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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF NIFUROXAZIDE AND ASCORBIC ACID EUTECTIC MIXTURE

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ABSTRACT

This study investigated the formation of a eutectic mixture comprising an active pharmaceutical ingredient (API), an oral antibiotic, and ascorbic acid as a coformer. The aim was to evaluate the nature of the resulting mixture and to explore strategies for enhancing its dissolution rate, ultimately improving bioavailability. The eutectic mixture was synthesized using the neat grinding method in an agate mortar, chosen for its ability to facilitate molecular interactions without the need for solvents. Characterization of the mixture was performed using powder X-ray diffraction (PXRD) to analyze crystallographic changes, infrared spectroscopy (IR) to detect functional group interactions, and nuclear magnetic resonance (NMR) to assess molecular-level interactions. Additionally, differential scanning calorimetry (DSC) was employed for thermal analysis to confirm the thermal behavior and stability of the mixture. To study its biological activity a test was made against Escherichia Coli, Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans.

Key words: Nifuroxazide, Ascorbic acid, eutectic mixture, DSC.

1. INTRODUCTION

Nifuroxazide (4-Hydroxy-N-[(E)-(5-nitrofuran-2-yl) methylene]-benzohydrazide) an oral nitrofuran antibiotic use both in human and veterinary medicine since 1966, active against the majority of causative agents of intestinal infections, Gram-positive and Gram-negative, it has been used successfully for many decades for the treatment of infectious colitis and diarrhea. It can be associated with other drugs for the treatment for other diseases like constipation with Plantago ovata mucilage [1-3].

Nifuroxazide is characterized by its poor water solubility, which restricts its absorption into the bloodstream and limits its therapeutic action predominantly to the gastrointestinal tract. While this property is advantageous for targeting intestinal infections, it significantly reduces the drug's systemic bioavailability and broader therapeutic potential. To overcome this limitation, cocrystallization with a suitable coformer has emerged as a promising strategy [2].

Cocrystallization involves the formation of a multi-component crystal where the active pharmaceutical ingredient (API) is combined with a coformer that interacts through non-covalent bonds, such as hydrogen bonding which modifies its original physicochemical and biological properties. This modification can enhance the dissolution rate of the drug, thereby improving its bioavailability and potentially expanding its clinical applications [4-9].

When APIs have no complementarity in the functional groups and the formation of homosynthons, solid solutions, eutectic solids or simply a physical mixture (PM) can be obtained. Eutectics can be defined according to IUPAC Gold Book: An isothermal, reversible reaction between two (or more) solid phases during the heating of a system, as a result of which a single liquid phase is produced [10]. And according to Encyclopedia Britannica: Relating to or denoting a mixture of substances that melts and freezes at a single temperature that is lower than the melting points of the separate constituents or of any other mixture of them [11-15].

Coformers are chosen based on their ability to interact with the drug through non-covalent bonds such as hydrogen bonds, π-π interactions, or van der Waals force sand including the presence of complementary functional groups, such as hydroxyl, carboxyl, or amine groups, which facilitate strong molecular interactions. Additionally, coformers must be non-toxic, pharmaceutically acceptable, and capable of improving the drug's solubility, stability, or dissolution rate, such as ascorbic acid, succinic acid, and nicotinamide [4,6,7,16,17].

This work studies a eutectic mixture of nifuroxazide with ascorbic acid, showing that the resulted compound is characterized by its low fusion point, synthetized by neat method. The eutectic mixture is characterized by IR, DSC, PXRD, and NMR.

2. EXPERIMENTAL

a. Materials and reagents

Nifuroxazide and ascorbic acid were purchases from an Algerian pharmaceutical industrial group, and the solvent is an analytical reagent grade without further purification.

b. Spectral and thermal measurement

FT-IR spectra were measured by NICOLET model 6700 Fourier transform infrared spectrometer coupled to a NICOLET CONTINUμM microscope in a wave number range between 600-4000 cm $^{-1}$ using a KBr tablet.

^{1}H-NMR and ^{13}C-NMR were recorded on a Bruker Ascend 400 MHz NMR spectrometer at 25 °C using a deuterated solvent DMSO-d_6.

