International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 8, 2025
https://theaspd.com/index.php

Microencapsulation Approach For Anti-Inflammatory Treatment: Drug Release Kinetics, Antibacterial Potential, And Intestinal Microbiota Evaluation

Rachida Bouteldja[1], Ilham Abdelmalek[2,*], Meryem Belabbas[3], Lamia Bennabi[2,4], Radhouane Doucene[5], Meryem Mouffok[6], Faiza Bennabi[3]
[1]Laboratory of hygiene and animal pathology, Institute of veterinary sciences, University of Tiaret, Algeria.
[2]Synthesis and catalysis Laboratory, Ibn Khaldoun University, Tiaret, Algeria.
[3]Laboratory of Ecodevelopment of Spaces, Faculty of Natural and Life Sciences, Djilali Liabes University, Sidi Bel Abbes, Algeria
[4]Department of matter sciences University of Chaib Bouchaib, Ain Temouchent, Algeria.
[5]Laboratory of farm animal reproduction, Institute of Veterinary Sciences, University of Tiaret, Algeria.
[6]Physical and Organic Macromolecular Chemistry Laboratory, Exacts Science Faculty, Sidi Bel Abbes, Algeria.
Email*: ilham.abdelmalek@univ-tiaret.dz

Received: 21/05/2025 **Accepted: 08/11/2025**

Abstract
The objective of this study was to evaluate the potential of a microencapsulated diclofenac formulation to prevent inflammation-induced alterations in the intestinal microbiota while minimizing systemic side effects. Diclofenac was encapsulated in an ethylcellulose-based microparticulate system using an oil-in-water (O/W) emulsion–evaporation method. The study examined the physicochemical properties of the microparticles, in vitro drug release behavior, antimicrobial activity, and in vivo anti-inflammatory efficacy, as well as their impact on gut microbiota composition. The resulting spherical microparticles, characterized by scanning electron microscopy (SEM), exhibited variable porosity, an average Sauter diameter of 292 µm, and a drug-loading efficiency of 32%. FTIR and XRD analyses confirmed the presence of the active compound. In vitro release kinetics followed a quasi-Fickian diffusion mechanism, consistent with the Higuchi model. The released drug demonstrated antimicrobial activity against Escherichia coli, Staphylococcus aureus, and Bacillus cereus In vivo testing in Wistar rats identified 6.42 mg as the effective anti-inflammatory dose in a paw-edema model. Fecal microbiota analysis revealed that treatment with the encapsulated drug modulated the abundance of specific bacterial populations, suggesting an influence on gut microbial balance. These results demonstrate that microencapsulation via O/W emulsion–evaporation can enhance diclofenac's anti-inflammatory efficacy, provide controlled drug release, and influence intestinal microbiota, offering a promising strategy for improved therapeutic interventions.
Keywords: *diclofenac, intestinal flora, antibacterial activity, anti-inflammatory activity.*

1. INTRODUCTION

In recent years, the problem of antibiotic resistance has been exacerbated mainly by the repeated use of this class of drug [1, 2]. One of the proposed solutions to this problem is the use of non-antibiotic drugs with antibacterial activity [3]. Non-antibiotic" compounds are all chemical drugs with powerful antimicrobial properties [4]. These mainly include non-steroidal anti-inflammatory drugs, which have demonstrated significant potential against both Gram-positive and Gram-negative strains [5]. Following extensive research into the antimicrobial action of non-steroidal anti-inflammatory drugs, diclofenac has been shown to exert significant activity against various strains via the production of a non-polysaccharide biofilm [6-8].

In galenic pharmacy, several routes of administration are used including the oral route, which is the most adopted for most active ingredients as it is the most convenient, least expensive and does not cause pain on administration [9].

In the case of orally administered drugs, the absorption phase may begin in the mouth or stomach, but most drugs are usually absorbed in the small intestine. The drug passes through the intestinal wall and liver before being transported to the target via the bloodstream [10].

The human intestinal microbiota constitutes a complex ecosystem, the microbiota is recognized for its effect on human health [11]. Intestinal flora is essential for regulating host physiology, such as glucose and energy homeostasis, educating the immune system and modulating sensory functions [12]. In addition, intestinal flora disorders promote chronic inflammation and metabolic dysfunction [13]. The imbalance of the bacteria that make up the intestinal flora is responsible for the prevalence of many modern diseases. These include allergies, inflammatory bowel diseases and metabolic and degenerative disorders [14].

This study sought to assess the protective efficacy of dichlofenac against changes in the intestinal microbiota during inflammation induction and therapy, by enumerating the main bacteria involved in the intestinal flora.

2. MATERIALS AND METHODS

2.1. Materials

Diclofenac (Dl) 98% was purchased from Acros organics. Ethyl cellulose (EC, ethoxylate at 48 % mass=22000 g/mole, viscosity 10 Cp) was purchased from Sigma-Aldrich (USA). Dichloromethane DCM >98 % purchased form Fluka and Tween 80 from Aldrich. (Escherichia coli ATCC 25922) and Gram positive (Staphylococcus Aureus ATCC 25923 and Bacillus cereus ATCC 10876) were purshased from pasteur institut of algiers. The culture media were purshased from Biochem-chemopharma, France.

2.2. Preparation of Microspheres

Diclofenac loaded microspheres were prepared using the usually water/oil emulsion-solvent evaporated method described in previously paper [15]. A dispersed solution containing 2g of EC, 1g of Dl and 100mL of DCM was prepared and emulsified with 100mL of aqueous solution (Tween80 at 1 %) using a four-blade turbine impeller stirrer for 2h. After total solvent evaporation solvent, the solidified microspheres were separated by vacuum filtration, washed with deionized water and dried at 40°C then under vacuum in a desiccator containing $CaCl_2$. The Obtained microspheres were named F1.

