DESIGN, CHARACTERIZATION AND BIOCOMPATIBILITY EVALUATION OF POLYMERIC NETWORKS AS CARRIERS FOR INDOMETHACIN MODIFIED RELEASE

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DESIGN, CHARACTERIZATION AND BIOCOMPATIBILITY EVALUATION OF POLYMERIC NETWORKS AS CARRIERS FOR INDOMETHACIN MODIFIED RELEASE (Abstract): Pharmaceutical nanotechnology's progression expands drug versatility, utilizing carrier systems to efficiently deliver active ingredients to targeted tissues for controlled release within effective concentrations. Integrating nonsteroidal anti-inflammatory drugs (NSAIDs) into nano-systems holds potential for enhancing pharmacokinetic properties and diminishing adverse effects. **Aim**: Our study concentrated on formulating nanoparticles embedding indomethacin (IND), characterizing them, exploring drug release dynamics, and assessing their biocompatibility in rats. **Materials and methods:** IND was loaded within copolymeric networks comprising poly(2-hydroxyethyl methacrylate-co-3,9-divinyl-2,4,8,10-tetraoxaspiro [5.5]-undecane) and poly(aspartic acid) (PAS) as a protective colloid, employing a dispersion polymerization approach. Fourier transform infrared spectroscopy (FT-IR) characterized the copolymeric matrices, and spectrophotometric analysis via dissolution method evaluated in vitro IND release. In vivo biocompatibility was gauged by monitoring hematological, biochemical, and immune parameters in rats. **Results:** Our developed nano-systems effectively loaded IND within polymeric matrices. The kinetics of IND release were influenced by copolymer composition, with lower comonomer concentrations extending release duration. The investigated copolymer networks incorporating IND did not elicit significant hematological, biochemical, or immune changes when administered to rats. **Conclusions:** the studied polymer samples exhibited promising in vivo biocompatibility, positioning them as potential candidates for IND modified-release systems with prospective biomedical applications. **Keywords:** INDOMETHACIN, COPOLYMERS, MATRIX, IN VIVO BIOCOMPATIBILITY, RATS.

Nanotechnology is the scientific domain focused on manipulating matter at an almost atomic level to create fresh structures, materials, and devices. This technology holds the potential for significant advancements across various sectors, including medicine, consumer products, and industry. Nanomaterials typically range in size from 1 to 100 nanometers (1), and at this scale, they exhibit distinct properties that profoundly affect their physical, chemical, and biological characteristics (2). Pharmaceutical technology is constantly evolving, engaging in activities such as the preparation and manufacture of medicines to enhance pharmacological properties and minimize adverse effects. This aims to improve patient compliance with therapy and overall satisfaction with medical care (3).

Nanoparticles exhibit a higher cellular uptake efficiency compared to larger micro molecules, making them attractive candidates for efficient transport and delivery systems. In therapeutic contexts, drugs can be either incorporated within the particle matrix or affixed to the particle's surface. An effective drug targeting system should have the capacity to regulate the drug's behavior within the biological environment, particularly its pharmacokinetics (4,5).

Inflammatory diseases impact a considerable global population, and NSAIDs are frequently used in their treatment. In recent years, the utilization of nano-systems incorporating NSAIDs has shown promise in amplifying anti-inflammatory effects while reducing adverse effects (6). However, conventional NSAID treatments often entail weeks of administration, leading to undesired side effects and necessitating repeated dosing. NSAIDs function by inhibiting cyclooxygenase enzymes responsible for converting arachidonic acid into prostaglandins (7). Due to their short half-life and high protein binding, substantial doses of NSAIDs are required for efficacy, leading to adverse effects such as an increased risk of gastrointestinal and cardiovascular complications (8, 9).

In order to address these limitations, there is a growing interest in utilizing systems with dimensions ranging from a few tenths to several hundred nanometers. Nowadays, it is feasible to create biocompatible formulations with precise control over their shape, size, and surface charge, and to obtain innovative drug carrier for modified the NSAIDs release (10-14).

