Research Article

Ciprofloxacin and Celecoxib: A Novel Micronized Formulation Strategy For Potential Amyotrophic Lateral Sclerosis Therapy

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ABSTRACT:

Objective: The objective of this study was to develop novel micronized particles of ciprofloxacin (CFL) and celecoxib (CCB) to improve drug delivery for patients undergoing amyotrophic lateral sclerosis (ALS) therapy.

Methods: Micronized particles were prepared by precipitating the dissolved drug substances through the addition of an antisolvent using a bottom-up technology approach. The developed micronized CFL and CCB particles were characterized for their physical properties, including particle size distribution, polydispersity index (PDI), surface morphology, and crystalline nature. In vitro drug release studies were also performed to evaluate their drug-release characteristics.

Results: The developed micronized particles exhibited a narrow particle-size distribution, with particle sizes below 13 µm. The drug-release studies demonstrated that approximately 96% of CFL and 85% of CCB were released over a period of 12 h. The developed micronized particles exhibited reduced particle size and altered crystallinity compared with the individual drug substances. These characteristics, together with the presence of HPMC, facilitated controlled drug release over an extended period.

Conclusion: The developed micronized CFL and CCB particles demonstrated reduced particle size, a narrow particle-size distribution, and modified crystalline characteristics, resulting in sustained drug release. The developed formulation may therefore provide a potential approach for improving the drug-delivery characteristics of CFL and CCB in ALS therapy.

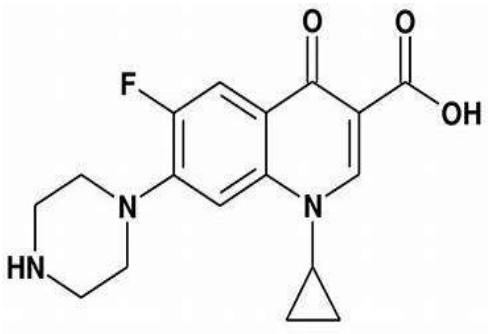
Keywords: Ciprofloxacin; Celecoxib; Micronized particles; Bottom-up technology; Particle size; Controlled drug release; HPMC; Amyotrophic lateral sclerosis.

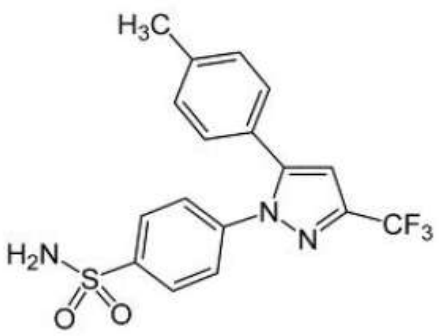
1. INTRODUCTION:

Combination of Ciprofloxacin and Celecoxib (CCB) has been proven to be useful for the treatment of Amyotrophic lateral sclerosis (ALS). ALS is a neurodegenerative disorder characterized by the deterioration of motor neurons thus causing muscle paralysis which ultimately leads to death if untreated [1].

Ciprofloxacin (CFL) (See Figure.1) is a broad-spectrum fluoroquinolone antibiotic widely used for the treatment of various bacterial infections, including urinary tract, gastrointestinal, respiratory, skin, bone, and joint infections. Beyond its established antibacterial activity, ciprofloxacin has attracted interest in its potential relevance to neurodegenerative disorders. Studies have reported that ciprofloxacin can bind to the RNA-binding protein TAR RNA-binding protein (TRBP), facilitating its association with the RISC-loading complex and potentially influencing microRNA processing [2]. Dysregulation of microRNA processing and related pathways has been observed in amyotrophic lateral sclerosis (ALS) and other neurodegenerative diseases [3]. Ciprofloxacin has also been reported to exhibit iron-chelating properties [4]. Since abnormal free iron accumulation and impaired iron homeostasis have been implicated in neurodegenerative disease progression, iron-chelating approaches have been investigated for their potential neuroprotective effects [5]. Celecoxib (CCB) (See Figure.2) acts as pain killer which comes under the class of non-steroidal anti-inflammatory agent acts by inhibiting the CoX-2 enzyme [6]. When given alone Celecoxib is not an ideal drug of choice for ALS treatment, however, when combining with Ciprofloxacin, it exerts augmented therapeutic benefits by improving the cells of central nervous system which is associated with ALS [7].

