Formulation of Green PGD Polymeric Nanoparticles of Curcumin: Physicochemical Characterization, Stability testing and Docking Study Targeting BTK

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Abstract. Polymers structured as nanoparticles for encapsulating the hydrophobic drugs is a strategy considered as a useful way to improve drug absorption and lower off-target activity. Curcumin is well-known for its anti-inflammatory properties, but it is not clinically used much because it does not dissolve well in water (600 ng/mL), this hampers its targeted delivery and bioavailability. Formulating a green, non-toxic and biocompatible nanoparticle system to retain curcumin utilizing poly(glyceryl succinate-adipate) polymer via a polycondensation process, tackled these challenges and made curcumin a powerful anti-inflammatory agent against many inflammatory diseases as arthritis. The formulation revealed a remarkable increment in solubility and stability in deionized water intended for intra-articular injection. Furthermore, the nanoparticle dimensions were optimized to enhance therapeutic penetration into intra-articular cells. This technology gives drugs the chance to stay in the body longer, release more efficiently, and spread out more evenly than the free drug. The accumulation of curcumin in deep synovial tissues facilitates its interaction with novel targets, such as Bruton's Tyrosine Kinase (BTK), suggesting a potential therapeutic role for curcumin as a BTK inhibitor. This study viewed a full physicochemical characterization for the produced formulation, tested its short-term stability by different concentrations of sodium sulphate, and did a molecular docking analysis to see how well it binds and how useful it is as a treatment.

Keywords. Bruton’s Tyrosine Kinase; Curcumin; Green Poly(glyceryl succinate-adipate); Polycondensation; Nanoparticles; Solubilization.

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1 Introduction

The structure of curcumin is two aromatic rings connected by a seven-carbon chain, its's a turmeric-derived compound recognized for the clear anti-inflammatory, immunomodulatory and antioxidant properties. Curcumin can decrease: i) the pro- inflammatory transcription factors as NF-κB; ii) the pro-inflammatory cytokines; iii) mitogen-activated protein kinases and pathways involved in nitric oxide synthase enzymes synthesis and down-regulate enzymes as LOX -5 and COX-2 [1].

Curcumin structured as pharmaceutical nanoparticles has the ability to reach the kinases deep in the synovial tissues in sufficient concentrations where it may interact with kinases as Bruton's Tyrosine Kinases. In inflammatory diseases as for example: Arthritis, curcumin targets inflammation and oxidative stress within the joint capsule, inhibiting the key inflammatory mediators and reducing the synovial inflammation. This action helps alleviate pain and improve joint function [2].

Unfortunately, these were challenged by curcumin's low bioavailability and water insolubility. To address this, polymeric drug delivery systems are being developed to enhance its stability and targeted release directly to the joint capsule. These systems aim to increase curcumin's efficacy by ensuring sustained and localized delivery, improving outcomes for arthritis treatment.

Formulating an anti-inflammatory agent in polymeric based nano particles is a desirable system for therapeutic nanoparticles to penetrate the superficial layer of the cartilage and allow the local control of the pharmacokinetics of the loaded therapeutic agents. When nanoparticles reach the articular cavity, nano– cartilage interactions occur throughout their transport and penetration within the matrix [3][4][5].

2 MATERIALS AND METHODS

2.1 Materials

Acetone, Adipic acid, Curcumin, Glycerol, Succinic acid, Sodium Sulphate.

2.2 Polymer Synthesis

Many polymers were tested with curcumin loading to reach the end with Poly(Glyceryl Succinate-Adipate) polymer, which shown a remarkable potential with curcumin. Poly(Glyceryl Succinate-Adipate) polymer is green, non-toxic and its reactants are biocompatible. Polyglycerol was synthesized according to Agach et al. [6] with a modification done by mixing Glycerol firstly with the mixture of the two diacids in an equimolar ratio then, the mixture was placed in a round-bottom flask and connected to a vacuum bump to ensure the forward direction by removing the water out of the reaction. Using a hotplate with stirring adjusted to less than 200 ◦C with constant stirring until first observed gelation as a phase change of the mixture to the pre-rubbery state. By reaching this point, this is the termination of the reaction, it was left to reach room temperature [1]. The polymeric product was weighted after cooling in order to determine the reaction yield by the following equation:

$$Polymer\ yield\% = \frac{Total\ Polymer\ weight\ after\ synthesis}{Total\ weight\ of\ all\ reactants} x100 \quad (1)$$

