical Co., Ltd (China). PVP® (338) were purchased from Lishui Nanming Materials and Chemical Co., Ltd (China). PVP® (K25), HPMC (E5), PVP (K25) and others provide stabilization for nanoparticles by surface binding as well as through the steric bulk of their three-dimensional structure.

Methods

Determination of melting point

Melting point was determined by digital melting point apparatus. A few quantity of AC sample was taken and placed in a thin walled capillary tube which placed and heated in the device, when the sample start to melt, the melting point was recorded.

Spectrophotometric analysis

AC powder (50mg) was weighed and dissolved in (50 ml) of each of ethanol, phosphate buffer (pH 7.4) and DW (in presence of ethanol 1% (V/V) as co-solvent) to get stock solutions with concentrations (1mg/ml). From which, serial dilutions were made and inspected spectrophotometrically. The λ<sub>max</sub> values were determined and matched with literatures values. The absorbance values of resultant diluted solutions were measured by UV-visible spectrophotometer using the respective blank solvents.

Determination of saturation solubility

Excess amounts of AC powder were put in (25ml-conical flasks) containing (20ml) of each of the involved media as reported by Mauvi et al. After that, the conical flasks were shaken with orbital shaking incubator at 37± 0.1°C for 24 hours, then kept in the incubator at 37°C for 24hours until equilibrium existed, the supernatant solution filtered through a 0.45μm filter paper and analyzed spectrophotometrically, three determinations were carried out.

Preparation of AC-Nanosuspensions

Solvent/anti-solvent precipitation process was used to prepare AC-NS. (100mg) of AC powder was dissolved in 2ml of ethanol, then added by syringe dropping into (50ml) of D.W. containing fixed quantities of one stabilizer or combined-stabilizers at room temperature and subsequently stirred by using magnetic stirrer at (800rpm) for (1hour) at (40°C) to allow volatile solvents to evaporate. The compositions of the prepared formulas (1-15) were illustrated in Table (1).

Evaluation of the prepared AC-NS.s

Measurement of particle size and polydispersity index (PDI)

Analysis of particle size was done using Malvern Mastersizer 2000 MS (Worcestershire, Great Britain). Average particle size and PDI of the prepared NS formulas were observed (The results of particle size was recorded as an average value depending on the device setting) to ensure that the particles are within Nano-range size and PDI is acceptable.

Measurement of % entrainment efficiency (%EE)

Freshly prepared drug-loaded NS.s were centrifuged at 12500 rpm for 20 minutes. The concentration of drug in the supernatant was spectrophotometrically determined after filtration through 0.45μm filter paper at the estimated λ<sub>max</sub>. The EE of AC was calculated as follows:

\[
\% \text{EE} = \frac{\text{Wt initial drug} - \text{Wt free drug}}{\text{Wt initial drug}} \times 100
\]
Table 1. Compositions of AC NSs formulas using different stabilizers at different drug: stabilizer ratios with constant volume of injected organic solution (2 ml).

| Formula code | Aceclofenac amount (mg) | PVP K25 Amount (mg) | HPMC (E5) Amount (mg) | Poloxamer (338) Amount (mg) |
|--------------|-------------------------|---------------------|-----------------------|-----------------------------|
| F1           | 100                     | 100                 |                       |                             |
| F2           | 100                     | 200                 |                       |                             |
| F3           | 100                     | 300                 |                       |                             |
| F4           | 100                     |                      | 100                   |                             |
| F5           | 100                     |                      | 200                   |                             |
| F6           | 100                     |                      | 300                   |                             |
| F7           | 100                     |                      |                       | 100                         |
| F8           | 100                     |                      |                       | 200                         |
| F9           | 100                     |                      |                       | 300                         |
| F10          | 100                     |                      | 100                   |                             |
| F11          | 100                     |                      | 200                   |                             |
| F12          | 100                     | 100                 | 100                   |                             |
| F13          | 100                     | 200                 |                       | 200                         |

Physical stability assessment
The prepared NS formulas were stored in a dark place at an ambient temperature for up to seven days. During this time, the particle size and visual appearance were monitored and determined (15).

Lyophilization of the prepared NS formulas
For further characterization, the best formulas were lyophilized using freeze dryer (Labconco USA). The powder yields were kept in a tight container at room temperature (16).

