Nano Innovation 2020 Rome, 15-18 September Conference & Exhibition

Pharmaceutical Technology & Drug Delivery Lab

Influence of chitosan on a polysaccharide blend *in situ* gelling powder for wound dressing

Chiara Amante

PhD Student in Drug Discovery and Development, Department of Pharmacy University of Salerno, Fisciano, (SA), Italy

18/09/2020

Wound dressing

Cutaneus ulcers

- Lower limb ulcers
- Diabetic foot
- Bedsores

Conventional dressings

- Local irritation, contact sensitization and immune reactions
- Frequent dressing changes
- Dehydration of the wound bed
- Traumatic removal

Ideal wound dressing

- Absorption of exudate
- Transpiration
- Adhesiveness to the wound site
- Easy application and atraumatic removal
- Drug/adiuvant release
- Inexpensive

Hydrocolloid dressings

Alginate dressings


Foams


Hydrogel dressings

Biological dressings

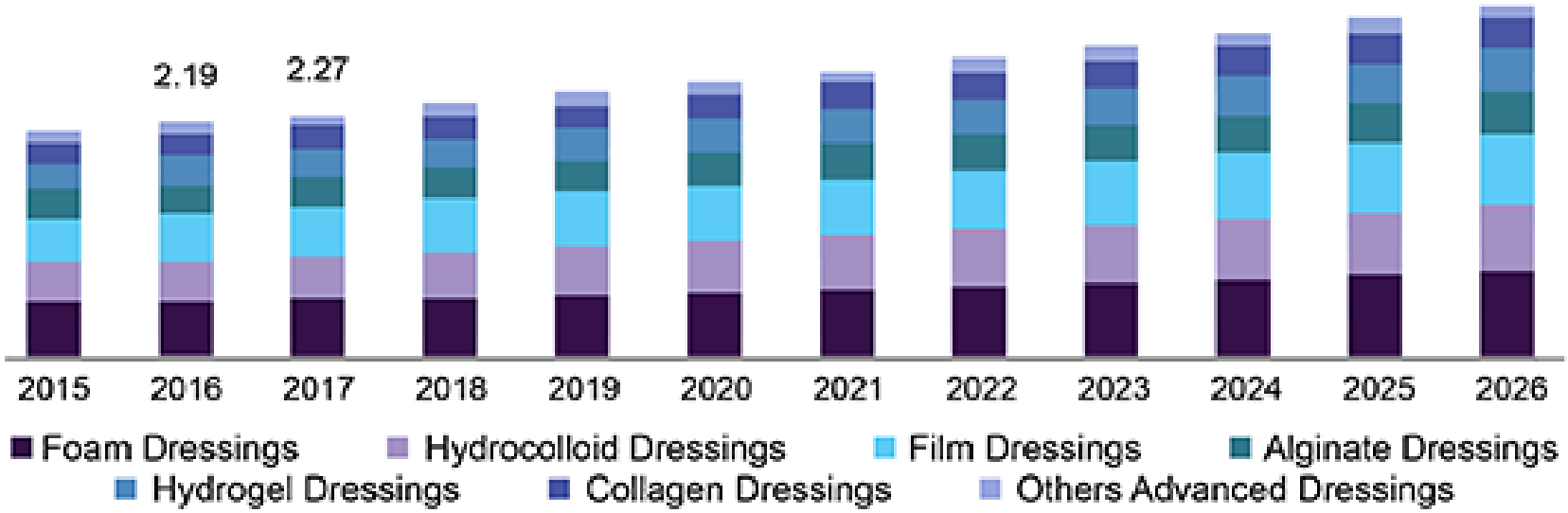

Semi-permeable adhesive films


Tissue engineered skin substitutes

Mayet, N., et al., (2014) . A comprehensive review of advanced biopolymeric wound healing systems. Pharm-Sci.

Wound dressing market

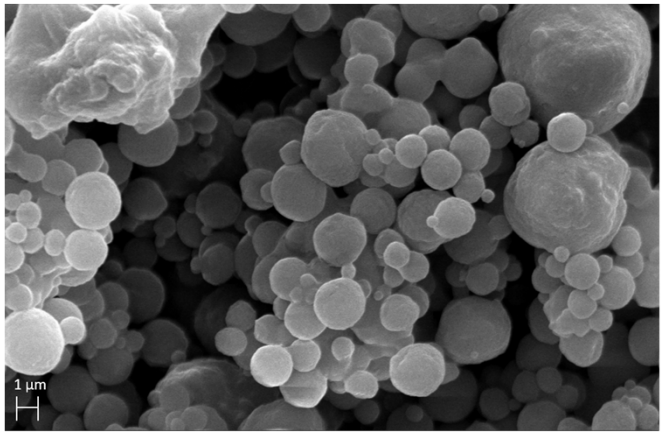
- The global advanced wound dressing market was valued at USD 6.32 billion in 2018 and it is predicted to reach an CAGR (Compound Annual Growth Rate) of 4.3% over the forecast period



Advanced Wound Dressing Market Size, Share & Trends Analysis Report By End Use (Hospitals, Home Healthcare), By Application (Chronic, Acute Wounds), By Product (Foam, Film, Hydrogel), And Segment Forecasts, 2019 – 2026. Grand view Research.