2.3 Polymer Characterization

Polymer characterization by NMR, FTIR, GPC, and GC–MS was not done in our laboratory. Instead, we ensured following the same steps and conditions during polymer production and characterization data and procedures were directly referenced from a previously published

study [7], which used the same polymer synthesis method and identical reagent ratios. NMR analysis was done by the use of acetone-d_6 as the solvent with a 400 MHz instrument.

FTIR spectra were collected by Bruker instrumentation (ranged between 4000 to 400 cm^{-1}, resolution: 4 cm^{-1}). Molecular weight and polydispersity were determined by the use of GPC with PLgel Olexis columns and THF as the eluent, and calibrated against polystyrene standards. The residual monomer analysis was performed by GC–MS after the derivatization with BSTFA and pyridine. These reference data were used to validate that the synthesized polymer under current conditions corresponds to previously confirmed structures [7].

2.3 Curcumin Loading in Polymeric Nanoparticles

The polycondensation nanoprecipitation method was used to load curcumin into PGD nanoparticles, curcumin dissolved in acetone amounts, PGD polymer also was dissolved in the same solvent, left to ensure incorporating curcumin with polymer then, the mixture was injected drop wisely into deionized water as the antisolvent [8]. The produced colloid was left above the stirrer for three hours to evaporate the acetone, leaving the polymeric loaded nanoparticles of curcumin dissolved in deionized water suitable for injection and stabilized without precipitation for a period of 6 months [9].

2.4 Nanoparticles Characterization

Particle Size, Polydispersity Index, Surface Charge and Zeta Potential were measured for all the produced formulations via Dynamic Light Scattering in order to compare between different formulations to determine the best performing formulation for the intended use [10].

2.5 Entrapment Efficiency Calculation

A solution of curcumin was produced using equal amounts of deionised water and high-grade ethanol as a solvent for curcumin, this solution was used to determine the calibration curve, trendline equation and corelation coefficient using different concentrations from the produced sample which measure different absorbances. These parameters will be used for the determination of formulations concentration [11]. The produced formulations were subjected to centrifugation for quarter an hour at refrigeration temperature with pelleting spin force, then the absorbance value for the supernatant was obtained by the spectrophotometer, which was used to determine the concentration of the un-entrapped drug to be incorporated in the following equation [12]:

$$EE\% = \frac{Total\ weight\ of\ curcumin\ added - \ weight\ of\ free\ curcumin\ in\ supernatant}{Total\ weight\ of\ \ curcumin\ added} x100 \quad (2)$$

2.6 Selection of the best formula

The best performing formulation was selected in accordance to many parameters, noting that the EE% value for all produced formulations ranged between 92% and 99% which confirm the extra successful loading of curcumin using the polycondensation method with PGD polymer, this means that EE% values could be omitted from the selection criteria. So, selection was done based on the Particle size, PDI values, Zeta Potential values, surface charges and the final concentration curcumin in the produced formulation.

2.7 Morphology of the selected formulation

Imaging was done on the selected formulation Transmission electron microscopy to confirm the production of the spherical nanoparticles and to reveal its morphology confirm the DLS measurements regarding the average particle size. Few drops of the selected formulation were

loaded on a carbon-coated copper grid after dilution with deionised water, after it was left to dry, the grid then was examined by a high-resolution TEM.

2.8 Stability test of the selected formula

Different concentrations of sodium sulphate solutions with increasing molar concentrations. Equal volumes of the selected formulation were mixed with these different concentrations, then it was incubated at room temperature away from light for half an hour, after that the extent of the turbidity was measured by the UV-Vis spectrophotometer. All measurements done three times; the mean average was calculated to determine the critical coagulation concentration in order to evaluate the salt-induced changes in colloidal stability [13].