Figure.1: Chemical Structure of Ciprofloxacin Figure.2: Chemical Structure of Celecoxib





Recent studies show that the combination of Ciprofloxacin and Celecoxib is promisingly effective against ALS [8], however not many marketed formulations are available.

From a biopharmaceutical perspective, ciprofloxacin (CFL) is generally classified as a Biopharmaceutics Classification System (BCS) Class III drug, characterized by relatively high aqueous solubility and low permeability [9], whereas celecoxib (CEL) is a BCS Class II drug with relatively low aqueous solubility and high permeability [10]. Ciprofloxacin is an antibiotic that is used to treat bacterial infections [11]. Ciprofloxacin belongs to the fluoroquinolone class of antibiotics [12]. The combination of these two drugs therefore presents a distinct physicochemical challenge for the development of a fixed-dose formulation, particularly because the poor aqueous solubility of celecoxib can limit its dissolution and consequently influence its drug-release and absorption characteristics. In contrast, ciprofloxacin exhibits pH-dependent solubility and is considerably more soluble under acidic conditions, which may result in rapid dissolution following oral administration. Such differences in the dissolution characteristics of CFL and CCB may complicate the development of a formulation capable of providing a more consistent and appropriately controlled drug-release profile as required by patients with ALS. Therefore, formulation strategies capable of modulating the dissolution and release characteristics of both drugs are of particular interest.

Micronisation or particle-size reduction using suitable stabilizers, such as hydroxypropyl methylcellulose (HPMC), polyvinylpyrrolidone K30 (PVP K30), sodium lauryl sulfate (SLS), and poloxamer 407, may increase effective surface area, improve dissolution behavior, and facilitate modulation of drug release. Accordingly, the present study aims to develop a novel nanoprecipitation-mediated micronisation approach for CFL and CCB using appropriate stabilizers, with the objective of optimizing particle size, invitro drug-release characteristics and thereby developing a formulation with improved and more consistent drug-delivery performance for further investigation in the context of ALS.

2.0MATERIALS AND METHODS:

2.1. Chemicals and Reagents: The Ciprofloxacin and Celecoxib were procured from the local vendor and other chemicals used for the preparation of Nanosuspension such as Ethanol and acetone were procured from Sigma - Aldrich, A polymeric stabilizer such as Hydroxy propyl methyl cellulose (HPMC), Poly vinyl pyrrolidone K30 (PVP K30) & Poloxamer 407 and the Surfactant namely, Sodium lauryl sulfate (SLS), were also procured from the local vendor.

2.1. Nanoprecipitation Mediated Micronisation

Nanoprecipitation (which is also called as bottom-up technology) method was adopted for the preparation of microparticles, since it is a simple conventional method and was first developed by List and Sucker in 1987 [13]. By this method the nanosized particles were achieved by precipitating the dissolved substances with the addition of another insoluble solvents. However, in the present study, micronized particles have been proposed to develop to minimize the limitations of mechanical processes. Ciprofloxacin 800mg and Celecoxib 400mg were dissolved in 16ml of organic phase (8 parts of ethanol and 2 parts of Acetone) and 1200mg of stabilizer was dissolved in 80mL of distilled Water (aqueous phase), with the help of 20ml syringe the organic phase was slowly and gradually poured into the aqueous phase at 1ml/min ensuring no organic phase adhere to the wall of the glass container and

the whole mixture was stirred constantly at ambient room temperature for about 24h ensuring no organic phase adhere to the wall of the glass container.

2.1. Formulation Optimization:

Optimization of better formulation was achieved by obtaining better micronized particles with proper stability; therefore, different concentrations of different stabilizers were tested using same amount of active ingredients such as CFL and CCB. The main aim of screening the stabilizer is to obtain different micronized particles with proper stability. Therefore, four different stabilizers were selected for the present study namely, HPMC, PVP K30, SLS and Poloxamer 407. Concertation of stabilizers plays a vital role in the formation of stable particles. The quantity of active ingredients kept constant, but varied amounts of stabilizers were used See Table.1.

Table.1: Several varied amounts of drug and stabilizers used for the formulation.

S.No	Quantity of active Ingredients (g) (0.8g CFL: 0.4g CCB)	Quantity of Stabilizers used (g)	Drug and stabilizer ratio
1	1.2	0.6	1:0.50
2	1.2	0.9	1:0.75
3	1.2	1.2	1:1
4	1.2	1.5	1:1.25
5	1.2	1.8	1:1.50

2.1. Characterization of Micronized Particles of Ciprofloxacin and Celecoxib

2.1.1. Microscopy: Longer physical stability of prepared micronized particles was chosen for the Particle characterization such as particle size, nature of particles and surface morphology studies using Confocal Microscope and a digital picture of the surface morphology was taken for the dried powder ensuring no traceable quantity of solvents.

