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RESEARCH ARTICLE

Development and Characterization of Indomethacin Quantum Dot Loaded Hydrogel

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SUMMARY

Inflammation occurring in the wound tissue plays an important role in wound healing. This situation can cause undesirable conditions such as pain and swelling in patients. Indomethacin (IM) is one of the most frequently preferred nonsteroidal anti-inflammatory (NSAID) active substances that suppress the inflammatory response. Indomethacin blocks prostaglandin synthesis by inhibiting the cyclooxygenase enzyme and thus helps support the wound healing process. This study includes hydrogel formulations of quantum dots (IMQDs) prepared from the indomethacin active substance for the first time. Characterization studies of the prepared IMQDs were carried out and particle sizes, % polydispersity indexes, and zeta potentials were measured as 8.53±0.096 nm, 19.49±0.550 %, -8.2±0.781 mV, respectively. Quantum yield % was calculated as 56.48%. Hydrogel formulations containing F1 (0.5%), F2 (0.75%), and F3 (1%) containing carbomer at different concentrations and F1-IMQDs, F2-IMQDs and F3-IMQDs containing 2% IMQDs were prepared. The prepared hydrogels' viscosity, pH, mechanical properties, and spreadability were measured. In vitro release studies of all formulations were performed, and it was seen that the gel drug containing IMQDs exhibited a higher release rate than traditional drugs. At the same time, the gel containing IMQDs showed fluorescent properties. Cell culture studies did not show toxicity. In line with the obtained data, the hydrogel formulations containing IMQDs prepared can be applied to dermal wound healing or anti-inflammatory treatments. This study can be a source for future studies and applications.

Key Words: *Indomethacin, quantum dots, hydrogel.*

İndometazin Kuantum Nokta Yüklü Hidrojelin Geliştirilmesi ve Karakterizasyonu

ÖZ

Yara dokusunda meydana gelen inflamasyon, yara iyileşmesi sürecinde önemli bir rol oynamaktadır. Bu durum hastalarda ağrı ve şişlik gibi istenmeyen durumlara neden olabilmektedir. Indometazin (IM), inflamatuvar yanıtı baskılayan ve sıklıkla tercih edilen nonsteroidal anti-inflamatuvar (NSAIDs) etken maddelerin başında gelmektedir. Indometazin, siklooksijenaz enzimini inhibe ederek prostaglandin sentezini bloke etmekte ve böylece yara iyileşme sürecini desteklemeye yardımcı olmaktadır. Bu çalışma ilk defa indometazin etkin maddesinden hazırlanan KN'lerin (IMQDs) hidrojel formülasyonlarını içermektedir. Hazırlanan IMQDs'lerin karakterizasyon çalışmaları gerçekleştirilmiş olup partikül büyüklükleri, % polidispersite indeksleri ve zeta potansiyelleri sırasıyla 8.53±0.096 nm, 19.49±0.550 %, -8.2±0.781 mV olarak ölçüldü. Kuantum verimi %56.48 olarak hesaplandı. Farklı konsantrasyonlarda karbomer içeren F1 (0.5%), F2 (0.75%) ve F3 (1%) ile %2 IMQDs içeren F1-IMQDs, F2-IMQDs ve F3-IMQDs içeren hidrojel formülasyonları hazırlandı. Hazırlanan hidrojellerin viskozite, pH, mekanik özellikleri, yayılabilirlikleri ölçüldü. Tüm formülasyonların in-vitro salım çalışmaları gerçekleştirildi ve IMQDs içeren jel ilacı geleneksel ilaçlardan daha yüksek bir salım hızı sergilediği görüldü. Aynı zamanda IMQDs içeren jel floresan özellik gösterdi. Hücre kültürü çalışmaları toksisite göstermedi. Elde edilen veriler doğrultusunda hazırlanan IMQDs içeren hidrojel formülasyonları, dermal yara iyileşmesi veya anti-inflamatuar tedavilere uygulanabilir. Bu çalışma daha sonra gerçekleştirilecek çalışmalar ve uygulamalar için bir kaynak olabilecektir.