2.3. Characterization of microspheres

Determination of the Encapsulation Efficiency EE: The drug loaded (DL) and encapsulation efficiency (EE) were calculated by the literature equations (1 and 2) [16]. They were determined experimentally by dissolving of 0.1 g of crushed microspheres in 100 mL of methanol under stirring for 24 h. The obtained solution was analyzed by UV-VIS spectroscopy.

$$\% \text{ DL} = \frac{\text{Drug mass in microspheres}}{\text{Mass of microspheres}} . 100$$

$$\% \text{ EE} = \frac{\text{Actual drug loading}}{\text{Theoretical drug loading}} . 100$$

Particle Size: By Optical Microscopy (OPTIKA 4083. B1), a least of 500 analyzed microspheres, the mean particle size and size dispersion (δ) was calculated using various equations [17].

Scanning Electron Microscopy (SEM): The surface morphology and size of the prepared microspheres were observed using Scanning Electron Microscopy (SEM Quanta 200 FEI) of Development and Technology Advanced Center of Algiers.

Infrared Spectroscopy: The simples were characterized by infrared spectroscopy using a FTIR-8300 Shimadzu spectrophotometer. Samples were prepared in KBr disks.

X-ray powder diffractometry: X-ray diffraction analysis was performed with D8 Advance BRUKER diffractometer of synthetic and catalysis laboratory of IBN Khaldoun University (Tiaret-Algeria) and at the diffraction angle 2θ from 5° to 70°.

2.4. In vitro drug release

The in vitro Dl release by prepared microspheres were carried out in heterogeneous medium pH=1.2/Methanol "70/30" using an appropriate dissolution reactor which takes samples without microspheres. Initially, 0.1g of microspheres was soaked in100 mL of study medium, then 1 mL was withdrawn at predetermined times, and analyzed by UV-VIS spectroscopy at 280nm for Dl concentrations.

2.5. Antibacterial activity

The antibacterial disk-diffusion assays [18, 19] were used to study the activity of DL against Gram negative bacteria (Escherichia coli ATCC 25922) and Gram positive (Staphylococcus Aureus ATCC 25923 and Bacillus cereus ATCC 10876). The petri dishes containing the autoclaved and hardened agar were incubated a 37°C to confirm their sterility. When no growth occurred after 24h, the plates were considered sterile and an inoculating with bacterial suspensions of the test microorganisms was released. Then, Whatman paper discs were placed on the surface of plate and they were soaked in the Dl release medium of prepared microspheres (20 mg and 40 mg) in "pH=7.3/methanol", the studied medium was also tested. The plates were incubated at 37 °C for 24h. After incubation, the resulting diameters of zones of inhibition were determined.

2.6. Anti-inflammatory activity

Adult rats of wistar were purchased from Pastor Institute of Algiers. These rats including weight in average 205±5 g were reared in the Reproduction of farm animals Laboratory with free access to food and water. All animals were injected in the left paw of rats. Five groups were been tested and three animals (n=3) per group were used:

GRT-: rats were injected by physiological water.

GRT+: rats were injected by formol 1%.

GRD1: rats were injected by formol 1% and treated by Dl-loaded microspheres (F1) "20 mg"

GRD2: rats were injected by formol 1% and treated by Dl-loaded microspheres (F1) "40 mg"

GRP: rats were injected by formol 1% and treated by commercial cream).

When edema developed after two hours of injection, the rats were given the oral medication, which they would receive every 24 hours for five days. The measures of diameters of edematous paws of rats were carried out and the augmentation percentage of edema was calculated by the following equation [20]:

$$\%AUG = Dt\text{-}D0/D0 \quad * 100$$

All experiments complied with the Algerian legislation (Law number 95-322/1995) inherent to protect animals designed for experiments or other scientific purposes, also the guidelines of the Algerian Association of Experimental Animal Sciences (AASEA approved under the agreement number 45/DGLPAG/DVA/SDA/14)".

2.7. Gut microbiota and anti-inflammatory drugs interact

In order to evaluate the effect of anti-inflammatory drugs on the composition of the intestinal flora, certain fecal bacteria were enumerated, namely Escherichia coli, Enterococcus Spp, Clostridium Spp and Lactobacillus Spp.

These consisted in dissolving 1 g of fresh fecal material in 9 mL of physiological water (0.9%). Decimal dilutions were then prepared in the same diluent (10^{-1} and 10^{-2}). A volume of 0.1mL of bacterial suspension was then spread onto the surface of MacConkey agar for E. coli and Bile-Esculin-Azide (BEA) agar for Enterococcus Spp. These Petri dishes were incubated at 37°C for 24h. For Clostridium Spp and Lactobacillus Spp, 1 mL of bacterial solution was poured into sterile Petri dishes, filled with TSC agar for Clostridium and MRS agar for Lactobacillus, followed by incubation at 37°C for 48h. Results were expressed in log10 units (CFU/g) of fecal matter.

Results are presented as mean ± standard error. STATISTICA software (version 6.1, Stat,Tulsa.OK) was used for statistical analysis of the data. Comparisons between the five experimental groups were made using a one-factor analysis of variance, supplemented by the LSD test. A p-value of <0.05 was chosen as the significance level.

3. RESULTS AND DISCUSSION

The surface morphology was determined by SEM, their SEM images presented in Figure 1, they showed that the prepared microspheres present spherical shapes with different sizes and their surface is smooth and porous. The means diameters of the microspheres were studied by optical microscopy using previously describes equations (Kaczmarski K, 2003) [17]. The obtained microparticles showed a mean diameter of Sauter (d_{32}) in the range of 247.90µm and an upright dispersity index equal to 1.23. The microspheres' shape and diameters are consistent with earlier research that shown that ethylcellulose derivatives and solvent evaporated encapsulation method produce a spherical shape, with particles' sizes depending on the parameters of formulation (agitation speed, polymer viscosity and mass ratio polymer/ active agent [15]**.** This consistency demonstrates that the encapsulation method's dependability and implies that adjusting these parameters can maximize the release and delivery characteristics of active agents in a range of applications. By adjusting these formulation parameters, researchers can enhance the therapeutic efficacy and stability of encapsulated agents, paving the way for more effective drug delivery systems. This

optimization not only improves the performance of existing drugs but also opens avenues for the development of novel therapeutic agents that can be tailored to meet specific medical needs [21,22].

Table 1: Microspheres composition and microencapsulation results.

