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Development and characterization of acemetacin nanosuspension-based oral lyophilisates

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Received: 20 May 2024 / Revised: 8 June 2024 / Accepted: 13 June 2024

ABSTRACT: Acemetacin is a non-steroidal anti-inflammatory drug (NSAIDs) which has an analgesic, antipyretic, and anti-inflammatory effect. The aim of the present study was to develop oral lyophilisates containing acemetacin nanosuspensions. The primary goal was to improve tablet disintegration in the mouth, make swallowing easier, and potentially improve patient compliance. Furthermore, formulating acemetacin as a nanosuspension was intended to improve dose-to-dose proportionality compared to using the drug in its naked form. The solvent-anti-solvent technique was used to develop the nanosuspension, and Soluplus® was added to stabilize acemetacin nanoparticles. The nanosuspension was concentrated using a rotary evaporator before other excipients were added. The excipients utilized were gelatin, polyvinyl pyrrolidone K90, glycine, and mannitol. They were dissolved in the concentrated nanosuspension, poured into tablet blister molds, and lyophilized to create acemetacin nanosuspension-based oral lyophilisates. The experiments were developed *via* a computer-based approach using Design Expert® software. For this purpose, the Box-Behnken design was used to study the effect of different formulation factors on tablet disintegration time and friability values. The selected formula F9 had the highest desirability value (0.913), and its disintegration time and friability values were 26.6 seconds and 0.938%, respectively. The dose-to-dose proportionality has substantially improved, and more than 85% of the formula F9 was released in only 8 minutes compared to the naked acemetacin-based oral lyophilisates, which only gave 24.5% in this time frame. Compatibility studies showed there was no chemical interaction between the tablet components.

KEYWORDS: acemetacin; nanoparticles; oral lyophilisates; Soluplus; solvent-anti-solvent.

1. INTRODUCTION

Orodispersible tablets (ODTs) are uncoated tablets that rapidly disintegrate in the mouth with saliva without the need for water. The ODT's fast disintegration rate may increase the absorption rate of drugs and facilitate a faster onset of action. Additionally, because many people have trouble swallowing ordinary tablets, especially children, the elderly, and patients with mental illnesses, incorporating the drug into ODTs could improve patient compliance. Numerous common techniques to prepare orodispersible tablets, such as molding, compaction, spray drying, and freeze drying [1,2]. In the freeze-drying method, a suspension or solution containing the drug and excipients is completely frozen and then transferred to the lyophilizer to be freeze-dried. The resulting tablets are known as oral lyophilisates (OLs) [3]. The primary advantage of oral lyophilisates is their great porosity, which facilitates fast salivary penetration and short disintegration times. Freeze-drying is an effective technique for heat-labile substances, and the resulting tablets have a pleasant mouthfeel. On the other hand, the freeze-drying method has many drawbacks, like a costly and time-consuming production process. It also produces tablets that require special packaging techniques because of their brittleness, low stability, and humidity sensitivity [4].

Drugs must meet a number of requirements in order to be manufactured as oral lyophilisates. Firstly, the dose of water-insoluble drugs should not exceed 400 mg to maintain the porous structure that is required for the fast disintegration of the manufactured tablet. On the other hand, the amount of water-soluble drugs should be less than 60 mg, as they have the potential to generate an amorphous, glassy solid during the freezing stage, which could lead to the dissolution of the tablet's supporting structure during the sublimation process. Additionally, the particle size of the drugs should be less than 50 µm to avoid

How to cite this article: Al-Gharani H, Al-Kinani K. Development and characterization of acemetacin nanosuspension-based oral lyophilisates. J Res Pharm 2025; 29(2): 626-638.

ISSN: 2630-6344

https://doi.org/10.12991/jrespharm.1664881

Journal of Research in Pharmacy
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sedimentation during manufacturing and the formation of a gritty texture. Also, the drug needs to be stable at room temperature for a whole day in order to store the drug solution or suspension before being poured into the blister sockets. Finally, the drug should have an acceptable taste [5]. Nanotechnology reduces drug particle size to 1–1000 nanometers (much smaller than 50 micrometers) [6]. In order to avoid drug sedimentation from the drug-excipient mixture, acemetacin can be formulated as a nanosuspension before being mixed with other excipients. Acemetacin (ACM) is a non-steroidal anti-inflammatory drug (NSAID). It is a glycolic ester of indomethacin, and it is converted into indomethacin in vivo. Due to its prostaglandin inhibitory activity, ACM has analgesic, antipyretic, and anti-inflammatory effects. The pharmacological activity is due to both the prodrug (acemetacin) and its major metabolite (indomethacin). Acemetacin has been indicated to treat osteoarthritis, rheumatoid arthritis, toothache, lower back pain, acute gout, dysmenorrhea, and postoperative pain [7]. Acemetacin is a yellow crystalline powder with a molecular weight of 415.8 g/mol. It is practically insoluble in water. Furthermore, it is light-sensitive and needs to be protected from light to maintain its optimal stability [8]. This study aimed to develop acemetacin nanosuspensions as oral lyophilisates. The primary goal was to improve tablet disintegration in the mouth, facilitate easier swallowing, and potentially enhance patient compliance. Additionally, the researchers aimed to achieve faster disintegration times for OLs, thereby accelerating the onset of acemetacin's therapeutic action. Furthermore, formulating acemetacin as a nanosuspension was intended to improve dose-to-dose proportionality during the lyophilisates production compared to using the drug in its naked form.

2. RESULTS

2.1. Particle size and polydispersity index of the prepared acemetacin nanosuspension before and after rotary evaporation

The acemetacin nanosuspension's particle size and PDI values were 55.86 nm and 0.1098, respectively. The particle size and PDI values after rotary evaporation were 84.39 nm and 0.07213, respectively. Every value fall within the acceptable ranges for pharmaceutical use. (particle size < 200 nm, and PDI < 0.2) [9,10].

