In-Vitro Wound Healing and Release Kinetics of β-Cyclodextrin Encapsulated Curcumin Loaded Carrageenan Hydrogel Film: An Efficient Wound Dressing Material

Aswni Sundara Rajan, Kerensa Miriam Sheen, Balaji Sadhasivam, and Nachimuthu Saraswathy(✉)

Department of Biotechnology, Kumaraguru College of Technology, Chinnavedampatti, Coimbatore 641049, Tamilnadu, India
{aswni.15bt, kerensa.15bt, balaji.17phd, saraswathy.n.bt}@kct.ac.in

Abstract. Curcumin has the potential as a therapeutic drug having wider pharmacological application. But due to its lipophilicity, it exhibits a deprived serum level absorption rate, resulting in rapid systemic elimination. Conjugation of curcumin in a polymeric micelle increase the bioavailability of curcumin as a drug delivery system. β-Cyclodextrins, being circular oligosaccharides with an α (1–4) linked α-D-glucopyranose structure, along with a lipophilic center and hydrophilic outer surface, entraps the curcumin in the central cavity. In this present work, encapsulated curcumin was prepared and characterized using FTIR, PSA, Zeta potential, EDAX and TEM. Carrageenan based hydrogel films loaded with β-CD/Curcumin were synthesized as a wound dressing material. Further, to understand release kinetics of encapsulated curcumin in hydrogel film, release behaviour of curcumin was carried out and the data was fitted in different models. Free radical scavenging activity studies showed the potency of β-CD/Curcumin loaded carrageenan hydrogel as a wound dressing material.

Keywords: Curcumin · Release kinetics · Encapsulation · B-cyclodextrin

1 Introduction

A wound is defined as a disruption in the epithelial lining of the skin or mucosal membrane as a result of either physical or thermal processes [1]. These disruptions in the skin membrane are a major risk for re-injury and the more severe reinfection, which is why wound healing is very important. Wound healing is a complex and dynamic process [2] that involves three major phases: the initial inflammatory phase the second proliferative phase and the final maturation phase [3, 4].

An ideal dressing material must be sterile, non-toxic and non-allergic, while also promoting angiogenesis and connective tissue synthesis [5]. Based on their function in the wound, they can be classifying as debridement, anti-bacterial, occlusive, absorbent and adhesive [6]; in case of the type of the material used to make the dressing, they are

M. Rajan et al. (Eds.): ICON 2019, SPM, pp. 107–119, 2019.
https://doi.org/10.1007/978-3-030-25135-2_11

classified as hydrocolloid, alginate and collagen [7] based on the physical form of the wound, they are classified as ointment, film, foam and gel [8].

Traditional wound dressings, such as gauze and cotton become too moist due to excessive wound drainage, adhering itself to the wound and making it difficult and painful to remove. Another disadvantage of gauze dressing is that they provide little occlusion which reduces moisture content, leading to a dehydrated wound bed, causing more injury to tissue [9]. Therefore, wound dressing materials are to be developed that are non-adhesive, more occlusive and more absorbent while also maintaining a good safety profile.

Modern wound dressing materials, like hydrocolloid, alginate and hydrogel-based materials are now more used than traditional dressing materials, because of their ability to create and more importantly, retain a moist environment around the wound, providing better and faster wound healing. But there is a lot of potential in hydrogel-based dressing materials, as they most resemble living tissues than other synthetically-derived systems, especially in their high moisture retentivity [10]. Other advantages in the use of hydrogels are elasticity and their ability to be adapted for the delivery of any drug molecule, regardless of size Hydrogels have also been deemed as "smart materials", because they are able to respond and adapt to changing stimuli in a living system, thus being able to release a drug in the body in a controlled manner [11]. Therefore, it is observed that hydrogels seem to obey all the characteristics of an ideal wound dressing, and because of their high moisture content, they also provide a cool feeling to the wound, enhancing the healing process while also giving comfort to the patient. In our study we have used carrageenan, a set of natural polysaccharides derived from the red seaweed class Rhodophyta [12], with 15 to 40% of ester-sulfate content and an average relative molecular mass well above 100 kDa, are finding novel applications in wound care and drug delivery. Carrageenans are now preferred for use because of their good pharmacokinetic properties, specifically its good biocompatibility, which is being used by researchers to improve drug formulation and prolong drug release for over 24 h [13] to 3 weeks [14]. Besides this, they also possess medicinal properties such as antibacterial, immuno-modulatory, and even anti-viral activity [15, 16], against Hepatitis A virus. Carrageenan is one of the most preferred natural polymers for formation of hydrogels and for encapsulation of pharmaceutical compounds because of its gelling properties. κ-carrageenans are the most commonly used class to be used for manufacturing hydrogels.