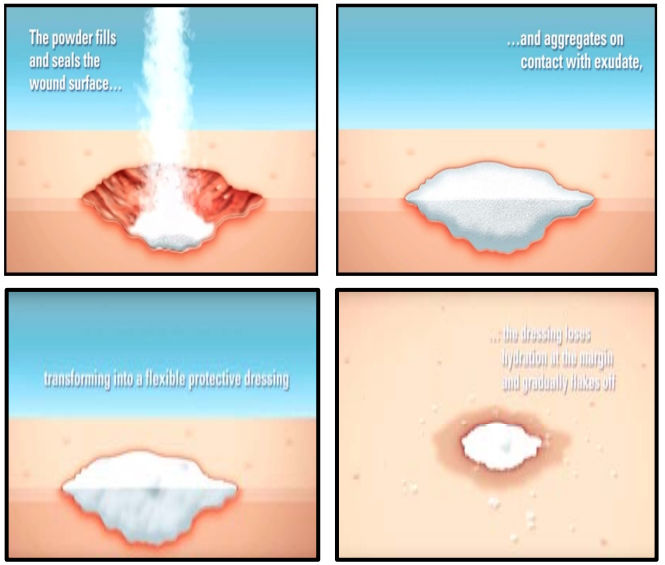
In situ gelling powders

Micro particle carriers
in form of dry powders



In situ

Hydrogel



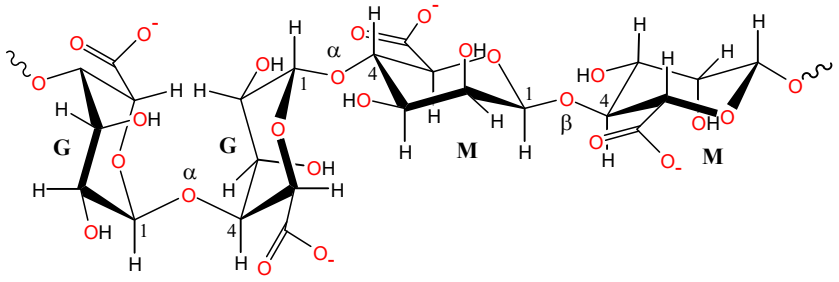
- Easy application
- Absorption of exudate
- Conformability to the surface of the wound
- Atraumatic removal
- Release of active pharmaceutical ingredients (API)

Production by mini spray drying

Economic
Biopolymer

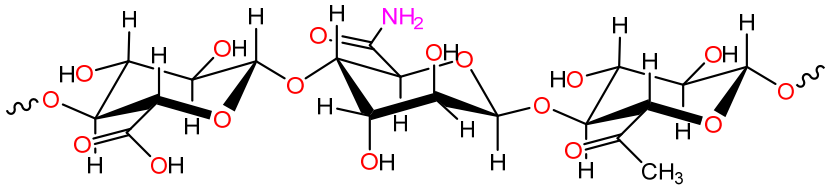
2019 Del Gaudio P., et al. PCT /IB2018/058742 May 16th 2019 IN SITU GELIFYING POWDERS (WO/2019/092608)

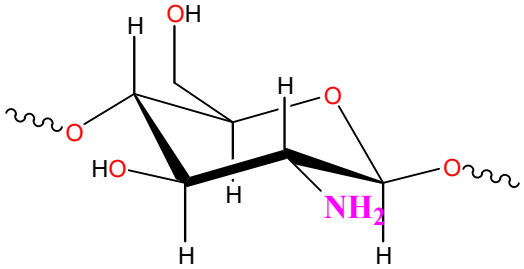
Polymers



Alginate with high content of M induce the production of cytokines by human monocytes, a very useful process in the healing of chronic wounds *

Amidated **pectin** with a low degree of methylation is able to increase in situ gel forming rate





Chitosan, antimicrobial agent, activates macrophages, stimulates cell proliferation and tissue organization.
Chitosan low molecular weight enhance *in situ* gelification *

Thomas et al., (2000). Alginates from wound dressings activate human macrophages to secrete tumour necrosis factor-alpha. Biomaterials. *
R.C. Goy, D.d. Britto, O.B.G. Assis, A review of the antimicrobial activity of Chitosan, Polymeros 19 (2009) 241–247 * *

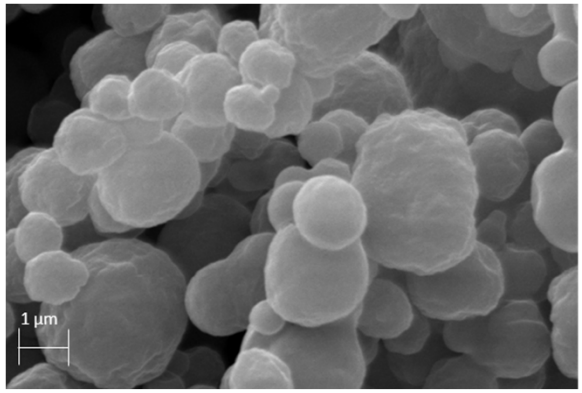
Alginate/pectin/chitosan powders

- Process yield depends on chitosan concentration
- Mean diameter is related to the concentration of chitosan

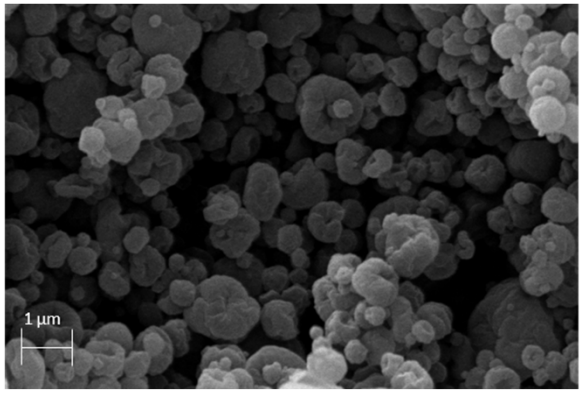
Sample code	Polymers concentration (w/V)	Polymers ratio	Yield (%)	Mean diameter (nm)
APC_111	0.15	1:1:1	62.12	7.23
APC_113	0.15	1:1:3	73.14	2.43
APC_117	0.15	1:1:7	73.57	2.74