2.2. Scanning electron microscopy of acemetacin and acemetacin-lyophilized nanosuspension

The scanning electron microscope (SEM) pictures of acemetacin and acemetacin-lyophilized nanosuspension are shown in Figure 1. Figure 1A shows the crystalline structure of acemetacin, which resembles a tetragonal prism with a particle size larger than 50 micrometers., Figure 1B shows the SEM of ACM lyophilized nanosuspension, which appeared as smaller, flakey particles in the nano-size range.

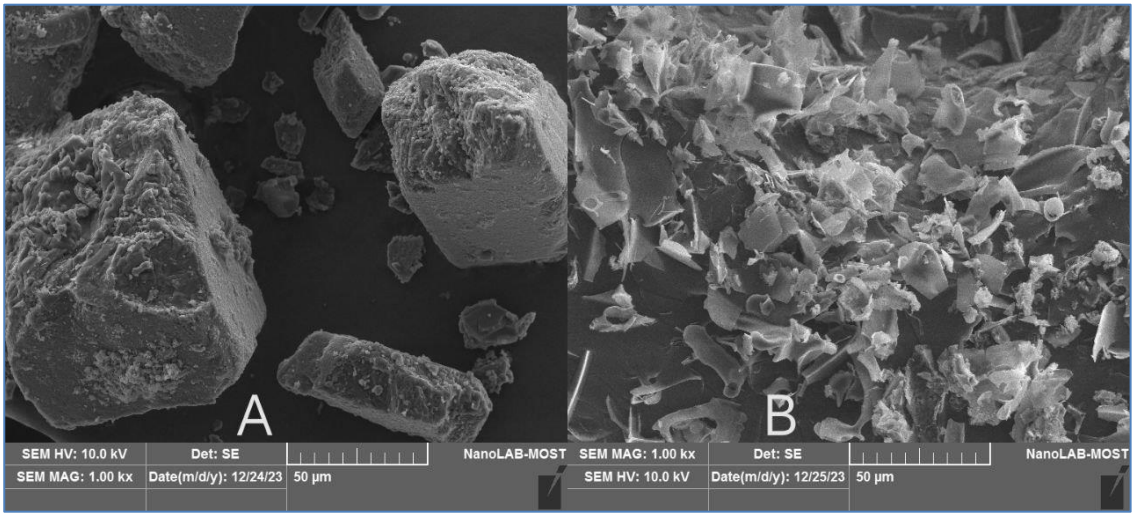

Figure 1. SEM of (A) acemetacin (MAG:1.00 kx), (B) acemetacin-lyophilized nanosuspension (MAG: 1.00 kx**).**

2.3. Mean disintegration time and friability

The mean disintegration times (D.T.) of the fifteen ACM nanosuspension-based OLs formulas, which were made with varying percentages of gelatin, polyvinyl pyrrolidone K90 (PVP K90), and glycine, range from 26.6 to 585 seconds (Table 1). The United States Pharmacopeia (USP 44) states that the

disintegration time of ODTs is limited to 30 seconds [11]. However, the European Pharmacopeia (Ph. Eur. 10) recommended that it should last no longer than 180 seconds. Additionally, Table 1 displays the friability values (%F), which varied from 0.167 to 1.05. Friability values of 1% and below are frequently considered acceptable for ODT (Ph. Eur. 10). Thus, of the fifteen prepared formulas, fourteen have good friability values [12].

Table 1. Mean Disintegration time and friability results

Formula	Mean disintegration time (seconds)	Friability (%)	Formula	Mean disintegration time (seconds)	Friability (%)
F1	145.1	0.291	F9	26.6	0.938
F2	53	0.472	F10	51.6	0.858
F3	237	0.254	F11	298	0.317
F4	43.6	0.325	F12	439	0.167
F5	55.6	0.412	F13	57.5	0.371
F6	192.16	0.315	F14	46.1	0.312
F7	61.5	1.05	F15	138	0.560
F8	186.1	0.465			

2.3.1. *The effect of gelatin on disintegration time and friability values*

Using the ANOVA test, gelatin has a significant effect (P value < 0.0001) on ACM nanosuspension-based OLs mean disintegration time values. As the gelatin percentage increases, the disintegration time increases, as illustrated in Figure 2. Furthermore, gelatin has a significant effect (P value =0.0001) on ACM nanosuspension-based OLs friability values. As the gelatin percentage increases, the friability decreases, as shown in Figure 2.

2.3.2. The effect of PVP k90 on disintegration time and friability values

Using the ANOVA test, PVP k90 has a significant effect (P value < 0.0001) on ACM nanosuspension-based oral lyophilisate disintegration time values. As the PVP k90 percentage increases, the disintegration time decreases, as illustrated in Figure 2. However, PVP k90 doesn't have a significant effect (P value > 0.05) on the friability values of ACM nanosuspension-based OLs.

2.3.3. The effect of Glycine on disintegration time and friability values

Using the ANOVA test, glycine has a significant effect (P value = 0.0015) on ACM nanosuspension-based OLs disintegration time values. As the glycine percentage increases, the disintegration time decreases, as illustrated in Figure 2. However, glycine doesn't have a significant effect (P value > 0.05) on the friability values of ACM nanosuspension-based OLs.

2.4. Optimization of the acemetacin nanosuspension-based oral lyophilisates formulas

The criteria used in the optimization of ACM nanosuspension-based OLs were a low mean disintegration time (below 180 seconds) and a friability value in the range of 0 to 1%. The formulas with higher desirability values are shown in Table 2.

2.5. Acemetacin nanosuspension-based oral lyophilisates selected formula

The formula F9, which has the highest desirability value (0.913), was determined as the selected formula based on its mean disintegration time value (26.6 seconds) and friability value (0.938%) to be subjected to further characterization.

