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SOLUBLE POLYMER-CURCUMIN ENCAPSULATION TO PROTECT AGAINST GAMMA IRRADIATION AND INCREASE THE WATER SOLUBILITY

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Abstract: Curcumin is a polyphenol derived from turmeric, a herbaceous plant native from Asia, which has been studying for medicinal properties. Over the years, different civilizations have used those plants to treat or prevent bacterial diseases. Technological advances have made it possible for scientists to study the activity mechanisms, as well as properties derived from these mechanisms, known as medicinal plants. These studies have confirmed that turmeric's medicinal properties are derived from its polyphenols, which in turn can be identified, isolated and used more efficiently. Despite curcumin's antimicrobial benefits, its highly hydrophobic molecule affects its use in biological systems, as well as its bioavailability in humans and animals. The process of modifying a molecule allows changes to be made to its characteristics, benefiting its use; in this context, encapsulation with polymers with amphiphilic characteristics, such as PVP K30, presents itself as a viable alternative for greater affinity with biosystems. The encapsulate curcumin, called C-PVP K30, proved to be possible and effective, keeping the molecule stable and in nanometric dimensions, based on results from DLS and ZETA analyses. Microscopy analysis (SEM-FEG) showed morphologically spherical and dispersed particles with small points of agglomeration. The successful encapsulation of this active substance allowed the solution to be studied under gamma radiation. The results obtained by FTIR and UV-Vis show that this process was unable to protect the curcumin molecule against ionizing radiation.

Keywords: Curcumin, gamma irradiation, nanoencapsulation.

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ENCAPSULAMENTO DE CURCUMINA EM POLÍMERO SOLÚVEL PARA PROTEÇÃO CONTRA A IRRADIAÇÃO GAMA E AUMENTO DA SOLUBILIDADE EM ÁGUA

Resumo: A curcumina é polifenol derivado da cúrcuma, planta herbácea originária da Ásia, que vem sendo estudada pelas suas propriedades medicinais. Ao longo dos anos, diferentes civilizações utilizam essas plantas com o objetivo de tratar ou prevenir enfermidades. O avanço tecnológico possibilitou que cientistas estudassem os mecanismos de ação, bem como as propriedades derivadas destes mecanismos, das plantas ditas como medicinais. Estes estudos comprovaram que as propriedades medicinais da cúrcuma são derivadas de seus polifenóis; estes por sua vez, puderem ser identificados, isolados e utilizados com maior eficiência. Apesar dos benefícios antimicrobianos da curcumina, sua molécula altamente hidrofóbica prejudica a utilização em sistema biológico, bem como a biodisponibilidade em seres humanos e animais. Processo de modificação de uma molécula permite que haja alterações em suas características, benefício de utilização; neste contexto, a encapsulação com polímeros de características anfifílicas, como o PVP K30, apresenta-se como uma alternativa viável para maior afinidade com biossistemas. A encapsulação da curcumina, denominado C-PVP K30, mostrou-se possível e eficaz, mantendo a molécula estável e em dimensões nanométricas a partir dos resultados provenientes de análises de DLS e ZETA. A análise por microscopia (MEV-FEG) mostrou partículas morfologicamente esféricas e dispersas com pequenos focos de aglomeração. O sucesso na encapsulação deste princípio ativo permitiu o estudo da solução frente à radiação gama. Os resultados obtidos por FTIR e UV-Vis comprovam que este processo não foi capaz de proteger a molécula de curcumina contra a radiação ionizante.

Palavras-chave: curcumina, irradiação gama, nanoencapsulação.

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1. INTRODUCTION

Several civilizations have a tradition of using plants for medicinal purposes in the treatment or prevention of pathologies and injuries. Among the most used plants and species, curcumin, the most abundant curcuminoid in turmeric shines out. The scientific approach to study this plant has shown that these properties are derived from its chemical composition. Among the most important for the current manuscript, the curcuminoids stand out: curcumin (~77%), desmethoxycurcumin (DMC, ~17%); and bisdemethoxycurcumin (BDMC, ~3%) [1, 2, 3, 4, 5].