Anahtar Kelimeler: *İndometazin, kuantum noktaları, hidrojel.*

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INTRODUCTION

The skin is the largest sensory organ that protects our body from external factors. The skin consists of many layers. These layers protect our body from impact, trauma, cold, heat, etc. Wounds occur because of the disruption of the tissue integrity of the skin. Wound healing includes a series of processes carried out by our body to restore skin integrity and repair the damage. Treatments and medications are used to improve this process in wound healing (Baron, Glatz, & Proksch, 2020). Non-steroidal anti-inflammatory drugs (NSAIDs) can be an example of groups applied topically in wound healing. Indomethacin is a non-steroidal anti-inflammatory drug. It is an anti-inflammatory drug that selectively inhibits the cyclooxygenase enzyme (Augusto de Castro et al., 2023). Indomethacin (IM) also has formulations that are applied topically to the skin and eyes in wound healing.

Hydrogels are gels in which the dispersion medium is water. They are structures that can hold significant amounts of water without losing their three-dimensional network properties (Aswathy, Narendrakumar, & Manjubala, 2020). Hydrogels can be produced from polymers with various chemical and physical properties depending on their areas of use (Yan Wang, Zhang, Zhang, & Li, 2012). Many natural, synthetic, and semi-synthetic polymers such as chitosan, alginate, hyaluronic acid, cellulose derivatives, polyvinyl alcohol, and polyethylene glycol can be used in hydrogels (Divyashri et al., 2022).

Quantum dots with dimensions ranging from 1 to 10 nm are semiconductor nanocrystals discovered in the 1980s (Yunqing Wang & Chen, 2011). Their unique optical properties make them more interesting than other nanomaterials. With the development of carbon-based quantum dots in 2004, their potential for use in the health field has also begun to be investigated (Nair, Haponiuk, Thomas, & Gopi, 2020). All attempts in the literature show higher effects and better permeabilities through biological membranes. Therefore, indomethacin was prepared in quantum dots as we already noted in our other studies (Camlik, Bilakaya, Ozsoy, & Degim, 2024).

In our study, hydrogel formulations of quantum dots synthesized from indomethacin active substances were developed. Preliminary characterization studies were completed by performing viscosity, tissue profile analyses, spreadability, *in vitro* release studies, and cellular cytotoxicity in the developed formulations.

MATERIALS AND METHODS

Materials

Indomethacin, urea, and triethanolamine were purchased from Sigma Aldrich (St. Louis, MO, USA). Carbomer was purchased from BioBasic (Canada). All pharmaceutical materials were used in analytical grade. A microwave reactor (Anton Paar Microwave 300, Anton Paar, St. Albans Hertfordshire, AL4 0LA, UK) was used to produce IMQDs. Additionally, particle size, size distribution, and zeta potential were determined using an Anton Paar LiteSizer 500. The optical properties of IMQDs were characterized using a spectrofluorometer (Model 229129, Agilent Technologies, Santa Clara, CA, USA). The formulations' pH measurement and viscosity values were performed using Mettler Toledo S220-K (Switzerland) and Brookfield DV1-LV (UK) viscosimeter, respectively. Texture analyses of the gels were completed on TAXT Plus.

Preparation of indomethacin quantum dots (IMQDs)

IMQDs were prepared by simple microwave synthesis (Monowave 300, Anton Paar, Austria) method. 0.01 g indomethacin, 0.005 g of urea, and 1 ml of distilled water were combined and allowed to react at 150 degrees for 20 minutes. The obtained quantum dots were purified using a 0.22 μm membrane filter.

Preparation of blank and loaded gel formulations

Hydrogels were prepared with various carbomer concentrations (0.5%, 0.75%, 1%). Carbomer and distilled water were added to the beaker and mixed. Cross-linking was achieved by adding 0,25 ml of triethanolamine. (Table 1) shows the formulation components and their quantities.

Table 1. Components of blank and loaded formulations

Components	F1(%)	F1-IMQDs (%)	F2(%)	F2-IMQDs (%)	F3(%)	F3-IMQDs (%)
Carbomer	0.5	0.5	0.75	0.75	1.00	1.00
Triethanolamine	0.25	0.25	0.25	0.25	0.25	0.25
Indomethacin (IM)	2.00	-	2.00	-	2.00	-
IMQDs	-	2.00	-	2.00	-	2.00
Methyl paraben	0.015	0.015	0.015	0.015	0.015	0.015
Distilled Water (q.s.)	100	100	100	100	100	100

Characterization of IMQDs

Particle Size, Polydispersity Index% (PDI%) and Zeta Potential

To characterize indomethacin quantum dots, structure and morphology analysis were performed. Anton Paar Lite Sizer 500 (Austria) was used for particle size, distribution, and zeta potential measurements. Particle size measurements were performed with six replicates, and zeta potential measurements were performed with 12 replicates (Camlik et al., 2024).