2.2.2. Particle size and Polydispersity index: The mean particle size and size distribution of the prepared micronized particles were measured by Malvern Particle size analyzer. Approximately 50-100 mg of the samples taken into the test tube and dispersed by adding about 2 mL of silicone oil to form a smooth paste without agglomeration of particles, further added about 20 mL of dispersion medium, mixed the solution using glass rod for uniform dispersion of particles. The dispersed solution was then transferred into Hydro 2000 S, until the desired obscuration reaches (10%-20%), measured the particle size distribution in triplicates.

2.4.3. PXRD:

Polymorphism was evaluated for CFL, CCB and the prepared micronized particles CFL and CCB was measured using powder X-Ray diffraction technique, Panalytical X'pert powder instrument. Enough representative sample was taken and filled and packed in the zero-background holder, prepared smooth surface using glass slide. Exposed the sample holder under the X-Ray, collected the diffraction pattern for the sample.

2.5. In-Vitro Drug Release Studies.

2.5.1 Preparation of Dissolution Medium (pH 6.8 Phosphate Buffer Preparation with 0.5% SLS):

Accurately weighs about 40.8 g of Sodium Dihydrogen Phosphate, 5 g of sodium hydroxide and 30 g of Sodium Lauryl Sulfate into a suitable container containing 6000-mL of water and mix well. Adjust the pH to 7.4 ± 0.05 with 1N sodium hydroxide solution (4 g in 100-mL of water).

2.5.2. Sample Preparation

Poured the prepared micronized particles of CFL and CCB into hard gelatin capsules Size #0 and the dissolution were run into individual six dissolution vessels (USP Distek Dissolution apparatus, Type II Paddle) containing 750ml of 0.1N HCl as dissolution medium followed by adjustment of the dissolution medium to pH 7.4. with 0.5%SLS for about 12h with intermittent time points.

Replace the media volume by adding 10-mL into a respective all the six (6) dissolution vessels and carefully withdraw 10 mL of the sample solution with specified time intervals and the collected solution was then filtered through a 0.45 µm Nylon filter by discarding the first 6-mL of the filtrate. Throughout the dissolution run the temperature was maintained at 37°C±0.5°C. The collected samples were then analyzed using RP-HPLC method.

3.0 RESULTS AND DISCUSSION:

3.1. Formulation optimization of Micronized Particles of CFL and CCB.

Several mechanical methods such as dry milling, wet milling, and high-pressure homogenization techniques are available for the particle size reduction, however, all the mechanical processes of reducing particle size have major limitations which includes, increased polydispersity index cluster of particles, particle agglomeration and reversible conversion of crystallization. To obtain a stable, non-agglomerated and low polydispersity index particles, the stabilizer drug ratio was optimized using various stabilizers and the selected stabilizer and drug for further evaluation.

3.1.1. Stabilizer optimization

To obtain a most suitable stabilizer for the preparation of micronized particles of CFL and CCB, best stabilizer drug ratio has been selected. The main objective of the optimization of stabilizer is to obtain lower particle size, longer stability, maintenance of amorphous nature throughout the shelf life and to decreases the factors that causes the agglomeration of particles by reducing the electrostatic forces between the particles, thus enhancing the powder flow by decreasing the cohesion of particle surface and equipment surface. Also, it reduces the chances of poor powder mixing. By considering above, we selected the most suitable stabilizer and the best stabilizer drug ratio See

Table.2 for Various Stabilizer drug ratio used in this study.

Table.2.: Various Stabilizer drug ratio used in this study:

Formulation Code	Name of the Stabilizer	Physical Stability Under 25°C/60%RH (Initial)	Physical Stability Under 25°C/60%RH (30 Days)
F1	HPMC	Stable	Stable
F2	SLS	Stable	Stable
F3	PVP K30	Stable	Unstable
F4	Poloxamer 407	Stable	Unstable

The quantity of stabilizers was then optimized based on their physical stability (See Figure.1 for the image of stable micronized particles of CFL and CCB). Based on the physical stability of the stabilizer which were seen visually after 30 days at 25°C/60% RH, we selected HPMC as most suitable stabilizer in the ratio of 1:1.25, therefore, evaluated for further investigation. Furthermore, HPMC is a water-soluble, non-ionic cellulose-based polymer widely used as pharmaceutical applications due to its versatile biodegradable nature, solubility enhancer and in a solid form of HPMC exhibits longer stability and durability, cost-effective and sustainable medium.