Differential scanning calorimetry (DSC): NETZSCH DSC 3500, temperature range between 20–250 °C with a scanning rate of 10 °C/min.

Powder X-ray diffraction (PXRD): PANalytical X'Pert diffractometer equipped with 2.2 KW Cu Anode.

c. Synthesis of the mixture

The eutectic mixture was prepared by neat grinding method with an equimolar ratio, the pure compounds Nifuroxazide and ascorbic, (Figure 1) acid were co-ground on an agate mortar for one hour and half until the obtention of a fine powder.

Molecular structures

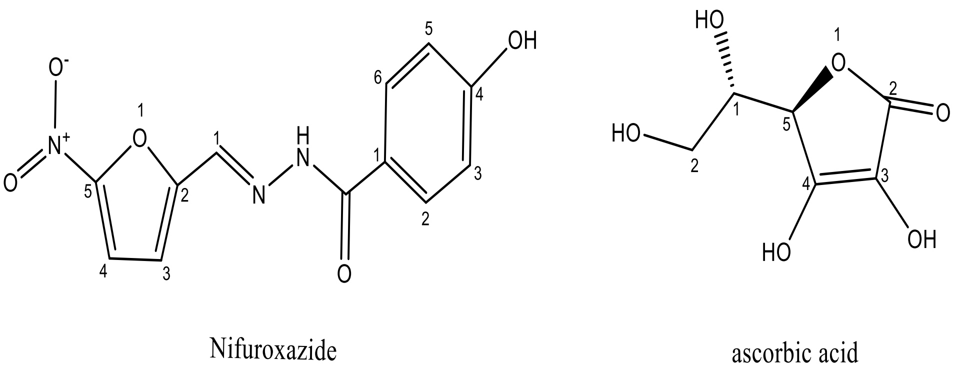

Fig.1. Developed structures of the pure compounds Nifuroxazide and Ascorbic acid

3. RESULTS AND DISCUSSION

a. Infrared spectroscopy

Figure 2 shows the spectra of the pure compounds and the resulted mixtures, the spectrum of nifuroxazide shows the characteristic peaks of the molecule, intense bands around 1530 cm^{-1} and 1340 cm^{-1} are observed corresponding to the asymmetric and symmetric stretching vibrations of the N=O bonds, vibration band of C=O bond around 1650 cm^{-1}, N-H vibration band at 1550 cm^{-1} and N-H stretching vibrations around 3350 cm^{-1} [18].

The important bands of ascorbic acid are shown in the IR spectrum which a broad band are observed around 3200-3600 cm^{-1}, corresponding to the stretching vibrations of the O-H bonds. A sharp band at 1680 cm^{-1}, attributed to the stretching vibration of the C=O group in the lactone ring. C=C bonds of the unsaturated ring show bands in the region of 1600-1650 cm^{-1} [19].

The appearance of the characteristic bonds in the spectrum of (Nifuro-Asc) mixture indicates the presence of the two molecules, a slight displacement is observed in some bands due to the influence of the environment.