- Increasing of chitosan leads to an higher surface roughness

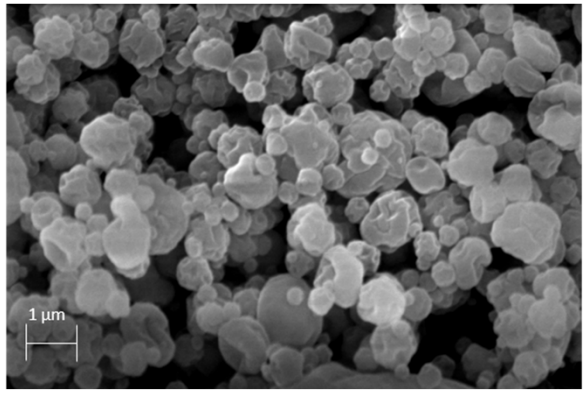
APC_111



APC_113

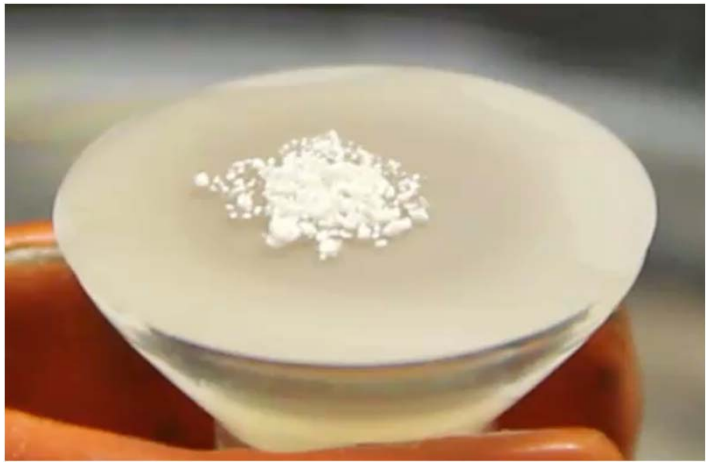


APC_117

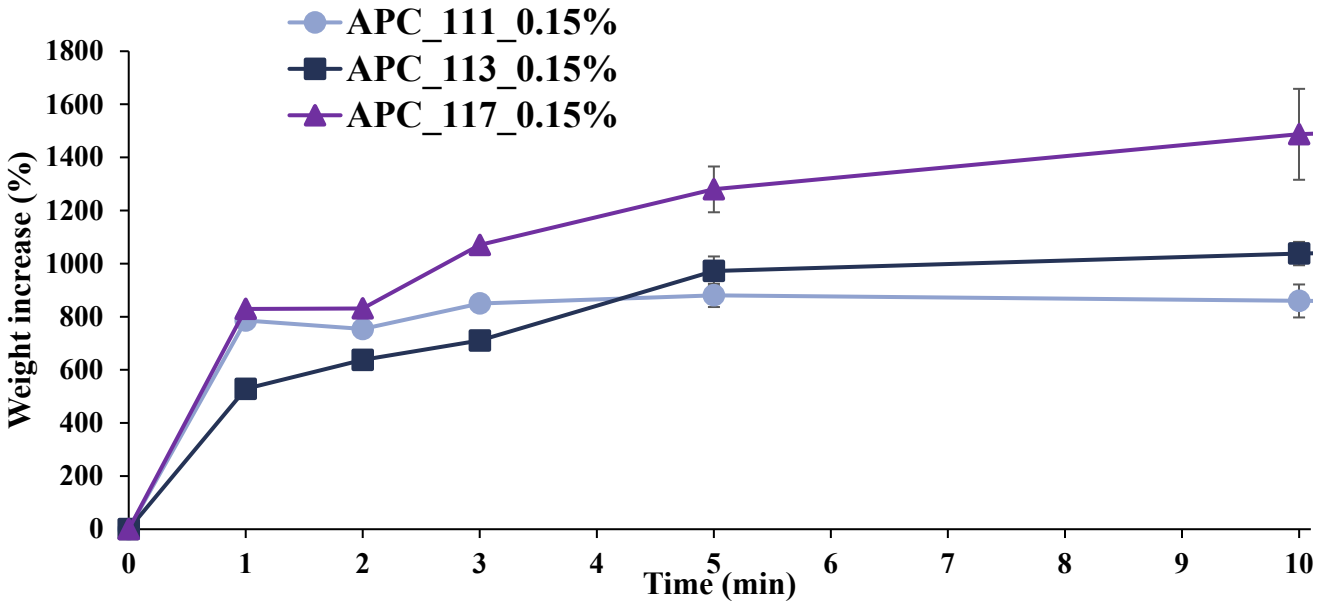


Alginate/pectin/chitosan powders

- **Fluid uptake ability:** maximum swelling in about 5 minutes