2.6. Criteria of acemetacin nanosuspension-based oral lyophilisates selected formula (F9)

2.6.1. Visual appearance and scanning electron microscopy

Figure 3A is a photograph of the formula F9, which appeared as slightly yellowish tablets with a uniform shape, no cracks or deformities, and they are easy to press out of the blister. The scanning electron microscope (SEM) of F9, which is displayed in Figure 3B, demonstrates the extremely porous structure of the OLs, which ensures fast water penetration, causing the tablet to quickly disintegrate and dissolve.

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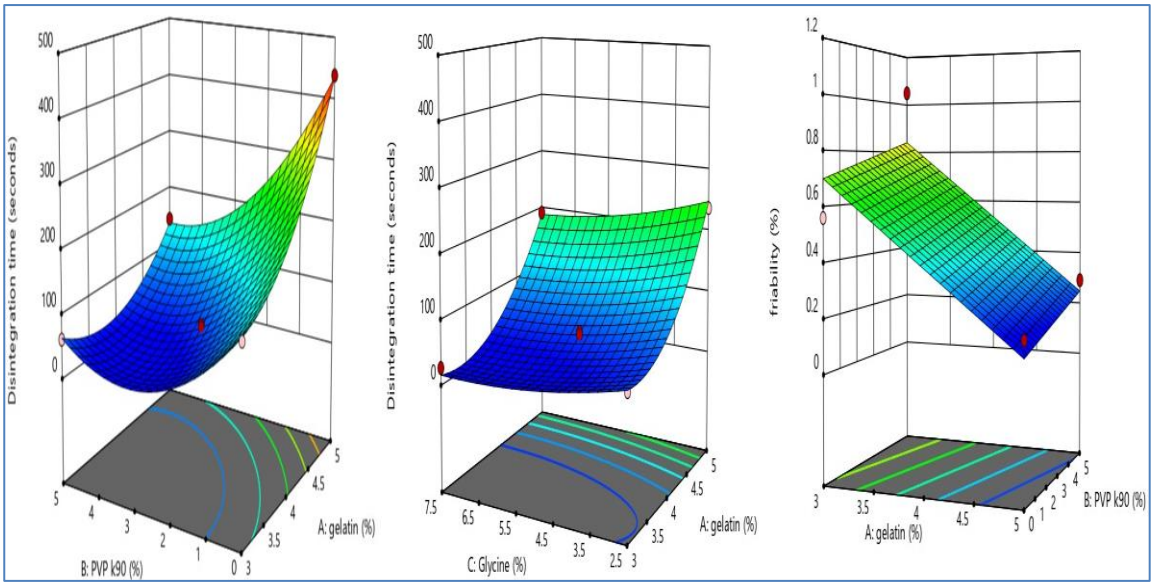

Figure 2. 3D surfaces describe the effect of gelatin, PVP K90, and glycine percentages on the disintegration time and the friability values of acemetacin nanosuspension-based oral lyophilisates formulas.

Table 2. The desirability values of the best oral lyophilisates formulas.

Formula	Desirability	Actual D.T. / Predicted D.T.	Actual %F / Predicted %F
F2, F13, and F14 (central points)	0.710	53,57, and 46 seconds / 52.2 seconds.	0.472%, 0.371%, and 0.312% / 0.4738 %.
F4	0.710	43.6 seconds / 52.17 seconds.	0.325% / 0.5702%.
F5	0.719	55.6 seconds / 50.66 seconds.	0.412% /0.5197%.
F9	0.913	26.6 seconds / 15.64 seconds.	0.938% / 0.7964%.
F10	0.699	51.6 seconds / 54.15 seconds.	0.858% / 0.7459%.

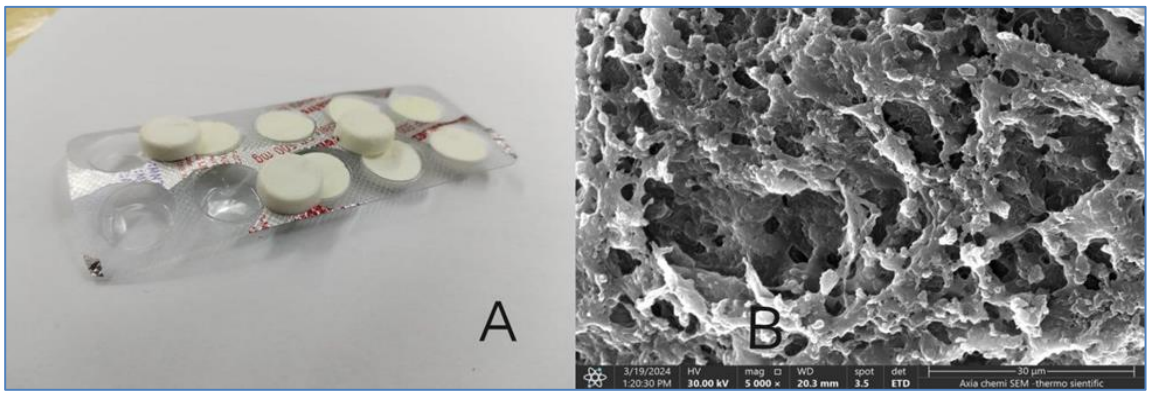

Figure 3. (A) visual appearance of F9 oral lyophilisates, (B) SEM of F9 oral lyophilisates (MAG 5.0 kx).

