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Controlled, pulsatile release of thermostabilized inactivated polio vaccine from PLGA-based microspheres

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Controlled, pulsatile release of stable inactivated polio vaccine from PLGA-based microspheres

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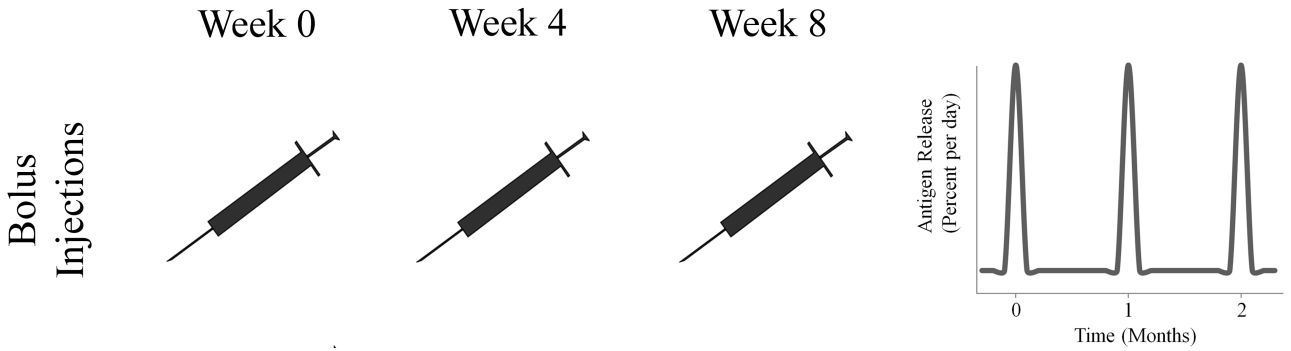
June 13, 2016

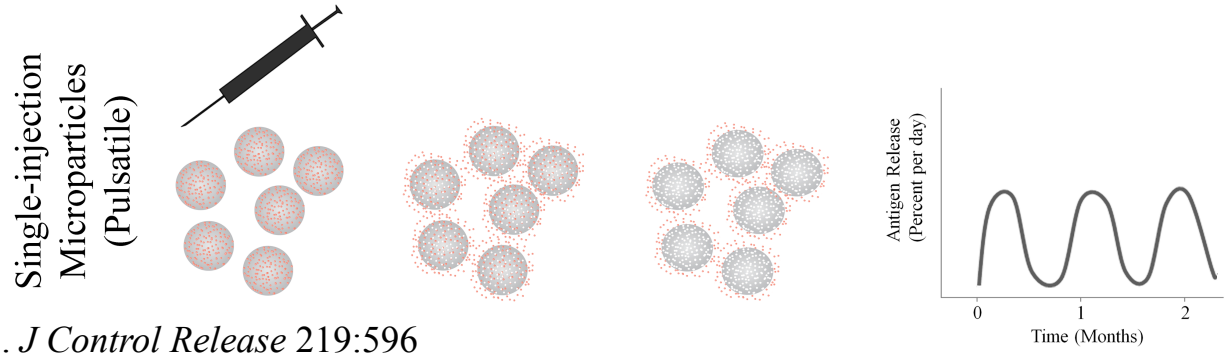
BILL & MELINDA GATES foundation

Inactivated polio vaccine (IPV): background

- Poliomyelitis is a potentially fatal infectious disease that can be prevented by vaccination
- For vaccine efficacy, IPV must be administered in 2-3 bolus injections (IM) spread over weeks or months

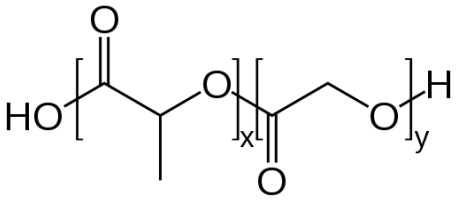


- Vaccine coverage is hampered in developing countries by difficulties of patient access in at-risk populations



McHugh et al. 2015. *J Control Release* 219:596

Poly(lactic-*co*-glycolic acid) (PLGA) microparticles for controlled release

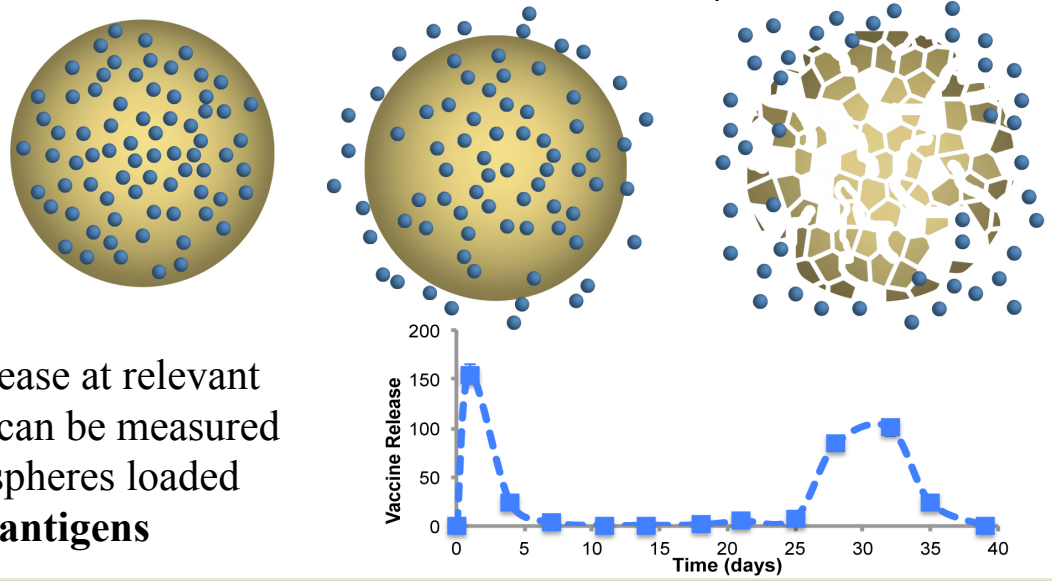


- Biodegradable polyester
  - Hydrolytically cleaved at ester linkages
  - Bulk-degrading
  - Degradation rate depends on lactide-to-glycolide ratio, molecular weight, and formulation parameters
- Polymer degradation leads to protein release

IPV encapsulated in PLGA matrix

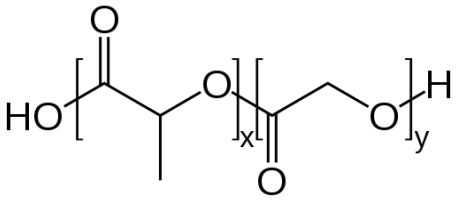
Burst #1: IPV near surface (diffusion)

Burst #2: PLGA degrades and forms pores that allow IPV release



Pulsatile release at relevant time points can be measured from microspheres loaded with **stable antigens**

Poly(lactic-*co*-glycolic acid) (PLGA) microparticles for controlled release



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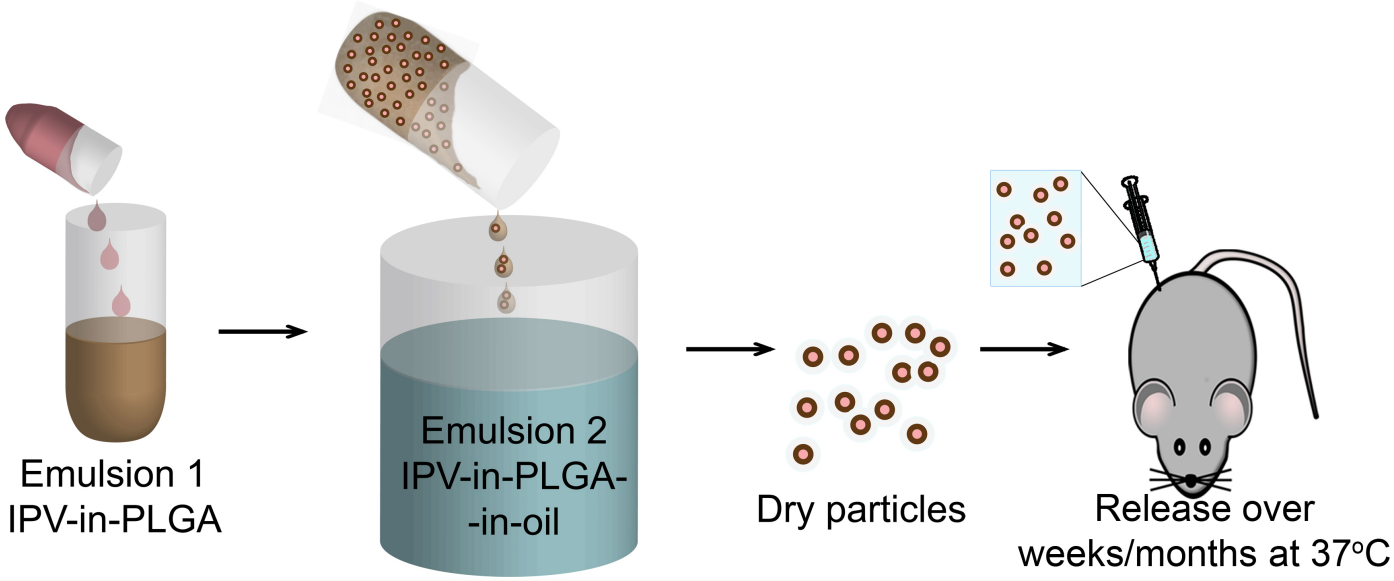
- All 3 serotypes of IPV must survive encapsulation, incubation, and release without denaturation



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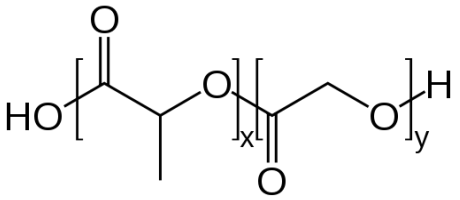