Curcumin is a polyphenol found in turmeric rhizomes and is one of the factors responsible for giving it antimicrobial, antibacterial and other characteristics; it is also responsible for its yellow and red color. Curcumin is a symmetrical molecule with IUPAC nomenclature (1E,6E)-1,7-bis-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-dien-3,5-dione and chemical formula $C_{21}H_{20}O_6$ and was isolated in 1815. Its properties linked to antimicrobial activities are derived from its molecular chemical structure, which contains the functional groups 1,3-diketone and enol present in a tautomeric equilibrium. Although its characteristics help to combat bacteria and microorganisms; its hydrophobicity hampers the development of drugs since curcumin does not interact well with biological fluids. Thus, the search of develop new paths to work with curcumin in aqueous solutions and with high concentration and stability shows multiple strands within its studies. The use of encapsulated curcumin is one of them [6, 7, 8, 9, 10].

In addition to modifying the hydrophobicity of the molecule, encapsulation can protect curcumin from external stresses, like irradiation, that can degrade or destroy the molecule, changing its medicinal properties. The vulnerability of plant-based foods has meant for over the centuries, human beings have used techniques and processes to increase the shelf life of perishable foods.

Gamma radiation belongs to the ionizing radiation group, which can ionize an atom by removing one of its electrons. Currently, gamma radiation from the ^{60}Co source is the most widely used for irradiating plant origin foods. In 1985, the process of food irradiation was regulated in Brazil by the *Divisão de Vigilância Sanitária* (ANVISA). This method sterilizes and preserves food by inhibiting pathogenic microorganisms, bacteria and fungi. Furthermore, this type of radiation slows down the ripening of vegetables, delaying the growth of sprouts and allowing for a longer shelf life [11,12]. This study evaluated the encapsulate curcumin process and the protection against gamma radiation from the ^{60}Co source at a dose of 25 kGy.

2. MATERIALS AND METHODS

The encapsulated curcumin, called C-PVP K30, was synthesized following [13] with a couple modifications. First, solubilizing 114.1 mg of sodium citrate and 10 mg of PVP K30 in 120 mL of distilled water. This solution was poured into a round-bottomed flask using a mechanical stirrer and a condenser and heated to boiling for 45 minutes. After this process, the solution was cooled to room temperature and added to 25 mL of ethanol containing curcumin at a concentration of 3 $mg.mL^{-1}$ and submitted to distillation for 3h. Furthermore, centrifugation was used to remove the unencapsulated curcumin and calculate the soluble curcumin after this process. The final solution must be stored at room without sunlight incidence. Afterwards, the solution was submitted to gamma irradiation process of 25 kGy dose at dose rate of 5.5 $kGy \cdot h^{-1}$. The techniques used to characterize the C-PVP K30 solutions were DLS & ZETA, MEV-FEG and TG; UV-Vis and FTIR techniques were required to analyze the protection of the encapsulation against gamma irradiation.

3. RESULTS AND DISCUSSIONS

3.1. Soluble curcumin encapsulated

The curcumin encapsulation proved to be efficient, making it soluble and stable in aqueous solutions. It can be classified as a colloid due to Tyndall Effect and nanometric diameter that will be clarified and discussed on next section. In addition, this route was able to change the solubility of curcumin from 0,013 $mg.mL^{-1}$ to 0.48 $mg.mL^{-1}$ [5,9,14]. This result provides different ways of applying curcumin for biomedical purposes. Together with the other data presented in this study, it opens new possibilities for studying this molecule.

3.2. DLS & ZETA

The techniques of DLS and Zeta Potential contribute to an explanation of the dimensions and stability of solutions that can be called colloids or nanosuspensions. Table 1 and 2 show the DLS and Zeta Potential values obtained from C-PVP K30 solutions, respectively.

Table 1 - DLS data.

C-PVP K30 1:50	
Intensity (PDI 0,587)	
Size (d.nm)	%
63,32	30,30%
391,7	67,60%
50532	2,10%

It was used the 1:50 dilution to read the DLS analyses. The results presented showed, for the most part, the nanometric dimensions of the solution. However, 2.10% of the particles in suspension had micrometric dimensions (>1000 nm). In addition, the solution had a high polydispersity index (>0.5), which shows the different populations in the sample.

Table 2 - Zeta Potential data.