Code	Dl/Polymer	% DL	%EE	d_{10} (µm)	d_{32} (µm)	d_{43} (µm)	Dispersion
F1	Dl/EC (1/2)(w/w)	32.11±0.09	84.14±0.13%	247.90	292.86	306.78	1.23

(± SD) : standard deviation; DL: Drug Loading; EE: Encapsulation Efficiency.

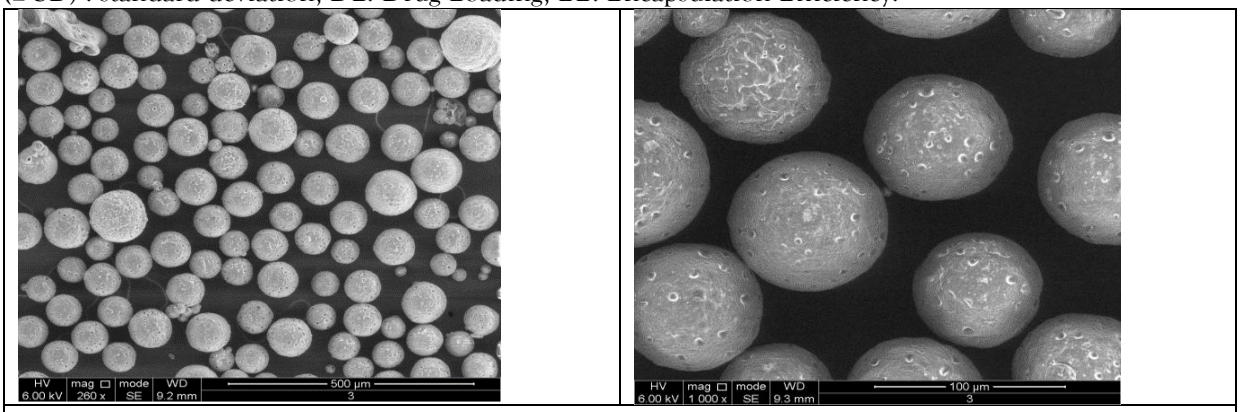

Figure 1: SEM of microspheres F1

The IR spectrum of microspheres was compared with the EC IR spectrum previously published [15]. In Figure 2**,** the presence of important significant IR bands of Dl in the microparticles spectrum was identified at the expected wave number: 3234.20cm^{-1} for the N-H band, 1686.81cm^{-1} for the C=O band, 1571.77cm^{-1} for aromatic C=C vibration, 744.14cm^{-1} for the C-Cl band and 1551.63cm^{-1} correspond to the C=O vibration of carboxylic acid. The FTIR analysis confirms the presence of DI in prepared microspheres and since the microspheres' IR spectrum appears as the sum of the Dl and polymer spectra with low shift and intensity indicating that the drug and polymer matrix are not interacting.

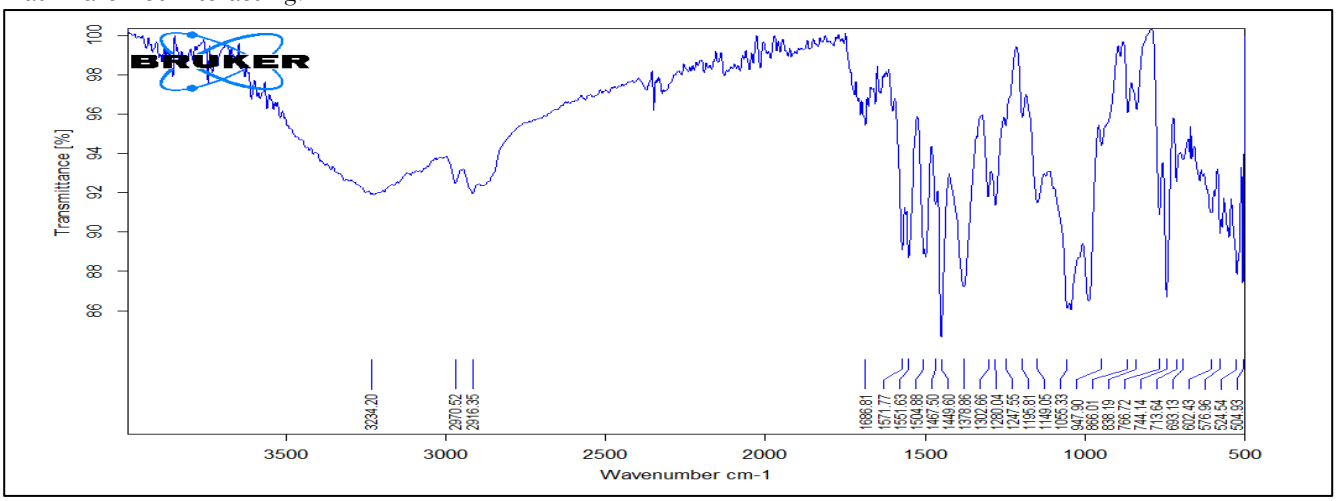

Figure 2: Infrared spectrum of loaded microspheres.

The XRD diffractogram shown in Figure 3 was used to study the physical states of microspheres. The prepared microspheres present a semi-crystal form. Some of crystal peaks of Dl were observed around 3.36°, 18.18° and 28.8°. These peaks appeared less intense compared to the Dl XRD diffractogram [23]. The XRD diffractogram of EC shows a semi-crystal form characterized by an intense peak at 13.63°, this peak is not observed in the XRD diffractogram of microspheres. For that, it can be concluded that the Dl is included in amorphous state of polymer. The

incorporation of the active agent into the amorphous regions that's validated by FTIR and DRX enhances its bioavailability and therapeutic efficacy, making it a crucial factor in drug formulation strategies. Optimizing the distribution of the active agent within these amorphous regions can lead to improved release profiles and greater patient compliance in therapeutic applications [24].