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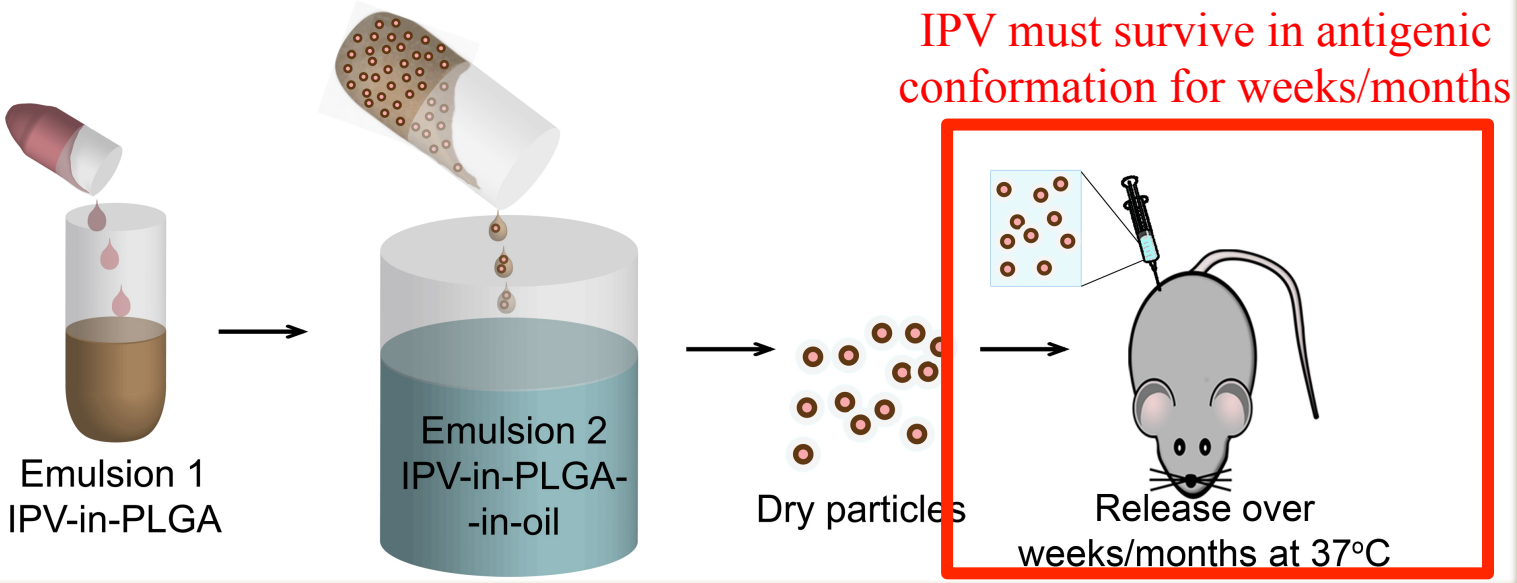
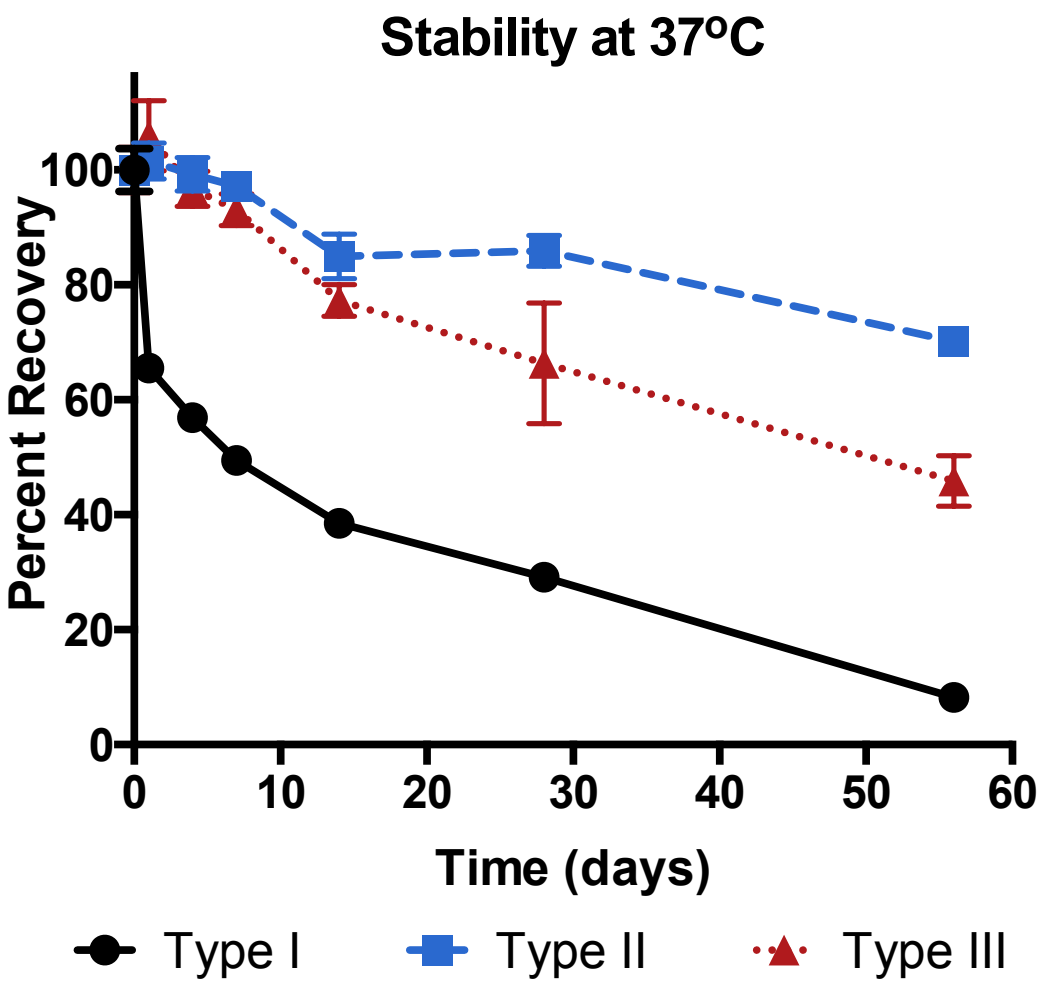


IPV stability: thermostability



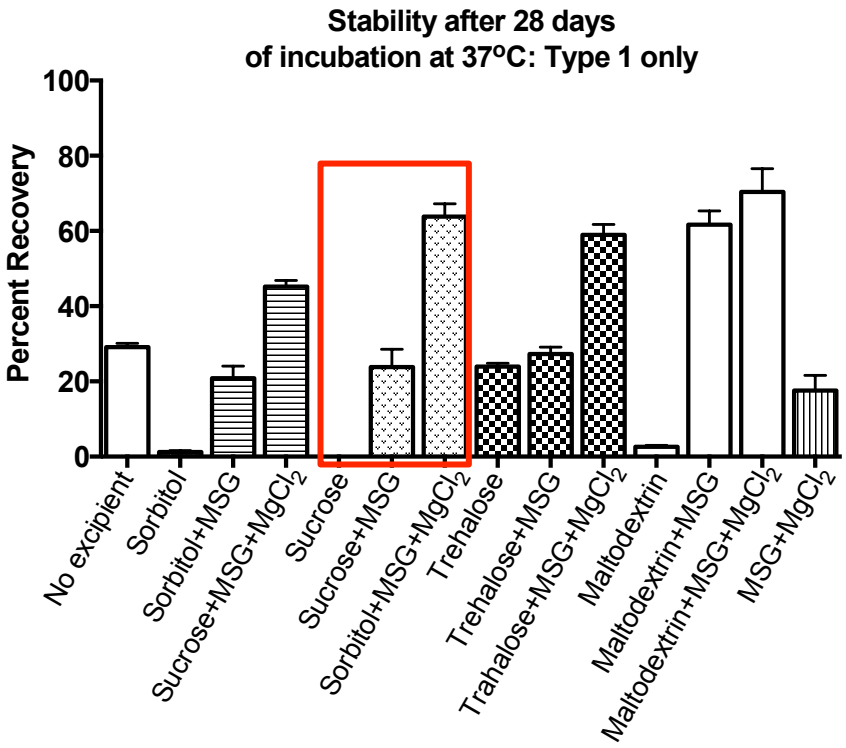
- IPV consists of three serotypes (types 1, 2, and 3), which have different stability properties
- Recovery was calculated from the D-antigenicity as measured by ELISA
- IPV was incubated at 37ºC in PBS (pH 7.4) buffer
- IPV is unstable at elevated temperatures, with >50% of serotype 1 denatured after 1 week at 37ºC.
- All initial thermostability studies were done with IPV in aqueous solution (not in microspheres)

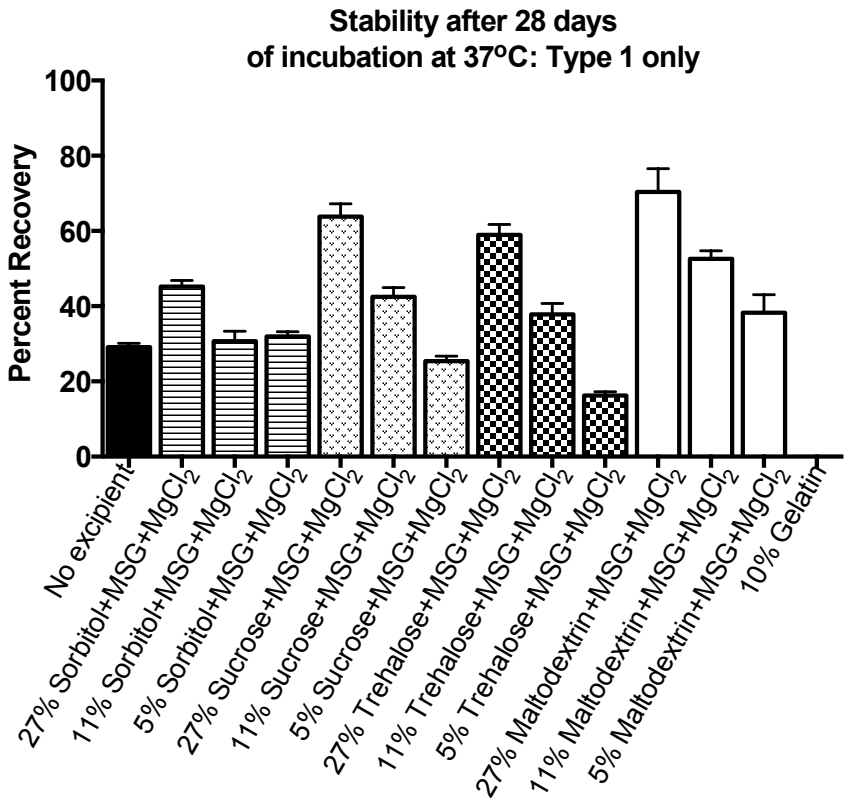
Types of excipients

Sugar	Protein	Amino acid(s)	Ions	pH modulator	Other
Sorbitol	Gelatin	**MSG (glutamate)**	**$MgCl_2$**	$Mg(OH)_2$	D_2O
Sucrose	BSA	Arginine		$Mg(CO)_3$	
Trehalose	Casein	Lysine		Arginine	
Maltodextrin		Methionine		EPO	
Inulin		Aspartate			
Chitin/chitosan		Glutathione			

Vaccine formulations often contain a mixture of multiple excipients. Some of these are remnants of the cell culture used for vaccine production, some are added deliberately to preserve stability, and some are a combination of the above.

IPV stability: incubation at 37°C





Sugars on their own did not confer improved stability on IPV during incubation at 37°C.

Sugars performed better when combined with **MSG** and **$MgCl_2$** and also performed better at higher concentrations. Of the sugars tested, **maltodextrin and sucrose** were the best.