Quantum yield % (QY%)

Quinine sulfate solution in 0.1 M sulfuric acid was used as a standard for calculating quantum yield. Measurements were carried out on a Shimadzu RF6000 (Japan) Spectrofluorometer. Equation (1) was used to calculate quantum yield (Alkian, Sutanto, & Hadiyanto, 2022).

$$QYc = QYs \times \frac{Ic}{Is} \times \frac{As}{Ac} \times \left(\frac{\eta c}{\eta s}\right)^2 \quad (1)$$

QY% represents the yield of carbon-based quantum dots, QYs represents the quantum yield of the reference quinine sulfate (0.54), I represents the fluorescence area, A represents the absorbance intensity at 360 nm excitation wavelength, η represents the refractive index, c represents carbon-based quantum dots, and s represents quinine sulfate.

Characterization of IMQDs loaded hydrogel

pH measurements of blank and loaded formulations were carried out in triplicate at ambient conditions using a Mettler Toledo S220-K (Switzerland) device. Viscosity measurements of the developed formulations were carried out using Brookfield DV1-LV (UK) viscosimeter.

Drug Contents

Drug content determination was performed on the Shimadzu RF6000 (Japan) device. 1 ml of gel sample was diluted in 50 ml of distilled water. The analysis was performed on a spectrofluorometer device (Chaudhary, Kohli, Amin, Rathee, & Kumar, 2011). Briefly, excitation was obtained at 560 nm, and emission was detected at 544 nm. The drug was analyzed in the gel using a calibration curve. IMQDs were sequentially diluted with distilled water across an 8-point concentration range from 9 mg/mL to 3 mg/mL. The calibration curve thus obtained had a linear response with a coefficient of determination (R^2) of 0.998.

TPA analysis

Texture analysis of the gels was conducted with a TAXT Plus texture analyzer. For the determination of mechanical properties such as hardness, compressibility, adhesiveness, cohesion, and elasticity of the gels, a 25 mm diameter Perspex probe (P/25P, θ: 25 mm) was utilized. Pre-test speed was 2.00 mm/s, and test and final test speeds were both 2 mm/s with a 0.001 N trigger force. The compression depth in each process was set to 10.00 mm, and the time interval between two compressions was set to 10 seconds. The experiments were all done in triplicate at 25 ± 0.5 °C.

Spreadability

The test sample was placed inside the female cone. The male cone was shifted towards the female cone by

up to 23 mm at a rate of 3 mm/s during the test and then at 10 mm/s for the post-test. The spreadability of the gels was identified in terms of firmness, stickiness, work of shear, and work of adhesion. The testing was conducted under room conditions.

***In vitro* release**

Franz diffusion cells and cellophane membranes were used to determine *in vitro* migration. Cellophane membranes were placed between the receptor and donor chambers of the Franz cells. Franz diffusion cells were used in triplicate for each formulation. Temperature was maintained at 37 °C, and pH 7.4 isotonic phosphate buffer solution was used as the receptor phase. 1 mL of the formulation was loaded into the donor chamber. The donor compartment's top was covered with parafilm to prevent evaporation. The receptor compartment samples were taken at 15, 30, 45, 60, 90, 120, 180, 240, and 300 minutes, and the volume taken was replaced with fresh solution to ensure sink conditions (Camlik et al., 2024).

Cellular toxicity

Cell culture

The immortalized human keratinocyte cell line (HaCaT) was grown in DMEM with 10% fetal bovine serum and 1% penicillin/streptomycin/amphotericin solution. The cell culture media was replaced every two days.

MTT cytotoxicity assay

Formulations F1, F1-IMQDs, F2, F2-IMQDs, F3, and F3-IMQDs were tested for cytotoxicity using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay. Per mL of formulation was dissolved in complete cell culture media, and $1x10^4$ cells/well in 96-well plates were incubated with the solutions at different concentrations. Following 24 h of exposure, the cell culture medium was changed, and cells were incubated with the MTT dye solution. 100 µL of DMSO solution was added to each well to dissolve formazan crystals generated by metabolically active cells. Optical density (OD) at 590 nm was detected using a multimode plate reader (Biotek, Agilent, USA). The absorbance of the control group was set to 100% to represent cell viability.