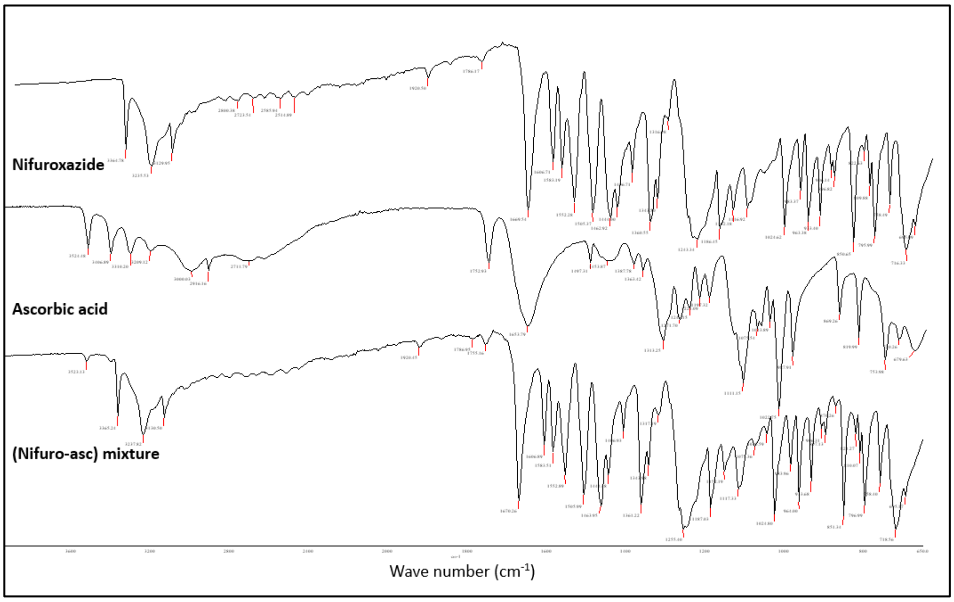

Fig.2. infrared spectra of Nifuroxazide, ascorbic acid and (Nifuro-asc) mixture

b. Differential Scanning Calorimetry (DSC)

The DSC curve of the mixture, Figure 3 shows a single endothermic peak at 184°C which represents the melting point which is lower than the melting points of the pure compounds and this is explained by the formation of a eutectic mixture. The mixture also exhibits an exothermic peak just after the melting peak, which is attributed to the decomposition temperature.

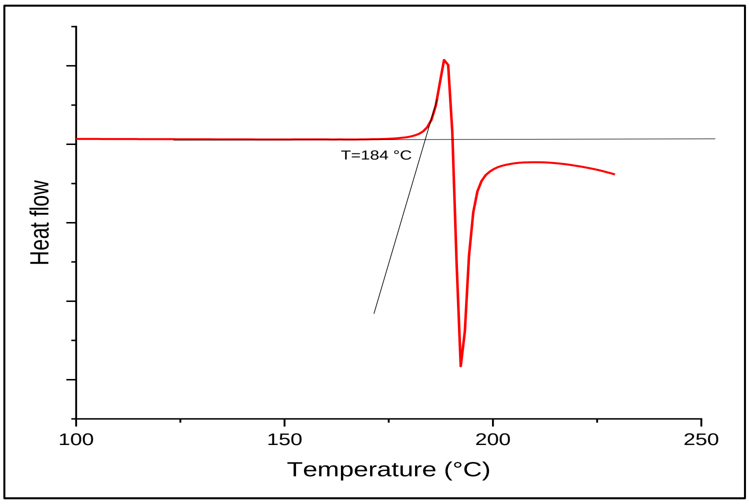

Fig.3. DSC thermogram of (Nifuro-asc) mixture

c. Powder X-ray diffraction

X-ray powder diffraction patterns of Nifuroxazide, Ascorbic acid and the mixture Nifuroxazide-Ascorbic acid are shown in Figure 4, the results shows that the spectrum of the mixture (Nifuro-asc) present the co-existence of the characteristics peaks of the pure compounds with a slight displacement and the absence of new peaks. This result indicates the formation of a eutectic mixture [20].

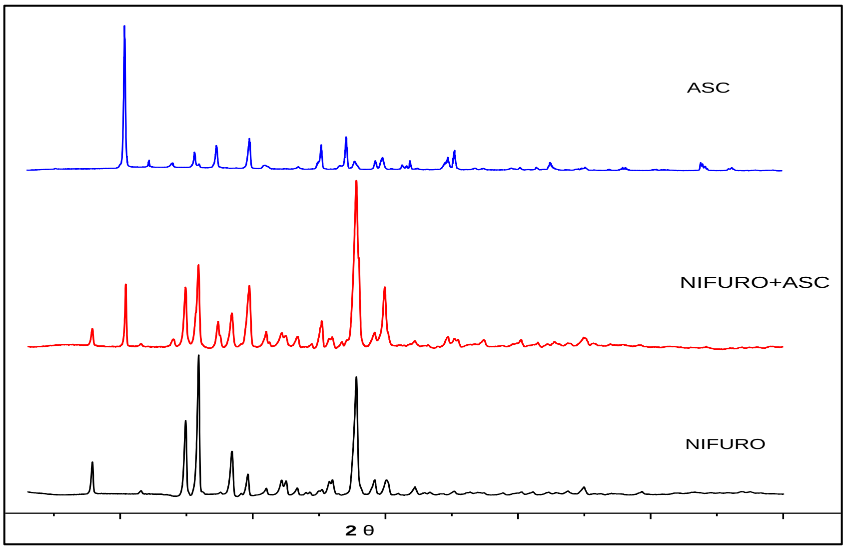

Fig.4. PXRD patterns of nifuroxazide, ascorbic acid and the mixture (Nifuro-Asc)