Tzeng et al. 2016. *J Control Release* 233:101

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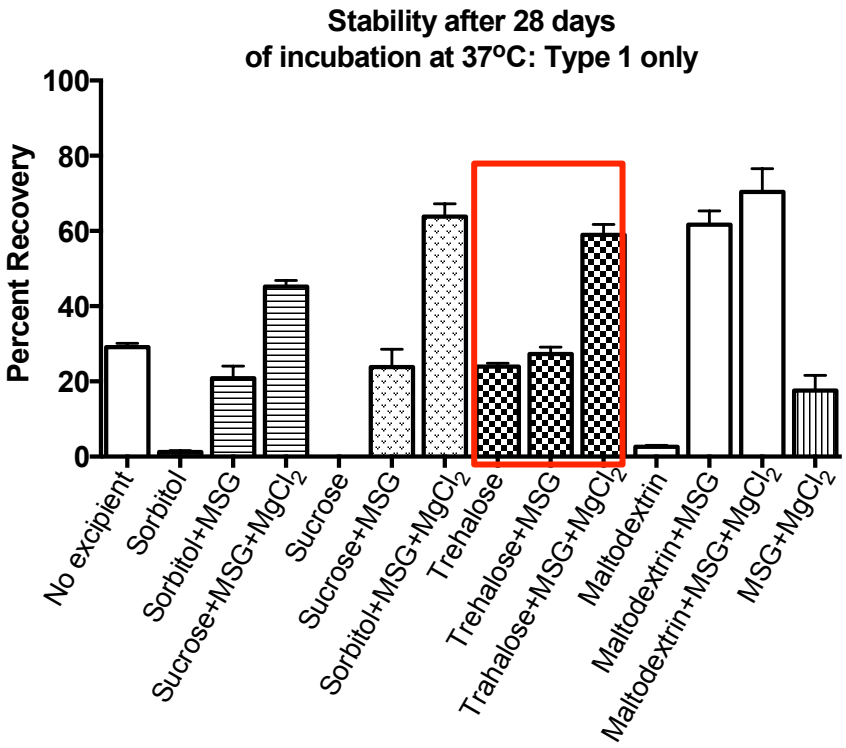

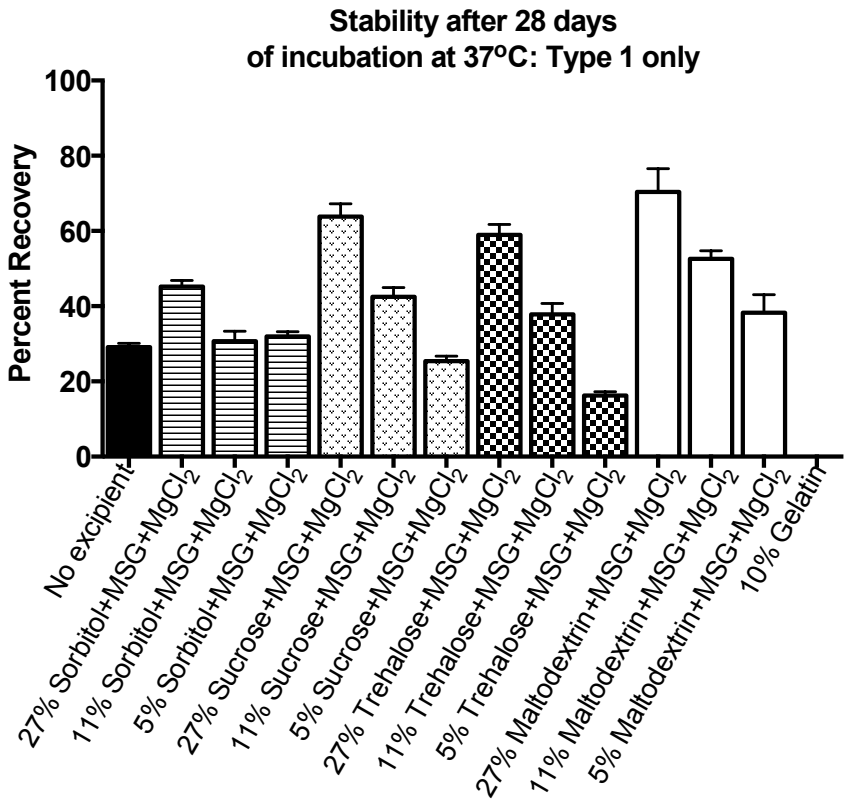

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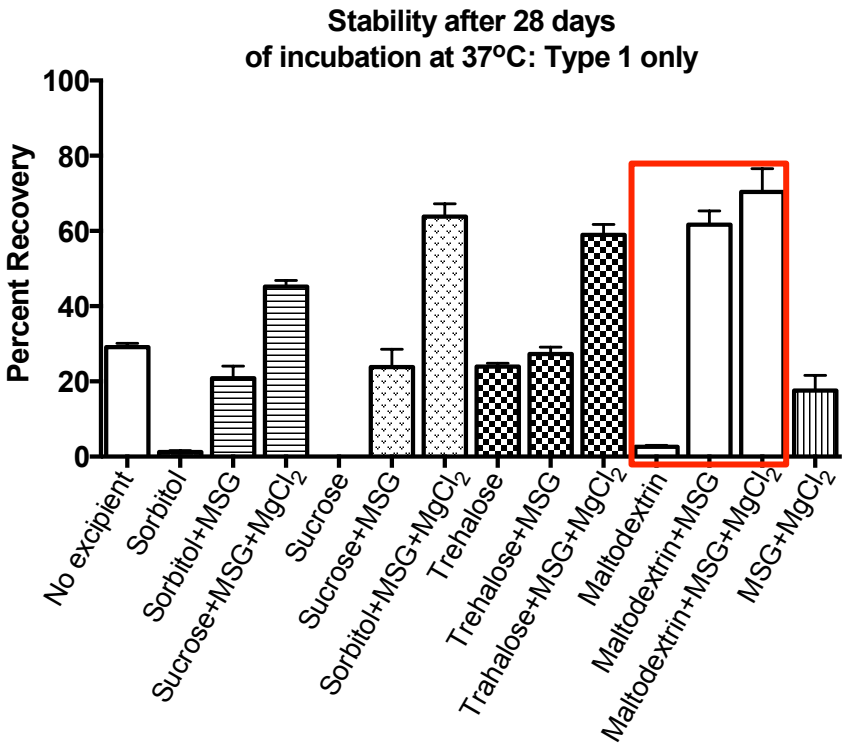

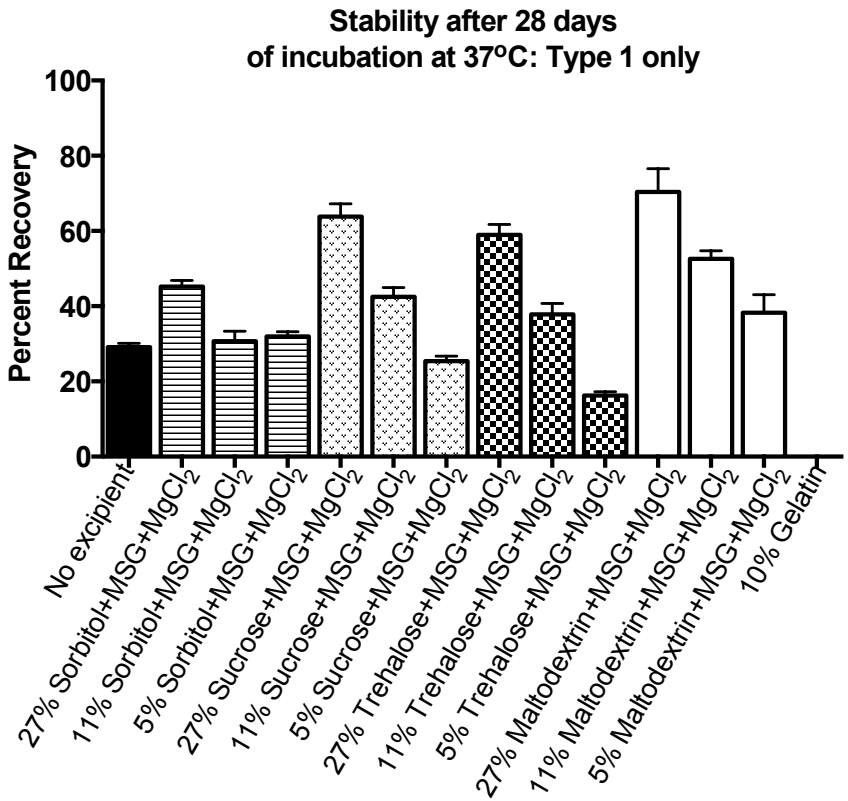

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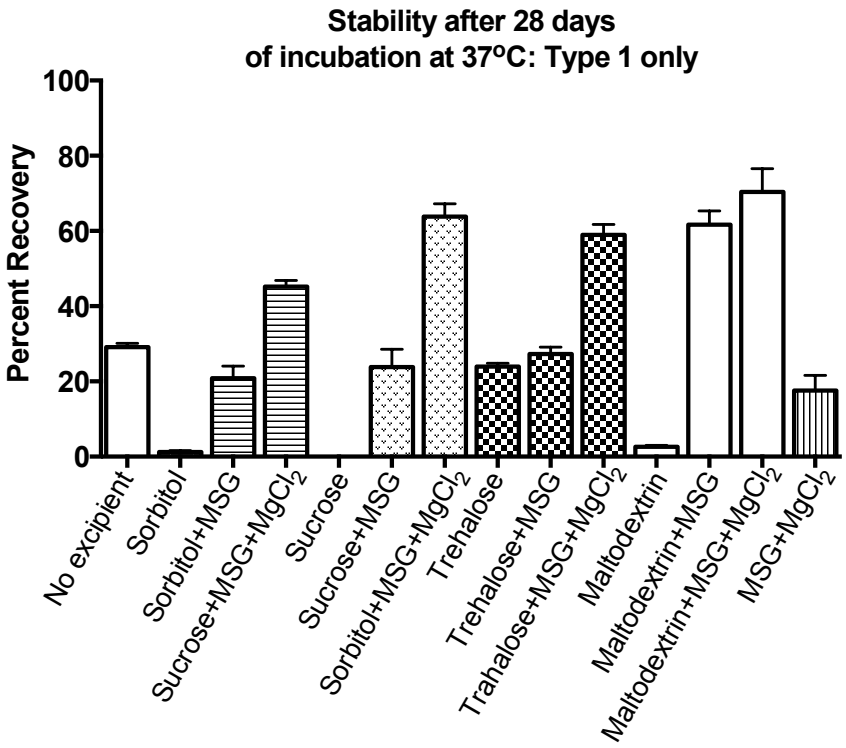