Stability

The prepared formulations were subjected to stability testing. F1-IMQDs formulations were stored in the refrigerator at 5 °C and at room temperature (25 °C ± 2 °C and 60% relative humidity) for 12 months. Stability measurements were performed using the Zeta Sizer 500 (Anton Paar, Austria).

Statistical evaluation

To evaluate the results in this study, experimental mean values were accepted as mean ± SD. A two-way ANOVA test was used for statistical analysis.

RESULTS AND DISCUSSION

IMQDs were synthesized in a microwave reactor using a bottom-up method. This method was chosen for its advantages, including high efficiency, short reaction time, simplicity, environmental compatibility, and high reproducibility (Hou et al., 2016). The initial and most rapid indication of quantum dot formation was determined through observation under 365 nm UV light (Elugoke, Uwaya, Quadri, & Ebenso, 2024). When examined under 365 nm UV light, IMQDs fluoresced yellowish green (Figure 1).

Figure 1. The physical appearance of IMQDs under UV light (365 nm).

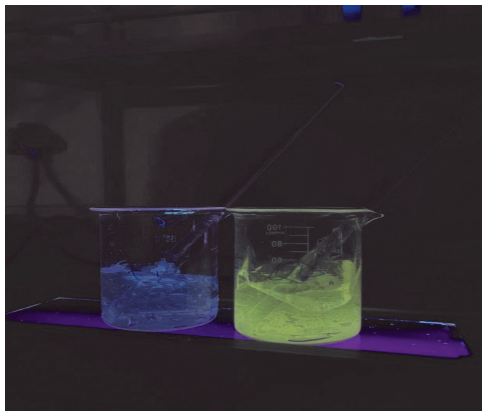

Figure 2. Physical appereances of gels with and without IMQDs under UV light (365 nm).

This study successfully developed hydrogel formulations containing IMQDs. The hydrogel formulations were prepared using a simple method (Figure 2), and IMQDs were subsequently added. Figure 2 presents the formation of IMQDs post-synthesis and the physical appearance of IMQDs in the gel. IMQDs were prepared in a gel that can be applied to the skin for various reasons, including wound healing (Huang, Dan, Dan, & Chen, 2021).

Carbon quantum dots (CQDs) are nanocrystals with unique photochemical and photophysical properties such as particle sizes smaller than 10 nm, continuous, long tunable emission wavelength, high fluorescence stability, low toxicity, and good biocompatibility (Smith, Gao, & Nie, 2004). Characterization studies were also carried out for IMQDs and particle size, percentage of polydispersity index (PDI%), and zeta potentials were found to be 8.53±0.096 nm, 19.49±0.550 %, -8.2±0.781 mV, respectively. The fact that the particle size is below 10 nm further confirms quantum dot formation (Camlik et al., 2024). The value of PDI% being between 10-30 indicates a narrow size distribution, which indicates a homogeneous distribution (Luan, Zheng, Yang, Yu, & Zhai, 2015; Özkahraman, Acar, Gök, & Güçlü, 2014). The QY% of the prepared IMQDs was then calculated as 56.48%, which was accepted as high enough (Albrecht, 2008).

Hydrogel formulations F1 (0.5%), F2 (0.75%), and F3 (1%) were prepared using three different carbomer concentrations (Table 1). Figure 2 shows the image of the empty gel formulation and quantum dot-added gel formulations under UV fluorescence.

Ideal gel formulations are expected to be easy to apply and not run after application. At the same time, the pH of the preparations to be applied to the skin should be suitable for the skin pH. The viscosity and pH values of the prepared formulations are given in Table 2.