Characterization of the lyophilized powder
Scanning electron microscopy (SEM) analysis
The particle morphology of lyophilized powder and unprocessed drug were characterized by using SEM. A small fraction was fixed on a double-sided conductive carbon tape and sputter-coated with 5 nm of a Pt–Pd alloy (17).

Compatibility studies
Differential scanning calorimetry (DSC)
This test was used to examine the physical compatibility between AC, additives and methods conditions. The samples were precisely weighed and sealed hermetically with aluminum lid. The thermograms of lyophilized AC formulas and pure drug powder were recorded (18).

Fourier transform infrared spectroscopy (FTIR)
FTIR scanning of KBr pellets containing powder samples of pure drug, lyophilized formulas in the wave number range 400–4000 cm⁻¹ at a resolution of 4 cm⁻¹ with speed of 2 mm/sec (19).

Powder x-ray diffraction (PXRD)
Using a Diano X-ray diffractometer (USA) equipped with Co–Kα radiation (45 kV, 9 mA, scanned from 3° to 50° at 2 angles), samples from pure drug and lyophilized formulas powders were analyzed (20).

Preparation of NS-based hydrogel
Carbopol®940 was used as gelling agent in concentration of (1%) to prepare NS-based hydrogel from the selected AC-NS formula. The calculated amount of carbopol was dispersed in water using a magnetic stirrer with a speed of (1000 rpm) for (1 hour) until getting uniform dispersion, then a freshly prepared AC-NS formula (the selected one) was gradually added to the aqueous dispersion of carbopol. After that (1.6) ml glycerin was added as a viscosity modifier. Finally, few drops of triethanolamine were added to initiate the hydrogel formation (21). The prepared hydrogel had been allowed to stand overnight to clear stuck air, then the prepared hydrogel formula was sealed in a tightly closed container at room temperature in a dark place for other tests (22). Additionally, by the same procedure, plain hydrogel was prepared using AC pure form.

Evaluation of the prepared hydrogel
Measurement of pH
The pH of the prepared hydrogel formula was determined using a pH meter at room temperature. This was done by fully placing the glass electrode in the gel system and recording pH (23).

Spreadability
Hydrogel formulas were found to be spreadable or not after (48 hour) of preparation, by measuring 2 g of gel spreading-diameter between two (10X10 cm) glass plates. Determination of diameter after 1 minute of applying the weight. The spreadability can be calculated using the following equation:
\[ S = \frac{m.l}{t} \quad \ldots \quad (2) \]

Where: S is spreadability, m is the weight tied to the upper slide, l is the length of the glass slide and t is time consumed (24).

**Rheological studies (Viscosity analysis)**

They were done by Drawell NDJ-8S viscometer. The hydrogel was filled in a wide mouth container, so that the viscometer’s spindle could be appropriately dipped without touching the bottom of the jar. Hydrogel samples were permitted to settle at constant temperature more than 30 min. The viscosity was measured by increasing the speed of rotation starting from 0.3 rpm to 60 rpm. This done by using a spindle (number 4). The measurements were recorded at temperature of 25°C (25).

**In vitro drug release study**

The NS-based hydrogel release analysis was performed by using assembly franz diffusion cell using a tube in which one end covered by dialysis membrane (8000-14000 Dalton cut-off) that is soaked in a freshly prepared diffusion medium (phosphate buffer pH 7.4) over night. A specific amount of hydrogel equivalent to (100 mg) of AC was taken and spread on a semi-permeable dialysis membrane, the tube was partially submerged in a jar containing buffer. The temperature was held at 32±0.5°C stirred at 100 rpm. Two milliliters-sample was taken at different time intervals and the drug concentration was spectrophotometrically determined at the determined λ_{max} against a suitable blank (27).

The obtained data were fitted to different mathematical expressions to describe the kinetic and mechanism of aceclofenac release from the selected nanosuspension formulas with the help of DDSolver: An add-in program in Microsoft Excel (28). The kinetic models (zero order, first order, Higuchi model and Korsmeyer – Peppas) were used (29). The model that produced the highest correlation coefficient was selected as the best fitted model.

**Stability studies (Effect of temperature)**

For the assessment of the stability, the prepared hydrogel formula was kept at different temperature degrees: a refrigerator (4°C), oven (40°C) and room temperature (25°C) for three months. Samples were periodically withdrawn and tested for the physical appearance, homogeneity and viscosity (30).