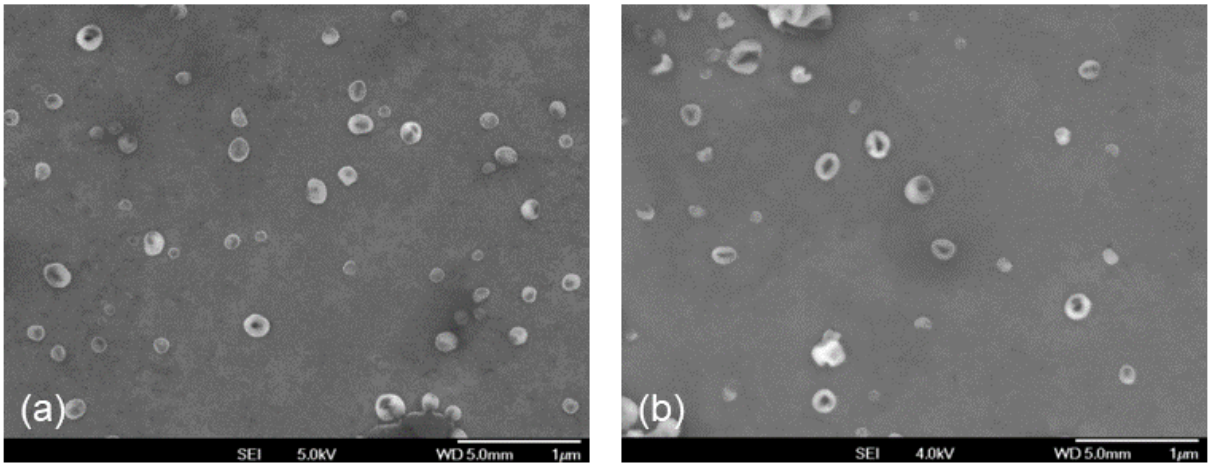
		Average	%	Standard deviation (mV)
Zeta Potential (mV) -29,6	**Peak 1**	-27,9	94,80%	6,99
	Peak 2	-51,7	5,20%	3,26
	Peak 3	0	0%	0,00

Table 2 shows that the C-PVP K30 solution had negative values, indicating anionic behavior on the double layer surface of the particles. This behavior is linked to the stabilizer (sodium citrate) used, since the sodium cations are solvated in solution while the citrate structure is interacting with the encapsulated curcumin particles. The data obtained shows that <94% of the particles have surface charges close to the region considered ideal for electrostatic repulsion, however, the standard deviation of 6.99 mV can modify the surface charge to values below -30 mV as well as values above -25 mV. The significance of the standard deviation is important to assess because, as shown in the DLS, around 2.1% of the particles are in micrometric ranges, and these values may be derived from an agglomeration resulting from the surface charge and not from capsule particles on the micrometer scale.

3.3. MEV – FEG

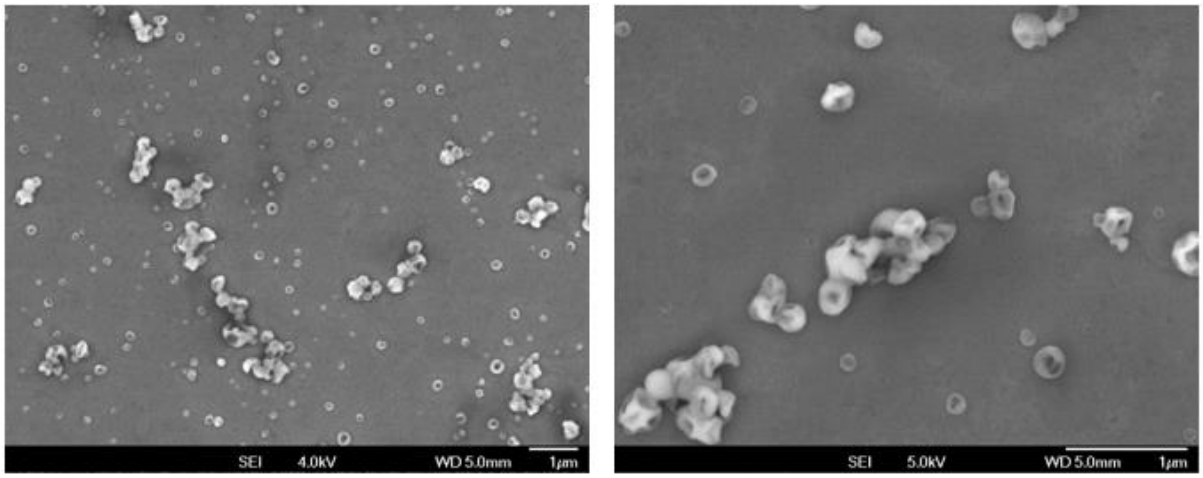
The microscopy technician was used to clarify the encapsulated curcumin morphology and check its dispersion. Figure 3a) and (b) shows general micrographs of the encapsulated curcumin nanoparticles.