Figure.1: **Image of stable micronized particles of CFL and CCB using HPMC as stabilizer:**



3.2. Size, PDI, Surface Morphology and Polymorphism:

Initial particle size distribution of Ciprofloxacin (CFL) and Celecoxib (CCB) (before micronisation) were determined individually using Malvern Mastersizer 2000 by 'Laser light diffraction technique'. Wet dispersion method used for

particle dispersion. The reported average particle size distribution for the individual CFL, CCB and Micronized CFL and CCB were presented in Table.3 and Figures.2, 3 and 4.

Table.3: Particle Size Results of individual CFL and CCB

S.No	Sample	Particle size results in µm			
		D (v,0.10)	D(v,0.5)	D(v,0.9)	Span
1	Ciprofloxacin (CFL)	108.4	197.4	369.8	1.324
2	Celecoxib (CCB)	101.7	149.7	223.2	0.812
3	Monolithic matrix of HPMC comprises CFL and CCB	6.115	8.821	12.565	0.731

Figure.2. Particle size graph of non-micronized CCB

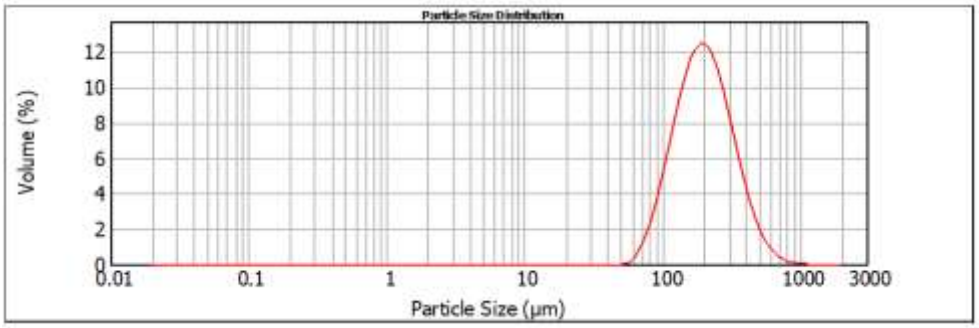


Figure.3. Particle size graph of non-micronized CFL

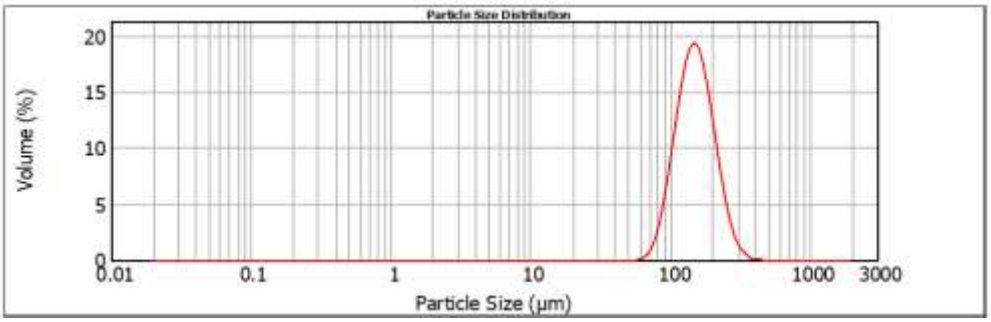
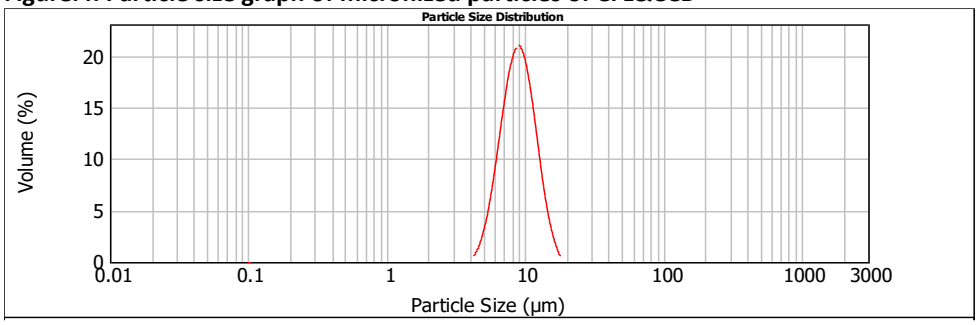