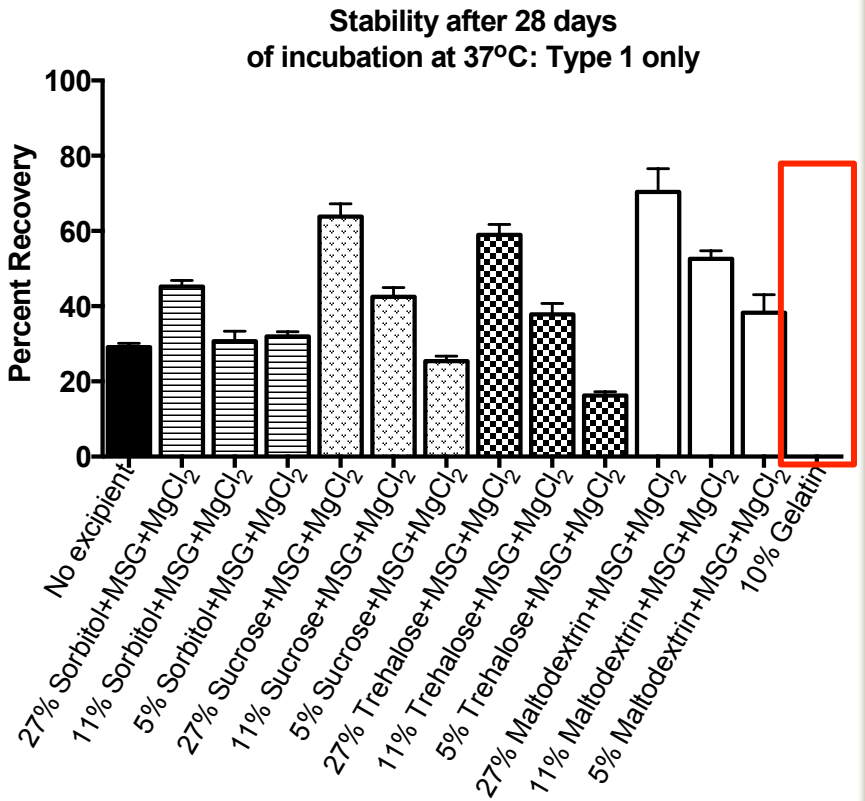

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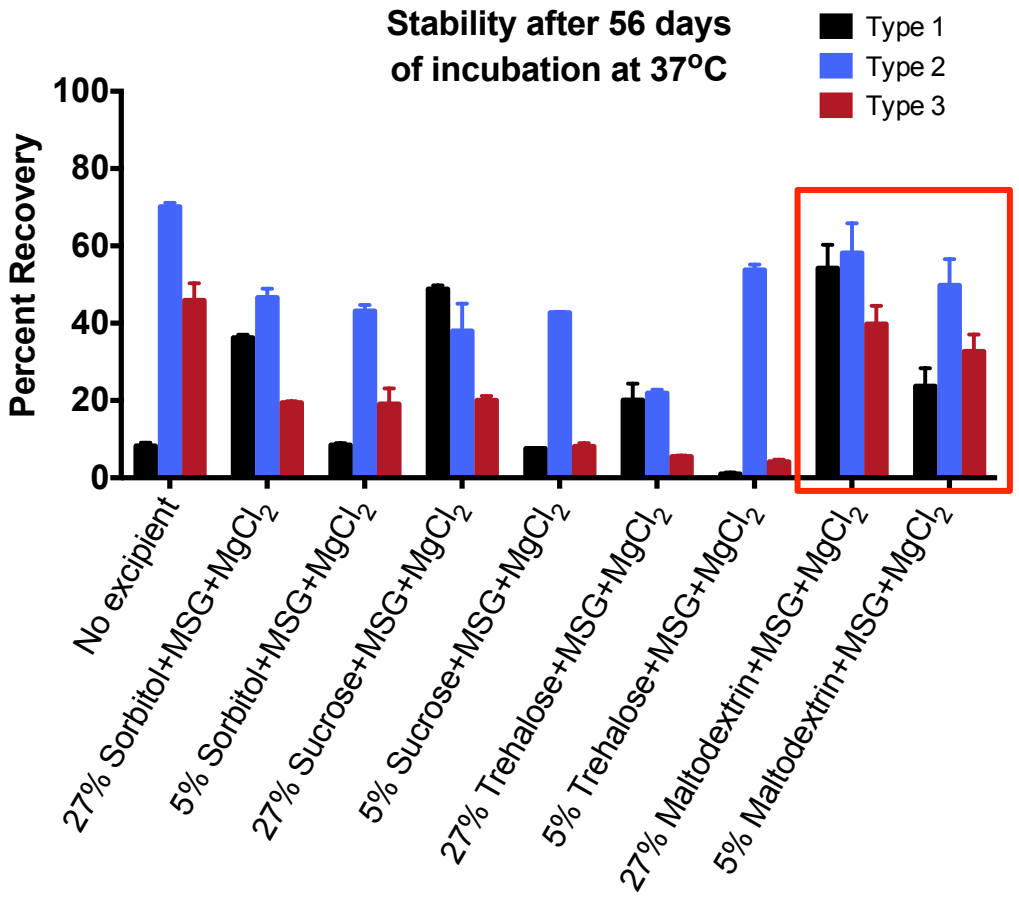

After two months of incubation at 37°C, IPV formulation with maltodextrin + MSG + $MgCl_2$ saw good recovery of all three serotypes, with 30-60% recovery of type 1 and 40-70% recovery of types 2 and 3.

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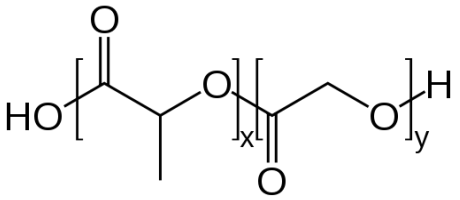

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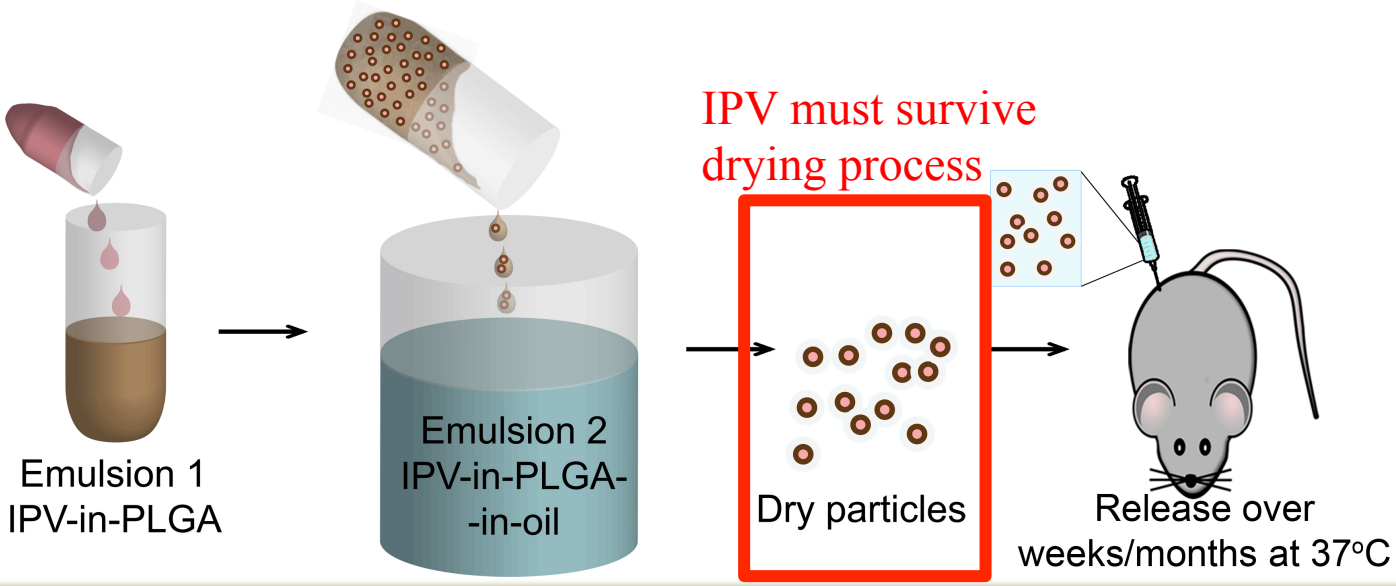
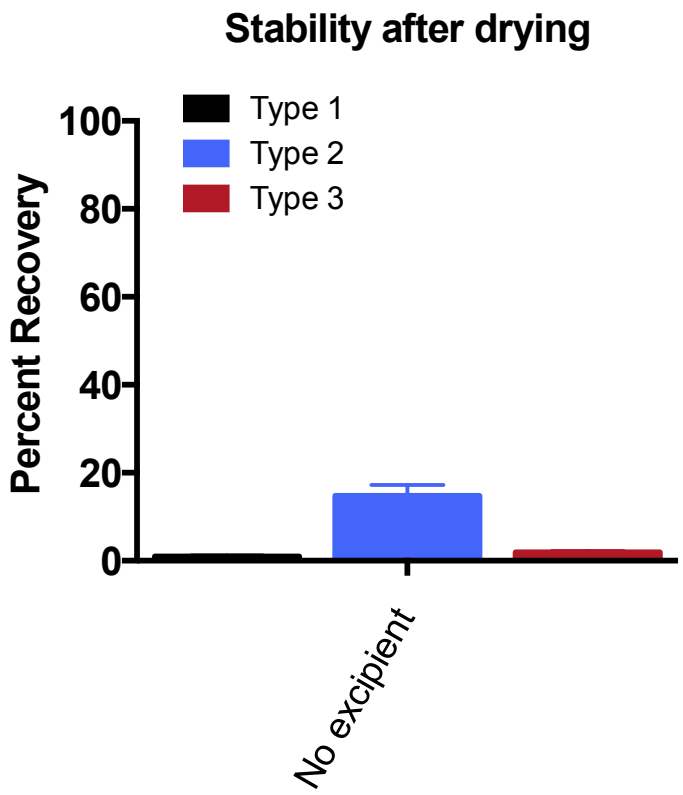


IPV stability:
Vacuum drying



An aqueous solution of IPV was dried under vacuum for 1 hr at room temperature.

IPV alone (without added excipients) was denatured by drying.

Excipients and excipient combinations were added to improve stability.

Tzeng et al. 2016. *J Control Release* 233:101

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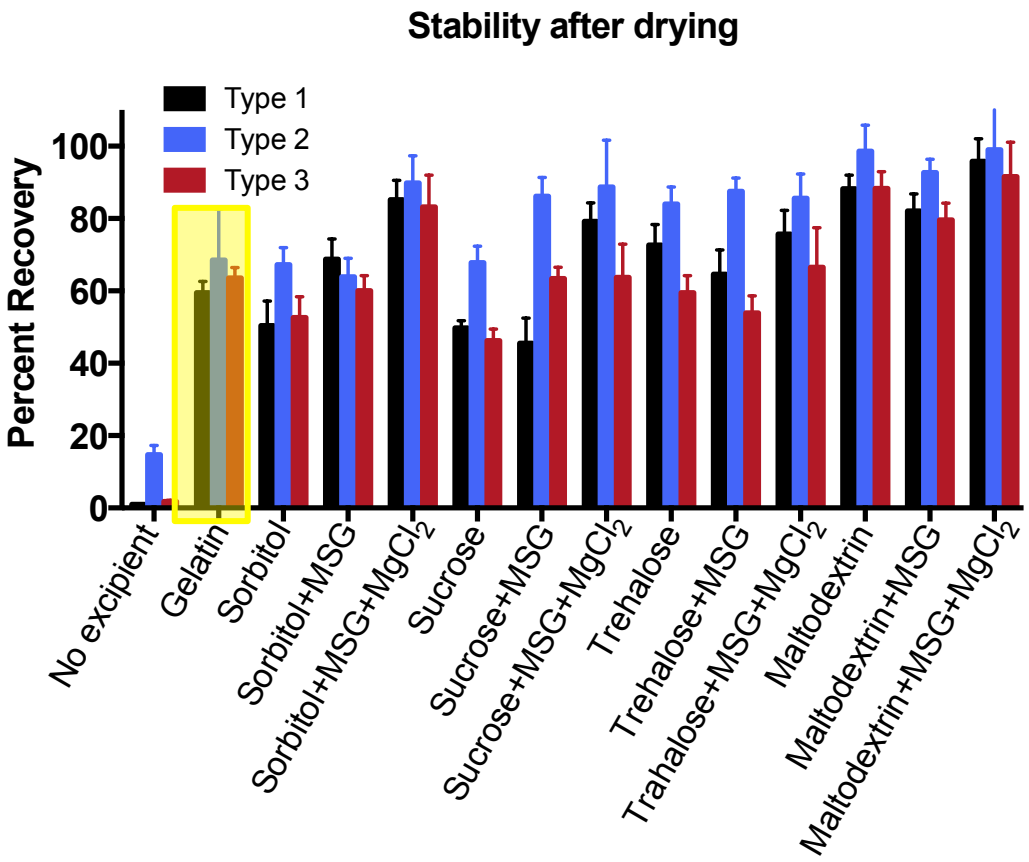

Gelatin and **sugars** both showed good results after drying.