Curcumin has the potential as a therapeutic drug having wider pharmacological application. But due to its lipophilicity, it exhibits a deprived serum level absorption rate, resulting in rapid systemic elimination. Curcumin has been shown to possess significant anti-inflammatory, anti-oxidant, anti-carcinogenic, anti-mutagenic, anti-coagulant and anti-infective effects. Due to these properties and many more it has good wound healing properties. While curcumin has medicinal benefits that make it suitable for wound healing, it faces one major drawback. Being hydrophobic in nature, it cannot be administered orally as the metabolites it forms in the body are not stable and get easily excreted. For it to be suitable as part of a wound dressing material, it must have the ability to prolong its release into the body. In our study we look at encapsulation of curcumin molecules in suitable drug carriers like β-cyclodextrin (BCD) as a suitable method for prolonging the drug release.

Although curcumin has many medicinal benefits, a great limitation of its use in drug delivery applications is its poor bioavailability. Aggarwal and Sung [17] reviewed studies performed in both humans and animals and observed that curcumin when administered orally was very poorly bioavailable with little to no levels of curcumin metabolites present in serum tissue. These metabolites have a very short release time and are quickly eliminated from the body. Curcumin's low bioavailability is due to its hydrophobic nature, so it cannot be taken orally without some modifications to its structure to make it more hydrophilic. Many approaches are available to increase curcumin's bioavailability in aqueous solutions. Chemically modified structures of curcumin have also been developed. Many studies have been performed to demonstrate the different types of modifications that can be made to the structure of curcumin, as Vyas et al. [18] illustrated in the following Fig. 1:

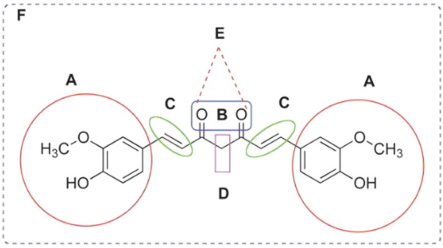


Fig. 1. Structural modification approaches to chemically modify curcumin.

Reviewing studies on chemical modifications of curcumin, there are five approaches that can be taken:

i. Modifying the aryl side chains on either side of the curcumin structure (A);
ii. Modifying the di-keto functionality (B);
iii. Modifying the double bonds (C);
iv. Modifying the active methylene functionality (D);
v. Modifying the complexing activity of curcumin by binding it to metal complexes (E).

All these approaches lead to structural analogues of curcumin, that have better bioavailability when compared to free curcumin.

Micelles are aqueous dispersions of self-assembled aggregates of surfactant or block co-polymers in the size range of 5–100 nm [19], making it suitable for oral administration of hydrophobic drugs such as curcumin in the body. They can be manufactured easily using various simple techniques such as dissolution, dialysis, and oil-in-water emulsions. This method is quite useful for curcumin to increase its bioavailability in the body [20]. The nano-micellar approach of increasing the bioavailability o can be considered as a better and more cost-economical approach as compared to the structural analogue approach explained previously. Nano-particle formulations (NP) can offer more effective drug delivery mechanisms than conventional drug delivery systems [21].

β-Cyclodextrins, are circular oligosaccharides with an α (1-4) linked α-D-glucopyranose structure, with a lipophilic center and hydrophilic outer surface, hence β-Cyclodextrins can form inclusion complexes with a wide variety of hydrophobic molecules. [22] For β-cyclodextrin (BCD), there has been much work on its encapsulation with curcumin being used to check its anti-cancer activity. Compatibility studies show that the self-assembly of BCD and free curcumin (BCD-Cur) shows great biocompatibility in aqueous solutions, with an uptake in cells being more than 0.6 mg/ml. This is because the self-assemblies are stable when produced in a solvent evaporation technique. In prostate cancer cell lines, a formulation of β-cyclodextrin encapsulated curcumin showed great promise, with cellular uptake of BCD-Cur being significantly more than free curcumin [23].