Figure.4. Particle size graph of micronized particles of CFL&CCB

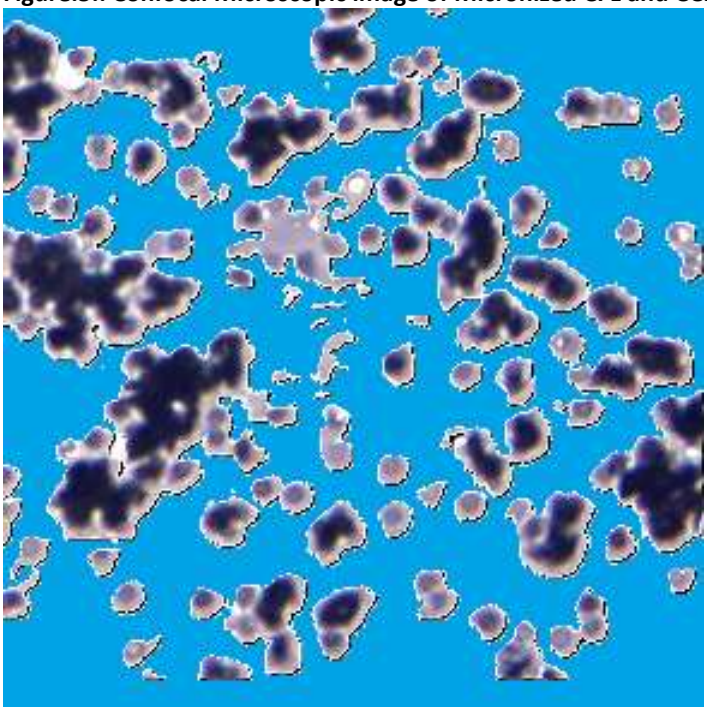


The particle size distribution results obtained using the Malvern particle size analyzer for CFL, CCB, and the micronized samples were evaluated. The results showed that 90% of the particles by volume were below approximately 370 µm for CFL and 230 µm for CCB. Following the micronisation of the CFL and CCB particles using the nanoprecipitation technique, a significant reduction in particle size was observed, with 90% of the particles being below 13 µm.

The microscopy images of the micronized samples were also evaluated, and most of the particles were observed to be less than 10 µm in size. The particle size results obtained from the Malvern particle size analyzer and

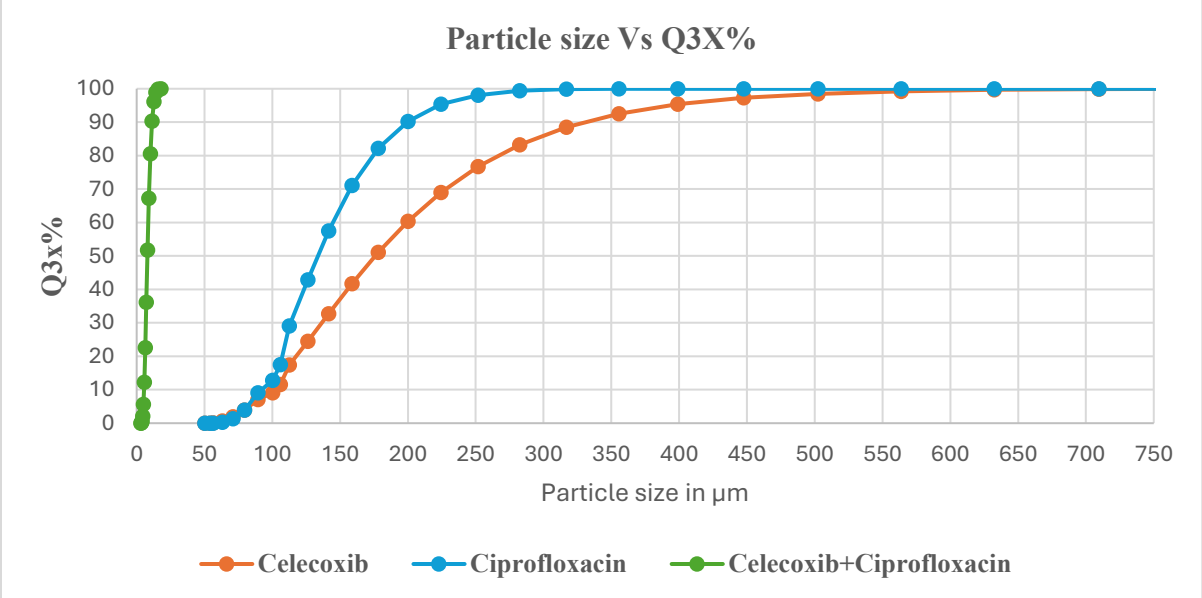
microscopic image were well correlated (Figure.5). Therefore, the Malvern particle size analysis confirmed that 90% of the micronized particles were below 13 µm.