IND is an indole derivative NSAID, possessing anti-inflammatory, analgesic, and antipyretic properties. These effects are achieved by reducing the production of prostaglandins through the inhibition of cyclooxygenases. Notably, it exerts a more pronounced inhibition of COX-1 compared to the COX-2 enzyme (15).

Due to its strong anti-inflammatory properties and moderate analgesic effects, indomethacin is approved for use in both articular and extra-articular inflammations (such as pericarditis, pleurisy, and uveitis), dysmenorrhea, chronic pelvic pain, migraines, persistent arterial canal in newborns, and managing fever from Hodgkin's disease (7, 8). Literature indicates that indomethacin suppresses small intestinal motility and can inhibit stretch-dependent responses in visceral smooth muscle (16, 17), particularly in the gastrointestinal tract, mediated by mechanosensitive interstitial cells of Cajal located in the circular and longitudinal muscle layers (18). Indomethacin has also demonstrated beneficial effects in COVID-19 patients by counter-

acting the virus's mechanisms that exacerbate the disease. These mechanisms involve the inhibition of cyclooxygenases, regulation of the renin-angiotensin system, blocking of pro-apoptotic proteins, reduction of the excessive inflammatory response due to elevated bradykinin levels, and antiviral activity (19, 20)

Indomethacin belongs to Biopharmaceutics Classification System class II, characterized by its low solubility and high permeability (21). Given the limited utility of IND due to its adverse effects, particularly gastric irritation (22), integrating it into nano-systems and optimizing its release could yield several pharmacological advantages. This approach holds promise for reducing gastrointestinal side effects and fine-tuning its anti-inflammatory and antinociceptive effects following administration in laboratory animals (15).

Our **objective** was to develop polymeric networks capable of loading IND as carrier systems to facilitate modified drug release.

MATERIALS AND METHODS

Our research team synthesized matrices through copolymerization of poly 2-hydroxyethyl methacrylate (p-HM) with varying proportions of other polymers. We utilized poly(2-hydroxyethyl methacrylate-co-3,9-divinyl-2,4,8,10-tetraoxaspiro [5,5]-undecane) to create carrier systems for IND, incorporating three different concentrations of undecane (IND-U-1.75, IND-U-3.5, IND-U-10), effectively embedding IND (10 wt% of the copolymer weight, dissolved in ethanol/phosphate buffer solution at pH = 7.4). These macromolecular systems were synthesized via dispersion polymerization using 4,40-azobis (cyanopentanoic acid) as a radical initiator, alongside sodium lauryl sulfate as a surfactant and poly(aspartic acid) as a water-soluble condensation biocompatible and biodegradable polymer, which acted as a colloidal protector (23). This addition enhanced the absorption capacity of the network, bolstered its stability, and conferred resistance to chemical degradation, owing to the highly cross-linked nature of poly(aspartic acid), characterized by an amine-terminated aliphatic polyurea structure. These copolymer systems, incorporating IND, underwent physicochemical characterization. The presence of IND within the copolymer matrices was assessed using FT-IR. The in vitro drug release profile was determined spectrophotometrically via the dissolution method.

The biocompatibility of these nano-systems was examined in vivo by assessing their impact on various hematological, biochemical, and immune parameters in rats. Four groups, each comprising five healthy, non-genetically modified white male Wistar rats, were utilized. These rats were administered the test substances orally in a single dose using an eso-gastric tube. The groups included: C (control, receiving distilled water at a dosage of 0.1 ml/100 g body weight); p-HM (receiving p-HM); IND-U-1.75 (receiving IND-U-1.75); and IND-U-10 (receiving IND-U-10). One day and one week following the administration of the substances, 0.3 mL of blood was drawn from the lateral tail vein for laboratory analyses. The following determinations were conducted: leukocyte formula (including polymorphonuclear - PMN, lymphocytes - Ly, eosinophils - E, monocytes - M, basophils - B), serum levels of aspartate aminotransferase (AST),

alanine aminotransferase (ALT), urea, creatinine, complement, and the phagocytosis capacity of peripheral blood polymorphonuclear neutrophils (PMN), assessed through the nitroblue tetrazolium reduction (NBT) test. These investigations aimed to evaluate the impact of the tested substances on liver and kidney function, as well as the immune defense capacity of the laboratory animals.