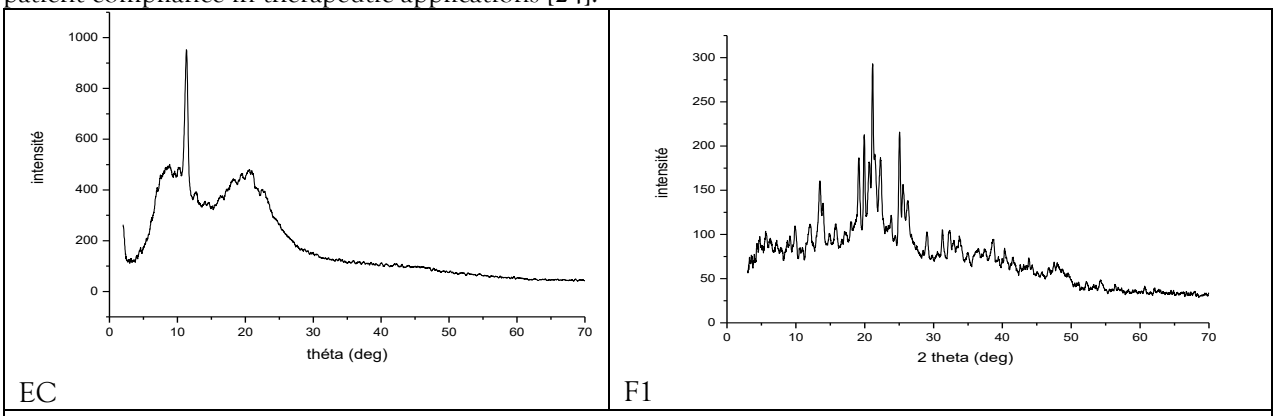

Figure 3: XRD Diffractogram of prepared microspheres.

The obtained results of drug release are shown in Figure 4. A biphasic profile was seen, with an average 50% burst release followed by a slower release phase that reached 10% for the remainder of the experiment. The Dl liberation process from microspheres is released slowly and steadily into the studied medium. Linking to other work [25], this is achieved a prolonged liberation system that the rate of diffusion is calculated using Higuchi and Korsmeyer-Peppas equations. Prolonged drug release is a critical aspect of pharmaceutical formulations at enhancing therapeutic efficacy. Various strategies have been developed to achieve sustained release, including transdermal patches, lipid nanoemulsions, sustained tablets, and microspheres. These formulations focus on controlling the release kinetics of drugs to maintain therapeutic levels over extended period [26].

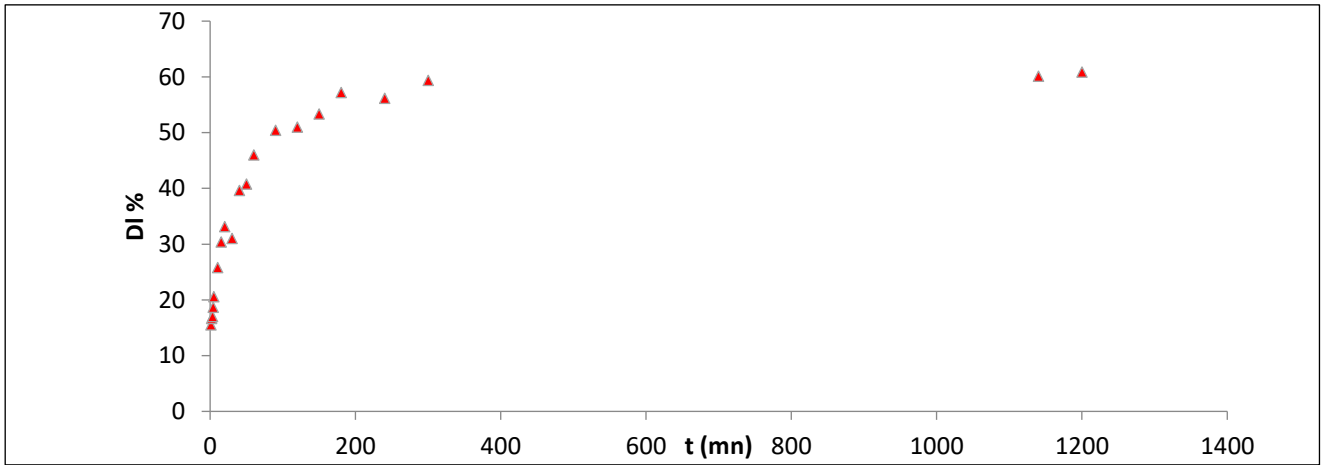

Figure 4: Release profile of Dl from microspheres.

To analyze the drug release kinetics, the percentage of drug release was fitted to various mathematical models [27], cited in Table 2. The calculated rates show that the highest correlation coefficient was obtained with Higuchi and Korsmeyer–Peppas models. When modeling kinetics to Korsmeyer–Peppas equation, the n-value of the formulation was < 0.5 suggesting quasi-Fickian diffusion mechanism.

Mathematic model	**Order 0**	**Order 1**	**Higuchi model**	**Korsmeyer et Pappas**
K	3. 10^{-7}	0.0026	0,1005	1.50 n= 0.4033
R^2	0,7568	0,5355	0,9898	0,9869

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 8, 2025
https://theaspd.com/index.php

Table 2: Results of drug release of Dl loaded microspheres, Qt and Q0 are the amount of drug at t and at t=0 respectively, Mt/M∞ is the fraction of released drug, t is the release time, n is the diffusional exponent. K_0, K_1, K_H and K correspond respectively to the rate constants of 0, 1, Higuchi and Korsmeyer-Peppas models.

The antimicrobial properties of prepared microspheres were evaluated against three bacterial strains E. coli (gram-) and S. aureus and Bacillus cereus (gram+). The inhibition of growth of bacteria after being incubated was measured in millimeter (mm) and the results are shown in Figure 5. After 24h, it can be observed that 40mg of microspheres inhibit the growth of all tested strains. It was noted that the inhibition zone changed according to the tested strains as well as the composition of microspheres. 20mg of microspheres can inhibit only staphylococcus aureus suggesting that this strain is sensitive to low liberated concentration but Escherichia coli and Bacillus cereus were resistant to this concentration.

The DI and EC blend indicates a good antibacterial activity through the high concentration, ethylcellulose (EC) in conjunction with others active agent has been explored and it serves as an effective matrix for microencapsulation, enhancing the stability of active agent with potentially influencing its antibacterial properties [28].