Figure 1 – (a) and (b) Encapsulated curcumin nanoparticles micrographs.



The encapsulated curcumin was spherical in shape with free and agglomerated particles and dimension < 0.2 microns. This result is in line with the polydispersity obtained through the DLS test. In Figure 4, the microscopy is focused on the agglomerates.

Figure 4 – (a) and (b) Encapsulated curcumin nanoparticles agglomerated micrographs.

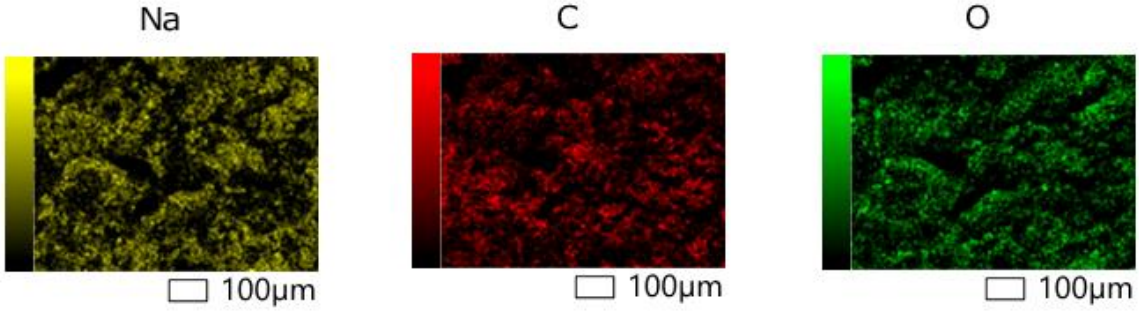


These figures show that the larger-scaled structures (>1 μm) are agglomerates of smaller particles that form micrometric particulates through electrostatic attraction, as discussed in the DLS test section. Encapsulation was effective in forming spherical curcumin particles and could be confirmed using Scanning Electron Microscopy.

The dispersion of the stabilizer sodium citrate is key to maintaining the colloidal stability of the nanoparticles. The elementary distribution mapping, from EDS analysis, was

carried out to check the dispersion of sodium (Na), which comes from the stabilizer, in the dry product of the C-PVP K30 solution. The Figure 5 presents the sodium EDS and the dispersion in comparison to carbon and oxygen, the two most abundant in the molecules that compose the solution.

Figure 5 – EDS elemental mapping.



This technique made it possible to verify that the sodium atoms, from stabilizer, are distributed throughout the dry C-PVP K30 sample. That result contributes with the good data from Zeta Potential and fit that a good distribution of the stabilizer helps maintain the colloid's stability.

3.4. TG

Thermogravimetry made it possible to analyze the thermal stability of the encapsulated curcumin and its reagents. This analysis will allow us to confirm the new interactions resulting from the encapsulation process through changes in thermal stability. Figure 6 presents the thermograms of encapsulated curcumin and its reagents in normalized form. Figure 7 shows the relationship between the TG and dTG of these compounds.

Figure 6 – Reagents and C-PVP K30 TG.