**Statistical analysis**

The results of particle size was recorded as an average value depending on the device setting, while others have been mostly provided as the mean value of triplicate readings ± standard deviation and have been assayed statistically using (ANOVA) test for estimation if changes of involved variables have been statistically significant at level (P ≤ 0.05) and non-significant at level of (p>0.05).

**Results and Discussion**

**Determination of melting point**

It was found to be (156-158 °C) which is the same as reported reflecting the purity of the powder used in the study (31).

**Spectrophotometric analysis**

The estimated λ_{max} is 275nm that is agreed with the reported values (32). Figures (2-A, 2-B and 2-C) showed the calibration curves in the involved media. A straight lines were obtained indicating that the calibration curves obeys Beer-lambert law within the range of concentrations used (33).

![Figure 2. Calibration curves of AC in ethanol, phosphate buffer pH 7.4 and D.W.](image1)

**Saturated solubility of AC powder**

The saturation solubility values of AC at different media were summarized in Table (2). From which, we conclude that AC is practically insoluble.
in water and soluble in ethanol. The aqueous solubility will be increased by increasing pH due to weakly acidic properties of drug as in phosphate buffer medium pH 7.4.

Table 2. Solubility of AC at 37± 0.1 °C in different media (n=3)

| Media               | Saturation solubility (mg/ml) ± SD |
|---------------------|-----------------------------------|
| D.W                | 0.07 ± 0.01                        |
| Phosphate buffer 7.4| 14.2 ± 0.04                        |
| Ethanol            | 40 ± 0.02                          |

Preparation of AC-NS

In this study, fifteen formulas of AC-NSs were prepared having particle size values within nanometer range as illustrated in Tables (3 and 4) which are exposed for several characterization methods to get the best one.

Table 3. Particle size, polydispersity index values of the prepared formulas using different types and amount of single stabilizer

| Formula code | Average particle size (nm) | PDI |
|--------------|---------------------------|-----|
| F1           | 270                       | 0.3 |
| F2           | 202                       | 0.3 |
| F3           | 164                       | 0.3 |
| F4           | 198                       | 0.09|
| F5           | 271                       | 0.07|
| F6           | 220                       | 0.07|
| F7           | 98                        | 0.3 |
| F8           | 112                       | 0.3 |
| F9           | 85                        | 0.3 |

Table 4. Particle size and PDI values for the prepared formulas (using combined stabilizers)

| Formula code | Average Particle size (nm) | PDI |
|--------------|---------------------------|-----|
| F10          | 72                        | 0.09|
| F11          | 43                        | 0.06|
| F12          | 242                       | 0.1 |
| F13          | 237                       | 0.07|
| F14          | 41                        | 0.1 |
| F15          | 45                        | 0.09|

Characterization of the prepared AC-NS formulas

Particle size and PDI measurements

The most important characterization parameters for the prepared NSs are the average particle size and PDI which governs the physicochemical properties like saturation solubility, dissolution velocity and physical stability. The usual range of PDI obtained values are (0.1-0.25) which indicates narrow size distribution while PDI value more than 0.5 refer to very broad distribution. The PDI values of prepared formulas were ranged from (0.06-0.3). The best formulas (F11 and F15) have Nano-sized particle with low PDI values (0.06 and 0.09) respectively as shown in Table (4) which indicates good uniformity of nanoparticles.

Stabilizers such as HPMC, PVP and poloxamer were incorporated to prevent sedimentation, agglomeration and crystal formation. In addition to the safety and regulatory requirements, the choice of stabilizer is based on their ability to provide particle surface wetting and a barrier to prevent agglomeration of nanoparticles. Formulas (F1-F3) give a good average particle size especially at drug: polymer ratio (1:3) with remarkable increase in average particle size, this may be due to role of PVP in inhibiting guest molecule crystallization and its excellent efficiency as agent for coating and high affinity of polymer to the nanoparticles surface.