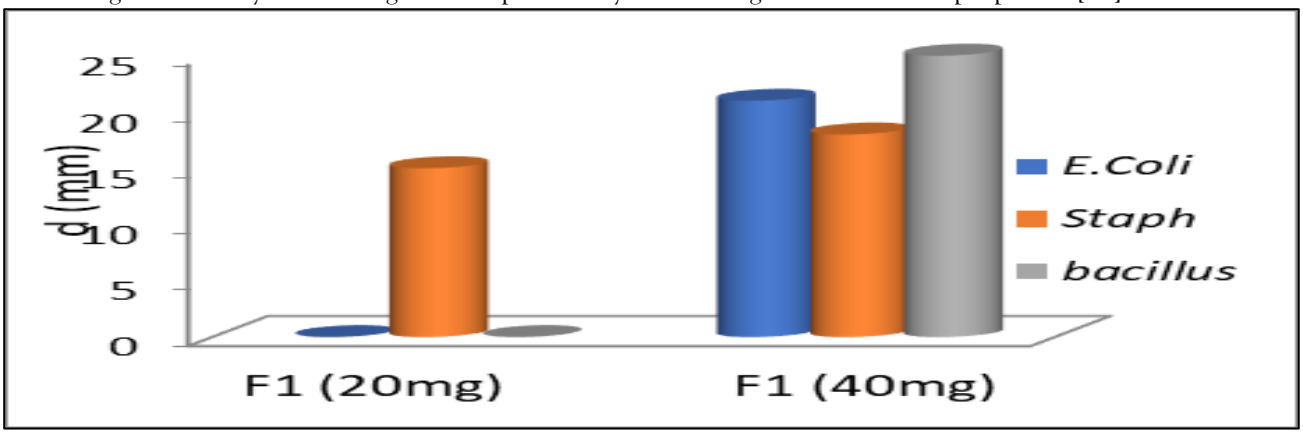

Figure 5. Inhibition zone diameters (mm) produced by drug solution against different bacteria after 24 h.

To evaluate the anti-inflammatory activity of prepared microspheres, the 1% of formaldehyde rat paw edema induction was performed and the size of the edema was measured regularly and the augmentation percentage was also calculated. The edema appeared after 2h of injection. For the witness group, the volume of edema increases over time until the 48hours and it decreases from the 72h due to the immune defense of rats. For group 3 and 4 treated with Dl-loaded microspheres, the results presented in Figure 6 show a decrease in paw diameter. It can be suggested that the treatment of the rats with two amounts of microspheres (20mg and 40mg) and commercial cream reduced the inflammatory compared with the control rats. Comparing the two amounts' percentages of augmentation, it's founded that 20mg reduce significantly the edema compared to the second amount (40mg). Nevertheless, both the commercial cream and the second dose(40mg) decreased the edema at 96h. Consequently, 20mg of microspheres wich contain around 6.42mg of Dl has a stronger inhibitory effect than 40mg and the commercial cream demonstrated that 20mg is an appropriate dosage.

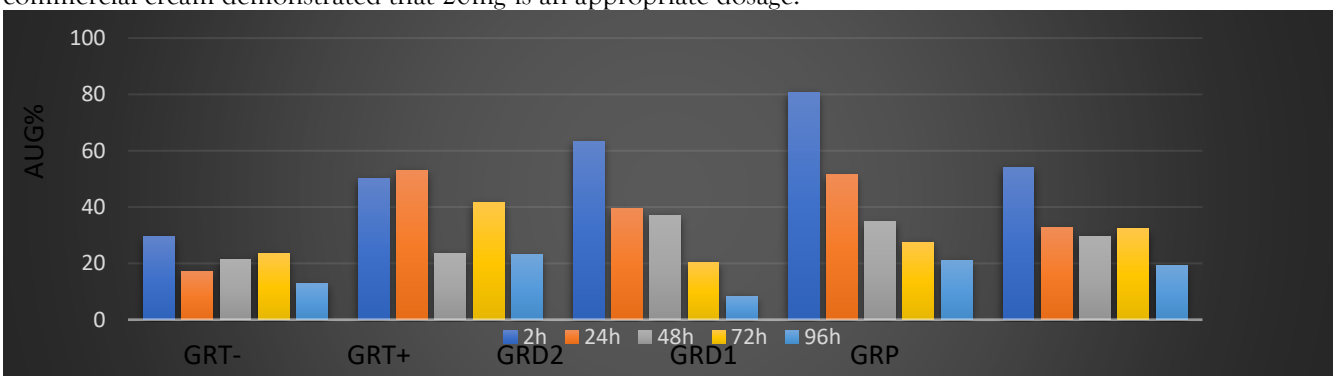

Figure 6: Evolution of the left paw diameter of different group.

The results of E. coli, Enterococcus spp., Lactobacillus spp. and Clostridium spp. counts during this experiment are shown in the Figure7.

Indeed, the number of E.coli at Tf showed a significant reduction ($p < 0.05$) in diclofenac-treated rats (GRD1) compared with the GRT+ control group (13.03%). On the other hand, no significant difference was observed between the negative control group and the GRD1; GRD2; DRP groups.

According to the obtained results, the D1 dose of diclofenac sodium reduces the number of E.coli in the intestinal flora. With regard to Enterococcus spp, a significant reduction ($p < 0.05$) of 13.60% was observed between the GRD2 and GRP groups. At the same time, no difference was reported between the GRT-, GT+ and GRD1 groups. Lactobacillus results revealed a significant increase against GRT- compared with GRD1 of 18. 78%. No significant difference was detected between the 05 groups at T0. The results of evaluating the number of Clostridium spp isolated from faecal material is shown at T0 and Tf, revealing no significant difference between the 05 experimental groups.