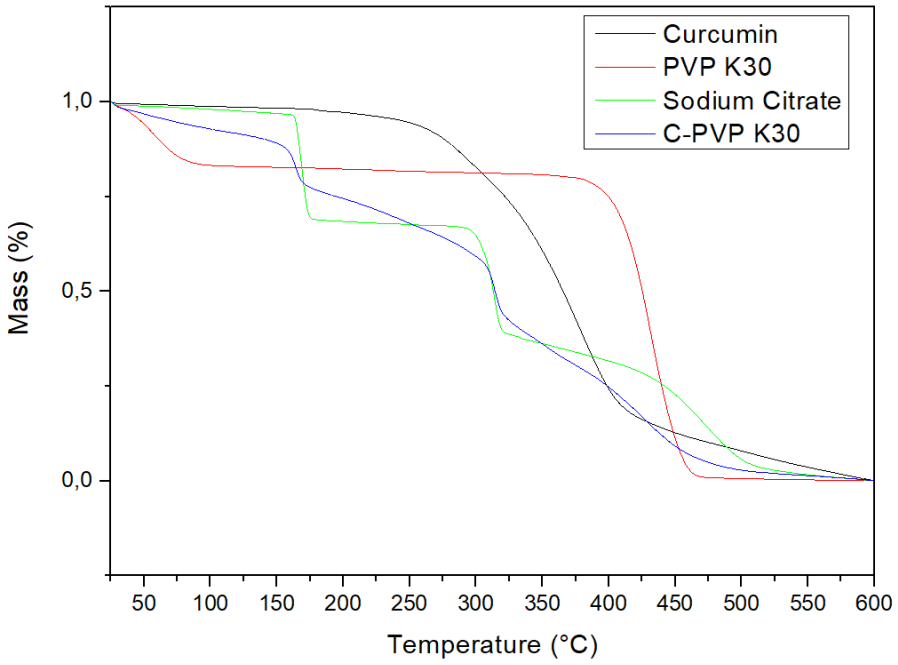


Figure 7 –TG and dTG of (a) Curcumin (b) PVP K30 (c) Sodium Citrate and (d) C-PVP K30.

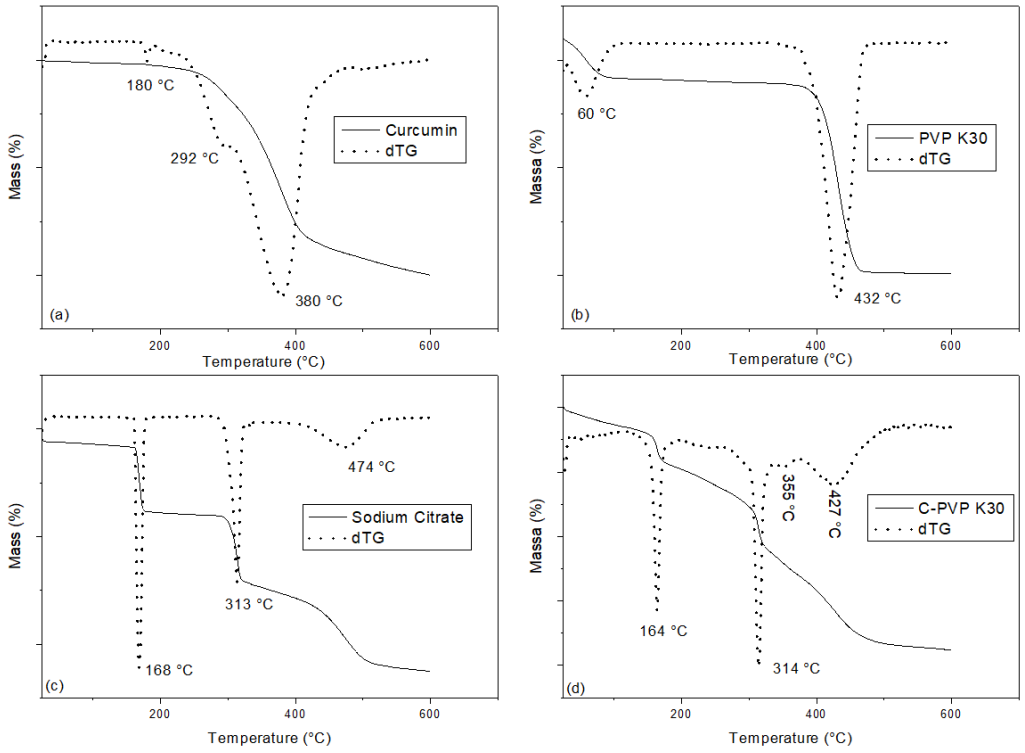


Figure 6 shows the predominance of the decomposition profile derived from the sodium citrate in the encapsulated curcumin nanoparticles due to higher concentration in the solution,