Table 2. Viscosity and pH values of formulations

Formulation Code	Viscosity (cP)	pH (24°C)
F1	8920±40.000	5.76± 0.044
F1-IMQDs	8026.67±23.094	5.81±0.165
F2	9333.33±23.094	5.41±0.061
F2-IMQDs	8480±40.000	5.32±0.042
F3	10426.67±23.094	4.97±0.015
F3-IMQDs	9306.67±23.094	4.98±0.084

The pH of the formulations should be in a range that will not irritate the skin and should be prepared in a way that will not alter the efficacy and stability of the drug in the formulation. IMQDs-gel formulations should be compatible with the pH of the application site and should not irritate the skin. The physiological pH of the skin is between 4.1 and 5.8 (Tottoli et al., 2020). It has been observed that the developed formulations are compatible with the skin pH. The pH of the developed F1, F1-IMQDs, F2, F2-IMQDs, F3, and F3-IMQDs gel formulations was between 4.97 ± 0.015 and 5.81 ± 0.165 (Table 2). Viscosity is a very significant parameter in the formulation of most topical products. Failure to achieve the appropriate and desired viscosity while formulating can lead to the drug being unable to achieve the desired effect under the conditions of usage (Jin, Imran, & Mohammed, 2022).

Ideal gel formulations should be easy to apply and not run after application. Viscosity measurements were performed to determine the viscosity and flow properties of the gels. Formulations with low viscosity had a short residence time, while those with higher viscosity had a longer residence time (Binder, Mazál, Petz, Klang, & Valenta, 2019). Therefore, viscosity influences the rate of skin penetration. The developed formulations exhibited viscosities suitable for topical applications.

The viscosity values of the developed formulations were found to vary between 8480±40 cP and 10426.67±23.094 cP. The solvent mixture in all formulations is held in the three-dimensional network of the bonded polymer chains in the prepared hydrogels, and polymeric fibril content increases due to the evaporation of the solvents after the application. In addition, due to the evaporation of the solvents and their absorption into the skin layers, a dense gel network is formed. In the measurements, it is seen that the viscosity increases parallel to the carbomer concentration. Moreover, the viscosity decreases with the addition of IMQDs to the formulation. The intermolecular forces in the polymeric chain network are disrupted by IMQDs, as a result, the viscosity decreases. Depending on the pH and viscosity results, it was found that F2 and F2-IMQDs gel formulation have the appropriate viscosity and pH values (Table 2).

When the active ingredient content of the formulations is examined, it is 96.68%, 94.33%, and 96.75% for F1-IMQDs, F2-IMQDs, and F3-IMQDs, respectively.

Indomethacin-loaded hydrogel formulations were developed with increasing carbomer concentrations. When the viscosity values were examined, it was observed that the viscosity increased with increasing carbomer concentration. The hardness value of the F2-IMQDs formulation was determined to be lower than that of the other formulations. When viscosity measurements were evaluated together, it was found that the F2-IMQDs formulation had lower viscosity than the others. In addition, it can be concluded that the quantum dots added to the blank formulations reduced the viscosity of the formulation and therefore reduced the hardness of the gels. It was found that the F1-IMQDs and F2-IMQDs formulations had similar hardness.

Table 3. Results of TPA

Formulation Code	Hardness (g)	Adhesiveness (g.sec)	Cohesion	Resilience (%)	Springiness (%)
F1	-4.876±1.706	-73.877±10.760	0.894±0.019	13.652±0.006	85.129±0.003
F1-IMQDs	-5.644±0.440	-78.246±3.101	0.878±0.045	14.090±0.015	85.510±0.010
F2	-6.535±1.346	-87.906±13.969	0.922±0.037	14.600±0.012	86.214±0.007
F2-IMQDs	-7.172±0.305	-97.233±7.164	0.894±0.031	14.763±0.014	83.977±0.003
F3	-3.895±2.161	-66.800±7.067	0.925±0.027	16.862±0.018	89.157±0.024
F3-IMQDs	-5.692±2.734	-95.930±25.361	0.887±0.040	15.139±0.027	85.878±0.026

The hardness value indicates the durability of the formulation. A high hardness value is very important for the stability of the formulation. The maximum positive value in the graph indicates the hardness of the formulations. The negative regions seen in the graph indicate cohesiveness (Çağlar et al., 2025).

The mechanical properties of the prepared hydrogels, such as adhesion, cohesion, flexibility, and springiness, were evaluated with TPA (Tian et al., 2024). These properties were assessed by subjecting the formulations to external compressive stress and measuring their capacity to undergo reversible and irreversible deformations. Gel hardness indicates the ease of application of gels to the skin surface, while at the same time, it can indicate how long the gel will remain in the application area. The hardness of the F1, F2, and F3 formulations decreased with the addition of IMQDs, suggesting that IMQDs intercalate

within the polymer network and form a complex. A lower hardness is desirable for easy application and spreadability. Compared to the other formulations, F3 and F3-IMQDs formulations exhibited higher firmness, which suggests that gel hardness increases with carbomer concentration. All the developed formulations showed good firmness, spreading, and stickiness for topical applications.