The particle size of the pure components and the eutectic mixture were calculated using Scherrer equation. The size of the mixture's particles is smaller than the parent compounds, this provides a good approach to enhancing the dissolution of the drug. The values of the calculated particle sizes are shown in Table 1.

Table 1. Particle sizes of the components.

	Nifuroxazide	Ascorbic acid	(Nifuro-asc) mixture
Particle size	33.0143874	38.8712541	29.4226318

d. Nuclear magnetic resonance NMR-^{13}C

The solid-state NMR spectra of the pure compounds present all the carbons existing in the molecules, and shows the purity of these molecules. and the mixture (Nifuro-Asc) shows the coexistence of all the signals without any changes, Figure 5 and Table 2 [21].

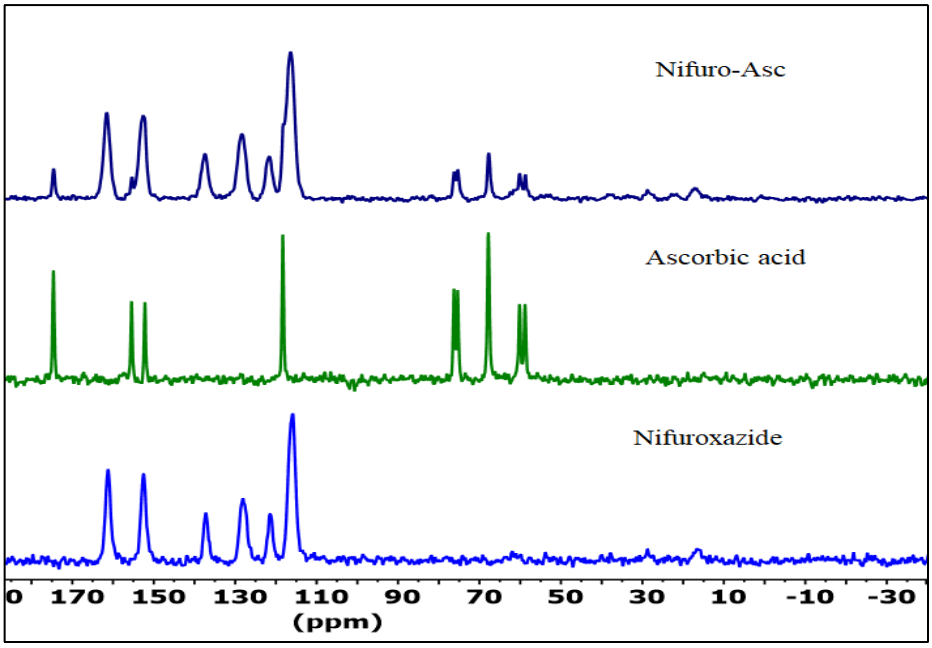

Fig.5. NMR-^{13}C spectra of nifuroxazide, ascorbic acid and the mixture Nifuro-Asc

Table 2. Displacements of the signals of NRM-^{13}C

Displacement (ppm)	Ascorbic acid	Displacement (ppm)	Nifuroxazide
59,8	C2 linear	115.92	C3, C4 (cycle à 5)
67.84	C1 linear	121.44	C3; C5 (phenyl)
76.03	C5	128.12	C1, C2, C6 (phenyl)
118.30	C3	137.21	C=N
154.2	C4	152.53	C2, C5 (cycle à 5)
174.65	C2	161.24	C=O, C-OH

e. Nuclear magnetic resonance NMR-^{1}H

The nuclear magnetic resonance spectrum of the active pharmaceutical ingredient Nifuroxazide, Figure 6 shows all the hydrogens that the molecule contains, the same for the ascorbic acid we observe all the hydrogens. The eutectic mixture presents all the hydrogens of the molecules with a slight shift, Table 3 [21].