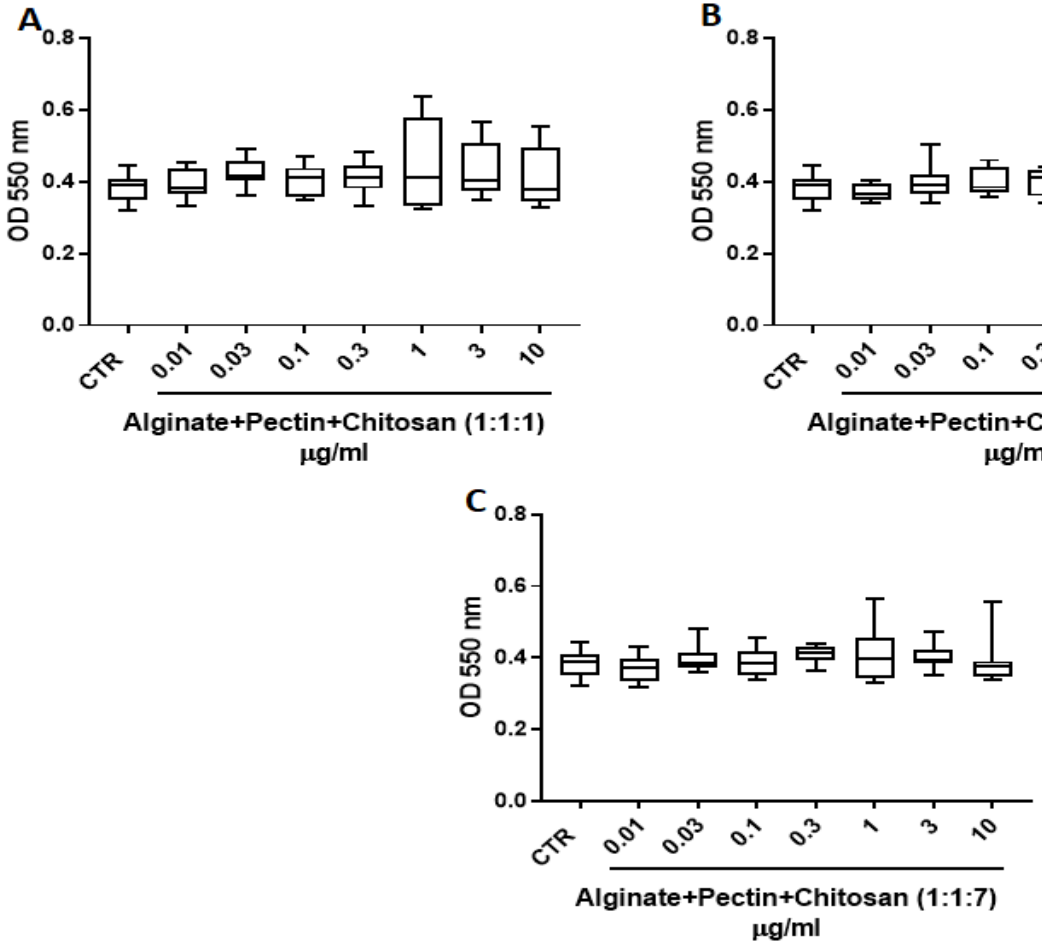
Conditions: Franz cell filled with SWF (simulated wound fluid), 37°C



Biological test

- **Cytotoxicity activity**

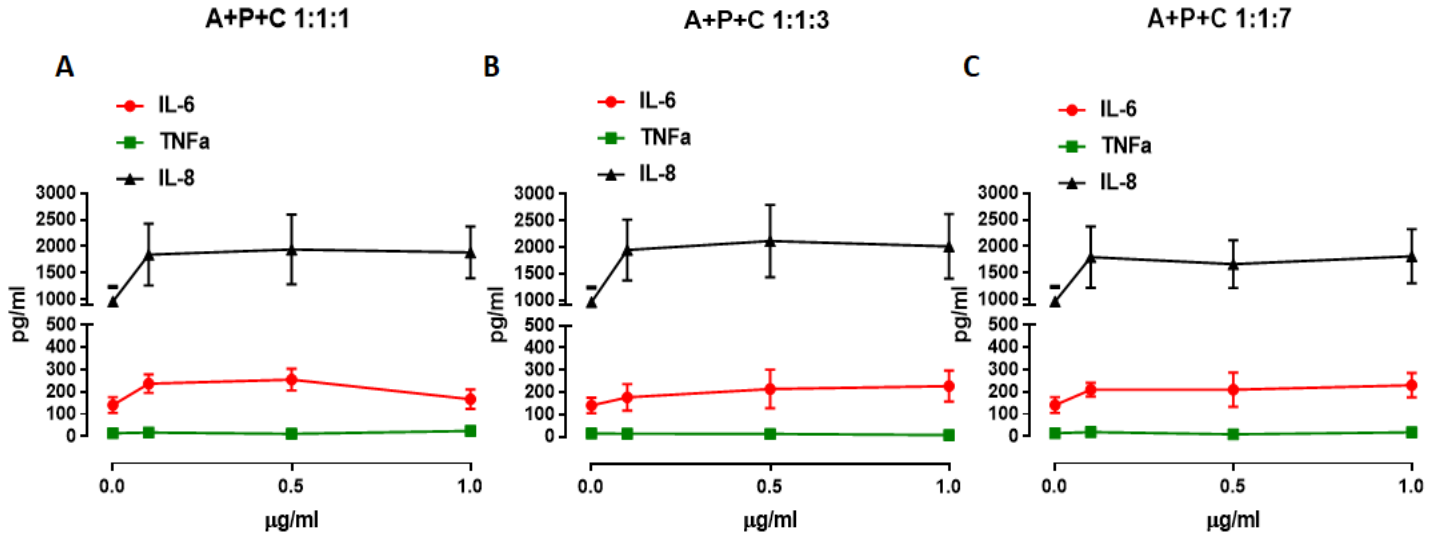
  - Cells used: human keratinocyte (HaCAT)
  - Concentration of powders tested = 0.01-10 µg/mL