2 Materials

Curcumin was purchased from Sigma-Aldrich, Beta Cyclodextrin and L-Carrageenan purchased from HiMedia labs. LR grade Carbinol, Tween®20 and Glycerol purchased from HiMedia. All tests were performed using autoclaved distilled water.

3 Methods

3.1 Preparation and Optimization of Gel Films

2% w/v and 1% w/v L-Carrageenan gels were prepared by sol-gel method. Stock solutions of 2% w/v and 1% w/v L-Carrageenan were first prepared in distilled water and heated till clear solution was obtained. One set of gels were prepared with 0.5% w/v glycerol solution and one set was prepared without glycerol. These solutions were then cast and left to set overnight. After the gels were set, they were left to dry in the hot air oven for 6 h. The dried films were then observed for differences in texture.

3.2 Encapsulation/Preparation of β-Cyclodextrin Curcumin Nano Micelle

The ratio for β-cyclodextrin (BCD) and Methanolic Curcumin (MC) was optimized to be 11.3:0.36 mg/ml. The first stock solution of 11.3 mg/ml BCD was prepared in distilled water for 50 ml, next stock solution of 0.36 mg/ml MC was prepared for 50 ml. The stock solution was transferred to a brown bottle and prepared to be mounted on magnetic stirrer. The MC solution was added dropwise whilst continuous stirring, after which 20 µl of Tween20 was added, and the bottle was recapped. The stirring continued for 24 h. After 24 h a colloidal solution appeared. This solution was centrifuged at 5000 rpm for 15 min, and the pellet was left to dry in hot air oven at 55 °C till it became dry. The pellet NMC was scraped out and stored in dry conditions.

3.3 Preparation of NMC-Hydrogels

2%w/v L-Carrageenan gels were prepared by sol-gel method. A stock solution of 2% w/v L-Carrageenan were first prepared in distilled water and heated till clear solution was obtained. 1 mg/ml β-cyclodextrin encapsulated curcumin (BCD-Cur) was suspended in distilled water. These suspensions were mixed with their respective gel solutions before casting. The castings were left to set overnight.

3.4 Encapsulation Efficiency

1 mg/ml stock solution of the NMC procured was prepared in methanol and OD checked at 420 nm in a UV-Visible spectrophotometer. The solution was serially diluted till an accepted OD value was reached. The following formula was used to calculate the encapsulation efficiency of βcyclodextrin,

$$Drug\ encapsulation\% = \left[\frac{Amount\ of\ curcu\ \min\ encapsulated}{Amount\ of\ curcu\ \min\ used}\right] X\ 100$$

3.5 Particle Size Analysis and Zeta Potential

The particle size and zeta potential of the NMC was evaluated by using Malvern® Mastersizer 2000-Particle Size Analyser and Nano-range Zetasizer.

3.6 Release Studies

The films were cut into 2 × 2 cm squares. Each square was suspended into 5 ml of distilled water. Samples of 1 ml were drawn every hour and the solutions were replenished with 1 ml of fresh distilled water. The samples were taken, and absorbance was measured via UV-visible spectrophotometer at 420 nm. This process was repeated until the gel lost its integrity. The release kinetics was studied by using the open-access software KinetDS3.

3.7 Swelling Studies

The films were cut into 2 × 2 cm squares. Each square was suspended into 5 ml of distilled water. Once every hour the gel was taken out of the solution, blotted dry and weighed. The gels were then immersed in fresh distilled water. This process was repeated until the gel lost its integrity.

3.8 Free Radical Scavenging Activity

Curcumin is a good anti-oxidant and will reduce 1,1-Diphenyl-2-picrylhydrazil (DPPH), hence it is a good free radical scavenger. Scavenging activity was found for both the compound as well as the film. Stock of 1 mg/ml of the compound was prepared in methanol. Different concentrations (20 μl–100 μl) were then pippeted into test tubes, 3 ml methanolic DPPH was added into each test tubes and incubated in dark

for 30 min. OD of each concentration were taken at 520 nm. The films were immersed in 3 ml of methanolic DPPH and OD were taken every 10 min for 1 h at 520 nm. The ant-oxidant activity of the NMC was calculated by using the formula,

$$Scavenging\ Activity = \left[\left(\frac{OD_{control} - OD_{sample}}{OD_{control}}\right) - 1\right]$$

4 Results and Discussion

4.1 FT-IR

Fourier Transform-Infra-Red (FT-IR) spectroscopy measures a sample's absorbance of infra-red light at various wavelength to determine the material's composition and structure.