The *in vivo* experiment protocol adhered to the guidelines set forth by the Ethics Commission of the "Grigore T. Popa" University of Medicine and Pharmacy from Iasi, aligning with current European ethical regulations (24).

RESULTS

Polymeric networks containing IND were synthesized earlier with varying ratios of comonomers (23). We selected two of these networks for examination concerning their in vivo biocompatibility in rats. The aim is to utilize them subsequently to explore their impacts on inflammatory responses in acute and subacute inflammation models, which are experimentally induced in the laboratory.

Polymeric networks loaded with IND have been synthesized and characterized. FT-IR spectroscopy confirmed the presence of both comonomers, the conformation of the bioactive compound, and the loading process of IND onto the polymeric matrix. The absorption bands at 532 cm^{-1} and 927 cm^{-1}, alongside the shifting of major peaks of the polymers, verified the uptake of the drug by the two distinct networks (fig. 1).

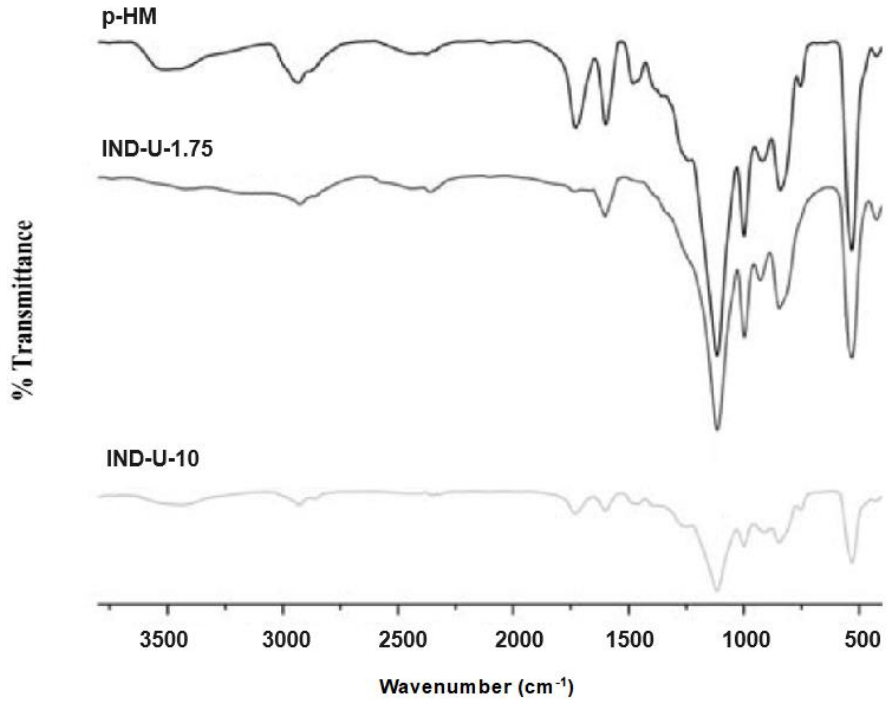

Fig. 1. FT-IR spectra of the copolymeric networks incorporating IND

DISCUSSION

Examining the in vitro kinetic profile of IND reveals that the percentage of undecane significantly impacts the drug release rate from the polymer matrices. Specifically, nearly 60% of the IND quantity is released from the IND-U-1.75 copolymer within the initial 120 minutes, and over 90% within 500 minutes, reaching a release plateau after 750 minutes. Conversely, in the system with a higher concentration of undecane (IND-U-10), the drug is released at a notably slower rate over time, with no observed release plateau even after 2500 minutes (fig. 2).