Figure.5.: Confocal Microscopic image of Micronized CFL and CCB.



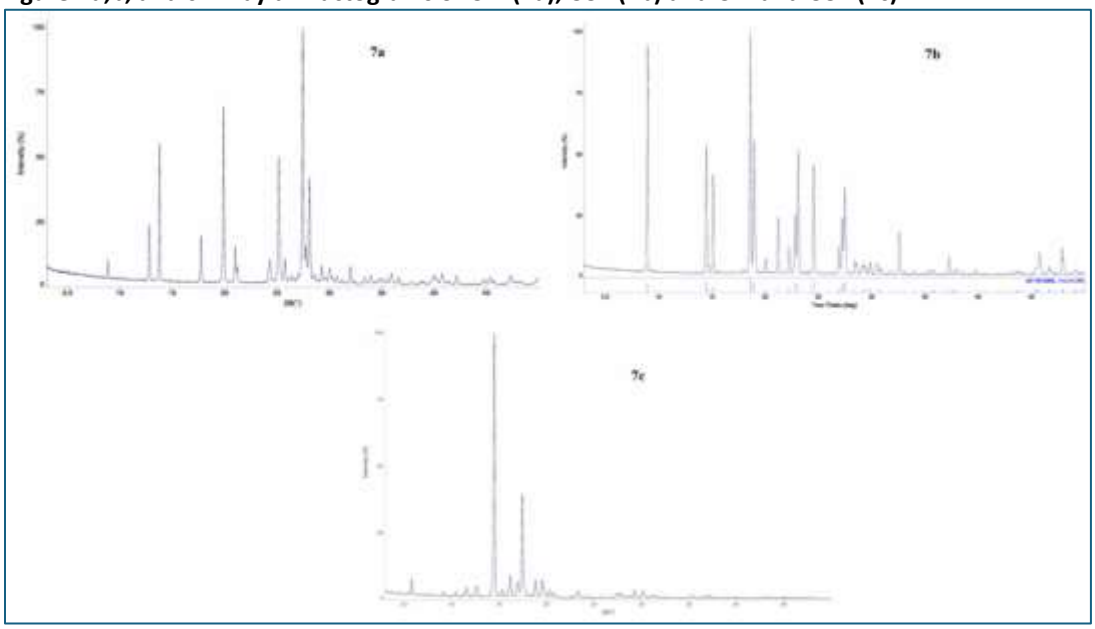
For a better comparison of the particle size distributions, a graph was plotted with particle size (µm) on the X-axis and cumulative undersize (%) on the Y-axis (Figure.6). The plot showed a wider particle size distribution for CFL and CCB, whereas a narrower particle size distribution was observed for the micronized CFL & CCB particles. The reduction in particle size and the narrower particle size distribution is expected to increase the specific surface area of the particles, which may enhance the dissolution rate compared with the initial particle size distributions of CFL and CCB.

Figure.6. illustrates the particle size distribution comparison chart



Polymorphic study of the micronized CFL and CCB were evaluated using X-Ray diffraction pattern by comparing with the individual particles of CFL and CCB. See Figures 7a for CFL diffractogram, Figure7b for CCB diffractogram and Figure7C for micronized CFL and CCB.

Figure.7a,b, and c: X-ray diffractograms of CFL (7a), CCB (7b) and CFL and CCB (7c)



Based on that evaluation, X-Ray diffractograms of CFL and CCB were observed to be crystalline nature. The XRD analysis of the micronized particles demonstrated a reduction in the intensity and sharpness of the characteristic crystalline peaks compared with the respective initial drug materials. This indicates a partial reduction in crystallinity accompanied by an increase in the amorphous fraction of the micronized particles of CFL and CCB.