2.6.2. In-vitro dissolution study

The *in-vitro* dissolution profile of F9 in phosphate buffer of pH 6.8 (Figure 4) reveals that 88.3% of acemetacin was released in eight minutes, compared to the naked ACM OLs, which only gave 24.5% in this time frame. According to the Food and Drug Administration, at least 85% of the active pharmaceutical ingredients in ODTs must be released within 30 minutes [13].

https://doi.org/10.12991/jrespharm.1664881
J Res Pharm 2025; 29(2): 626-638

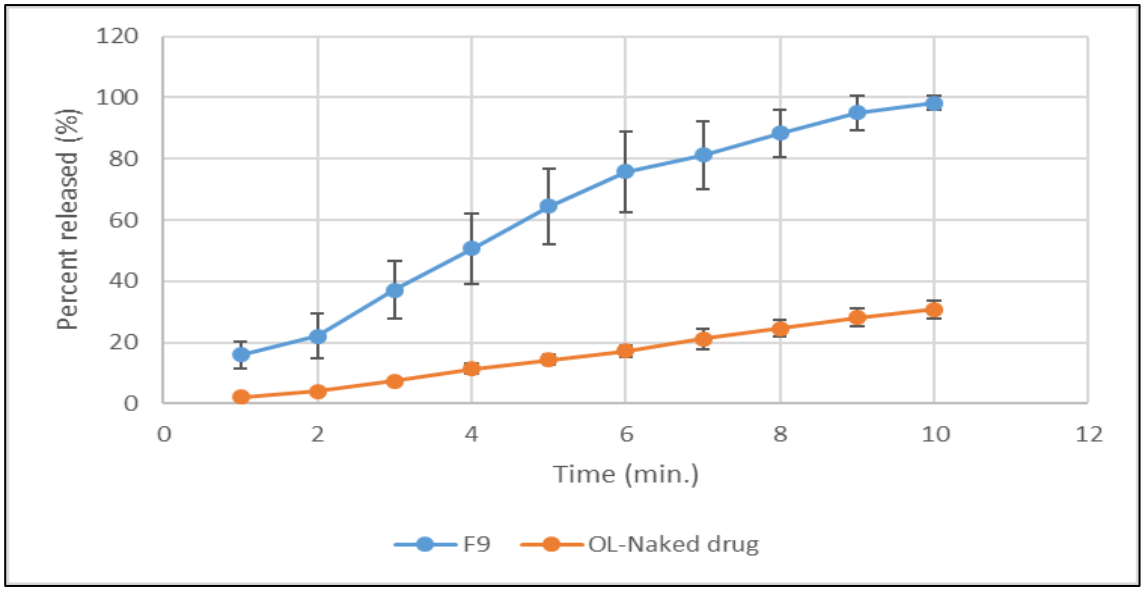

Figure 4. The *in-vitro* dissolution profile of F9 in phosphate buffer of pH 6.8 as compared to the release profile of the naked ACM-based oral lyophilisates**.**

2.6.3. Content uniformity and dose to dose proportionality

The content uniformity results of both naked ACM-based OLs and ACM nanosuspension-based OLs demonstrate a higher improvement in dose-to-dose proportionality in ACM nanosuspension-based OLs (91%–105%) as compared to naked ACM-based OLs (68.3%–124.6%). All ten units of the ACM nanosuspension-based OLs fell within the USP-accepted range (90%–110%) [14], as shown in Table 3.

Table 3. The content uniformity **results** of both naked acemetacin-based oral lyophilisates and acemetacin nanosuspension-based oral lyophilisates.

Tablet	Naked ACM oral lyophilisates		ACM nanosuspension-based oral lyophilisates	
	ACM amount	Content %	ACM amount	Content %
1	20.5 mg	68.3%	28.8 mg	96%
2	24.8 mg	82.6%	28.5 mg	95%
3	27.1 mg	90.3%	31.5 mg	105%
4	26.4 mg	88%	31.2 mg	104%
5	25.1 mg	83.6%	30.5 mg	101%
6	26.6 mg	88.6%	29.1 mg	97%
7	33.9 mg	113%	31.4 mg	104.6%
8	36.5 mg	121%	29.6 mg	98.6%
9	37.4 mg	124.6%	30.9 mg	103%
10	34.6 mg	115.3%	27.3 mg	91%

2.7. Drug-excipients compatibility tests

2.7.1. Fourier transform infrared spectroscopy

The Fourier transform infrared spectroscopy (FTIR) of the naked ACM, ACM lyophilized nanosuspension, F9 physical mixture, and F9 oral lyophilisates formula are shown in Figure 5. The most characteristic peaks of the naked ACM were nearly similar to the reference ACM FTIR spectrum [15].