The formulations also show that all formulations are suitable for skin applications when the adhesiveness, cohesion, flexibility, and durability results are evaluated (Carvalho et al., 2013).

The spreadability values of the developed formulations are shown in Table 4 and Figure 3.

Table 4. Results of spreadability

Formulation Code	Firmness (g)	Work of Shear (g.sec)	Stickiness (g)	Work of Adhesion (g.sec)
F1	474.63±3.920	431.03±12.470	-45.89±4.370	-138.86±3.230
F1-IMQDs	449.41±0.650	430.09±1.060	-425.68±1.190	-113.55±13.060
F2	508.01±11.140	471.84±7.860	-478.41±6.720	-130.42±9.490
F2-IMQDs	477.19±6.830	435.92±13.390	-455.69±5.640	-128.30±6.500
F3	559.79±9.670	513.94±21.060	-518.83±2.010	-144.94±4.570
F3-IMQDs	540.66±4.310	484.89±8.160	-506.55±1.850	-143.92±5.290

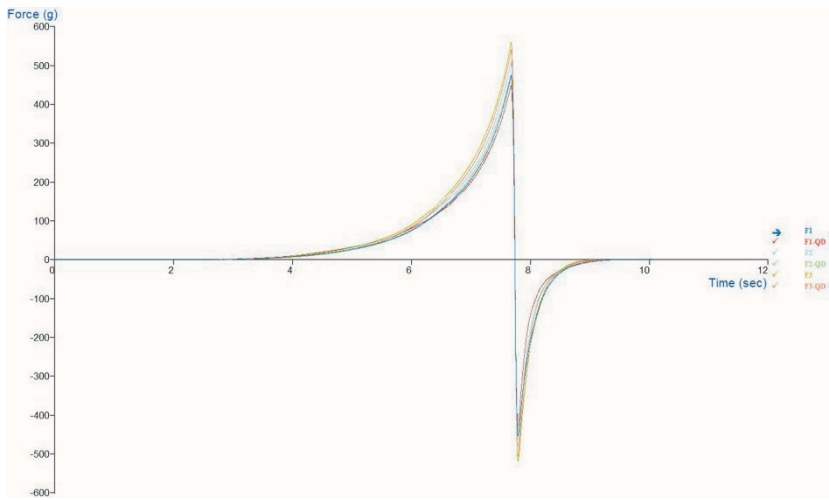

Figure 3. Graphical results of spreadability tests

Spreadability is the ease with which a gel spreads when applied to the skin. The more spreadable the product, the larger the area of skin surface covered by the product when topically applied, and therefore the larger the therapeutic effect of the active ingredient (Kashyap, Das, & Ahmed, 2020).

In vitro release of the formulations was conducted in Franz diffusion cells using phosphate buffer (pH 7.4). Cumulative drug release percentages for the formulations are shown in Figure 4.

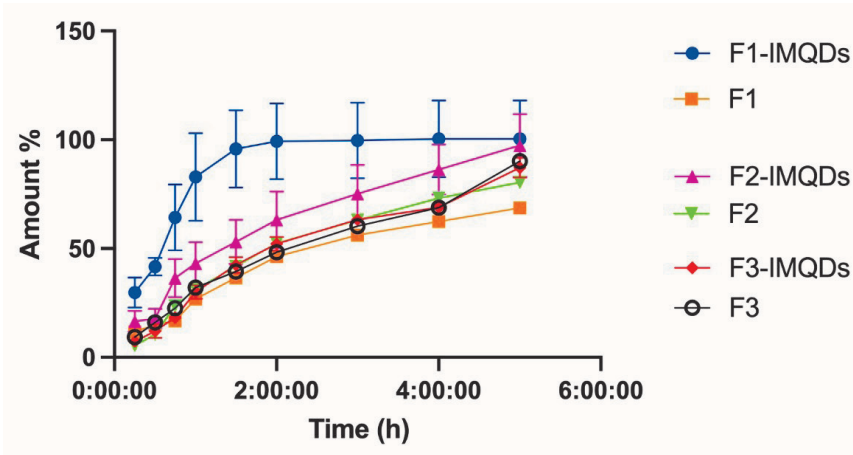

Figure 4. *In vitro* drug release using franz diffusion cells and cellophane membranes.