3.3. In-Vitro Drug Release Studies

The in vitro drug release studies of individual CFL and CCB were performed using 0.1 N HCl as the dissolution medium for the initial 2 h, followed by adjustment of the dissolution medium to pH 7.4 with SLS. The in vitro drug release results are presented in Table 3.

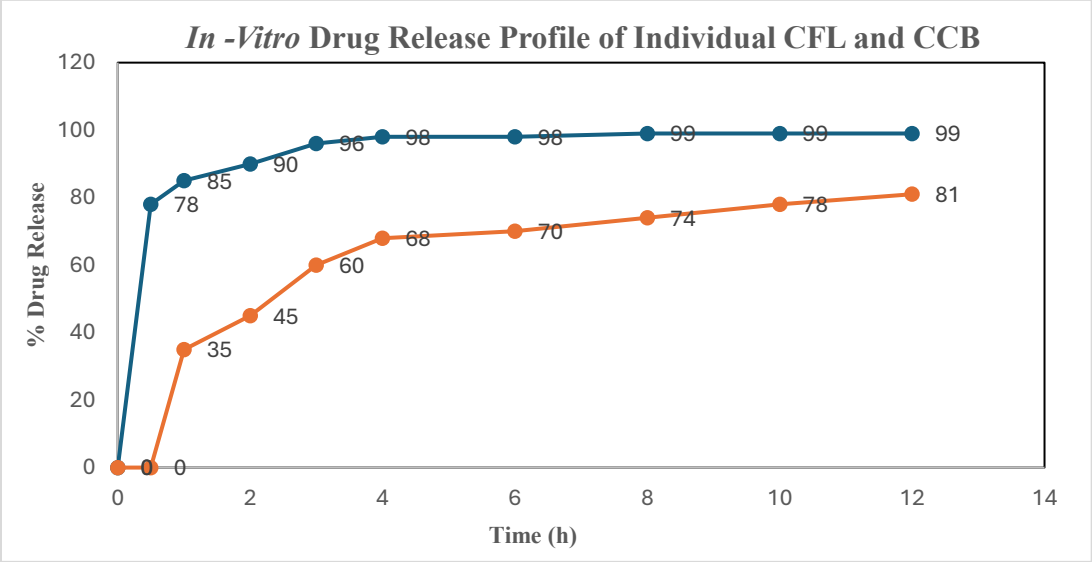
Table.3: Drug release profiles of individual CFL, CCB and Micronized CFL and CCB

Time (h)	Individual %CFL	%RSD	Individual %CCB	%RSD	%CFL in Micronized Particles	%RSD	%CCB in Micronized Particles	%RSD
0.0	0	0	0	0	0	0	0	0
0.5	78	13.5	0	0	8	18.0	4	15.0
1.0	85	10.0	35	18.5	12	15.0	5	12.0
2.0	90	8.5	45	14.2	18	12.0	10	11.0
3.0	96	4.2	60	10.0	24	10.0	17	9.2
4.0	98	3.1	68	7.6	36	9.4	31	9.1
6.0	98	2.8	70	6.5	54	5.5	44	5.6
8.0	99	2.2	74	5.2	70	4.9	55	4.5
10.0	99	2.0	78	5.1	84	4.5	66	4.2
12.0	99	1.8	81	4.6	96	3.8	85	3.4

Approximately 80% of CFL was released within 0.5 h, followed by a maximum release of approximately 85% at the end of 2 h. The drug release gradually increased thereafter, reaching approximately 99% at 12 h. In contrast, CCB exhibited a markedly different drug-release profile. Because CCB is highly insoluble in aqueous media, its initial