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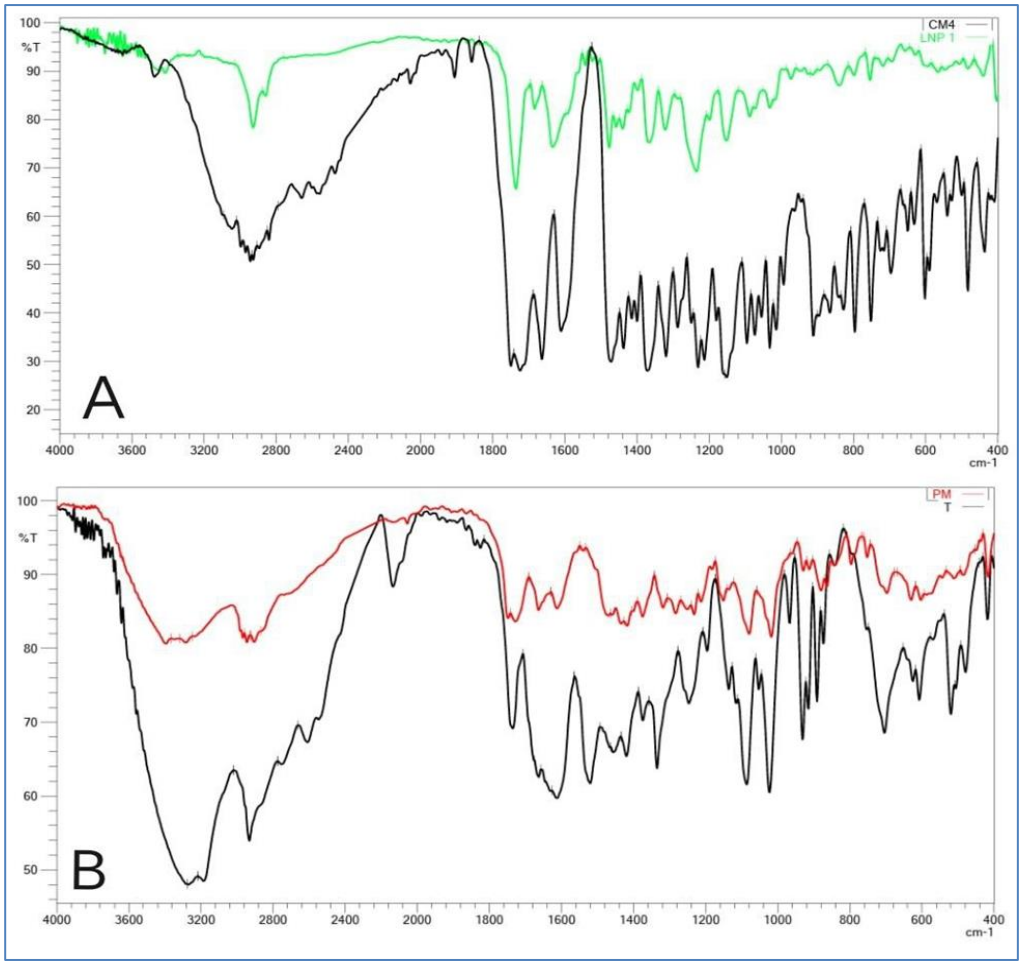

Figure 5. FTIR spectra of (A) naked ACM, and ACM lyophilized nanosuspension (green spectrum) (B) F9 oral lyophilisates formula, and F9 physical mixture (red spectrum).

2.7.2. Differential Scanning Calorimetry

The Differential Scanning Calorimetry (DSC) thermograms of the F9 physical mixture and F9 oral lyophilisates formula are shown in Figure 6.

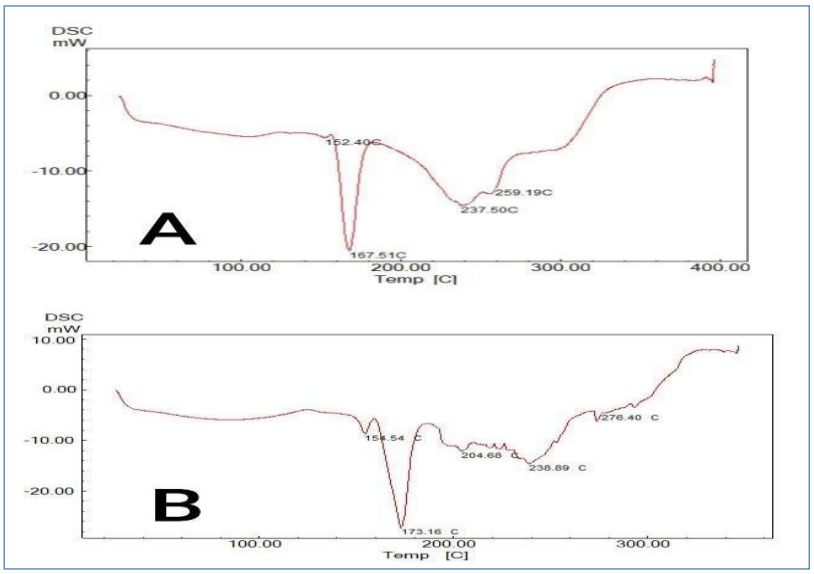

Figure 6. DSC thermograms of (A) F9 Physical mixture and (B) F9 oral lyophilisates formula.

3. DISCUSSION

3.1. The effect of independent variables on disintegration time and friability values of acemetacin nanosuspension-based oral lyophilisates.

According to the results, OLs with higher concentrations of gelatin produce stronger and more compact gels that slowly dissolve in water. Increasing the percentage of gelatin usually causes the oral lyophilisates to become more rigid. This is because more gelatin fibers form crosslinks and H-bonds across chains, increasing the overall hardness of the oral lyophilisates. Conversely, fewer crosslinks between the gelatin strands occur at lower gelatin concentrations, making the tablets more friable [16].

Because of its hydrophilic and complexing characteristics, PVP K90 is used to bind the lyophilisate together during the freeze-drying process and to enhance the solubility, stability, and even the absorption of the active pharmaceutical ingredient. PVP K90 has the ability to hold all ingredients together and ensure faster disintegration and dissolution of the OLs [17].

Due to its polar surface and strong affinity for water, glycine creates aqueous channels, which speed up the diffusion of the dissolving media into the tablet. This improves the tablets' in vivo disintegration and accelerates the rate of dissolution [18].

3.2. Criteria of acemetacin nanosuspension-based oral lyophilisates selected formula (F9)

The hydrophilic characteristics of the excipients account for the rapid rates of disintegration and dissolution of the ACM nanosuspension-based OLs. Additionally, as stated in the Noyes-Whitney equation, the extremely porous structure of the OLs and the nano-size range of the ACM results in a greater surface area that accelerates the rates of disintegration and dissolution. As shown in Figure 4, the F9 oral lyophilisates formula exhibited faster release compared to the naked ACM-based OLs. This is attributed to ACM nanoparticles' larger surface area, which enhances wettability and contact with the dissolution medium, as per the Noyes-Whitney equation [19].

Because smaller particles tend to diffuse more uniformly and precipitate less frequently than larger particles, ACM nanosuspension-based OLs have a far higher dose-to-dose proportionality than naked ACM OLs, which minimizes variations in tablet efficacy. Additionally, the mouth's perception of grittiness can be minimized by decreasing the size of the drug's particles [20,21].