Sugars generally showed increased protective ability in combination with amino acids (glutamate, MSG) and/or ions ($MgCl_2$).

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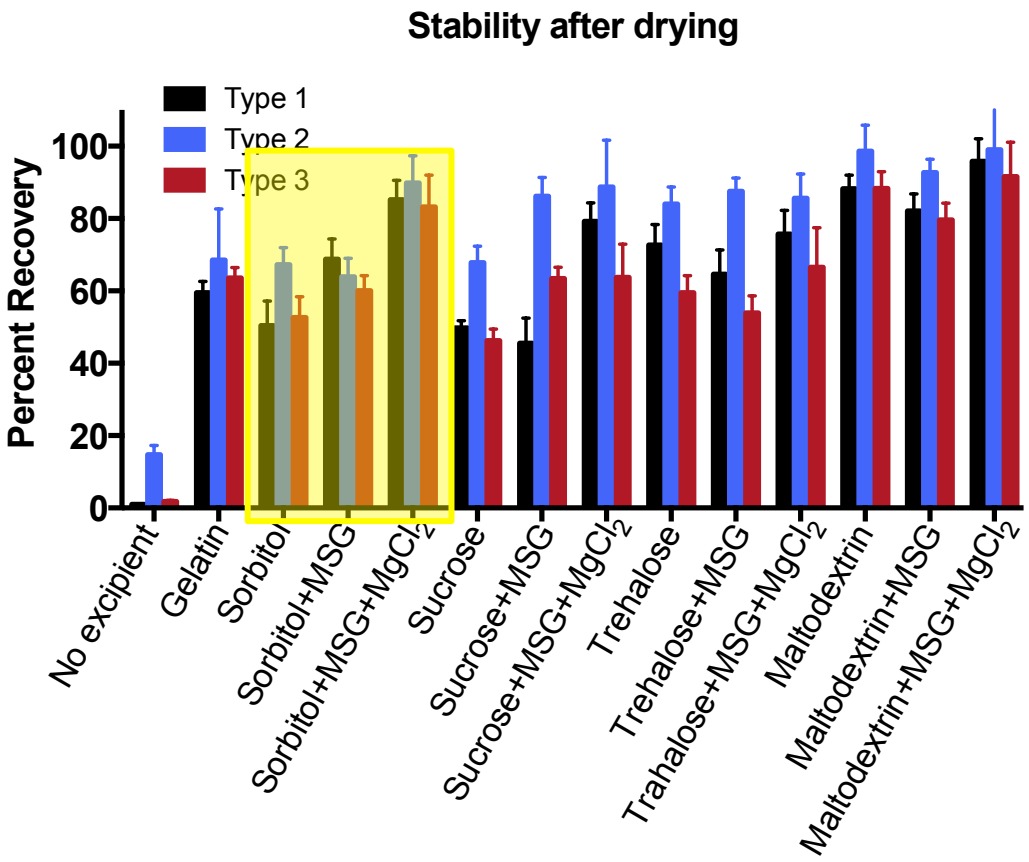

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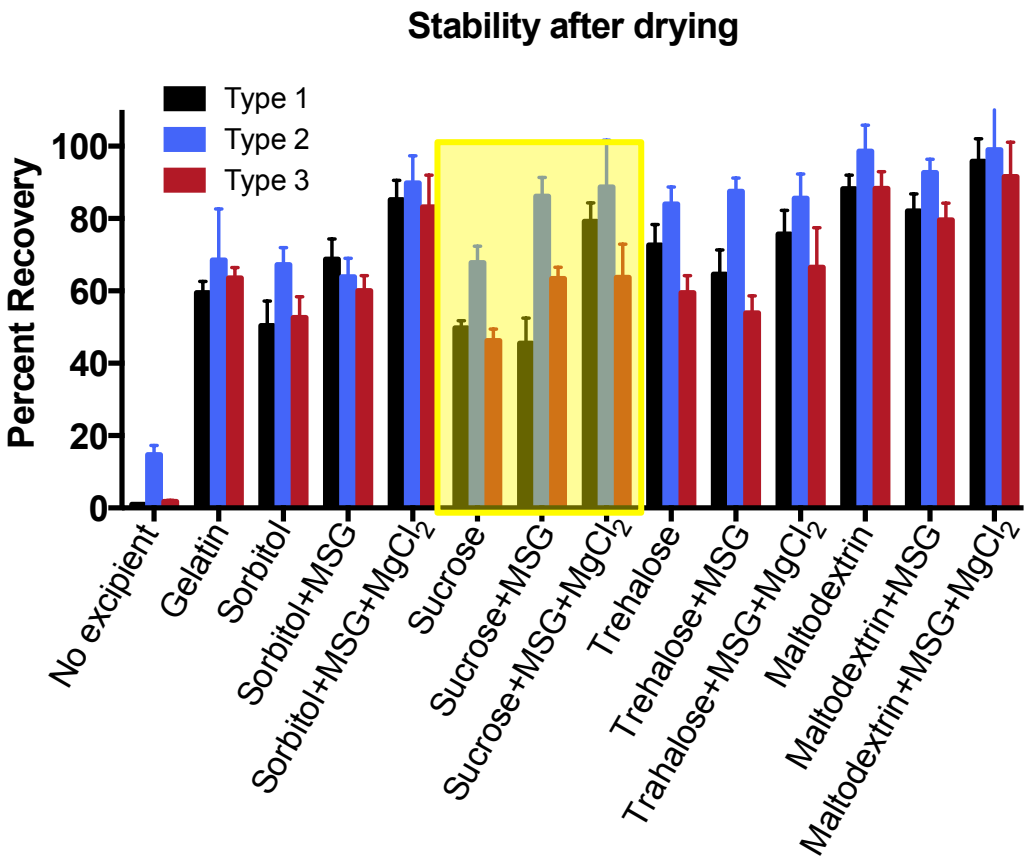

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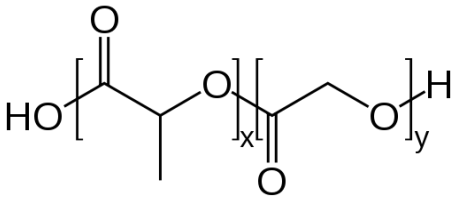

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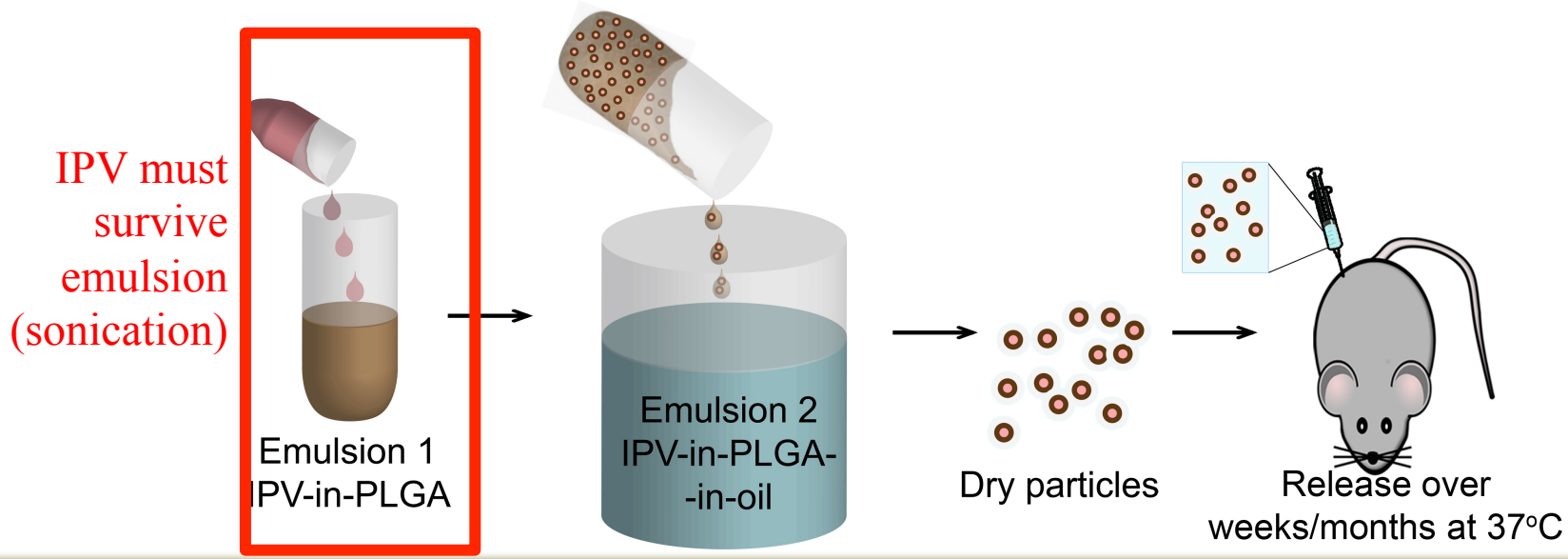
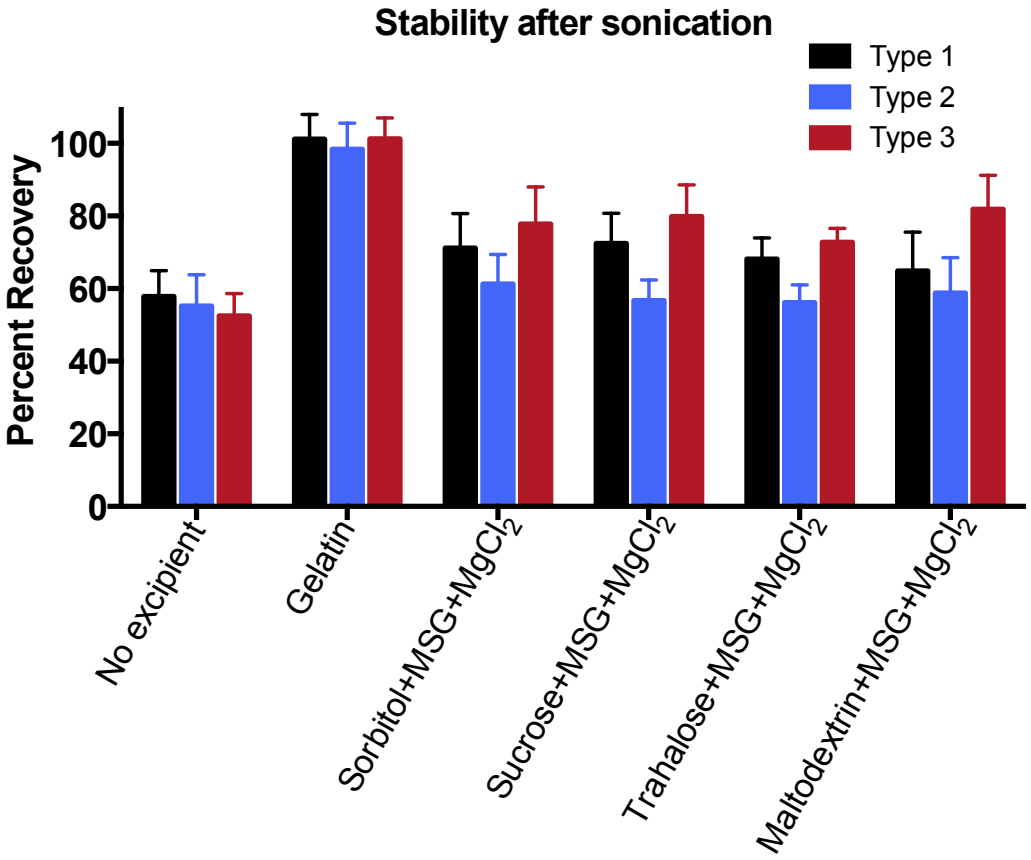


IPV stability:
Emulsification (sonication)



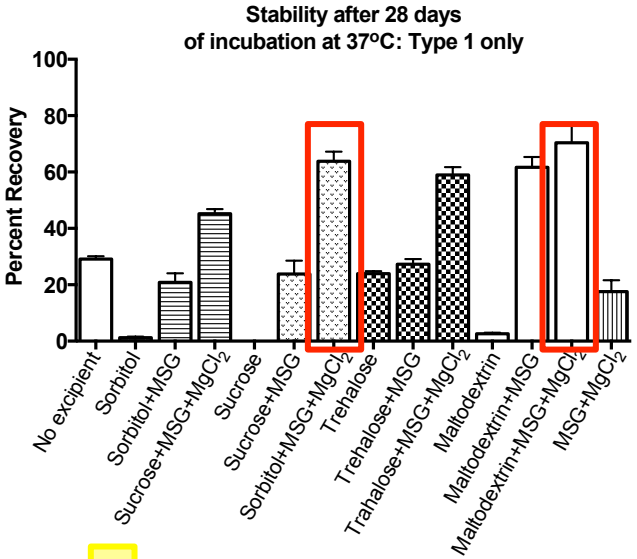
Gelatin and showed the best protection of IPV during the sonication step.