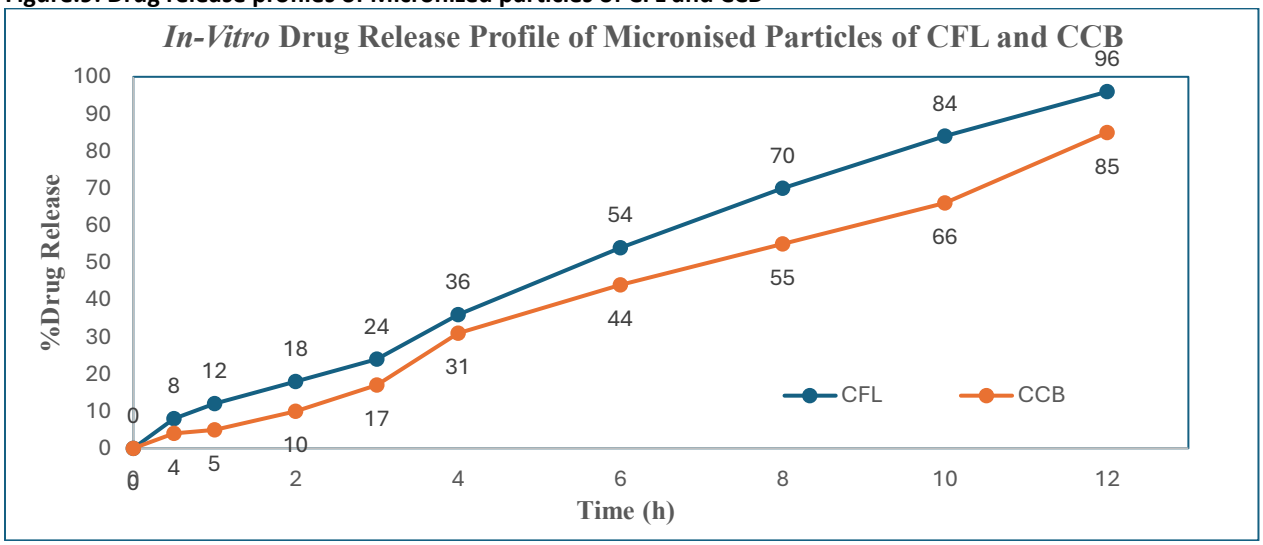
drug release was considerably slower than that of CFL, which can be attributed to its inherent physicochemical properties. At the end of 12 h, the cumulative drug release of CCB was approximately 81% (Figure 8).
The rapid release of CFL and the comparatively slow and inconsistent release of CCB from the individual drug preparations may not provide an optimal and consistent drug-release profile for maintaining the desired drug exposure in patients with ALS.

Figure.8: Drug release profiles of individual CFL and CCB



In contrast, the micronized particles of CFL and CCB exhibited distinctly different drug-release profiles. At the end of 2 h, the cumulative drug release from the micronized particles of CFL and CCB was approximately 18% and 10%, respectively. Compared with the individual drug preparations, the micronized particles exhibited substantially slower drug release during the initial 2 h in 0.1 N HCl, despite the high aqueous solubility of CFL. This behavior can be attributed to the presence of HPMC, which was used as a stabilizer during the preparation of the micronized particles and also contributes to sustained drug release. Thus, the presence of HPMC effectively retarded the release of both CFL and CCB compared with their respective individual drug preparations. The drug-release profiles of the micronized CFL and CCB particles are presented in Figure 9.

Figure.9: Drug release profiles of Micronized particles of CFL and CCB



Following the change to pH 7.4 dissolution medium, the micronized particles of CFL and CCB exhibited slow, consistent, and sustained drug release throughout the 2–12 h period. The sustained drug-release behavior may be

associated with the reduced crystallinity of the micronized particles, as evidenced by the PXRD diffractograms, as well as the predominantly amorphous characteristics observed in microscopic images. Furthermore, HPMC, which was used as a stabilizer during the preparation of the micronized particles, can also function as a sustained-release polymer and contribute to the controlled release of both drugs at pH 7.4.

Compared with the individual CFL and CCB preparations, the micronized particles demonstrated a more controlled, consistent, and sustained drug-release profile over the 12-h period. This release behavior may provide a more suitable drug-release profile for maintaining consistent drug exposure and may therefore be advantageous for the intended application in ALS therapy.

4.0. CONCLUSIONS:

Overall, it was concluded that the developed micronized particles of Ciprofloxacin (CFL) and Celecoxib (CCB) were successfully characterized for their particle size distribution and polydispersity index, confirming the formation of particles with sizes below 13 µm. The physical characteristics and surface morphology of the developed micronized particles indicated a reduction in crystallinity compared with the individual CFL and CCB particles, which was further supported by microscopic imaging and PXRD analysis. Furthermore, the in vitro drug-release profiles of the micronized CFL and CCB demonstrated a slow, consistent, and sustained release pattern over the study period. These findings suggest that the developed micronized particles may have potential for providing controlled drug release and could be further investigated as a drug-delivery approach for ALS therapy.

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6.0. CONFLICT OF INTEREST:

The authors declare no conflict of interest.

7.0. REFERENCES:

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