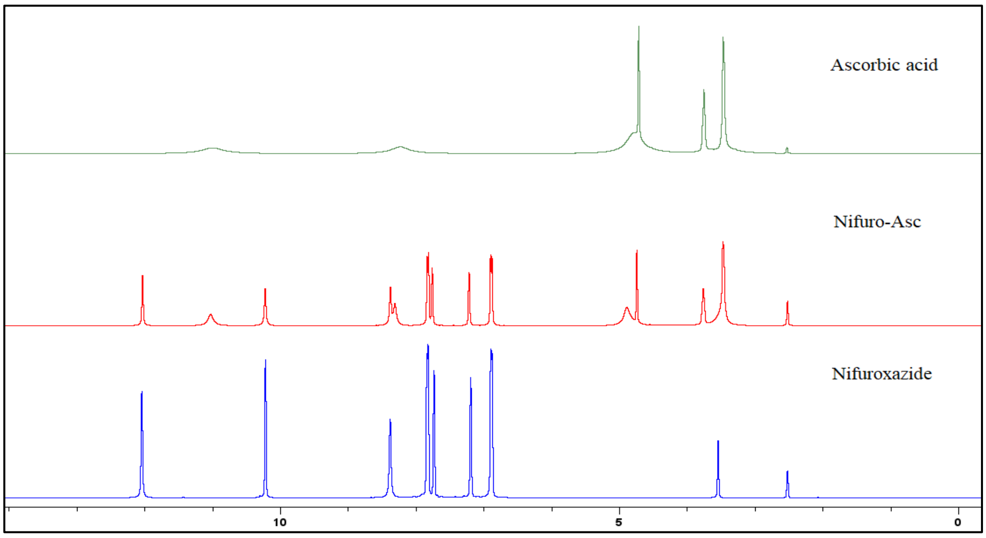

Fig.6. NMR-^{1}H spectra of nifuroxazide, ascorbic acid and the mixture Nifuro-Asc

Table 3. Displacements of the signals of NMR-^{1}H

Displacements (ppm)	Nifuroxazide	Displacements (ppm)	Ascorbic acid
6.9	2H (C_3. C_5 benzene)	3.47	2H(CH_2-OH)
7.18	H (C_3 ring)	3.75	H (CH)
7.72	H (C_4 ring)	4.72	H (CH)
7.82	2H (C_2. C_6 benzene)	4.81	H (OH-CH_2)
8.38	H (C_1=N)	8.23	H (OH-ring)
10.22	H (OH)	10.99	H(OH-ring)
12.04	H (NH)	3.47	2H(CH_2-OH)

4. BOLOGICAL ACTIVITY

The antimicrobial activity was tested on the pure drug and (Nifuro-asc) eutectic mixture against the aerobic bacteria Escherichia Coli, Staphylococcus aureus, Pseudomonas aeruginosa. The antifungal activity was also tested on Candida albicans. The results shows that the pure pharmaceutical ingredient Nifuroxazide is more active against Escherichia Coli and Staphylococcus aureus than the eutectic mixture with a slight deterioration in activity, Table 4.

Table 4. Biological activity of Nifuroxazide and (Nifuro-asc) mixture.

Compound	Staphylococcus aureus	Escherichia Coli	Pseudomonas aeruginosa	Candida albicans
Nifuroxazide	11.7	15.6	/	/
(Nifuro-asc) mixture	9.74	11.26	/	/

5. CONCLUSION

In this study, a eutectic mixture composed of an active pharmaceutical ingredient Nifuroxazide and ascorbic acid was investigated. Thermal analysis revealed that the mixture behaves like a single pure compound, exhibiting a single endothermic peak corresponding to its melting point, which is lower than the melting points of the two individual pure components. This finding suggests the potential to conduct dissolution tests in the future to evaluate the improvements a eutectic system can offer over a pure API.

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