Sugars and salts generally had minimal effects on the stability during the emulsification step.

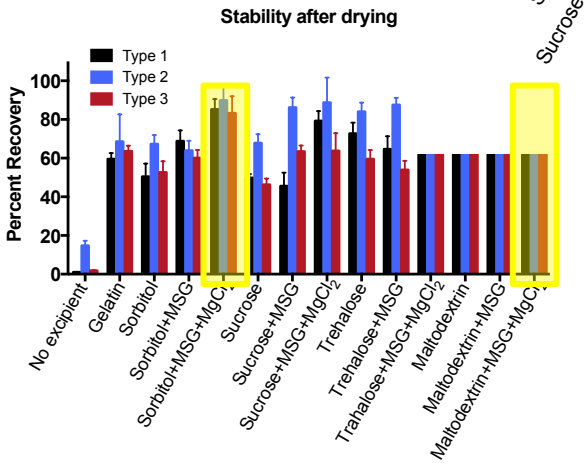
Tzeng et al. 2016. *J Control Release* 233:101

IPV stability: Emulsification (sonication)

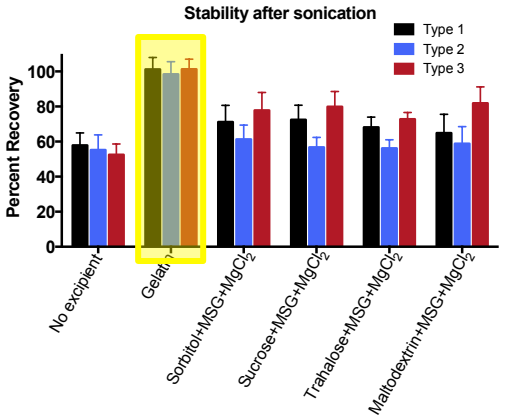
- Incubation at 37°C:
  - Sucrose/MSG/$MgCl_2$
  - Maltodextrin/MSG/$MgCl_2$



- Drying:
  - Sorbitol/MSG/$MgCl_2$
  - Maltodextrin/MSG/$MgCl_2$

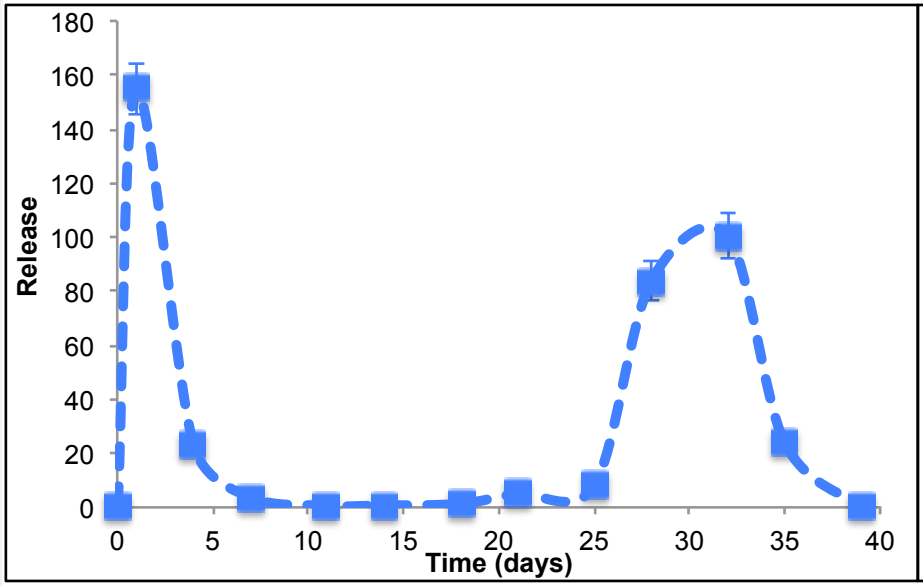


- Emulsification:
  - Gelatin

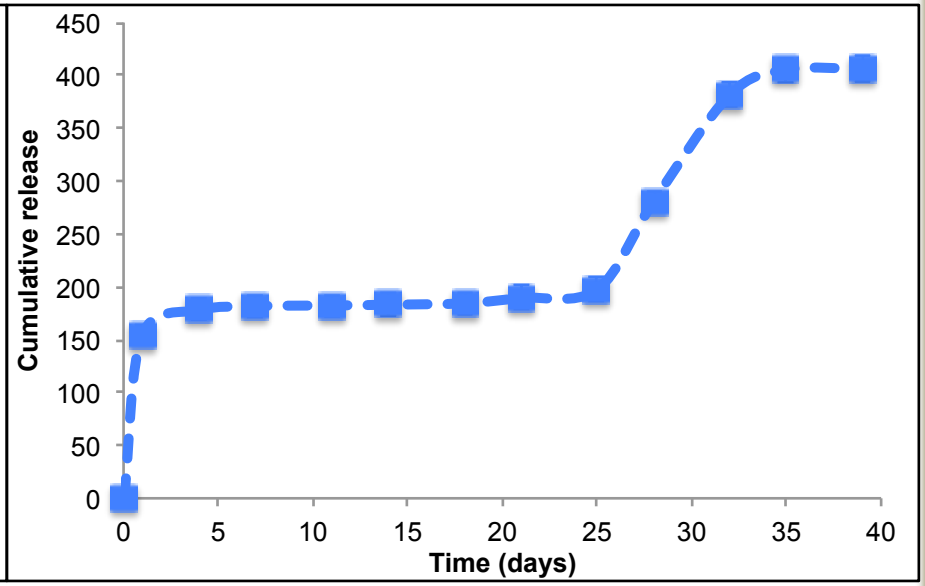


IPV release from PLGA particles

Pulsatile release

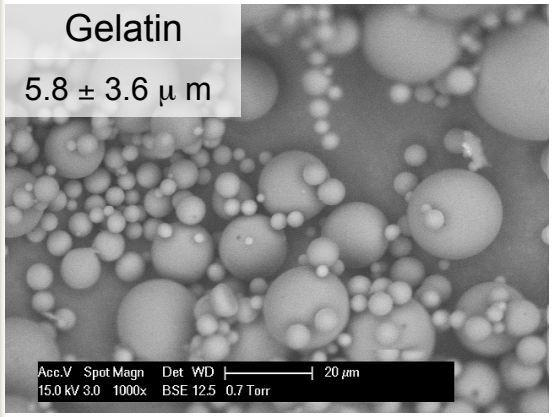


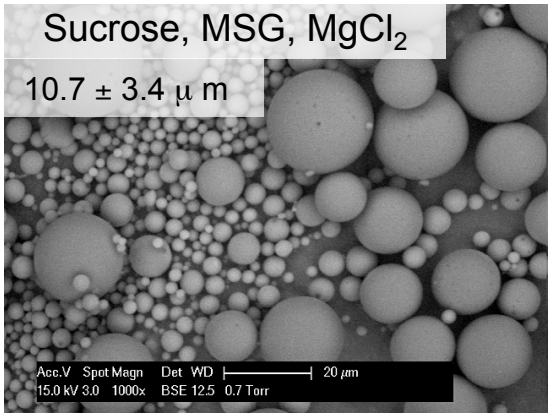
Cumulative release

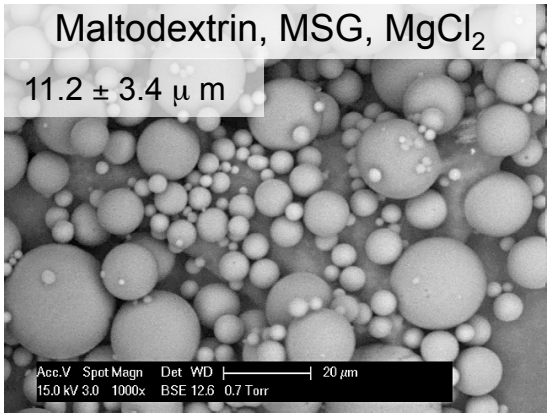


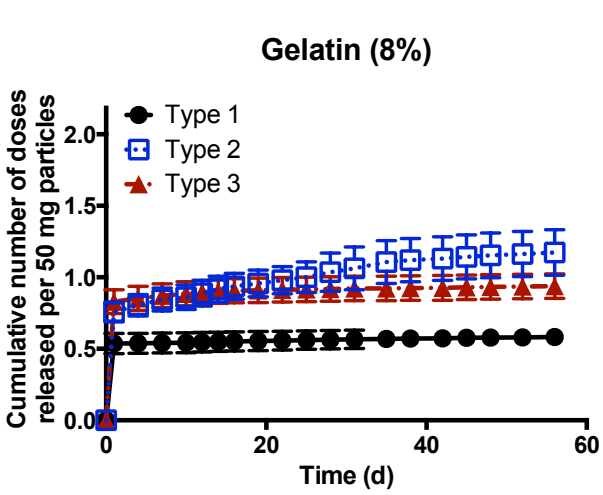
In vitro cumulative release results are plotted as percentages of the human dose (40 DU type 1, 8 DU type 2, 32 DU type 3)