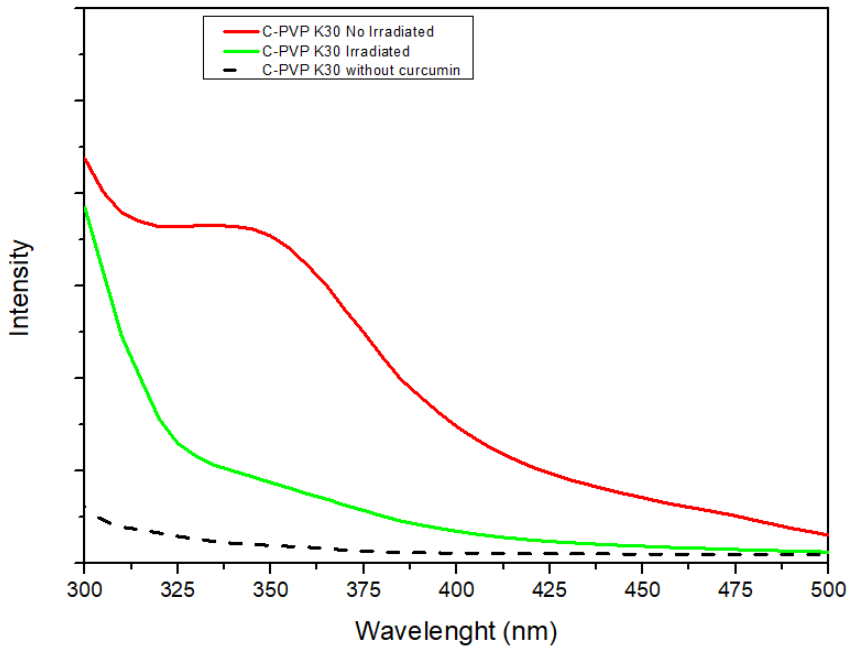
which loses mass throughout the process. Its initial losses (up to 200 °C) can be attributed to the degradation of PVP K30 and the first loss of mass of sodium citrate; meanwhile, curcumin begins its degradation at 160 °C, but a significant loss of mass occurs at 250 °C.

Figure 7(a), 7(b), 7(c) and 7(d) shows peak shifts and intensity changes in the decomposition of encapsulated curcumin, which can be attributed to new interactions derived from the encapsulation process. The increase in intensity of the second peak of the C-PVP K30 derivative (313 °C) refers to the modification with the presence of curcumin in this event. In addition, the dTG of C-PVP K30 has peaks at 355 °C and 427 °C which refer to the remaining mass of curcumin and PVP K30, respectively.

3.5. UV-Vis Spectroscopy

The Figure 8 presents the UV-Vis spectroscopy of curcumin encapsulated irradiated and no irradiated.

Figure 8 - UV-Vis Spectroscopy of C-PVP.



The characteristic absorption wavelength of curcumin in keto-enol equilibrium is around 420 nm, meanwhile, this molecule it's able to assume different isomeric forms and, consequently, absorbs on different wavelength. If curcumin is in the β-diketone form and