The FTIR spectra of ACM (Figure 5A) showed the carbonyl stretching peaks at 1749.44 cm^{-1} and 1724.36 cm^{-1} which were obscured by the Soluplus® carbonyl stretching peak at 1735.93 cm^{-1} in the ACM nanosuspension IR spectrum (green spectrum). The carbonyl stretching peaks of amide and carboxylic acid at 1662.64 cm^{-1} and 1610.56 cm^{-1}, the hydroxyl stretching peak at 3473.8 cm^{-1}, and the C-O-C ether stretching peak at 1230.58 cm^{-1} were appeared in the ACM nanosuspension IR spectrum, which means there was no chemical interaction between ACM and Soluplus®. In Figure 5B, the IR spectra of both the F9 physical mixture (red spectrum) and the F9 oral lyophilisate formula are nearly the same. All peaks appearing almost in the same positions indicate there were no chemical interactions between all the components of ACM nanosuspension-based oral lyophilisates. The small shift in the peak positions in the formula F9 FTIR spectrum could be due to hydrogen bond formation [22].

The DSC thermogram of the F9 physical mixture (Figure 6A) showed endothermic peaks for the drug and mannitol, respectively, at 152.4 °C and 167.51 °C. The endothermic peaks of mannitol and ACM can also be seen in the thermogram of the F9 oral lyophilisates formula (Figure 6B), appearing at 154.54 °C and 173.16 °C, respectively. These findings suggest that the drug and the excipients utilized in the formulations had no interaction [23].

4. CONCLUSION

The study successfully produced ACM nanosuspension-based oral lyophilisates through the addition of gelatin, PVP K90, glycine, and mannitol. The disintegration time of oral lyophilistaes increased with increasing gelatin percentages. However, it is decreased with increasing PVP K90 and glycine percentages. The friability value of oral lyophilisates decreased with increasing gelatin percentages, and it is not affected by changing PVP K90 and glycine percentages. Formulating acemetacin as a nanosuspension greatly improved the dose-to-dose proportionality compared to using the drug in its naked form. Compatibility studies showed there was no chemical interaction between the tablet components.

5. MATERIALS AND METHODS

5.1. Materials

Acemetacin was obtained from Bidepharm, China. Soluplus® was obtained from BASF SE, Germany. Potassium Dihydrogen Phosphate (KH_2PO_4) and Disodium Hydrogen Phosphate (Na_2HPO_4) were obtained from Panreac, Spain. Ethanol was obtained from BDH Chemical Ltd., England. Gelatin and mannitol were obtained from Thomas Bakers, India. PVP k90 was obtained from Hyperchem, China. Glycine was obtained from Qualikems, Fine Vhem Pvt Ltd., India.

5.2. Methods

5.2.1. Preparation of acemetacin nanosuspension

The solvent-antisolvent method was used to prepare ACM nanosuspension. The organic phase was prepared by dissolving 300mg of ACM in 30 milliliters of ethanol. The aqueous phase was prepared by dissolving 600 mg of Soluplus® in 200 milliliters of distilled water, then stirred on a hot plate magnetic stirrer at 1000 rpm. The organic phase was added drop by drop to the whirling aqueous phase using a syringe pump at a rate of 0.5 milliliter per minute. The suspension was left on the magnetic stirrer for a time that was long enough for the entire organic solvent to evaporate [24].

5.2.2. Preparation of concentrates of the nanosuspension using rotary evaporation

The ACM nanoparticles were dispersed in 200 ml of distilled water, and since the tablet pocket volume in the blister pack is only 0.5 ml, the volume of the ACM nanosuspension dispersion medium needs to be decreased to nearly 5 ml in order to make ten units (one blister) of OLs. The water was allowed to gradually evaporate over four hours using the rotary evaporator (Buchi Rotavapor, Switzerland). The device was initially set up with a pressure of 300 mbar, a temperature of 40 °C, and a rotating speed of 40 rpm. The pressure was lowered from 300 mbar to 150 mbar at a pace of 10 mbar every three minutes to avoid nanosuspension foaming, which might cause the formula to become damaged due to the separation of Soluplus® from ACM nanoparticles. The rotation speed was increased by 10 rpm every 10 minutes from 40 to 70 rpm, and the temperature was raised from 40 °C to 60 °C at a rate of 5 °C every 10 minutes [25].

5.2.3. Measurement of particle size and polydispersity index (PDI) of acemetacin nanosuspension before and after rotary evaporation

The particle size and PDI of the ACM nanosuspension were measured before and after rotary evaporation using the dynamic light scattering method [26]. The particle size analyzer nanolaser (Malvern Zeta sizer), manufactured by Spectris Company in the United Kingdom, was the device used. One milliliter of each sample was poured into a polystyrene cell. The light scattering was recorded at 25 °C with measurement angles of 13°, 90°, and 173°.

5.2.4. Freeze-drying of acemetacin nanosuspension for characterization

The ACM nanosuspension formula was freeze-dried using a Christ Alpha 1-2 LDplus freeze dryer. Initially, liquid nitrogen was used to freeze the nanosuspension formula. Then, the frozen formula was transferred to the freeze dryer to be lyophilized. Ice was sublimated from the frozen formula during the lyophilization process in two stages: primary drying (a pressure of 0.021 millibars at -50°C) and secondary drying (a pressure of 6.1 millibars at 0 °C). The lyophilization process continued until a dry, light, and easily crushed powder was obtained. The dried ACM nanoparticles were utilized in the subsequent experiments [27].

5.2.5. Scanning electron microscopy (SEM) of acemetacin and acemetacin lyophilized nanosuspension

The surface morphologies of both acemetacin and the ACM lyophilized nanosuspension were examined by the scanning electron microscope (Nano-Lab, USA) to study the shape and size of ACM drug particles. When examining the surface morphology of the naked drug and the lyophilized nanosuspension formula, the powder was placed directly on double-sided adhesive carbon tape and coated with gold. Several magnification levels were used to conduct the examination [28].

