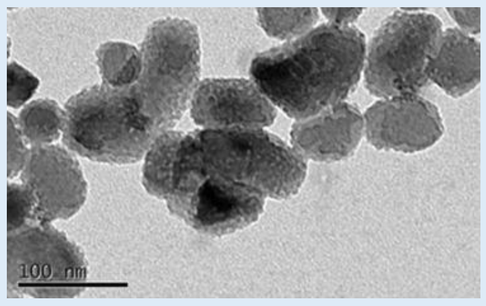
Pomegranate peel extract adsorbed on calcium carbonate nanocrystals: enhanced bioactivity and controlled release

F. De Castro [a], F. Baldassarre [a,b], V. Vergaro [a,b], G. P. Suranna [b,c], D. Rongai [d], F. P. Fanizzi [a] and G. Ciccarella [a,b]

[a] Department of Biological and Environmental Sciences and Technologies (DiSTeBA), University of Salento, Via Monteroni, I-73100 Lecce, Italy
[b] Institute of Nanotechnology, CNR NANOTEC, Consiglio Nazionale delle Ricerche, Via Monteroni, 73100 Lecce, Italy
[c] Dipartimento di Ingegneria Civile, Ambientale, del Territorio, Edile e di Chimica (DICATECh), Politecnico di Bari, Via Orabona 4, 70125 Bari, Italy.
[d] CREA Research Centre for Plant Protection and Certification, via C.G Bertero, 22, 00156 Rome, Italy

Introduction

Pomegranate (Punica granatum) is an ancient fruit, widely used in traditional medicine for its protective and therapeutic effects. Different studies, focusing on the isolation and characterization of the active component of pomegranate, have further demonstrated the high therapeutic potential of the pomegranate peels, often discarded as waste.



TEM characterization of nanoCaCO$_3$

Pomegranate peel extracts (*pae*) contain the highest concentration of phytochemicals, mainly represented by phenolic compounds, such as tannins, ellagitannins and anthocyanins, including ellagic acid and punicalagin. These metabolites are responsible of the *pae* high beneficial properties such as antimicrobial, anti-cancer, antiobesity, anti-diabetic, anti-ulcerogenic and anti-hypertensive[1].

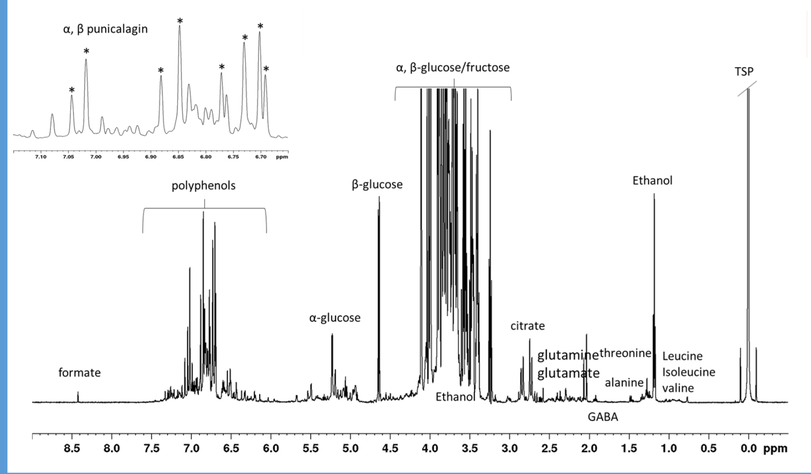
The purpose of this work was the adsorption of ***pae*** on carbonate nanocrystals (nanoCaCO$_3$), coated or not with chitosan, in order to modulate the release of the different components (metabolites) of *pae* and improve the bioactivity.

nanoCaCO$_3$ have previously showed their potential as carriers with a great affinity towards different biomolecules and drugs, interacting both with human cells and bacteria[2]. For this reasons nanoCaCO$_3$ was chosen as delivery system for this study.