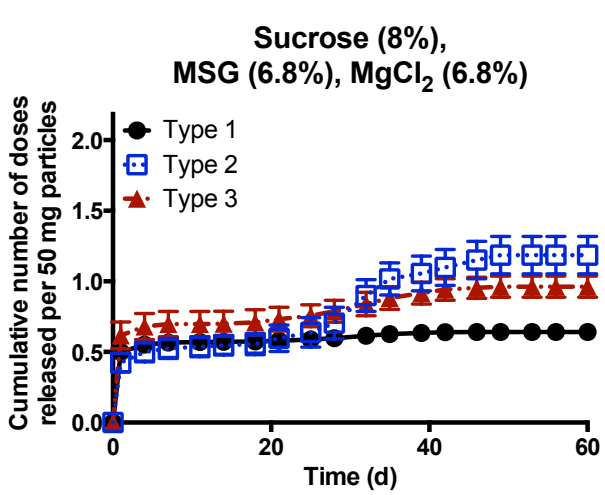
IPV release from PLGA particles

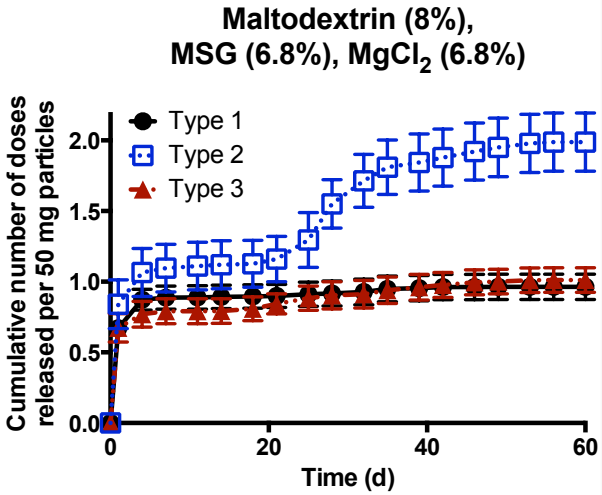










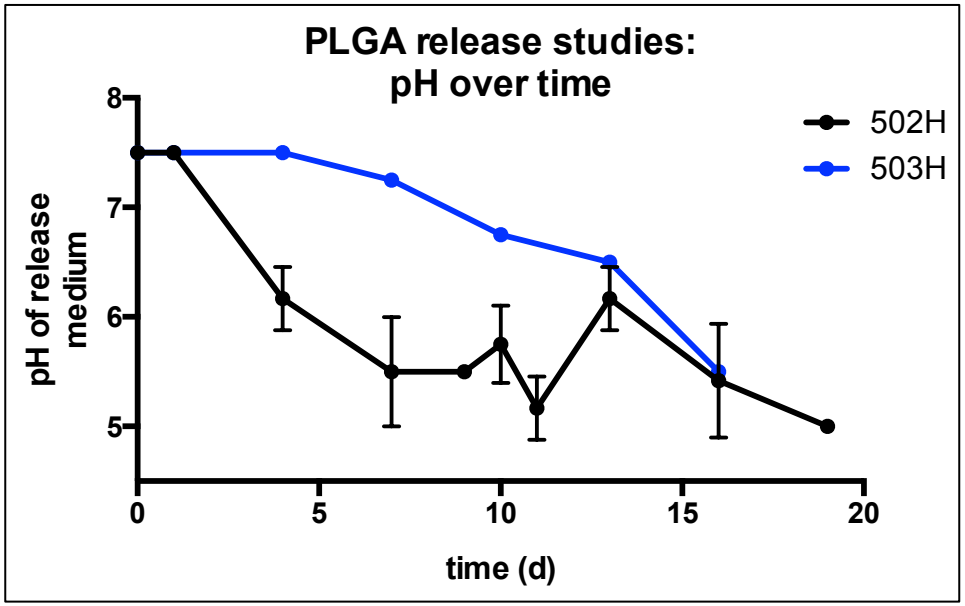


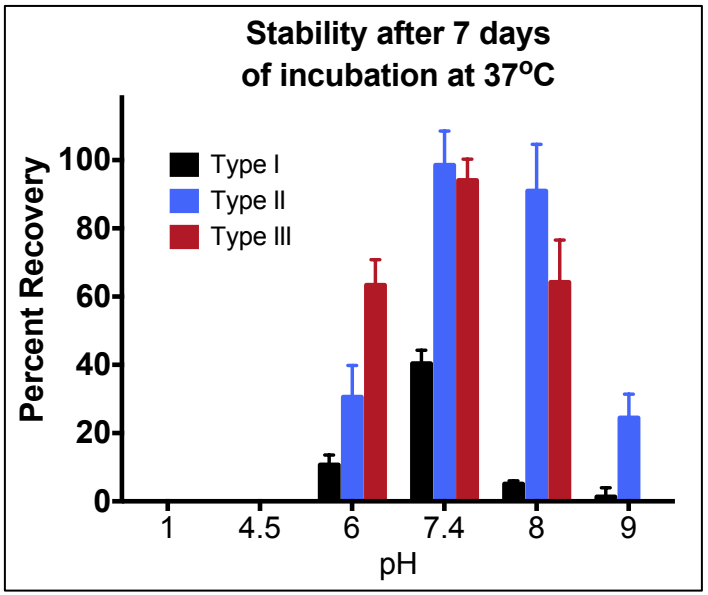
- Sugar-based excipients co-encapsulated in PLGA particles promote IPV release in two bursts; however, very little later release is seen for types 1 and 3.

Tzeng et al. 2016. *J Control Release* 233:101

PLGA microspheres: pH

- PLGA degrades into acidic molecules
- PLGA degrades by bulk erosion
  - Acid buildup inside microspheres
- pH of release medium (outside particles) over time suggests acid is also building up inside particles over time

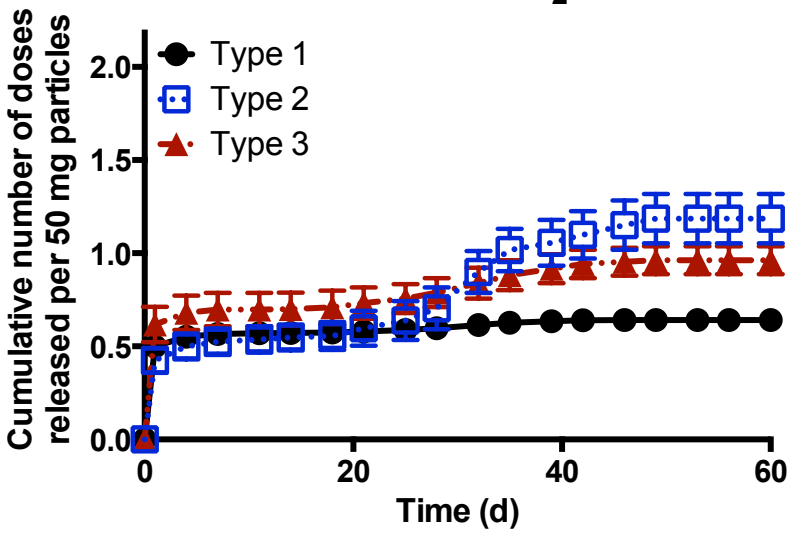




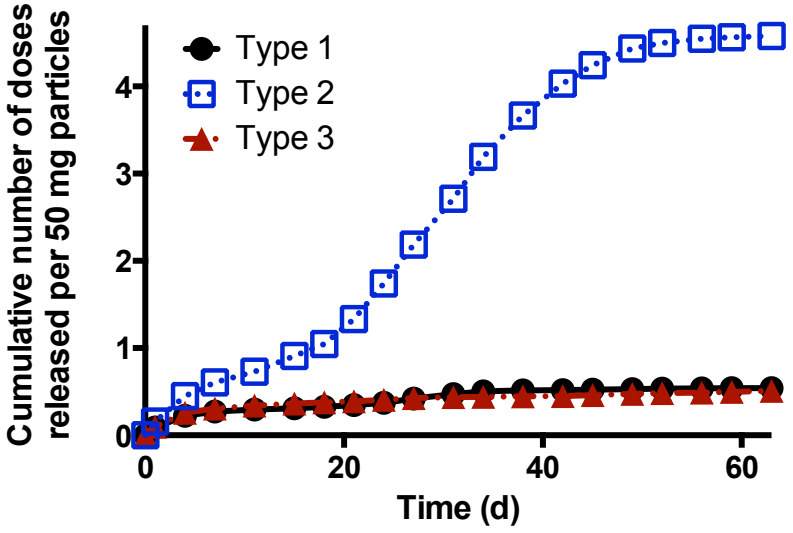
PLGA microspheres: Insoluble excipients buffer acidic products

- Water-soluble bases raise the pH of the IPV environment and cause denaturation
- Small molecule bases diffuse out of the microparticle faster than the antigen, depleting the basic or buffering component before PLGA degradation is completed
- $Mg(OH)_2$ is an insoluble base that can be dispersed in the polymer maxtrix

Sucrose (8%), MSG (6.8%), $MgCl_2$ (6.8%)



Sucrose (8%), MSG (6.8%), $MgCl_2$ (6.8%), $Mg(OH)_2$ (5%)



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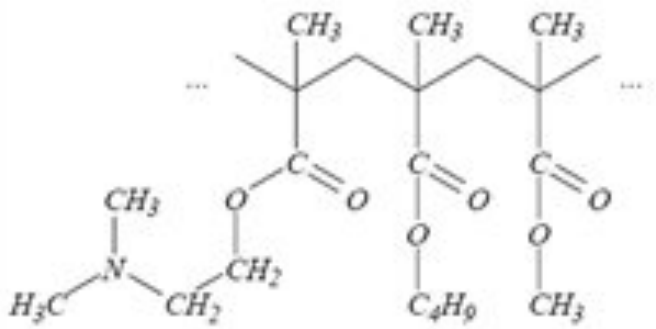
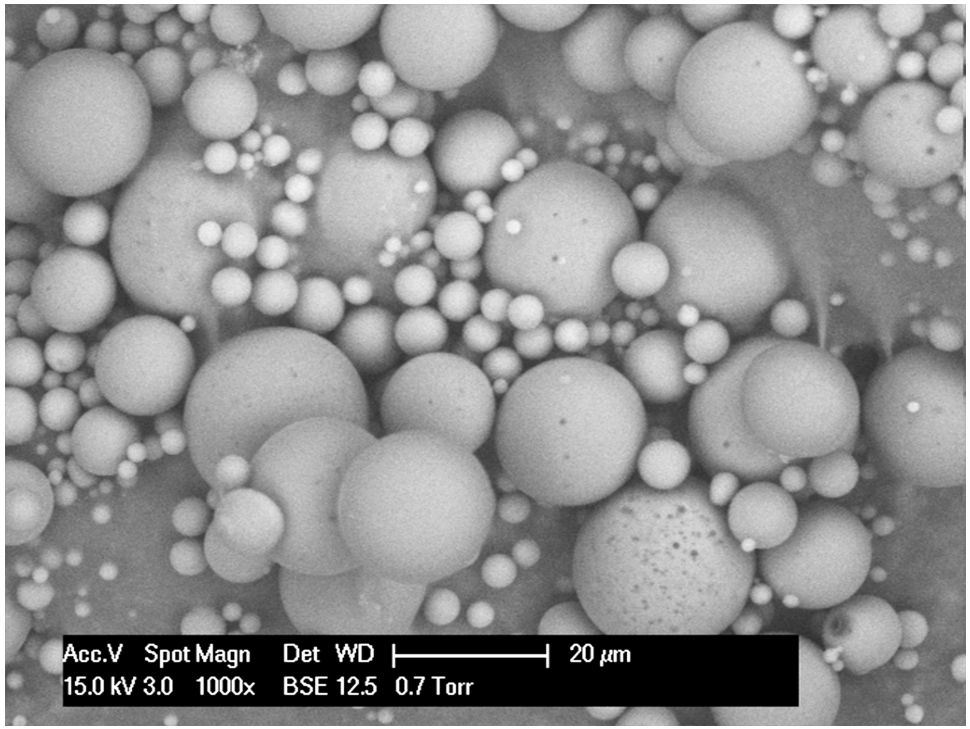
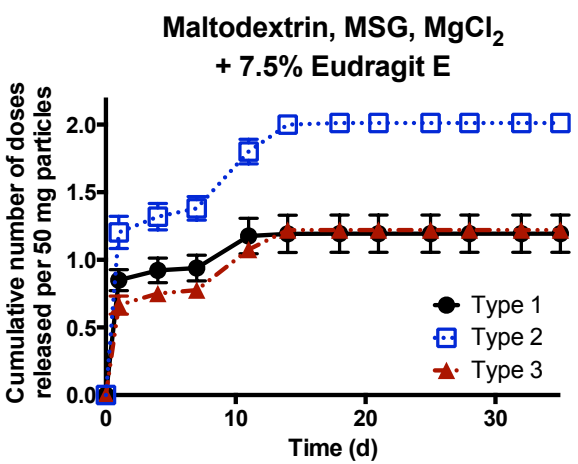
PLGA microspheres: Insoluble excipients buffer acidic products