The FT-IR spectrum of methanolic curcumin showed a broad peak at 3319.49 cm^{-1}, indicating the phenolic OH stretch long; broad peak at 1450.47 cm^{-1} indicates the C-O stretch in the aromatic ring. The FT-IR showed signature peak at 3280.92 cm^{-1} indicating the OH stretch an arrow peak at 2108.20 cm^{-1} and a broad peak at 1645.28 cm^{-1} representing the C-C stretch in the aromatic ring. All peaks of the β-cyclodextrin-curcumin coincide the peaks of β-cyclodextrin. Peak at 1014.56 cm^{-1} and 112.43 cm^{-1} coincide with the peaks of curcumin. There is modification at peaks 3319.49 cm^{-1}, 2831.50 cm^{-1}, 114.86 cm^{-1} and 1024.20 cm^{-1} of curcumin in the encapsulated curcumin FT-IR spectrum, indicating the bonding of β-cyclodextrin and curcumin (Fig. 2).

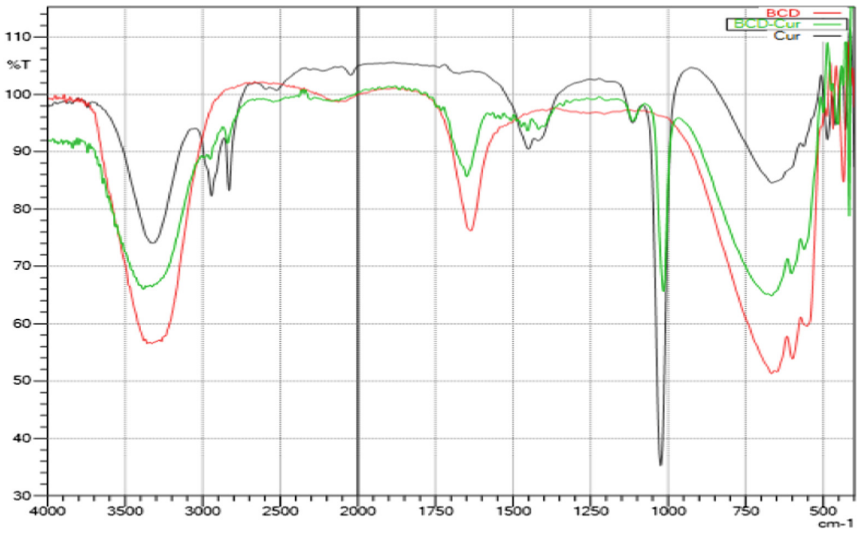


Fig. 2. FT-IR spectrum of β-cyclodextrin-curcumin

4.2 Texture Studies

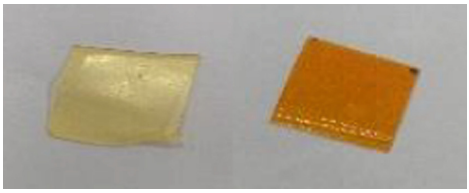
After the gels were set, they were left to dry in the hot air oven for 6 h. The dried films were then observed for differences in texture (Table 1).

Table 1. Texture Studies of the prepared gel films

Hydrogel film	Observation
1% carrageenan	Brittle, may crack on touch, feels light, is not elastic, very thin, clear, smooth
1.5% carrageenan	Brittle, is not cracked when touched, is slightly elastic, thin, clear, smooth
2% carrageenan	Slightly brittle, slightly elastic, clear, smooth
1% carrageenan + 0.5% glycerol	Flexible, does not crack when touched, crease formation when bent, elastic, clear, smooth
1.5% carrageenan + 0.5% glycerol	Flexible, more elastic than previous, elastic, clear, smooth
2% carrageenan + 0.5% glycerol	Most flexible, thicker than all, most elastic, clear, smooth

4.3 Film Formation

The hydrogel prepared from L-carrageenan and encapsulated curcumin, was left to dry for 6 h in a hot air oven at 55 °C (Fig. 3).