Mazumdar et al. [29] verified the E.coli enumeration results and demonstrated that diclofenac sodium inhibits E.coli. Ahmed et al. confirmed in their study that the effect of diclofenac on E. coli is due to membrane destruction through the formation of pores in the plasma membrane [30]. In addition, other research reveals a strong ability of diclofenac to inhibit E.coli and explains this ability by the anti-inflammatory effect of E.coli sliding clamp protein, which is essential for DNA replication [31]. Our results are supported by the work of Ferrer-Luque et al. [32], who report the significant antibacterial effect of dichlofenac against Enterococcus. Furthermore, a comparative study of the antibacterial effect of diclofenac, ibuprofen and amoxicillin against Enterococcus shows that diclofenac has a significant effect against the latter [33]. Abu- El- Azayem et al. report that treatment of isolated Enterococcus strains with diclofenac showed an inhibitory effect, which can be explained by a reduction in the expression of efflux pump genes [34]. At the same time, a significant reduction in Enterococcus is evidenced by inhibition of bacterial DNA, impermeability and altered membrane function, modification of genes encoding transport proteins, binding [35, 36]. The increase in the number of Lactobacillus spp can be explained by the antagonistic effect of the matrix used in the drug formulation “Ethylcellulose” [37].

Contrary to our result, Jimenez-Serna and Sanchez showed reliable inhibition of diclofenac against Lactobacillus spp, explained by the presence of a non-specific multidrug efflux of this bacterium [38]. Oral administration of probiotic Lactobacillus strains in diclofenac-induced inflammation enhances intestinal antimicrobial activity to prevent pathological invasion and maintain intestinal homeostasis [39].

The majority of studies indicate that Firmicutes levels, particularly Clostridium species, are reduced during NSAID treatment [40]. The composition of the microbiota is influenced differently by the use of anti-inflammatory drugs combined or not with antisecretory agents. They directly affect the relative abundance of Gram-positive and Gram-negative bacteria [41]. Various large-scale experimental studies have highlighted correlations between gut microbiota and the absorption of different drug classes, including anti-inflammatory drugs [42, 43], reveal in their study that diclofenac sodium has a highly bactericidal action against Gram-positive and Gram-negative bacteria by inhibiting bacterial DNA synthesis, either by binding directly to DNA or by causing its degradation [44]. In addition, several studies suggest that the antibacterial mechanism of dichlofenac is inhibition of bacterial DNA synthesis or modification of bacterial membrane activity [45, 46].

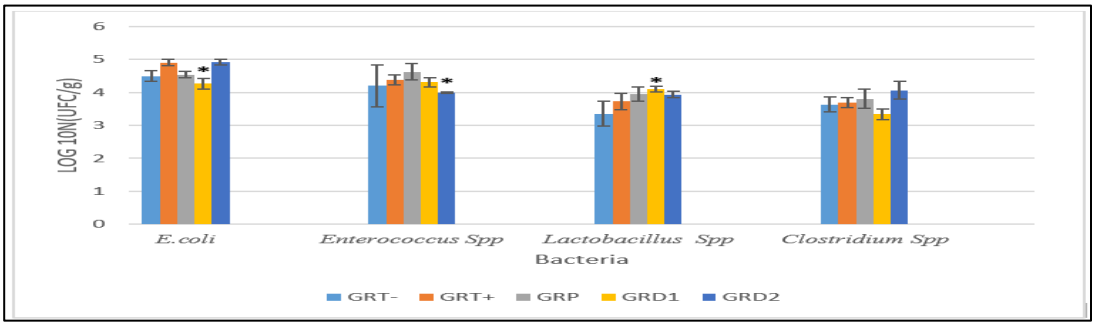

Figure 7: Bacteria count in the experiment.
Results are expressed as mean ± SEM with n= 4).

*Significant difference between GRD1 and GRT+; GRD 2 and GRP+; GRD1 vs GRT- groups.

CONCLUSION

The study suggests that the formulated microspheres not only provide a controlled release of diclofenac but also enhance its therapeutic efficacy against inflammation and bacterial infections. It highlights the potential of ethylcellulose-based microparticles as a delivery system for non-antibiotic drugs. Biocompatible and biodegradable microparticles were successfully prepared using ethylcellulose to encapsulate diclofenac. The drug release profile was analyzed and best fitted the Higuchi model, indicating a quasi-Fickian diffusion mechanism. This confirms that the drug is released in a sustained and controlled manner from the microspheres.

The released diclofenac exhibited significant antimicrobial activity against various bacterial strains, including Escherichia coli, Staphylococcus aureus, and Bacillus cereus. The study further confirmed that the antibacterial mechanism of diclofenac involves inhibition of bacterial DNA synthesis and disruption of membrane integrity. In vivo assays demonstrated that administration of diclofenac-loaded microspheres significantly reduced paw edema in rats. The optimal dose was determined to be 20 mg of microspheres (equivalent to ~6.5 mg of diclofenac), which proved more effective than a higher dose (40 mg of microspheres). Moreover, diclofenac administration influenced certain bacterial populations in the intestinal microbiota. Specifically, the number of E. coli in feces at the final time point (Tf) was significantly reduced in the treated group compared to the GRT+ group. Similarly, a reduction in Enterococcus spp. was observed in the GRD2 group compared to the GRP group. Conversely, the count of Lactobacillus spp. increased in the treated group compared to the GRT− group. No significant differences were observed in Clostridium spp. counts among the five experimental groups. These findings suggest that diclofenac sodium has a measurable impact on gut microbial composition. This study only looked at a small number of intestinal microbiota (Lactobacillus, Enterococcus, and Clostridium) and bacterial strains (E. coli, S. aureus, and B. cereus). Future research should use more extensive microbial profiling methods, like 16S rRNA gene sequencing or metagenomic approaches, to evaluate long-term effects on the diversity and functional dynamics of the gut microbiome in order to obtain a more thorough and accurate understanding of the microbiota changes brought about by diclofenac-loaded microspheres.