Formulas F4-F6, also give a good average particle size with increase in size when changing the concentration of the polymer reaching to drug: polymer ratio (1:3). This is attributed to the fact, HPMC has both a hydrophobic alkyl chain and hydrophilic OH in its side chain. On the Nano crystalline surface, the hydrophobic alkyl chain can be adsorbed, while the hydrophilic OH groups are exposed to increase the wettability of the Nano crystals. Formulas F7-F9 had the best average size, at drug: polymer ratio 1:3, it gave (85 nm) size, this because of its high molecular weight and a greater polypropylene oxide (PPO) region of poloxamer. Therefore, because of adsorption occurs via this hydrophobic PPO block, it has more 'anchoring capacity' to the nanoparticle surface. Optimum stabilizer concentration is needed where the use of insufficient amount of stabilizer may not provide complete coverage of the drug surface, thus jeopardizing steric repulsion between particles.

In our study, we see that as the concentration of stabilizer increases, the average particle size was decreased as seen in Table (3) in which the formulas coded (F3 and F9) give smaller particle sizes (164 and 85nm) respectively at which highest amount of polymers use. According to the above result a smaller particle size (85nm) was seen when using poloxamer 338 in higher amount, since this polymer show reduction in average particle size because of the high molecular weight of polymer.

Smaller average particle size values were obtained for F11 about (43 nm) and F15 about (45 nm).
nm) when using combination of (PVP K25 + Poloxamer 338) and (HPMC + Poloxamer 338) respectively at ratio of (1 drug: 4 polymer) as shown in Table (4). So these combinations showed a significant reduction in the average particle size (p<0.05). Also these formulas (F11 and F15) have PDI values (0.06 and 0.09) respectively which indicates a good polydispersity. These results may be obtained because of an excellent surface affinity of stabilizers toward drug molecules forming a large mechanical and thermodynamic barrier at the interface (43). The ratio of drug to polymer (1:4) looked to be optimum due to the effect of amount of polymer on the properties of the nanoparticles. The reason may be due to the fact, the viscous polymer solution has more difficulty to break up into smaller droplets at the same mixing input strength, leading to a rise in particle size (44).

**Determination of %EE**

The %EE values of the prepared formulas were ranged from 86.3(F9) to 94.2 % (F15) as showed in Table (5). There is no significant difference (p>0.05) between the prepared AC-NS.s which may give an impression of suitability of stabilizer mechanism (steric stabilization) especially when used in combination that give synergistic effect for stabilization of AC nanoparticles(44). It is clear that the increase in stabilizer concentration can increase the %EE, but the study revealed that the concentration of stabilizers at ratio (1 drug: 4 stabilizer) was sufficient to give the optimized %EE. This may be due to the presence of optimum stabilizer type and optimum stabilizer concentration (45).

**Table 5. EE values of the prepared formulas (n=3)**

| Formula code | EE% ± SD |
|--------------|----------|
| F1           | 90.2 ± 0.06 |
| F2           | 92.4 ± 0.03 |
| F3           | 92.6 ± 0.04 |
| F4           | 87.6 ± 0.01 |
| F5           | 90 ± 0.1 |
| F6           | 92.3 ± 0.11 |
| F7           | 86.3 ± 0.1 |
| F8           | 90.2 ± 0.08 |
| F9           | 93.5 ± 0.06 |
| F10          | 89.4 ± 0.005 |
| F11          | 92.7 ± 0.01 |
| F12          | 86.4 ± 0.01 |
| F13          | 93.5 ± 0.17 |
| F14          | 91.2 ± 0.02 |
| F15          | 97.5 ± 0.05 |

**Physical stability of AC-NS**

The prepared formulas were tested for their physical stabilities for one week at ambient temperature. Formulas F11 and F15 were seemed to be clear after (1 weak) as seen in Table(6). Other formulas look to be precipitated due to increase particle size. The improved stability may be due to the increased thickness of layer of adsorbed polymer when Poloxamer 338, HPMC E5 and PVP K25 were used in combinations, thus preventing the particles from aggregation and/or agglomeration by providing steric hindrance. NS stabilization can be done by electrostatic, steric, or electrostatic modulation (a combination of them) (46).