Results and discussion

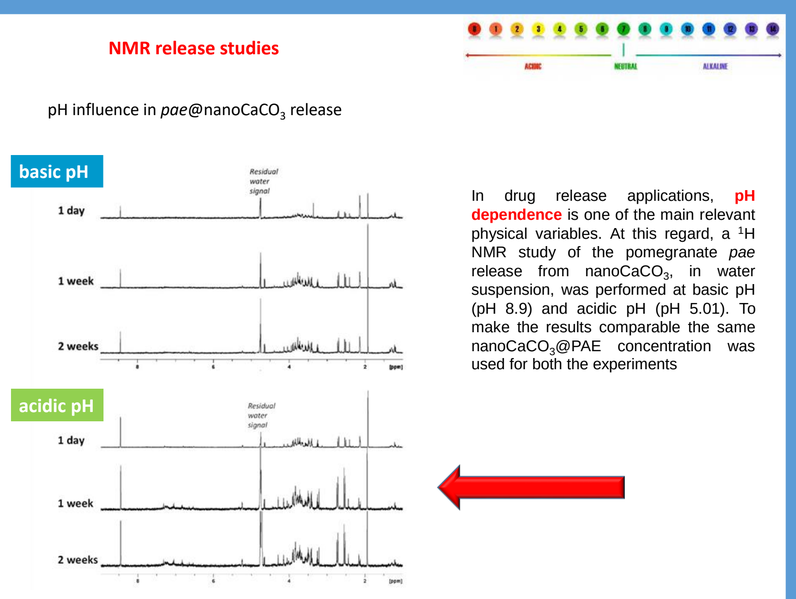
NMR characterization of pomegranate peel extract

1H zgcppr NMR spectrum of *pae* in D_2O (T=300K; pH=4.46)



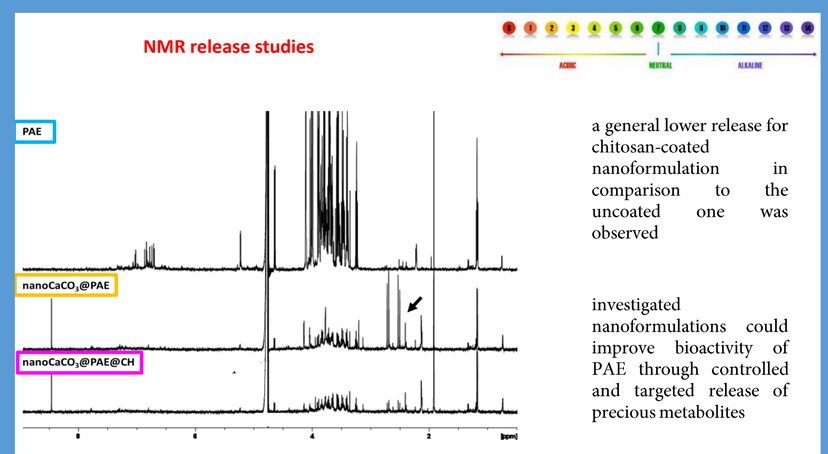
NMR release studies

pH influence in *pae*@nanoCaCO$_3$ release



In drug release applications, **pH dependence** is one of the main relevant physical variables. At this regard, a ^{1}H NMR study of the pomegranate *pae* release from nanoCaCO$_3$, in water suspension, was performed at basic pH (pH 8.9) and acidic pH (pH 5.01). To make the results comparable the same nanoCaCO$_3$@PAE concentration was used for both the experiments

NMR release studies



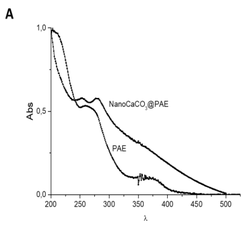
a general lower release for chitosan-coated nanoformulation in comparison to the uncoated one was observed

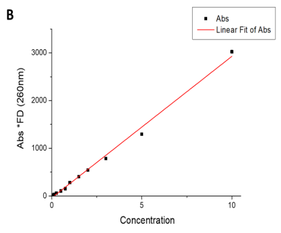
investigated nanoformulations could improve bioactivity of PAE through controlled and targeted release of precious metabolites

*^{1}H zgcppr NMR spectra of released PAE from nanoCaCO$_3$@PAE and nanoCaCO$_3$@PAE@CH at **acidic pH** (citrate release is indicated by black arrow).*

nanoCaCO$_3$ based formulation of *pae*: adsorption and release study

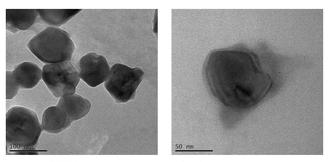
In figure **A** we can observe the adsorption spectra of PAE and nanoCaCO$_3$@PAE suspension that showed the splitting of indicative peak, probably due to phytochemicals interaction with crystals surface. *Standard curve from UV-absorption measurements at 260nm peak*. Standard curve by Origin software is reported in **B**





Organic layer of PAE is evident observing TEM nanocrystals images.The nanoCaCO$_3$@pae have lost the characteristic cubic shape showing a more irregular morphology.