(i) (ii)

Fig. 3. 2% Carrageenan hydrogel films incorporated with encapsulated curcumin. (i) β-cyclodextrin encapsulated curcumin (ii) free curcumin.

4.4 Drug Encapsulation Efficiency

The main motive behind encapsulation of curcumin was to increase its hydrophilicity. β-cyclodextrin has a hydrophilic exterior and a polar cavity that is slightly hydrophobic. The curcumin is hypothesised to interact with the β-cyclodextrin molecule inside its cavity. The molecular weight of β-cyclodextrin is 1134.9 g/mol and the diameter of the inner cavity is approximately 60 nm [22]. For a working volume 50 ml each of the compound and the drug the encapsulation efficiency was calculated using the above-mentioned formula, equalled to 88.76% for β-cyclodextrin-curcumin.

4.5 Particle Size

The smaller size of a particle higher the surface area for reaction. The particle size of the NMC was identified using Malvern® 2000- Particle Size Analyser. The range of particle size of β-cyclodextrin encapsulated curcumin is 110–800 nm in diameter, with one peak observed at 311.5 nm, and the average particle size is 345.2 nm in diameter (Fig. 4).

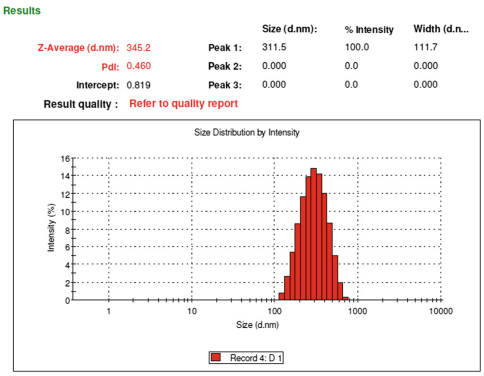


Fig. 4. Report of particle size of β-cyclodextrin-curcumin

4.6 Zeta Potential

Zeta potential indicates the electrostatic interactions of the particles in a fluid environment. This term is used to for determining the surface charge of nanoparticles. The zeta potential of the NMC was identified using Malvern® Nano-range Zetasizer. The zeta potential for β-cyclodextrin encapsulated curcumin was found to be −24.9 mV. The negative charge indicates that at the surface of the molecule, aligning of the amphilic β-cyclodextrin are such that the un-substituted −OH groups are pointing upwards towards the aqueous surrounding, rendering a potential surface hydrophobicity (Fig. 5).

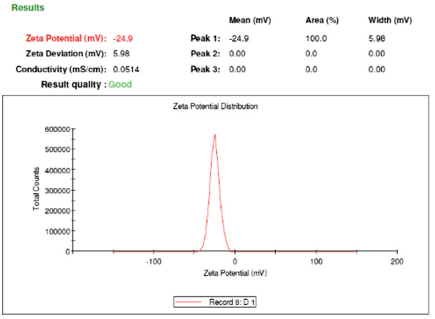


Fig. 5. Report of zeta potential of β-cyclodextrin- curcumin

4.7 Release Kinetics

As mentioned in the methodology the release kinetics for both the films were recorded and plotted to find the trend, the data were then put into the open-access software KinetDS3 to identify the model of release. Michaelis Menten (refer Fig. 7) was the best fit for the release of encapsulated curcumin from film. The dissolution rate for β-cyclodextrin-curcumin is slow and sustained (Fig. 6).

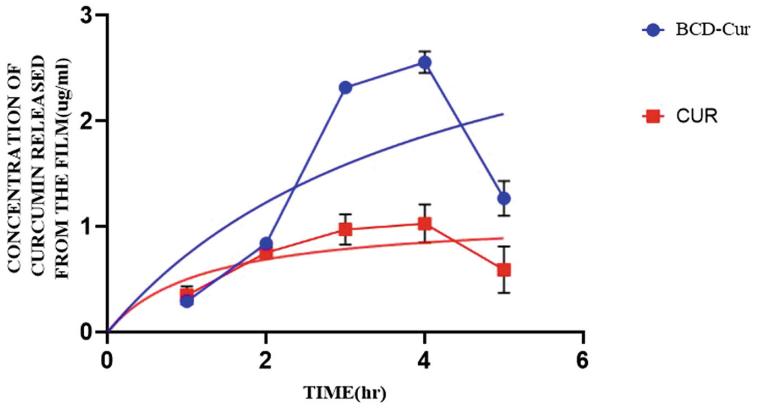


Fig. 6. Graph showing the trends in release kinetics of β-cyclodextrin encapsulated curcumin and free curcumin incorporated in 2% carrageenan hydrogel film