**Table 6. Physical stability study for F11 and F15 formulas**

| Measurement time | F11 (Particle size in nm) | F15 (Particle size in nm) |
|------------------|---------------------------|---------------------------|
| Initial time     | 43                        | 45                        |
| 2nd day          | 50                        | 54                        |
| 7th day          | 67                        | 80.2                      |

**Characterization of the lyophilized powder SEM**

The morphological analysis and particle size values of (F11 and F15) in compare to AC row powder were performed by SEM showing nanoparticles with a size of (43 and 45nm) as seen in figure (5). No aggregation of particles could be observed.
DSC

DSC thermogram of pure AC powder (figure 6-A) showed a sharp characteristic endothermic peak at (156.30 °C) that is agreed with references. This gives an indication that the drug has crystalline nature with high purity. The DSC thermograms of the lyophilized formulas (figure 6-F11) was showed shifting of the peak while figure (6-F15) showed loss of the melting endotherm which may be due to presence of small quantity of aceclofenac. Changing in the peak was reflecting the changing in the crystalline state of aceclofenac to amorphous [47, 48].

Figure 6. DSC thermograms of pure AC powder, F11 and F15.
**FTIR spectra**

The characteristics absorption bands of AC were: 3318 cm⁻¹ (N–H stretching or O–H stretching), 3277 and 2936 cm⁻¹ (C–H stretching) due to both aromatic and aliphatic stretching vibrations respectively in addition to 1771 cm⁻¹ (–C=O stretching), 1585 and 1506 cm⁻¹ (–C=C stretching of aromatic), and some prominent bands as explained in figure (7).

The spectrum of lyophilized powder showed the same characteristic peaks, albeit of smaller intensity as seen in figure (7). The results showed no major differences in the peaks of AC relative to the pure drug in the prepared formulas, suggesting the absence of any interaction between drug and polymers. A broad peak may be attributed to hydrogen bond between drug and polymer (49).

![FTIR spectra](image_url)

**Figure 7.** FTIR spectra for pure AC powder, F11 and F15.
**PXRD**

PXRD assays were done for pure drug and the lyophilized formulas as showed in figure (8). The PXRD patterns of AC pure drug showed a sharp diffraction peaks indicating the crystalline nature of the drug as a consequence of different arrangement of the molecules in the crystal lattice.

In the lyophilized formulas, lack of distinctive drug peaks or a significant reduction in the characteristic peaks indicating that AC was trapped in an amorphous or molecular shape within the mixture, this is agreed with the results obtained from the DSC assay (50).

![Intensity vs. 20 degree for AC](image1)

![Intensity vs. 20 degree for F11](image2)

![Intensity vs. 20 degree for F15](image3)

**Preparation of hydrogel**

F15 was selected to be incorporated in hydrogel system. For comparison, blank hydrogel containing unprocessed AC powder was also prepared.

**Characterization of NS based-hydrogel**

**Physical appearance**

Visual inspection of the prepared AC-NS based-hydrogel revealing a good homogeneity, free of grittiness and no phase separation. The gel of F15 with carbopol concentration (1%) was appeared to be white in appearance.

**pH determination**

The pH values of the prepared AC hydrogels were (6.83 to 6.87) as seen in Table (7).

| Formula number | spreadability (g.cm²/sec)± SD | PH ± SD       |
|----------------|-------------------------------|---------------|
| F15            | 14.3±0.01                     | 6.83±0.02     |
| blank          | 10.6±0.015                    | 6.87±0.025    |

**Spread-ability measurement**

The prepared hydrogel was easily spreadable by applying little shear as seen in Table (7). Good spread-ability value is one of the essential properties for gel dosage form. Spread-ability suggests gel propagation capability to a part of the skin. The therapeutic efficiency of gel determined according to spreading value (51, 52).

**Determination of the viscosity**

The viscosity was measured at different shear rates and the data are represented in Table (8). It can be seen in (figure-9) that the viscosity of hydrogel of F15 was ranged (180456-11306 cp), while for the blank hydrogel is (168435-10524cp). It was found that as the shear rate increased as the viscosity was decreased. Therefore, it is a non-Newtonian flow behavior (53). The results showed that carbopol was a good gelling agent for preparation of hydrogels.