After one day, as well as after seven days in the experiment, no significant differences were observed in the percentage values of PMN, Ly, E, M, and B between groups C, p-HM, IND-U-1.75, and IND-U-10 (tab. I).

No notable changes were observed in the activity of ALT, AST, urea, and creatinine serum levels among the groups of animals studied. This indicates that the administration of the tested substances did not evidently impact the liver and kidney function at any of the assessment time points (tab. II).

Serum complement activity and phagocytosis capacity of PMN remained relatively stable after both 24 hours and one week in all studied groups. This implies that the investigated nano-systems had minimal impact on the immune defense capacity of the rats (tab. III).

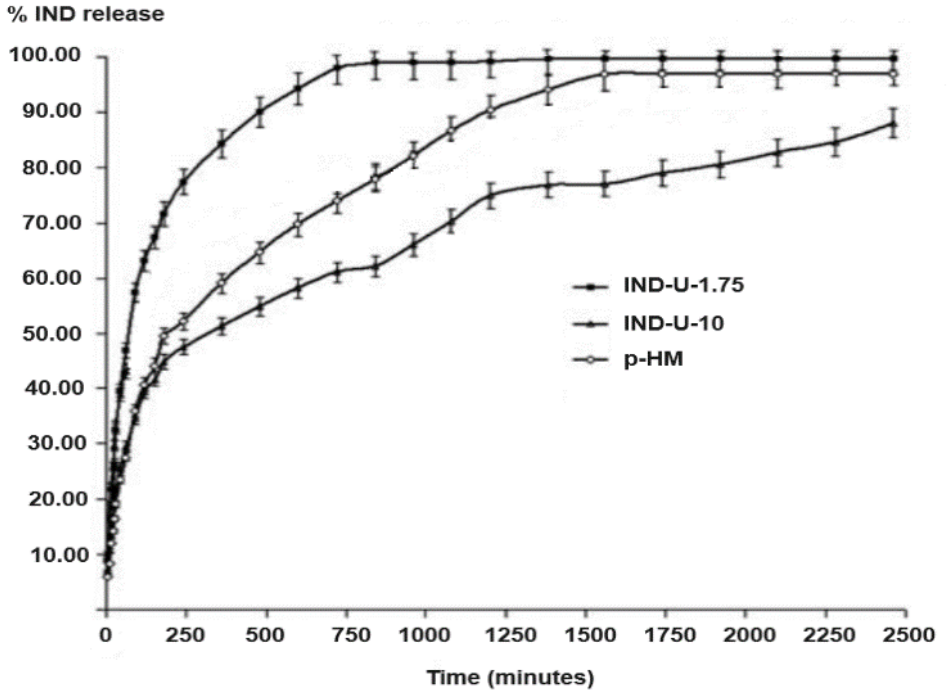

Fig. 2. The in vitro kinetic profile of IND from copolymeric carriers

TABLE I.

Evaluation of the polymeric networks-loading IND influence on the percentages of leukocyte formula elements

		White blood count (%)				
		PMN	Ly	E	M	B
C	1 day	26.32±6.5	67.55±9.1	0.4±0.03	5.34±0.5	0.1±0.01
	7 days	27.16±5.1	66.42±9.5	0.3±0.05	5.38±0.3	0.2±0.01
p-HM	1 day	27.06±5.9	67.38±8.7	0.3±0.05	5.44±0.5	0.2±0.01
	7 days	27.23±5.7	66.46±9.3	0.3±0.03	6.06±0.5	0.2±0.03
IND-U-1.75	1 day	26.59±6.3	67.23±9.5	0.4±0.03	5.23±0.5	0.1±0.01
	7 days	27.63±6.1	67.16±9.1	0.3±0.03	5.32±0.3	0.2±0.03
IND-U-10	1 day	26.38±5.5	67.44±8.7	0.3±0.05	5.46±0.7	0.2±0.01
	7 days	27.47±5.3	66.63±9.3	0.4±0.03	5.14±0.5	0.1±0.01

TABLE II.