In vitro release studies are a simple and reproducible method used to examine the release characteristics of a drug from a dosage form (Shah, Elkins, & Williams, 1999). This test provides information about the *in vitro* performance of the drug and allows comparison of its equivalence with other products (Weng, Tong, & Chow, 2020).

Literature reports that indomethacin, a BCS class II NSAID, exhibits low solubility and high permeability. Our study reformed it as carbon quantum dots (CQDs) to improve its dissolution properties. The solubility was not determined because the *in vitro* dissolution properties were the first concern. Therefore, a series of experiments was conducted to assess the dissolution rate, properties, and effectiveness. The obtained dissolution profiles indicated that the CQDs formulation substantially enhanced the rate and extent of indomethacin dissolution, with the max plateau values reflecting the increased solubility.

In *in vitro* release studies, cellophane membranes are preferred because they enable to separate nanoparticles and drug molecules without representing a barrier function from the gels and the passage through the membrane is similar to biological membranes, and also provide consistency between experiments by providing a standard and reproducible barrier, while their inert structure does not interact with formulations, allowing the observed release profile to be attributed directly to the formulation; these release studies are usually performed using a pH 7.4 buffer solution, which mimics the behavior of the drug in the body, its proximity to human physiology, are enable us to predict *in vivo* behavior of the drug and formulation stability, and offers an environment close to blood pH, especially in evaluating systemic absorption (Bhuyan, Saha, & Rabha, 2021; Makarov et al., 2022; Salamanca, Barrera-Ocampo, Lasso, Camacho, & Yarce, 2018).

In vitro release studies are critical for evaluating the safety, efficacy, and quality of the product (Çobanoğlu & Şenel, 2023). All release profiles were compared, and it was observed that the release rate slowed down as the carbomer percentage increased. It can be concluded that the F1 formulation released the drug faster than the F2 and F3 formulations; F2 and F3 released the drug about the same. Therefore, the F1 formulation was selected for further experiments.

Indomethacin was formulated as carbon quantum dots to improve its dissolution properties (Topal, Köse Özkan, & Özkan, 2023). *In vitro* dissolution experiments were conducted to assess the effectiveness of this approach. The obtained dissolution profiles indicated that the carbon quantum dot formulation led to a substantial enhancement in both the rate and extent of indomethacin dissolution, with the plateau values reflecting the increased solubility.

The IC50 value of formulations cannot be calculated, cells showed a minimum of 60% viability even at the highest concentrations.