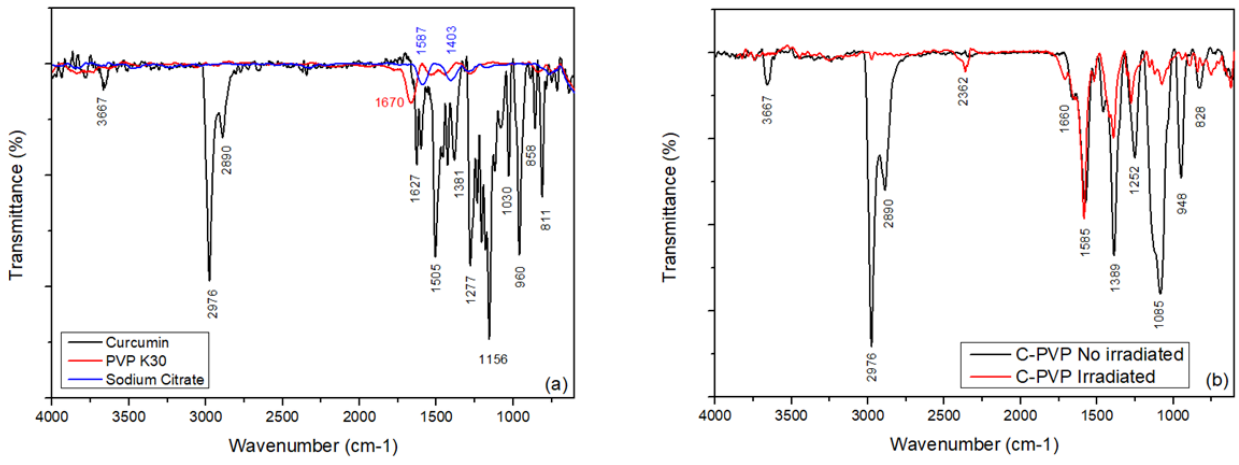
has an angular torsion due to this conformation, the absorption of the molecule changes from 420 to around 340 nm [15]. In this spectroscopy it's possible to notice the difference between both curves. The absorbance on red curve is derived from isomeric form β-diketone of curcumin which absorbs with more intensity between 320-345 nm, while the green curve, from irradiated curcumin, shows less absorbance on this wavelength. The encapsulation process was unable to fully protect the curcumin at the 25 kGy. The UV-Vis spectroscopy it's a very important characterization of curcumin because it could be understood what type of isomeric form it's present on solution and if the radiation changes the molecule.

The results showed that the wavelengths obtained are characteristic of the diketone form of the molecule, however, in the conditions presented, the keto-enol tautomeric equilibrium would have to be predominant (water solution). This fact suggests that the coating (PVP K30) and/or the stabilizer (sodium citrate) are modifying the curcumin molecule by means of physical or chemical interactions.

3.6. FTIR

In this paper, the FTIR it was used characterize the modification on curcumin under gamma irradiation. The Figure 9 presents spectra of (a) reagents utilized in the synthesis and (b) C-PVP K 30 irradiated e no irradiated.

Figure 9 - (a) C-PVP K30 reagents spectra; (b) comparison between irradiated and non-irradiated samples.



Firstly, curcumin has a peak in its spectrum at 3667 cm-1 derived from the hydroxyls present in the molecule. The peaks at 2890, 1627, 1505 and 1277 cm^{-1} are linked to the functional groups (OC-H) enol, C=O ketone, C=C and C-O of the aromatic ring, respectively. After encapsulation, the encapsulated curcumin showed peak shifts and an increase in absorbance intensity. Two shifts can be associated with the encapsulation process, namely the band at 1660 cm^{-1} (C=O) and 1574 cm^{-1} (N-H), both derived from the interactions between curcumin and the PVP K30 coating polymer [13,16,17,18]. The effects of gamma radiation from the 60Co source can be seen in Figure 9 (b). It can be noted that the bands belonging to the hydroxyls and the keto-enol equilibrium, 3667 and 2890 cm^{-1}, respectively, have no absorption intensity in the irradiated sample. The bands after 1085 cm^{-1}, which may be derived from the sigma present in the aromatic ring, were also affected by the radiation with a decrease or loss of absorption intensity.

The results presented here show that the encapsulation process with PVP K30 was unable to minimize and protect curcumin from the effects of gamma radiation in solution.

4. CONCLUSIONS

Curcumin is an organic molecule widely investigated by the scientific community with the aim of analyzing, studying and confirming its antimicrobial activity. Despite the benefits presented by this molecule, its use is restricted due to its high hydrophobicity, which results in different methods of use in biological fluids.

In this study, modification through nanoencapsulation proved to be efficient in solubilizing and stabilizing it in aqueous solutions, with concentrations greater than 35x compared to natural curcumin. However, this process was unable to protect the curcumin from the 25 kGy radiation exposure. The FTIR and UV-Vis results were able to show the degradation of the molecule compared to the non-irradiated one.

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CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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