2.9 Molecular docking

As established, the formulated curcumin nanoparticles achieve sufficient concentrations in deep tissues to enable effective interaction with Bruton's tyrosine kinase. Structure of Bruton's tyrosine kinase (BTK) was obtained from the RCSB Protein Data Bank, a preparation step for the protein was done by removing the water molecules, adding polar hydrogens, and assigning Gasteiger charges the use of AutoDock Tools. The 3D structure of curcumin was retrieved from PubChem and subjected to molecular energy minimization. Subsequently, the structure was converted to PDBQT format for molecular docking simulations. The molecular docking was performed using Auto Dock Vina. The output of docking included the binding energies, the predicted binding of many poses and comparing the interactions and binding energy of curcumin with the BTK inhibitor Ibrutinib.

3 Results

3.1 Polymer synthesis and characterization

All different polymers produced of different reactant ratios; their reaction yields were around 80%. Because the polymer used in this study was synthesized under the same conditions as those that were reported in [7], the characterization data were used directly. According to the study, the ^{1}H NMR spectrum confirmed the esterification of glycerol with both succinic and adipic acids, showing the characteristic peaks: δ = 2.61 and 2.67 ppm for succinic esters, δ = 1.65 and 2.4 ppm for adipic moieties, and a broad range of peaks from δ = 3.5–4.5 ppm for glycerol units.

FTIR analysis supported the polyester structure, showing key peaks at ~1720 cm^{-1} (C=O stretching) and ~1150 cm^{-1} (C–O–C stretching), along with broad O–H stretching between 3200–3400 cm^{-1}. GPC analysis reported a molecular weight range between 12–13 kDa for PGSA, with a narrow polydispersity index, which is an indication for controlled polymerization. GC–MS analysis showed minimal residual unreacted monomers, supporting efficient conversion. These data were used for reference aims only and were not performed experimentally in this study.

3.2 Curcumin Loading in Polymeric Nanoparticles and Characterization

The continuous stability of the produced formulas was a relevant indictor for the loading success. Multiple polymer batches were evaluated in triplicate across varying drug concentrations to generate a broad range of formulations. These were analyzed using Dynamic Light Scattering (DLS) via a Zetasizer to determine particle size, polydispersity index (PDI), and zeta potential. The optimal formulation was selected based on achieving the highest drug loading capacity while maintaining minimal particle size and maximum

colloidal stability. Among the obtained sizes, it ranged from 100 nanometres to 1 micron, and a PDI values ranged from more than 0.1 to less than 0.8, omitting the values of zeta potential because it recorded a highly acceptable range for drug delivery aims.

3.3 Best formula selection

By neglecting the EE% and the zeta potential values and considering the final drug concentration used in the produced formulation, it would be clear that the best formula should has the smallest size and the highest curcumin concentration. A formula with a size less than 110 nm, a PDI value less than 0.18 which is majorly monodisperse which is acceptable in drug delivery applications, a zeta potential value less than -21 which has a good stability with reduced aggregation risk over time that is suitable for intra-articular injection and finally a formula with highest used drug amount among the three different amounts, this formula will have a strong potential to give the effect that it was designated for.

3.4 Morphology of the selected NPs formulation

In good agreement with DLS measurements, TEM images of the best formula chosen revealed similar particle size as it's presented in Fig. 1. shows that the selected polymeric NPs appeared as spherical structures with an average particle size around 100 nm.

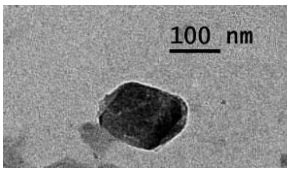

Fig. 1. TEM Image for the selected nano formulation

3.5 Stability test for the selected formulation

As shown in table 1. there was a slight rise in the absorbance values of the formulation with increasing the sodium sulphate concentration until around 0.75M, after that spectrophotometer read an aggressive increment indicating reduction of stability and increasing turbidity suggesting a threshold level of ionic strength that triggered aggregation or structural disruption. Beyond 0.8 M, absorbance values continued to rise, and this an indication of the sustained instability under high salt conditions.