5.2.6. Preparation of acemetacin nanosuspension-based oral lyophilisates

The acemetacin nanosuspension-based oral lyophilisates formulas, which were designed using a computer-based experimental model as described in the next section, were prepared as highlighted in Figure 7. Initially, the concentrated ACM nanosuspension was mixed with a certain weight-to-volume percentage

(w/v) of gelatin (as a matrix-forming agent). After that, the mixture was placed on a hot plate magnetic stirrer set to 400 rpm at 40 °C until the gelatin completely dissolved. Next, glycine (as a bulking agent and disintegration accelerant) and PVP K90 (as a binder) were added to the mixture in varying percentages. Mannitol was then added as a bulking agent at a 15% (w/v) percentage. The resulting mixture was evenly distributed among the ten tablet pockets in a blister pack, each measuring 13 mm in diameter and 3 mm in depth (0.5 ml in each pocket). The blister was placed inside the freeze dryer after being wrapped in aluminum foil, which was then punctured several times. The frozen tablets were dried for twelve hours at a temperature of -50 °C and a pressure of 0.021 mbar during the primary drying phase. The secondary drying stage of the procedure was then carried out for six hours at 0 °C and a pressure of 6.1 mbar [29].

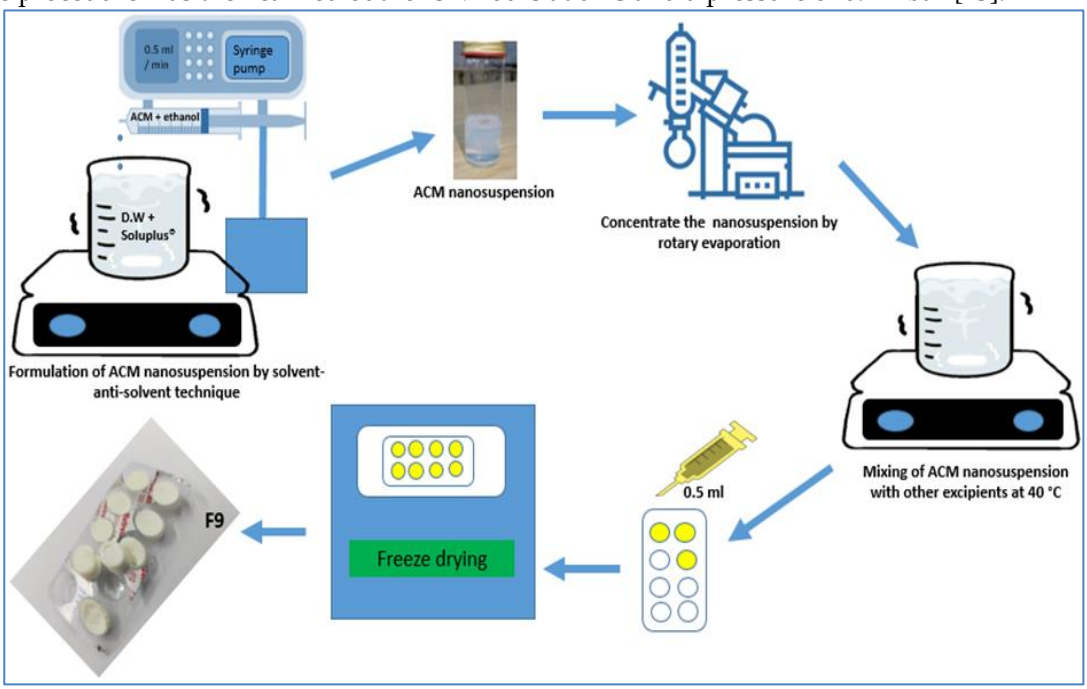

Figure 7. Steps in the production of ACM nanosuspension-based oral lyophilisates.

5.2.7. Computer-based experimental model

The ACM nanosuspension-based OLs formulas were designed using Design-Expert®, a software tailored for scientific experimentation across various models. We utilized the Box-Behnken model aided by preliminary empirical formulation. The independent variables and responses are shown in Table 4. The Box-Behnken model has suggested fifteen OLs formulas that are illustrated in Table 5.

Table 4. Factors and responses used in the Box-Behnken model of acemetacin nanosuspension-based oral lyophilisates.

Independent variables	Levels	Responses
A: Gelatin percentage.	3%, 4% and 5% (w/v).	1-Mean Disintegration time (seconds). 2-Friability (%).
B: PVP K90 percentage.	0, 2.5 %, and 5% (w/v).	
C: Glycine percentage.	2.5% , 5%, and 7.5% (w/v).	

Table 5. Formulas suggested by the Box-Behnken design of acemetacin nanosuspension-based oral lyophilisates.

Formula	ACM nanosuspension amount (mg), equivalent to 30 mg ACM	A: Gelatin percentage (w/v)[a]	B: PVP k90 Percentage (w/v)[a]	C: Glycine percentage (w/v)[a]	Mannitol [b] percentage (w/v)[a]
F1	90 mg	5%	5%	5%	15%
F2	90 mg	4%	2.5%	5%	15%
F3	90 mg	5%	2.5%	2.5%	15%
F4	90 mg	4%	5%	7.5%	15%
F5	90 mg	4%	5%	2.5%	15%
F6	90 mg	5%	2.5%	7.5%	15%
F7	90 mg	3%	5%	5%	15%
F8	90 mg	4%	0	7.5%	15%
F9	90 mg	3%	2.5	7.5%	15%
F10	90 mg	3%	2.5%	2.5%	15%
F11	90 mg	4%	0	2.5%	15%
F12	90 mg	5%	0	5%	15%
F13	90 mg	4%	2.5%	5%	15%
F14	90 mg	4%	2.5%	5%	15%
F15	90 mg	3%	0	5%	15%

[a] (w/v) percentage of the lyophilisates liquid mixture (5 ml/ blister)
[b] Mannitol is added as a fixed ratio.