Evaluation of the polymeric networks-loading IND influence on the blood values of ALT, AST, urea and creatinine

		ALT (U/ml)	AST (U/ml)	urea (mg/mL)	creatinine (mg/dL)
C	1 day	44.42±5.5	95.52±8.3	38.44±3.5	0.34±0.01
	7 days	44.38±5.3	95.33±9.5	39.14±3.3	0.33±0.02
p-HM	1 day	44.63±5.7	96.48±9.7	39.23±3.7	0.34±0.02
	7 days	45.06±6.1	95.63±9.5	39.63±2.9	0.35±0.01
IND-U-1.75	1 day	44.63±5.3	96.16±8.9	38.63±4.3	0.35±0.01
	7 days	44.87±6.3	96.44±9.7	40.48±3.3	0.36±0.01
IND-U-10	1 day	44.46±5.5	95.59±9.3	39.23±3.7	0.35±0.02
	7 days	45.23±5.7	96.38±9.1	40.06±3.5	0.35±0.01

TABLE III.

Evaluation of the polymeric networks-loading IND influence on the serum complement activity and the phagocytic capacity of PMN

		NBT (%)	Complement (UCH50)
C	1 day	14.33±3.3	56.76±6.37
	7 days	14.59±2.7	57.63±7.29
p-HM	1 day	14.48±3.5	58.44±9.17
	7 days	14.23±3.1	58.16±8.83
IND-U-1.75	1 day	14.06±2.9	57.33±8.29
	7 days	14.63±3.7	58.42±8.32
IND-U-10	1 day	14.16±2.3	57.59±7.22
	7 days	14.42±3.5	58.38±8.47

Considering the restricted application of IND owing to its adverse effects, notably gastric irritation, incorporating it into nano-systems and refining its release mechanism could offer several pharmacological benefits. This strategy shows potential for mitigating gastrointestinal side effects and optimizing its anti-inflammatory and antinociceptive effects upon administration in laboratory animals. Literature sources have revealed diverse strategies for developing nano-formulations incorporating IND, employing either preformed polymers or lipids (25).

Over time, a variety of strategies have been employed to develop nano-systems for the transport and release of IND, including:

- incorporation of phospholipids or lipid emulsions with IND, utilizing lipid, surfactant, and co-solvent mixtures (26,27);
- formulation of liposomal IND, encompassing plurilamellar monophasic vesicles with components such as stearylamines, cholesterol, or chitosan, as well as sterically stabilized liposomes (28,29);
- development of bioconjugates of IND, including triacylglycerides containing indomethacin, bioconjugates with phosphatidylcholine, palmitic acid, and stearic acid (30,31);
- development of enteric nanoparticles containing IND, utilizing materials such as EUDRAGIT® L100, polyethylene glycol, and polysorbate 80 (32);
- preparation of amorphous nanosuspensions containing IND stabilized with polyvinylpyrrolidone, achieved through the aqueous wet milling method (33).
- obtaining of chitosan nanoparticles with IND through the ion gelation method by adding an aqueous solution of tripolyphosphate anions (34).

CONCLUSIONS

Polymeric matrices composed of poly(2-hydroxyethyl methacrylate-co-3,9-divinyl-2,4,8,10-tetraoxaspiro [5,5]-undecane) and poly(aspartic) acid were synthesized as platforms for the controlled release of IND. The kinetic profile of IND is contingent upon the copolymer composition, wherein a lower concentration of comonomer leads to a prolongation in drug release duration. Treatment with both types of copolymer networks incorporating IND did not induce significant hematological, biochemical, or immune alterations, indicating favorable in vivo biocompatibility. This suggests that these polymeric systems hold potential for controlled drug release, with potential applications in the biomedical field.

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All the authors equally contributed to this work.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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