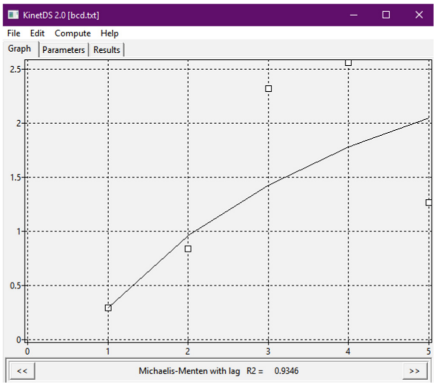


Fig. 7. Output of the KinetDS3 for β-cyclodextrin encapsulated curcumin

The equation governing the rate of dissolution is y = ymax*(x-lag)/(Km + x-lag).

4.8 Swelling Studies

Hydrogels have the excellent ability to retain high amount of water into them. Swelling of the hydrogel films were carried out as mentioned in the methodology. There was 6 h stability for a 2 × 2 cm film. Carrageenan hydrogel are composed of polymer chains containing charged groups, mainly sulfate groups [24]. The sulfate groups are ionizable and take up negative charge. The same negative charges repel each other and increase the distance between the chains and thereby increasing the network of the gel to take in more water. The hydrogel attained its equilibrium at the third hour and lost its integrity at the sixth hour. The β-cyclodextrin encapsulated curcumin film follows a stable swelling rate with a peak at the 3rd hour, unlike free curcumin incorporated film which sharply declines as the gel integrity is lost (refer Fig. 8).

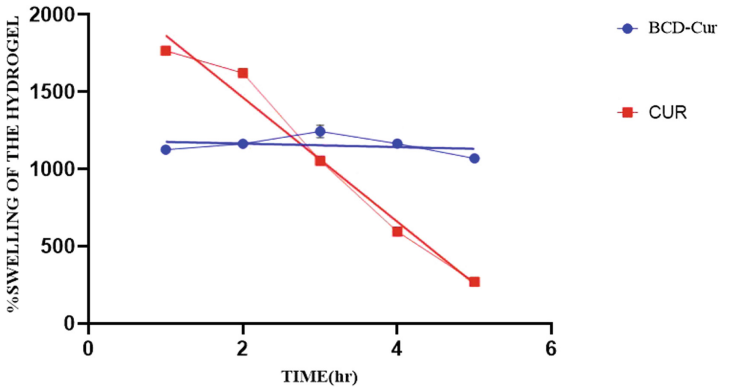


Fig. 8. Graph showing the trend of the swelling studies of β-cyclodextrin-cyclodextrin curcumin and free curcumin compounds.

4.9 Free Radical Scavenging Activity

1,1-Diphenyl-2-picrylhydrazil (DPPH) is a stable free radical that accepts an electron or hydrogen radical to become a stable dimanetic molecule. Decolorization of stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) in the presence of anti-oxidant was noted to evaluate the radical scavenging activity of the test compounds. Curcumin exhibits ant-inflammatory activity by generating reactive oxygen species. This property of curcumin is due to the presence of functional groups like two each of hydroxyl, methoxy and phenyl groups. β-cyclodextrin-curcumin has a lowered anti-oxidant activity compared to curcumin ($p < 0.005$) (Figs. 9 and 10).

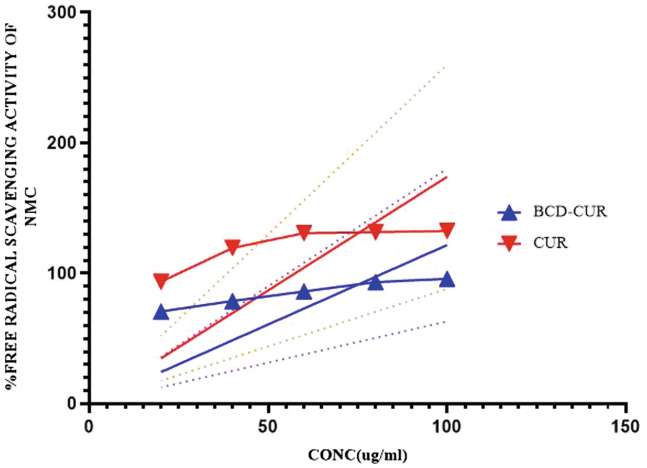


Fig. 9. Graph showing the trend in anti-oxidant activity for β-cyclodextrin and free curcumin compounds. *CONC (μg/ml)—Concentration of test compounds in μg/ml