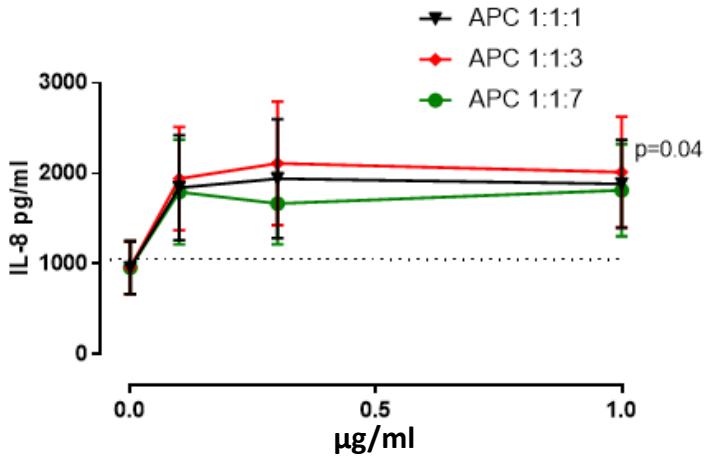


MTT test did not show any significant cytotoxic activity

Biological test

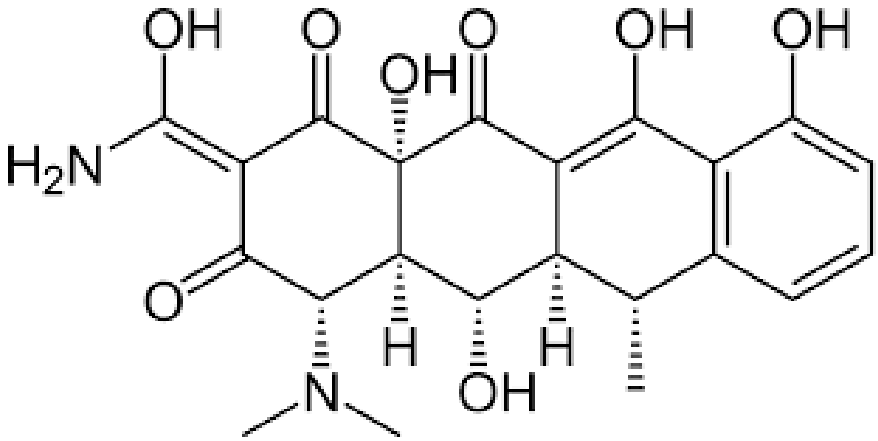
- **Proinflammatory activity**
  - Cells used: Human Epidermal Keratinocyte, adult (HEKa)
  - Concentration of powders tested = 0.1;0.5;1 µg/mL





APC induced a higher release of Interleukin-8

In situ gelling powders loaded doxycycline



- Wide antibacterial spectrum against Gram-positive and Gram-negative bacteria *
- Inhibition of host matrix metalloproteinases hyper expressed in chronic wounds **

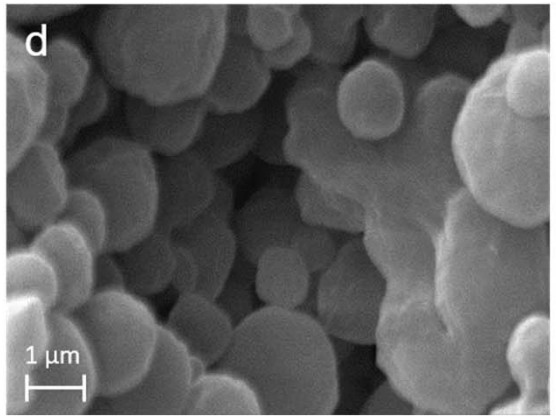
* Chopra I, Roberts M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol Mol Biol Rev*. 2001;65(2):232-260. doi:10.1128/MMBR.65.2.232-260.2001

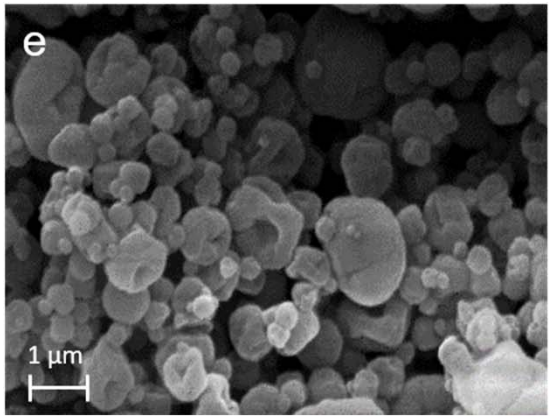
* * García, R. A., et al., (2005). Molecular Interactions between Matrilysin and the Matrix Metalloproteinase Inhibitor Doxycycline Investigated by Deuterium Exchange Mass Spectrometry. 67(4), 1128-1136.

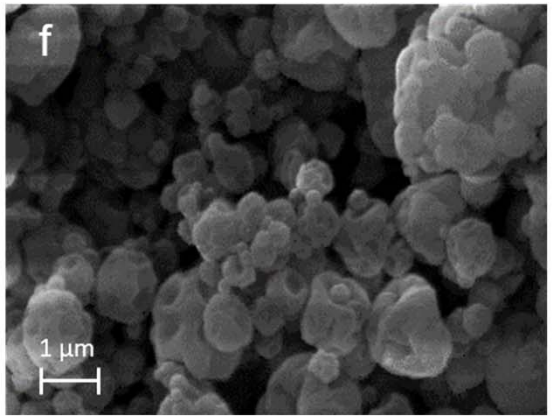
Alginate/pectin/chitosan powders loaded doxycycline

- **Encapsulation efficiency (e.e.)** depending on the relative amount of chitosan into the feed

Sample code	Polymers concentration (w/V)	Polymers ratio	Doxycycline concentration % (w/w)	Yield (%)	Mean diameter (nm)	Drug content (%)	E.E. (%)
d) APCD_111_2D	0.15	1:1:1	2	62.1	9.64	1.32	67.39
e) APCD_113_2D		1:1:3	2	70.82	3.09	1.42	71.73
f) APCD_117_2D		1:1:7	2	73.88	2.43	1.51	77.24