![Intensity vs. 20 degree for F15](image4)

Figure 8. PXRD graphs for AC powder, F11 and F15
Table 8. Viscosity values for the prepared AC hydrogels

| Speed (rpm) | shear rate (1/sec.) | Gelled F15 viscosity (cp) | Blank hydrogel viscosity (cp) |
|-------------|---------------------|---------------------------|----------------------------|
| 1.5         | 0.32                | 178456                    | 168435                     |
| 2           | 0.41                | 140786                    | 138576                     |
| 2.5         | 0.49                | 99800                     | 95723                      |
| 3           | 0.6                 | 83350                     | 80836                      |
| 6           | 1.32                | 60566                     | 58243                      |
| 12          | 2.48                | 48270                     | 46387                      |
| 30          | 6.4                 | 24546                     | 27534                      |
| 60          | 13.16               | 13785                     | 10524                      |

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In vitro drug release study

In vitro drug release study was done in phosphate buffer (pH 7.4). Figure (10) showed the release pattern of drug from the prepared hydrogels. At 10 hour period of the release assay, the cumulative percentages of drug release were in the order: gel of F15 > plain hydrogel with 84.8% and 32.6% respectively. At 12 hr., the release of gel F15 reached to 100%, while plain hydrogel had the lowest release percentage, as we see there was a significant difference (p<0.05) between F15 hydrogel and plain hydrogel, this is due to improved dissolution rate and permeation due to small size of nanoparticles, i.e. the reduction in particle size leading to increased dissolution velocity. As drug particles are smaller, the corresponding surface area is greater, according to the Noyes-Whitney equation, so the nanocrystals have a significant elevation area. In addition, the diffusion distance decreases for very small particles. The increased surface area and the simultaneous decrease in diffusion distance could therefore significantly increase the velocity of dissolution of the substance (54).

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Figure 9. Viscosity versus shear rate for gelled F15 and the blank hydrogel.

Figure 10. The cumulative release of AC for gelled F15 and blank hydrogel.

The release of AC from hydrogel of F15 is best fit with higher correlation (R²) with zero order equation which show zero order release profile. While the blank hydrogel (R² = 0.969) fitted with first order kinetics. It is worth mentioning that the diffusional exponent (n) obtained from Korsmeyer-Peppas can be utilized by calculating 60% release of the drug. When the liquid diffusion rate is slower than the relaxation rate of the polymeric chains, the diffusion is Fickian or called case–I transport (n ≤ 0.5). When liquid diffusion rate and polymer relaxation rate are of the same order of magnitude, non-Fickian diffusion or anomalous transport (0.5 < n < 1) is observed. When liquid diffusion rate and polymer relaxation rate are of the same time but diffusion rate more than relaxation rate are, super case–II transport (n > 1.0) is observed. While the relaxation process is very slow compared with the diffusion, the case – II transport occurs (Zero order kinetic model) (29).

The value of (n) for hydrogel of F15 (1%) was (0.822) and blank hydrogel was (0.873) that both of them were > 0.5 and < 1 which indicates that the drug release mechanism was non Fickian, and suppose that aceclofenac hydrogel delivered their
active ingredients by non Fickian diffusion (see Table 9).

Table 9. In vitro release kinetics of AC hydrogel formulas

| Formula code | zero order R² | first order R² | higuchi R² | kosmeyrer-peppas (n) |
|--------------|--------------|----------------|------------|---------------------|
| 15           | 0.974        | 0.943          | 0.917      | 0.822               |
| blank        | 0.951        | 0.969          | 0.88       | 0.873               |

Stability test
The results of stability test were illustrated in Table (10). The drug content was reduced at elevated temperature and this can be diminish by storage in refrigerator (55).

Table 10. Stability tests of gelled F15 (n=3)

| Stability parameter | 25 °C | 40 °C | 4 °C |
|---------------------|-------|-------|------|
| appearance          |        |       |      |
| homogenous          | 6.83±0.02 | 6.57±0.05 | 6.62±0.03 |
| pH ± SD             |        |       |      |
| Drug content ± SD   | 97.5±0.05 | 96.43±0.0 | 97.5±0.01 |

Conclusions
AC-NS.s have been successfully prepared using various stabilizers in different stabilizer: drugs ratios (1:1, 2:1, 3:1 and 4:1). The data indicate that the process of solvent/anti solvent precipitation is an efficient and a cost-effective method for preparing drug NS.s, and easily to implement for the manufacturing of drug nanoparticles. The results confirm that when AC particles are Nano sized, both the solubility and dissolution are improved. AC-NS based hydrogel showed enhanced release rate of drug as compared with blank hydrogel.

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