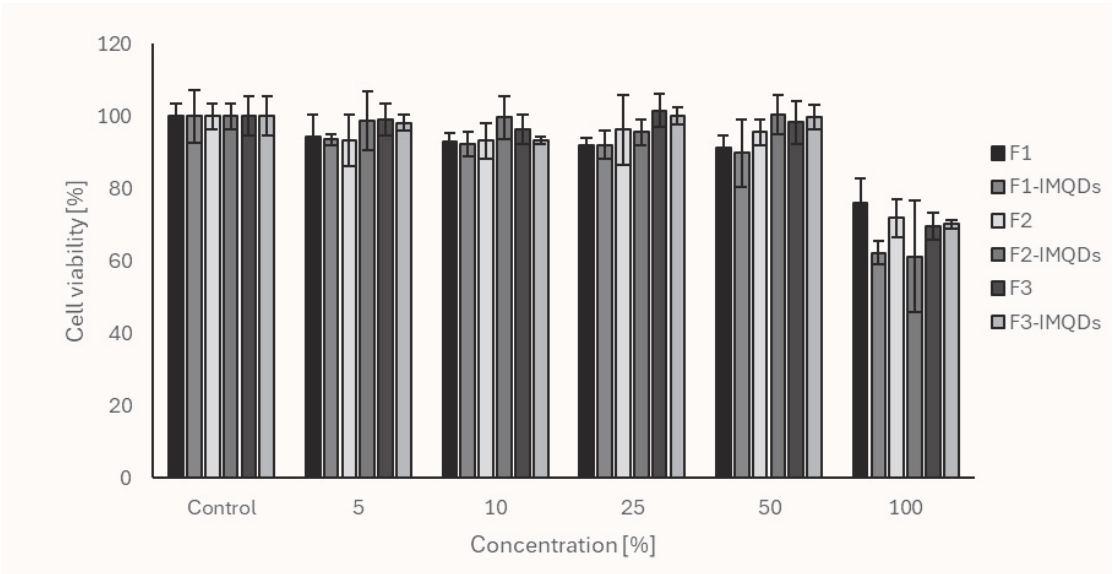

Figure 5. Changes in cell viability on immortalized human keratinocyte cell line (HaCaT) after F1, F1-IMQDs, F2, F2- IMQDs, F3, F3-IMQDs formulations exposure for 24 h.

Indomethacin is a reported NSAID active constituent with cytotoxicity in addition to its therapeutic effect (Harras et al., 2021). Cytotoxicity studies of the formulated preparations were conducted on the human keratinocyte cell line (HaCaT). The results obtained after 24 hours of exposure are shown in Figure 5. The IC50 value of the formulations cannot be calculated since the cells maintained at least 60% viability even at the maximum concentration. The graph obtained indicates the effect of indomethacin-loaded F1, F2, F3, and indomethacin-quantum dot-loaded F1-IMQDs, F2-IMQDs, and F3-IMQDs gel formulation on the cell viability. In Figure 5, no significant toxic effect is generally observed on the cell viability of F1, F2, and F3 formulations. However, there was a notable decline in cell viability when concentrations were greater than 50% for all formulations. It was observed that there was no significant shift in cytotoxicity when concentrations were at 50% levels in F1-IMQDs and F1 formulations. F2-IMQDs formulation was found to be less cytotoxic than F2 formulation when concentrations were 50% or lower. F3-IMQDs formulation was found to be less cytotoxic than the F3 formulation at a 50% concentration. The results depict that quantum dots formed from the active compound are less toxic than the active compound of the same concentration.

Stability tests of F1-IMQDs were carried out in a refrigerator at 5 °C and at 25 °C ± 2 °C and 60% relative humidity. Particle size, zeta potential, and changes in PDI values were checked. Measurements were made at 0, 3, 6, 9, and 12 months. The results showed that IMQDs were stable for 12 months (Table 5, Table 6). No significant differences were observed between the groups. The obtained results showed that the quantum dots were stable for 12 months.

Table 5. Stability test results of 25°C±*2 60% relative humidity*

	0 Month	3 Months	6 Months	9 Months	12 Months
Partical size	8.53±0.095	8.62±0.045	8.55±0.09	8.58±0.081	8.6±0.085
Zeta potential	-8.20±0.781	-8.24±0.201	-8.1±0.625	-8.3±0.745	-8.4±9.687
PDI%	19.49±0.55	19.58±0.25	19.55±0.48	19.62±0.68	20.05±0.72
Drug content %	96.68		96.48		96.38

Table 6. Stability test results of 5°C

	0 Month	3 Months	6 Months	9 Months	12 Months
Partical size	8.53±0.081	8.75±0.092	8.57±0.084	8.6±0.075	8.62±0.078
Zeta potential	-8.20±0.652	-8.34±0.542	-8.21±0.652	-7.56±0.794	-7.86±0.795
PDI%	19.49±0.550	19.70±0.250	19.71±0.670	19.85±0.470	19.96±0.430
Drug content %	96.68		96.50		96.32

CONCLUSION

IMQDs are the first carbon-based quantum dots prepared solely from IM in the literature. IMQDs were successfully prepared by one-step microwave synthesis and showed yellowish-green fluorescence in UV (365 nm) and very small particle sizes under 10 nm. The IMQDs-containing gel demonstrated suitability for dermal applications. This gel exhibited fluorescence and released the drug at a higher rate compared to conventional drug solutions. Cell culture studies show no toxicity, which makes it applicable to dermal wound healing or anti-inflammatory treatments. This study can be a shed for further studies and applications.

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AUTHOR CONTRIBUTION STATEMENT

Hypothesis development (NUO), (ITD), (GC), experimentation (GKG), (BB), (TB), (ESC), (GC), (NUO), preparation of the working text (GKG), (GC), text review (GKG), (GC), (ITD), analysis and interpretation of data (GC), (TB), (NU), (ITD), literature search and reference writing (GKG), (BB), (GC). All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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