Particles size of about 100nm



TEM nanocrystals images

In vitro Antifungal activity

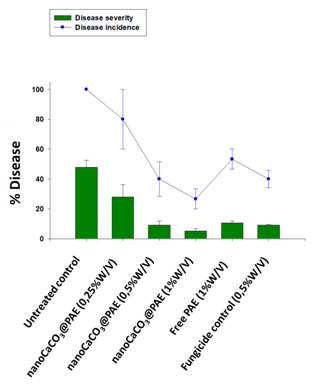
Treatment	Dose	Mycelial growth of *B. cinerea* 4th day	Mycelial growth of *F. oxysporum* 4th day
		mm	*mm*
Control		50.0 ±0.00a	50.0 ±0.00a
nanoCaCO$_3$@PAE@CH	(0.25%)	40.1 ±0.18b	40.5 ±0.09b
nanoCaCO$_3$@PAE@CH	(0.5%)	36.0 ±0.29d	40.4 ±0.08bc
nanoCaCO$_3$@PAE@CH	(1%)	17.0 ±0.08e	40.3 ±0.05c
PAE	(1%)	39.5 ±0.05c	18.2 ±0.17d
Fungicide	(0.5%)	16.8 ±0.09e	17.2 ±0.19d
		F=385.76 P<0.001	F=456.88 P<0.001

We have tested PAE nanoformulations against **two pathogenic fungi**, *Botrytis cinerea* and *Fusarium oxysporum*

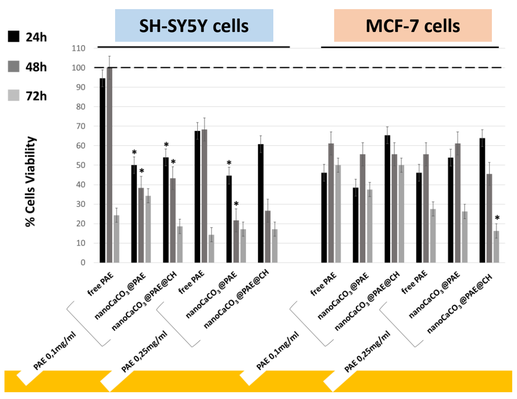
In vivo

No statistically significant difference was found in treatments between nanoCaCO3@PAE@CH (0.5%) and PAE (1%), both for the DS value and for the DI value, however, as just explained, nanoformulation is made up of half its weight of extract.

Increasing doses of nanoCaCO$_3$@PAE@CH on disease incidence (DI) and disease severity (DS) of gray mould (*Botrytis cinerea*) on strawberry.



Antiproliferative activity on human cancer cell lines



Free PAE at lower concentration reduced SH-SY5Y cells viability after 72 hours of treatment, instead respective nanoformulations about 50-60% already after 24 hours.

Nanoformulation has been a higher effect than respective free PAE treatment only after 72 hours and in presence of chitosan coating. Chitosan provided a less negative charge to nanoCaCO$_3$@PAE that has allowed a more effective interaction with cell membrane

Cells viability by MTT tests for SH-SY5 and MCF7 cells after 24-48-72h of treatment with nanoCaCO$_3$, free PAE, nanoCaCO$_3$@PAE and nanoCaCO$_3$@PAE@CH at different concentrations. Treatment with PAE formulations have been made at equal quantity of extract, as verified though LC data. Percentage viability data referred to respective not treated control condition at each time point (100%, indicated by dashed line). Values represent mean from three independent experiments. Statistically significant value $p \leq 0.05$ (*) respect to free PAE condition, from T-test.

Conclusion

This work confirmed the versatile nature of CaCO$_3$ nanocrystals and chitosan. PAE adsorption on nanoCaCO$_3$ is very efficient, and its release could be tuned by changing pH that is a crucial factor for drug delivery systems, such as in cancer therapy. Chitosan has been investigated also for its intrinsic antimicrobial activity that has been studied for two phytopathogenic fungi. A great increment of antimicrobial activity for nanoCaCO$_3$@PAE@CH has been quantified for *Botrytis Cinerea*, a very aggressive post-harvest pathogen. Efficient control of this fungus has been showed treating strawberries. Nanoformulations improved PAE antiproliferative effect, both on SH-SY5Y and MCF-7 cells.

In particular, a high SH-SY5Y cells viability reduction has been induced already after 24 hours treating with PAE nanoformulations. Our results demonstrated the great potential of PAE nanoformulation in different applications ranging from human health and crops protection. Other nanoencapsulation strategies will be investigated in order to improve PAE multifunctionality.

References

[1] Akhtar, S., Ismail, T., Fraternale, D. & Sestili, P. Pomegranate peel and peel extracts: Chemistry and food features. *Food Chem.* **174**, 417–425 (2015).

[2] Baldassarre, F. *et al.* Application of calcium carbonate nanocarriers for controlled release of phytodrugs against Xylella fastidiosa pathogen. *Pure Appl. Chem.* **92**, (2020).