Table 1. Effect of increasing molar concentration of Na_2SO_4 on the stability of the selected formulation

Na_2SO_4 (M)	Absorbance (500 nm)
0.1	0.100
0.2	0.115
0.3	0.135
0.4	0.158
0.5	0.164
0.6	0.204

0.7	0.240
CCC = 0.8	0.692
0.9	0.760
1.0	0.835

3.6 Molecular Docking

The 10 poses of ibrutinib yielded a binding energy range of -11.1 kcal/mol to -9.4 kcal/mol and the binding energy of curcumin with the same target ranged – 8.7 kcal/mol to -7.6 kcal/mol which is a favourable range. Among these ten poses, pose number 2 in Fig 2. was the best and more pharmacophoric, it engages with Glu475 and Thr474 which are critical residues also connected by the BTK inhibitor Ibrutinib. the binding energy of pose 2 which was comparable to competitive BTK inhibitors and within the active binding range of pharmacologically relevant compounds [14].

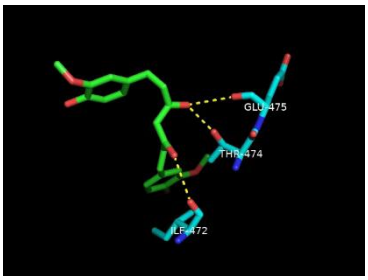

Fig. 2. Pose 2 of curcumin interaction with BTK

4 Discussion

Our investigation into polyglycerol diacid polymers-based nanoparticles for delivering curcumin directly into joints reveals a critical connection between the formulation's physical attributes and its potential therapeutic effect. Take the particle size, for example: it's not merely a lab measurement. Instead, it's fundamental to the system's viability for joint-specific treatment. Particles within the dimensions we achieved are small enough to prevent irritation to the delicate joint lining, yet sufficiently sized to linger in the synovial fluid for an extended duration, avoiding swift clearance. Striking this precise balance is crucial, as it ensures curcumin can target the inflamed area effectively and persist there longer, significantly boosting its ability to quell inflammation.

The selection of polyglycerol diacid polymers is equally important. These materials are known for their excellent compatibility with biological systems and their ability to naturally degrade over time – qualities indispensable for any treatment delivered directly into a joint. Beyond simple biocompatibility, the polymer matrix itself actively modulates the rate at which curcumin is released. Our expectation is that this controlled release will provide a continuous, sustained presence of the anti-inflammatory compound, a significant advantage over the fluctuating concentrations typical of single, rapid doses. This extended action, directly stemming from the polymer's inherent characteristics, represents a key advance towards optimizing curcumin's therapeutic impact within the joint, particularly in overcoming its typical issues with systemic absorption and rapid breakdown.

Ultimately, the physicochemical properties of these nanoparticles – their size and the composition of their polymer casing – are far from incidental. They are integral design

choices that directly dictate how the formulation interacts with the biological environment, thereby influencing its capacity to deliver curcumin efficiently, durably, and safely to arthritic joints. While this study establishes a robust groundwork, our subsequent work will involve rigorous testing of these principles in preclinical models to thoroughly confirm the anticipated therapeutic benefits.

5 Conclusion

This investigation successfully established the foundational principles for utilizing polyglycerol diacid polymers-based nanoparticles as a viable platform for an injectable curcumin formulation, specifically targeting intra-articular administration. The achieved particle size range, critically, falls within the optimal parameters for this route, underscoring its suitability for direct joint delivery. This meticulous control over particle dimensions is paramount, as it directly influences drug localization, residence time, and potential for sustained release within the synovial cavity. The implications of these physicochemical properties extend directly to the anticipated biological efficacy, suggesting an enhanced therapeutic profile over conventional delivery methods. While this work lays a robust practical starting point, the ultimate validation of this product's therapeutic potential will be rigorously pursued and confirmed through comprehensive preclinical studies using induced arthritis models.

Acknowledgments

The authors would like to gratefully acknowledge Egypt Japan University of Science and Technology University, Alexandria, Egypt.

Fundings

This research was funded by Egypt Japan University of Science and Technology University, Alexandria, Egypt.

Data Availability Statement

The data presented in this study is available on request from the corresponding author.

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