5.3. Characterization of the prepared oral lyophilisates formulas

5.3.1. Disintegration test

All acemetacin nanosuspension-based OLs formulas were subjected to the disintegration test in a phosphate buffer of pH 6.8 using the tablet disintegration testing instrument (Pharma Test, Germany). The device consists of six tubes, each with a mesh end where the tablet is placed. Six tablets were tested in each run, and the time it took for each tablet to completely disintegrate in the buffer and fade from the mesh was recorded [30].

5.3.2. Friability test

The ACM nanosuspension-based OLs friability test was performed according to the European Pharmacopoeia (4th edition) requirements. Twenty tablets of each formula were carefully weighed using a sensitive balance (Kern ACS 120-4, Germany) before being put into the friability tester's drum (Erweka type, GmbH, Germany). The tablets were rotated at 25 rpm for four minutes. Then, they were removed, cleaned, and precisely weighed a second time. Friability could be determined by calculating the percentage of weight loss [31].

5.4. Determination of acemetacin nanosuspension-based OLs selected formula

The selected ACM nanosuspension-based OLs formula was determined based on its mean disintegration time and friability values, and this formula is subjected to further testing as shown in the subsequent sections.

5.5. Characterization of acemetacin nanosuspension-based oral lyophilisates selected formula

5.5.1. Visual appearance and scanning electron microscopy

Visual inspection was carried out to detect any physical imperfections, lack of similarity between different units, and signs of structural collapse in the selected ACM nanosuspension-based OLs formula. The

surface morphology was further examined using a scanning electron microscope (Axia ChemiSEM, USA). Before being tested, the tablet was placed on double-sided adhesive carbon tape and coated with gold [32].

5.5.2. In-vitro dissolution study of the selected formula compare with naked ACM-based lyophilisates

The dissolution rates of the selected ACM nanosuspension-based OLs formula and the naked ACM-based lyophilisates (prepared by dispersing thirty milligrams of the naked ACM and the other excipients in five milliliters of distilled water) were measured using the USP type I dissolution apparatus (basket technique). Each tablet was placed into the dissolution basket and submerged in 1000 milliliters of the dissolution medium (phosphate buffer of pH 6.8) at 37 degrees Celsius. After turning on the dissolution device (Copley dissolution 8000, UK), the basket revolved at a speed of 100 rpm. Five milliliters of the sample were taken out and replaced with five milliliters of freshly prepared buffer at different intervals of time (1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 minutes). Spectrophotometric measurements were made at the ACM lambda max (318 nm) to determine the concentration of ACM released into the medium at each time point. Dissolution profiles were obtained by plotting the measured concentrations against time intervals [33]. The profiles were compared based on the percentage of acemetacin released within eight minutes.

5.5.3. Content uniformity and dose-to-dose proportionality

Both the selected ACM nanosuspension-based OLs formula and the naked ACM-based lyophilisates were subjected to the content uniformity test. Ten tablets of each formula were analyzed to determine dose-to-dose proportionality. Each tablet was dropped into a 100-milliliter beaker of ethanol, and a magnetic stirrer was used to agitate it for an hour. After that, one milliliter of the mixture was extracted, filtered through a 0.45-micrometer syringe filter, and analyzed using a UV spectrophotometer [34].

5.6. Drug-excipients compatibility tests

5.6.1. Fourier Transform Infrared study

The Fourier transform infrared spectroscopy spectra of the naked drug, ACM lyophilized nanoparticles, a physical mixture of the selected formula, and the selected ACM nanosuspension-based OLs formula were obtained using a Shimadzu FTIR spectrophotometer, Japan. Each material was mixed with potassium bromide powder (KBr) and compressed into a thin film disc, then the sample was analyzed using infrared radiation at a wavenumber of 4000-400 cm^{-1} [35].

5.6.2. Differential Scanning Calorimetry

The DSC thermograms of the physical mixture of the selected OLs formula, and the selected ACM nanosuspension-based OLs formula were obtained using Shimadzu DSC-60, Japan apparatus. About 5mg of each sample was placed into an aluminum pan, sealed by crimping and heated at a constant heating rate of 10 °C/min. Nitrogen gas was pumped at a flow rate of 20 ml/min to maintain an inert environment [36].

5.7. Statistical analysis

Statistical analysis done using ANOVA by Design-Expert® version 11 software, P values smaller than 0.05 were considered statistically significant whereas values equal to or larger than 0.05 were considered statistically insignificant.)

An analysis model called the quadratic model has been suggested by the software, and it was a significant model (P value < 0.0001) to analyze the mean disintegration time results. The adequate precision value was greater than four (47.106), which means the selected analysis model was effective. Also, the predicted R2 value (0.9914) was in reasonable agreement with the adjusted R2 value (0.9555), as the difference was less than 0.2. In the analysis of the friability values, the linear model was also suggested by the software, and it was a significant model (p-value = 0.0005). The adequate precision value was 9.704, and the predicted R2 value (0.5226) was in reasonable agreement with the adjusted R2 value (0.6944) as the difference was less than 0.2 [37].

Acknowledgements: The University of Baghdad's College of Pharmacy is acknowledged by the authors for providing the resources needed to complete this work.

Author contributions: Concept – H.A., K.A.; Design – H.A., K.A.; Supervision – K.A.; Resources – H.A., K.A.; Materials – H.A.; Data Collection and/or Processing – H.A; Analysis and/or Interpretation – H.A.; Literature Search – H.A.; Writing – H.A., K.A.; Critical Reviews – H.A., K.A.

Conflict of interest statement: The authors declare that there are no conflicts of interest.

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