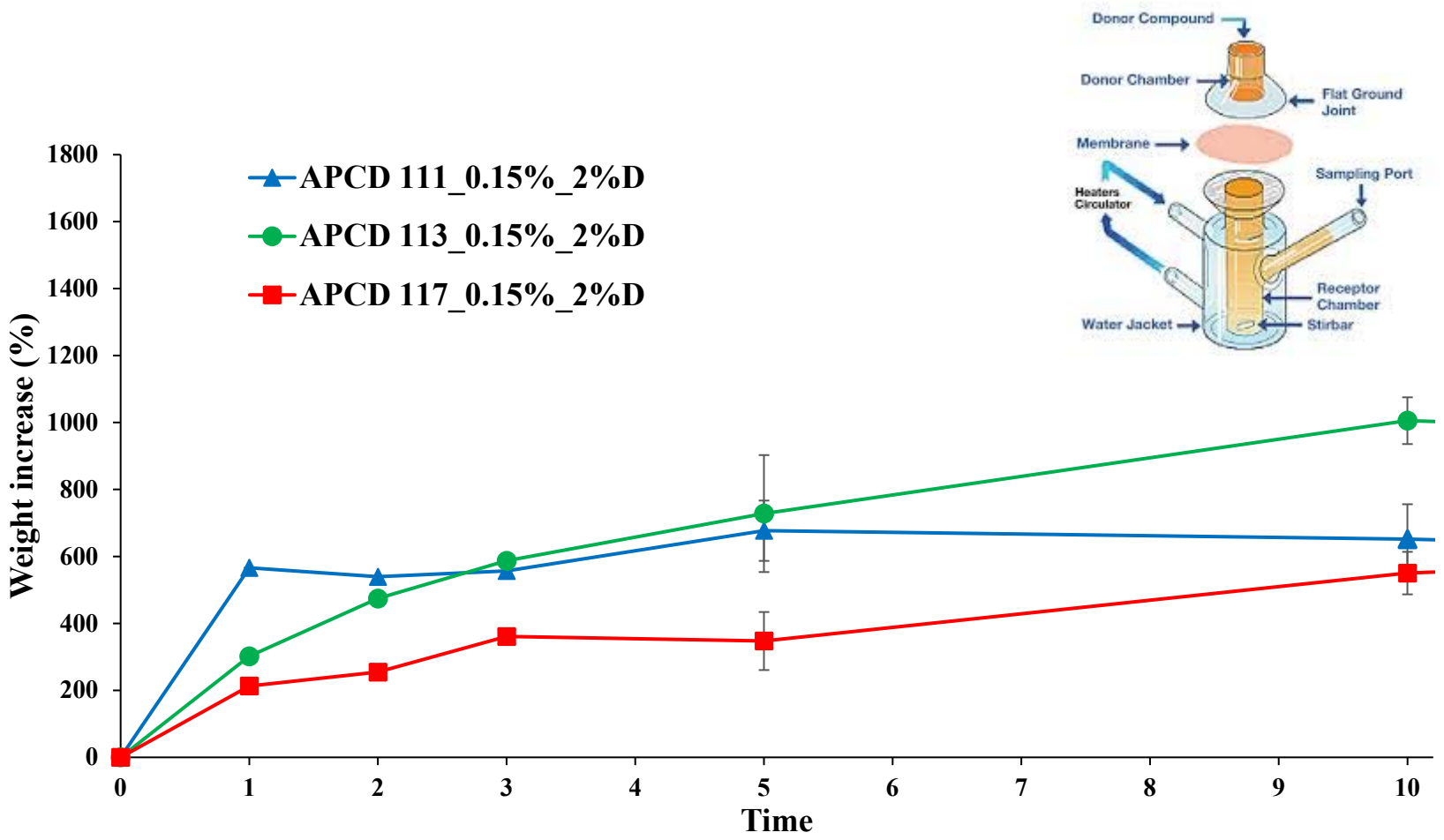






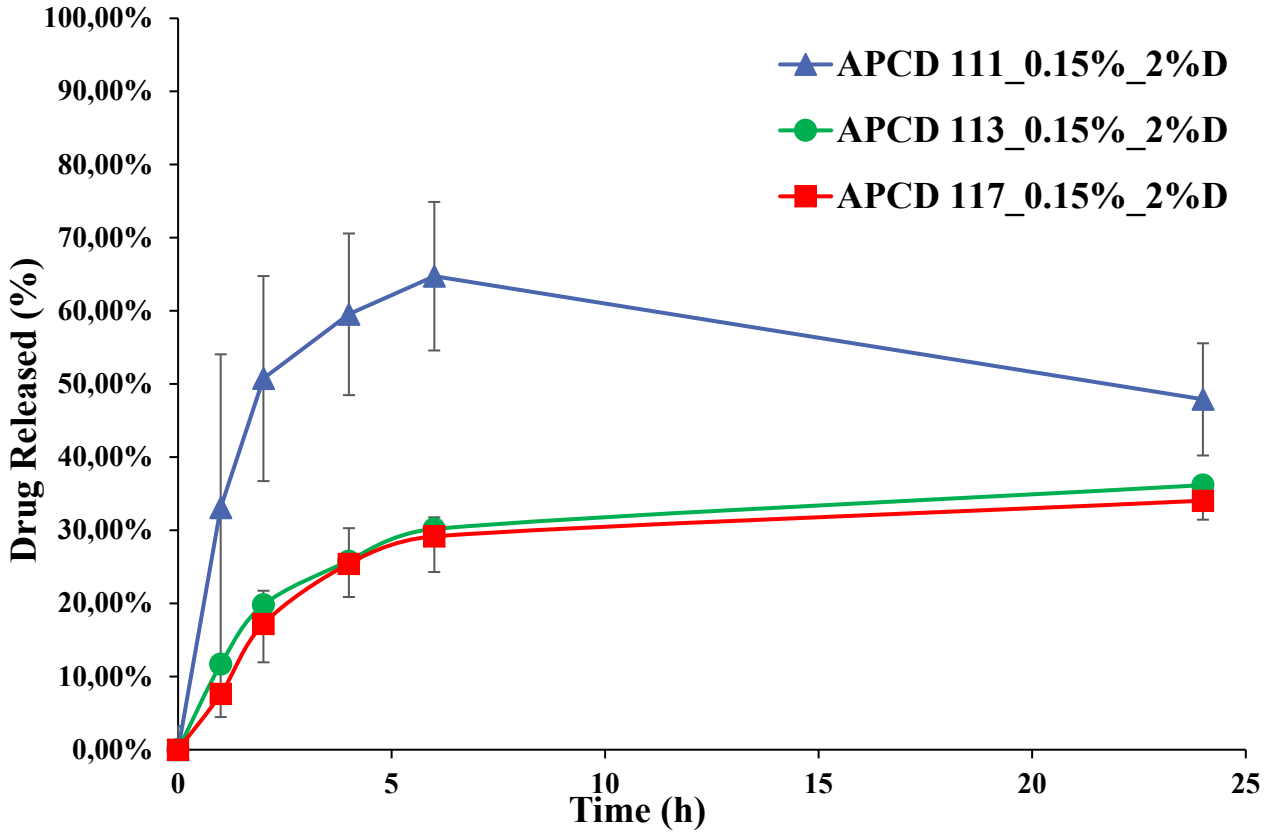
Alginate/pectin/chitosan powders loaded doxycycline