REFERENCES

1.Recep, T., Tuba, D.F., Bayram, F., 2014, Risk factors for nosocomial burn wound infection caused by multidrg resistant Acinetobacter baumannii. J. Burn Care Res., 35(1), pp. e73–e80, https://doi.org/10.1097/BCR.0b013e31828a493f.
2.Lin, L., Jin-Xiu, D., Hui-Yuan, D., Qin-Di, L., 2018, Antimicrobial resistance and pathogen distribution in hospitalized burn patients. Med. 97(34), pp. e11977, https://doi.org/10.1097/MD.0000000000011977.
3.Anaïs, P., Laurent, P., 2015, Emerging broad-spectrum resistance in Pseudomonas aeruginosa and Acinetobacter baumannii: mechanisms and epidemiology. Int. J. Antimicrob. Agents, 45(6), pp. 568–585, https://doi.org/10.1016/j.ijantimicag.2015.03.001.
4.Salem-Milani, A., Balaei-Gajan, E.-M., 2013, Antibacterial effect of diclofenac sodium on Enterococcus faecalis. J. Dent., 10(1), pp. 16–22, https://doi.org/10.30476/DENTJODS.2013.41674.
5.Marzena, M., Piotr, O., 2020, The study of biological activity of transformation products of diclofenac and its interaction with chlorogenic acid. J. Environ. Sci., 91, pp. 128–141, https://doi.org/10.1016/j.jes.2020.01.022.
6.Nongnuj, M., Pongtanapiwat, P., 2004, Structure determination of diclofenac in a diclofenac-containing chitosan matrix using conventional X-ray powder diffraction data. J. Appl. Cryst., 37, pp. 288–294, https://doi.org/10.1107/S0021889804002353.
7.Reslinski, A., Dabrowski, S., 2015, The impact of diclofenac and ibuprofen on biofilm formation on the surface of polypropylene. Hernia, 19, pp. 179–185, https://doi.org/10.1007/s10029-013-1200-x.
8.Agostinho, A.L.S., Silva, A., 2021, Diclofenac may induce PIA-independent biofilm formation in Staphylococcus aureus strains. Int. J. Microbiol., pp. 1–11, https://doi.org/10.1155/2021/8823775.
9.Kanselier, J.P., Piau, M., 2004, Procédés d'émulsification. In Mécanismes de formation des émulsions; Tech. Ing. Gén. Proc., Vol. J2152, pp. 1–12, https://doi.org/10.1051/techeng_j2152.
10. Rowland, M., Tozer, T.N., 2011, Clinical pharmacokinetics and pharmacodynamics. In Clinical Pharmacokinetics and Pharmacodynamics, 4th ed.; Lippincott Williams & Wilkins: Philadelphia, USA, pp. 1–800, https://doi.org/10.1007/978-1-59745-503-3.
11. Landman, C., Quévrain, E., 2016, Le microbiote intestinal : description, rôle et implication physiopathologique. Rev. Med. Interne, 37(6), pp. 418–423, https://doi.org/10.1016/j.revmed.2015.12.012.
12. Zoltán, S.Z., Katalin, K.-K., 2023, Interactions between NSAIDs, opioids and the gut microbiota – Future perspectives in the management of inflammation and pain. Pharmacol. Ther., 241, pp. 108331, https://doi.org/10.1016/j.pharmthera.2022.108327.
13. Wang, J., Chen, W., 2020, The relationship between gut microbiota and inflammatory diseases: the role of macrophages. Front. Microbiol., 11, pp. 1065, https://doi.org/10.3389/fmicb.2020.01065.