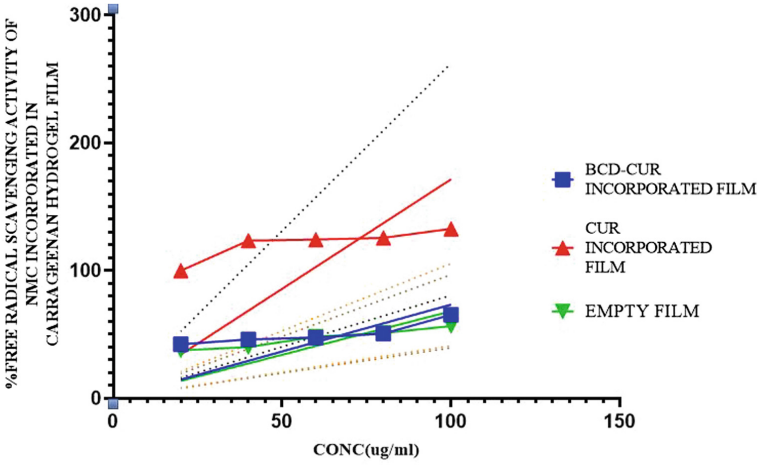


Fig. 10. Graph showing the trend in anti-oxidant activity for β-cyclodextrin and free curcumin when incorporated in carrageenan hydrogel film. *CONC (μg/ml)—Concentration of test compounds in μg/ml

5 Conclusion

The main motive of this research was to increase the hydrophilicity of curcumin. Previous researches have shown that hydrophilicity can be increased by either chemical modification, encapsulation or nano micelle formation. Curcumin is a novel drug which has wide therapeutic uses, the only drawback being less bio-available and hydrophobic. The need for the hour would be to overcome these problems in a cost effective and

efficient manner. β-cyclodextrin is a seven membered glucopyranose, toroidal shaped molecule with hydrophobic interior and hydrophilic exterior. It is a natural product derived from the enzymatic conversion of starch. It is assumed that the curcumin is encapsulated inside the cavity of the β-cyclodextrin. It showed impressive encapsulation of the curcumin, 88.76% with a yield of 16.42 mg/ml for a working volume of 50 ml. The particle size is the significant factor when it comes to nano micelle, it was reported good quality, within 500 nm, also the stability of the NMC is good with negative surface charge (within −30 mV). The chemical reaction between the two compounds and curcumin were analysed from the FT-IR spectrum. β-cyclodextrin-curcumin incorporated carrageenan hydrogel film showed slow and sustained release but there was not much effect in the swelling of hydrogel.