- **Fluid uptake ability:** APCD_117_2%D showed a lower swelling than blank formulation

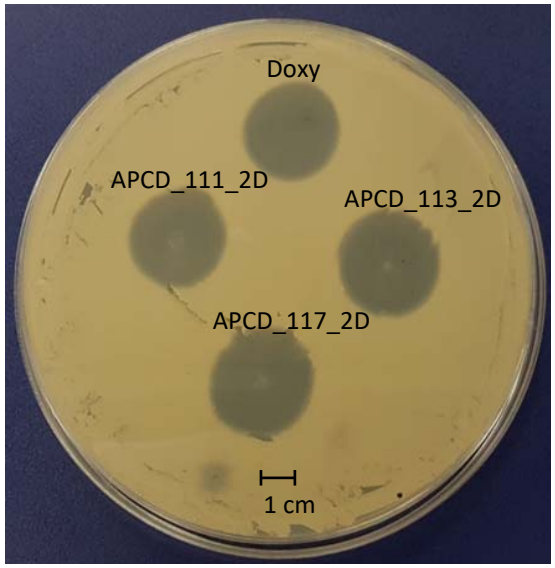


Doxycycline release

- **In vitro doxyclycine release**
  - Similiar trend for APCD 117_2%D and APCD 113_2%D
  - Higher amount of doxycycline released for APCD 111_2%D