14. Doré, J., Corthier, G., 2010, Le microbiote intestinal humain. Gastroenterol. Clin. Biol., 34(4), pp. 7–16, https://doi.org/10.1016/S0399-8320(10)70002-6.
15. Mouffok, M., Abdelmalek, I., 2023, Investigation of factors affecting particle size distribution and sustained release of a water-soluble drug from cellulose derivatives microspheres. Chem. Afr., 6, pp. 163–176, https://doi.org/10.1007/s42250-022-00323-6.
16. Putriana, R., Nurlela, L., 2025, Influence of polymer and binder on extended-release ondansetron HCl granules incorporated within a polymer matrix. Farmacia, 73(1), pp. 112–120, https://doi.org/10.31925/farmacia.2025.1.16
17. Kaczmarski, K., Baczek, J., 2003, Effect of particle-size distribution and particle porosity changes on mass-transfer kinetics. Acta Chromatogr., 13, pp. 22–37, https://doi.org/10.1556/AChrom.13.2003.1.3.
18. Grăţiana, R., Toma-Antonie, D., 2024, Evaluation of anti-melanoma, antibacterial and antioxidant effects of a phytochemically characterized water-soluble fraction from green coffee. Farmacia, 72(6), pp. 1261–1271, https://doi.org/10.31925/farmacia.2024.6.18.
19. Evans, D.W., 2024, Impact of MIC breakpoint changes for Enterobacterales on trends of antibiotic susceptibilities in an academic medical center. Antimicrob. Steward. Healthc. Epidemiol., 4(1), pp. S54–S56, https://doi.org/10.1017/ash.2023.408.
20. Alwashli, H., Al-Khulaidi, L.K., 2021, In vivo anti-inflammatory activity of aqueous extract of Carthamus caeruleus L. rhizome in mice. Jordan J. Biol. Sci., 14(3), pp. 453–458, https://doi.org/10.54319/jjbs/140304.
21. Gupta, A.K., Dey, K.B., 2024, Microencapsulation for controlled drug delivery: a comprehensive review. Update Dent. Coll. J., 1(1), pp.
22. 48–54, https://doi.org/10.3329/updcj.v1i1.73438.
23. Choudhury, A., Das, A., 2019, Microsphere: a promising approach for drug delivery. J. Appl. Pharm. Res., 7(2), pp. 1–6,
24. https://doi.org/10.18231/2348-0335.2019.0001.
25. Palomo, M., Bonet, M., 1999, Analysis of diclofenac sodium and derivatives. J. Pharm. Biomed. Anal., 21, pp. 83–94,
26. https://doi.org/10.1016/S0731-7085(99)00132-1.
27. Seftian, M.L., Lestari, M., 2024, Drug solubility enhancement strategies using amorphous solid dispersion: examination on type and the amount of the polymer. Indones. J. Pharm. Sci. Technol., 186–195, https://doi.org/10.20885/ijpharst.2024.1.2.8.
28. Saboji, J.K., Patil, S., 2012, Development of oral colon specific pH dependent microcapsules of NSAID drug Naproxen. J. Appl. Pharm. Sci., 2(5), pp. 201–211, https://doi.org/10.7324/JAPS.2012.2535.
29. Ahmed, S.M., Ali, A.A., 2016, Design and in vitro/in vivo evaluation of sustained-release floating tablets of itopride hydrochloride. Drug Des. Devel. Ther., 10, pp. 4061–4071, https://doi.org/10.2147/DDDT.S116139.
30. House, J.E., 2007, Principles of Chemical Kinetics, 2nd ed.; Elsevier: Amsterdam, Netherlands, https://doi.org/10.1016/B978-012356787-1/50000-9.
31. Julaeha, E., Putri, N.-A., 2023, Ethyl cellulose-based microcapsules of Citrus aurantifolia essential oil with an optimized emulsifier for antibacterial cosmetotextiles. Polymer, 283, pp.126265, https://doi.org/10.1016/j.polymer.2023.126265.
32. Mazumdar, J., Deyev, V., 2009, Hypoxia-inducible factors in stem cells and cancer. J. Cell. Mol. Med., 13(11–12), pp. 4319–4328, https://doi.org/10.1111/j.1582-4934.2008.00491.x.
33. Ahmed, E.F., El-Baz, A.A., 2016, Evaluation of antibacterial activity of some nonsteroidal anti-inflammatory drugs against Escherichia coli causing urinary tract infection. Afr. J. Microbiol. Res., 10(34), pp.1408–1416, https://doi.org/10.5897/AJMR2016.8127.
34. Padma, R., Yadav, P., 2015, Screening of diclofenac for antibacterial activity against pathogenic microorganisms. Int. J. Adv. Pharm. Biol. Chem., 4(3), pp. 554–558, https://doi.org/10.5281/zenodo.818144.
35. Ferrer-Luque, C.M., 2003, Efficacy of mixed diclofenac solutions against root canal biofilms. Aust. Endod. J., 49(3), pp. 530–536, https://doi.org/10.1111/aej.12684.
36. Ahmed, E.F., El-Baz, A.A., 2017, Antibacterial activity of some non-steroidal anti-inflammatory drugs against bacteria causing urinary tract infection. Am. J. Infect. Dis. Microbiol., 5(1), pp. 66–73, https://doi.org/10.12691/ajidm-5-1-12.
37. Abu-El-Azayem, A.K.M., Khalil, M.I., 2021, Antibiofilm and antibacterial activity of diclofenac against clinical enterococcal isolates. Int. J. Clin. Exp. Med., 14(3), pp. 1507–1515, https://doi.org/10.12998/ijcem.2021.031507.
38. Chockattu, S.J., Deepak, B.S., 2018, Comparison of anti-bacterial efficiency of ibuprofen, diclofenac, and calcium hydroxide against Enterococcus faecalis in an endodontic model: An in vitro study. J. Conserv. Dent., 21(1), 80–84,
39. https://doi.org/10.4103/JCD.JCD_237_17.
40. Abu Naeem, F.M., Mostafa, D., 2022, Evaluation of the antibacterial activity of ibuprofen, triple antibiotic paste and calcium hydroxide as intracanalmedicaments against Enterococcus faecalis in single rooted teeth. A comparative in-vitro study. Egypt. Dent. J., 68(2), pp. 1867–1874, https://doi.org/10.21608/edj.2022.121004.1990.
41. Zięba, A., Zięba, Z., 2022, Materials used for the microencapsulation of probiotic bacteria in the food industry: A review. Eur. J. Nutr. Food Saf., 14(5), pp. 20–35, https://doi.org/10.9734/ejnfs/2022/v14i530503.
42. Jiménez-Serna, A., Hütt, P.H., 2011, Effect of different antibiotics and non-steroidal anti-inflammatory drugs on the growth of Lactobacillus casei Shirota. Curr. Microbiol., 62, pp. 1028–1033, https://doi.org/10.1007/s00284-010-9801-3.
43. Monteros, M.J.M., Galdeano, C.M., 2021, Probiotic lactobacilli as a promising strategy to ameliorate disorders associated with intestinal inflammation induced by a non-steroidal anti-inflammatory drug. Sci. Rep., 11(1), pp. 571, https://doi.org/10.1038/s41598-020-79925-4.
44. Xue, Y., Zhao, H., 2014, Host inflammatory response inhibits Escherichia coli O157:H7 adhesion to gut epithelium through augmentation of mucin expression. Infect. Immun., 82(5), pp. 1921–1930, https://doi.org/10.1128/IAI.01555-13.
45. Otani, K., Tanigawa, T., 2017, Microbiota plays a key role in non-steroidal anti-inflammatory drug-induced small intestinal damage. Digestion , 95, pp. 22–28, https://doi.org/10.1159/000448541.
46. Maseda, D., Ricciotti, E., 2019, Prostaglandin regulation of T cell biology. Pharmacol. Res, 146, pp. 104456,
47. https://doi.org/10.1016/j.phrs.2019.104456.

48. De Freitas RP, G. V.,2017, Effect of the association of nonsteroidal anti-inflammatory and antibiotic drugs on antibiofilm activity and pH of calcium hydroxide pastes. J Endod., 43(1), pp. 131-134. https://doi.org/10.1016/j.joen.2016.09.014
49. Dastidar SG, G. K., 2000, The anti-bacterial action of diclofenac shown by inhibition of DNA synthesis. Int J Antimicrob Agents, 14, pp. 249-251. https://doi.org/10.1016/S0924-8579(99)00159-4
50. Sowmya MR, A. P., 2020, Antimicrobial efficacy of diclofenac sodium against Candida albicans by determining minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) – An in vitro study. J. Pharm. Res. Int., 13(7), pp. 227-233.
51. https://doi.org/10.21786/bbrc/13.7/38
52. Sarwar M.N. U. A., 2021, Evaluating antibacterial efficacy and biocompatibility of PAN nanofibers loaded with diclofenac sodium salt. polymers, 13(4), pp. 510. https://doi.org/10.3390/polym13040510