References

1. Robson, M.C., Steed, D.L., Franz, M.G.: Wound healing: biologic features and approaches to maximize healing trajectories. Curr. Probl. Surg. **2**, 72–140 (2001)
2. Shankar, M., Ramesh, B., Kumar, D.R., Babu, M.N.: Wound healing and it's important—a review. Der Pharmacol. Sinica **1**, 24–30 (2014)
3. Vanwijck, R.: Surgical biology of wound healing. Bull. Mem. l'Acad. Med. Belgique **156**, 175–184 (2001)
4. Tarnuzzer, R.W., Schultz, G.S.: Biochemical analysis of acute and chronic wound environments. Wound Repair Regener. **4**, 321–325 (1996)
5. Dhivya, S., Padma, V.V., Santhini, E.: Wound dressings–a review. BioMedicine **5** (2015)
6. Purna, S.K., Babu, M.: Collagen based dressings–a review. Burns: J. Int. Soc. Burn Injur. **26**, 54 (2000)
7. Queen, D., Orsted, H., Sanada, H., Sussman, G.A.: Dressing history. Int. Wound J. **1**, 59–77 (2004)
8. Falabella, A.F.: Debridement and wound bed preparation. Dermatolog. Ther. **19**, 317–325 (2006)
9. Boateng, J.S., Matthews, K.H., Stevens, H.N., Eccleston, G.M.: Wound healing dressings and drug delivery systems: a review. J. Pharmac. Sci. **97**, 2892–2923 (2008)
10. Gupta, P., Vermani, K., Garg, S.: Hydrogels: from controlled release to pH-responsive drug delivery. Drug Discov. Today **7**, 569–579 (2002)
11. Traitel, T., Goldbart, R., Kost, J.: Smart polymers for responsive drug-delivery systems. J. Biomater. Sci. Polym. Ed. **19**, 755–767 (2008)
12. Liu, J., Zhan, X., Wan, J., Wang, Y., Wang, C.: Review for carrageenan-based pharmaceutical biomaterials: favourable physical features versus adverse biological effects. Carbohyd. Polym. **121**, 27–36 (2015)
13. Pavli, M., Vrečer, F., Baumgartner, S.: Matrix tablets based on carrageenans with dual controlled release of doxazosin mesylate. Int. J. Pharmac. **400**, 15–23 (2010)
14. Grenha, A., Gomes, M.E., Rodrigues, M., Santo, V.E., Mano, J.F., Neves, N.M., Reis, R.L.: Development of new chitosan/carrageenan nanoparticles for drug delivery applications. J. Biomed. Mater. Res. Part A: Off. J. Soc. Biomater. Jpn. Soc. Biomater. Aust. Soc. Biomater. Korean Soc. Biomater. **92**, 1265–1272 (2010)

15. Mert, T., Sahin, M., Sahin, E., Yaman, S.: Anti-inflammatory properties of Liposome-encapsulated clodronate or Anti-Ly6G can be modulated by peripheral or central inflammatory markers in carrageenan-induced inflammation model. Inflammopharmacology 1–10 (2019)
16. Koenighofer, M., Lion, T., Bodenteich, A., Prieschl-Grassauer, E., Grassauer, A., Unger, H., Fazekas, T.: Carrageenan nasal spray in virus confirmed common cold: individual patient data analysis of two randomized controlled trials. Multidisc. Respirat. Med. **9**, 57 (2014)
17. Aggarwal, B.B., Sung, B.: Pharmacological basis for the role of curcumin in chronic diseases: an age-old spice with modern targets. Trends Pharmacol. Sci. **30**, 85–94 (2009)
18. Vyas, Alok: Perspectives on new synthetic curcumin analogs and their potential anticancer properties. Curr. Pharm. Des. **19**, 2047–2069 (2013)
19. Lu, Y., Park, K.: Polymeric micelles and alternative nanonized delivery vehicles for poorly soluble drugs. Int. J. Pharm. **453**, 198–214 (2013)
20. Ipar, V.S., Dsouza, A., Devarajan, P.V.: Enhancing Curcumin Oral Bioavailability ThroughNanoformulations. Eur. J. Drug Metabol. Pharmacokinet. 1–22 (2019)
21. Yadav, P., Bandyopadhyay, A., Chakraborty, A., Sarkar, K.: Enhancement of anticancer activity and drug delivery of chitosan-curcumin nanoparticle via molecular docking and simulation analysis. Carbohydr. Polym. **182**, 188–198 (2018)
22. Rachmawati, H., Edityaningrum, C.A., Mauludin, R.: Molecular inclusion complex of curcumin–β-cyclodextrin nanoparticle to enhance curcumin skin permeability from hydrophilic matrix gel. AapsPharmscitech **14**, 1303–1312 (2013)
23. Yallapu, M.M., Jaggi, M., Chauhan, S.C.: β-cyclodextrin-curcumin self-assembly enhances curcumin delivery in prostate cancer cells. Colloids Surf. B: Biointerfaces **79**, 113–125 (2010)
24. Distantina, S., Fadilah, F., Kaavessina, M.: Swelling behaviour of kappa carrageenan hydrogel in neutral salt solution. World Acad. Sci. Eng. Technol. Int. J. Chem. Mol. Nuclear Mater. Metall. Eng. **10**, 998–1001 (2016)
25. Irie, T., Otagiri, M., Sunada, M., Uekama, K., Ohtani, Y., Yamada, Y., Sugiyama, Y.: Cyclodextrin-induced hemolysis and shape changes of human erythrocytes in vitro. J. Pharmac.-Dyn. **5**, 741–744 (1982)