Biological test



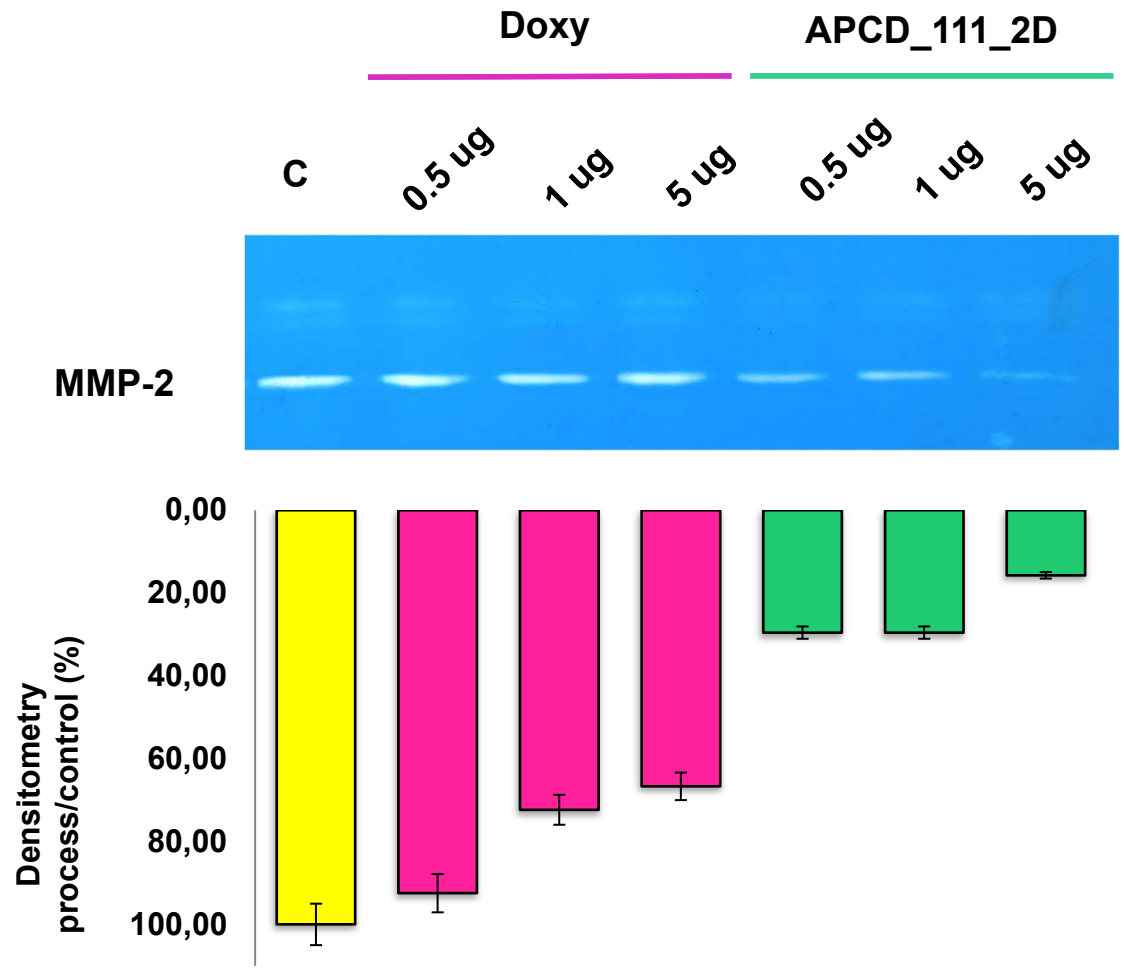
- **Antimicrobial test**

o Disc diffusion assay on *Staphylococcus aureus (ATCC 6538)*

Sample	Amount of doxy (µg)	Area (mm^2)	Amount of doxy N (µg)	Area N ($mm^{2)}$	Δ area
Doxy	1.55	759.36	1.55	759.36	
APCD _111_2D	1.59	780.83	1.55	761.18	0.24%
APCD_ 113_2D	1.58	878.88	1.55	862.19	13.54%
APCD_117_2D	1.55	934.29	1.55	934.29	23.04%

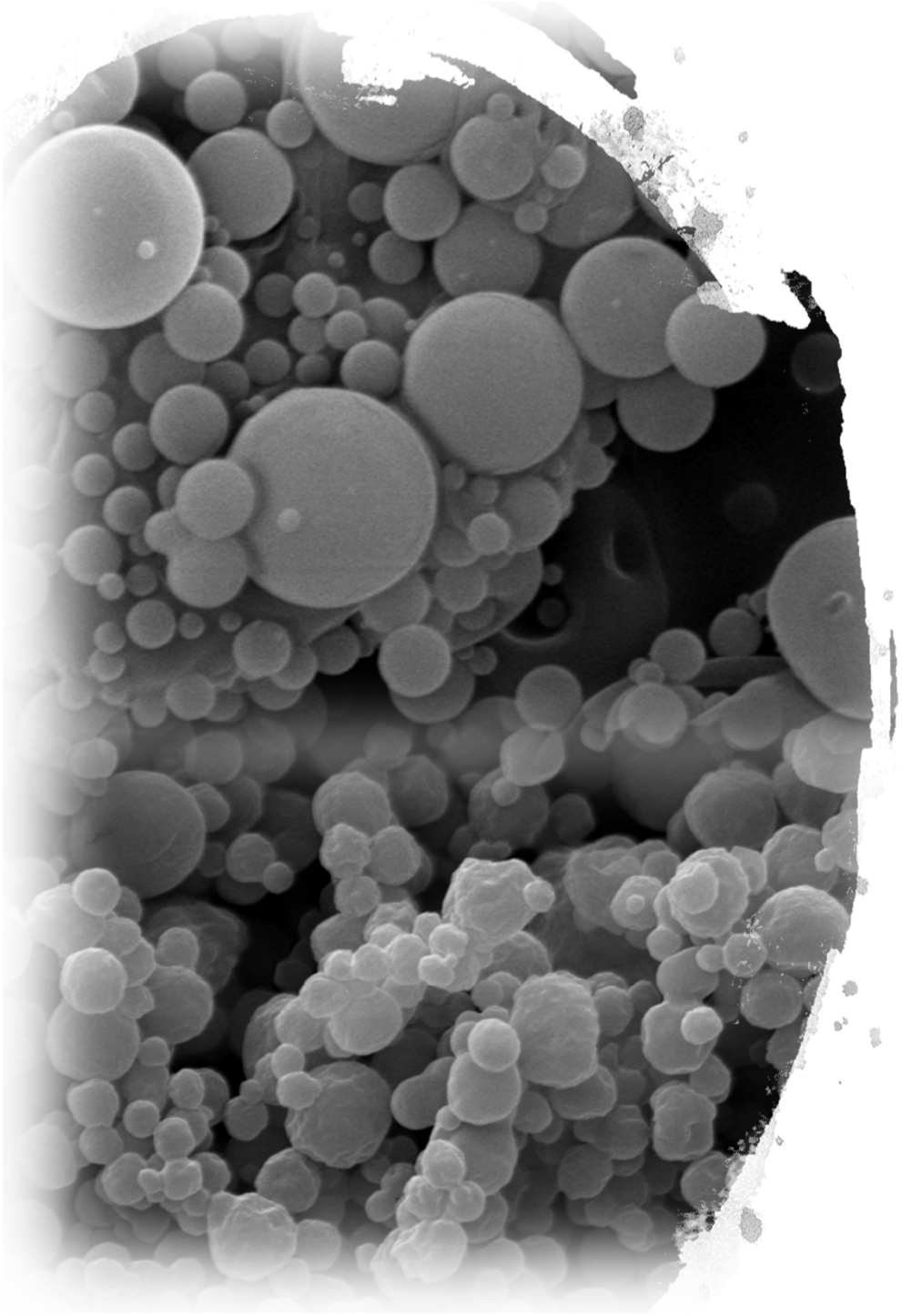
Biological test

- ***SDS-PAGE gelatin zymography**: d*oxycycline released from hydrogel inhibited MMP-2 even at 0.5 µg and its effect was stable up to 72 h



Conclusions

- Chitosan affected the particles properties leading to better characteristics
- Formulations did not show cytotoxic activity inducing IL-8 release at the application site
- All formulations showed a prolonged release of doxycycline enabling higher efficacy against bacteria and inhibiting MMP-2 activity



Thank you for the attention

Chiara Amante
E-mail: camante@unisa.it