- Cationic polymer Eudragit E PO is insoluble in water at neutral pH but soluble at < 3 pH
  - Basic functional groups raise the local pH of PLGA
  - The local basic pH accelerates PLGA degradation, forming acid components that are buffered by the basic Eudragit E



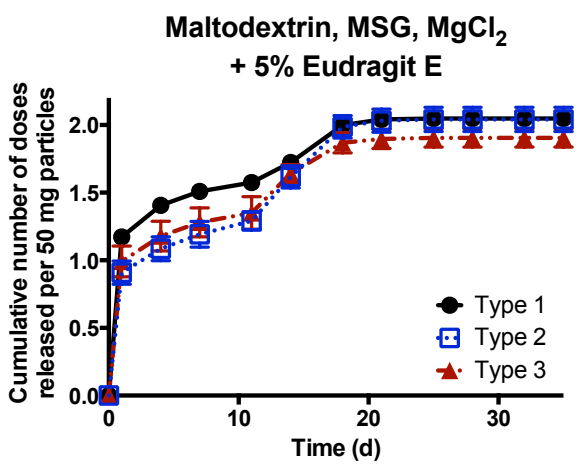


PLGA microspheres: cationic polymers as pH-modulating dopants



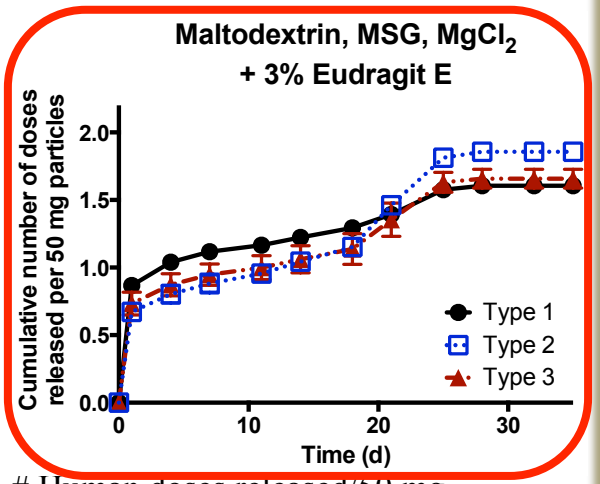
Human doses released/50 mg

Burst Time (d)	Type 1	Type 2	Type 3
0-4	0.92	1.32	0.75
7-14	0.25	0.62	0.44



Human doses released/50 mg

Burst Time (d)	Type 1	Type 2	Type 3
0-4	1.41	1.09	1.18
11-21	0.47	0.74	0.55



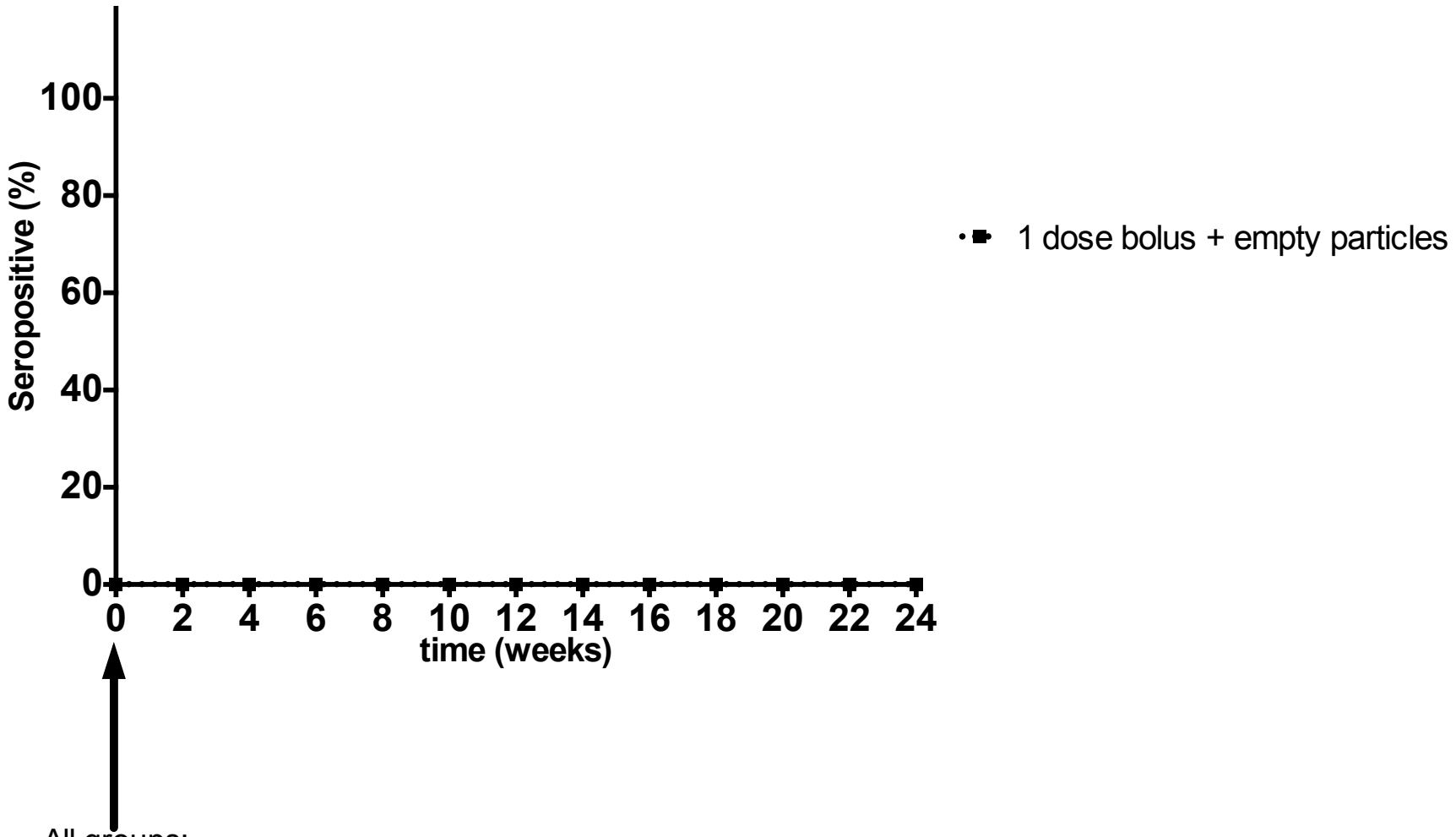
Human doses released/50 mg

Burst Time (d)	Type 1	Type 2	Type 3
0-4	1.04	0.80	0.87
18-28	0.32	0.97	0.52

- The amount of Eudragit E in the particles controls the spacing of burst release
- The formulations shown above release a total of ~2 human doses spread between 2 distinct bursts, mimicking multiple bolus injections

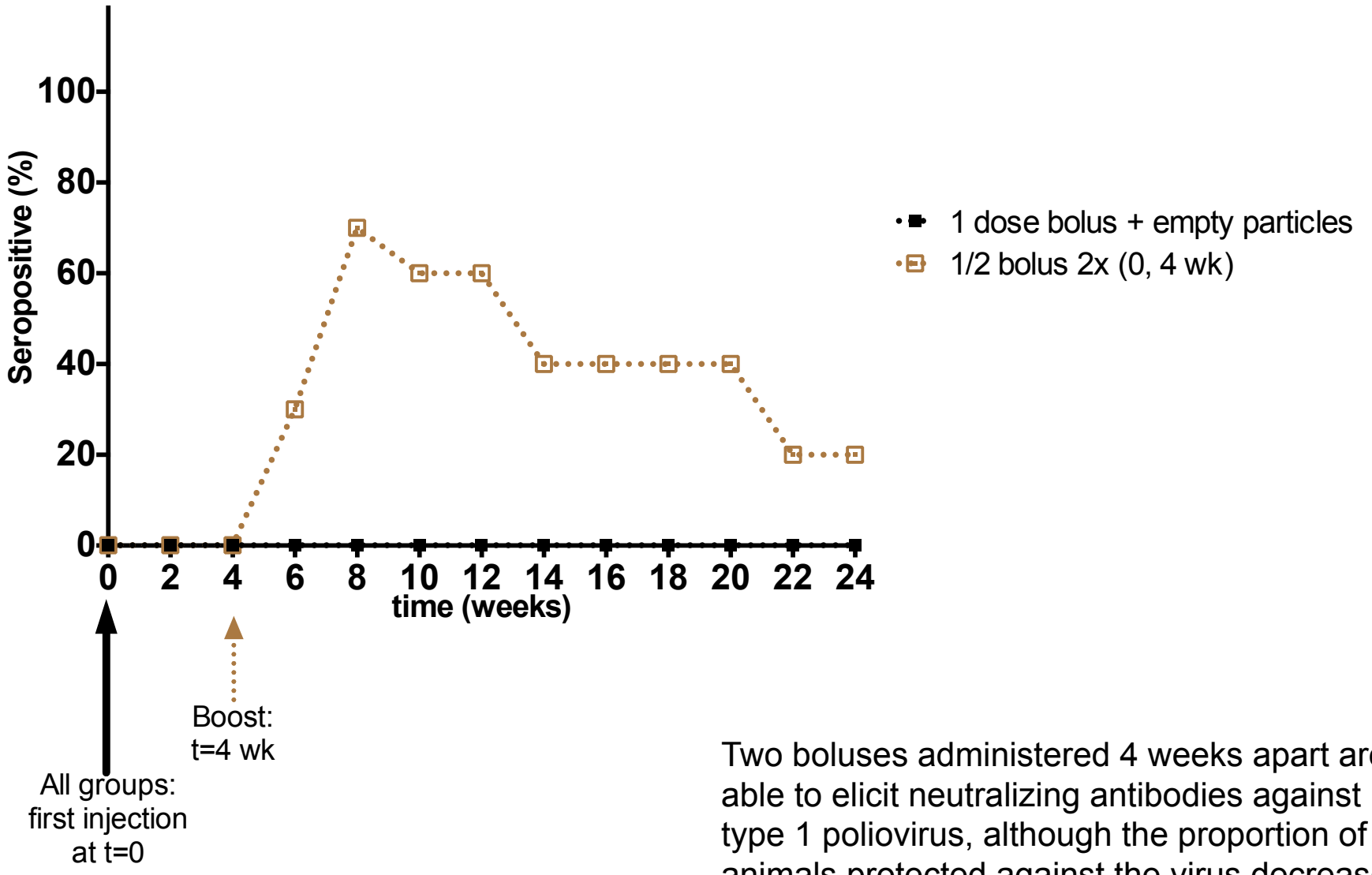
Tzeng et al. 2016. *J Control Release* 233:101

IPV Type 1 Neutralizing Antibodies in Serum



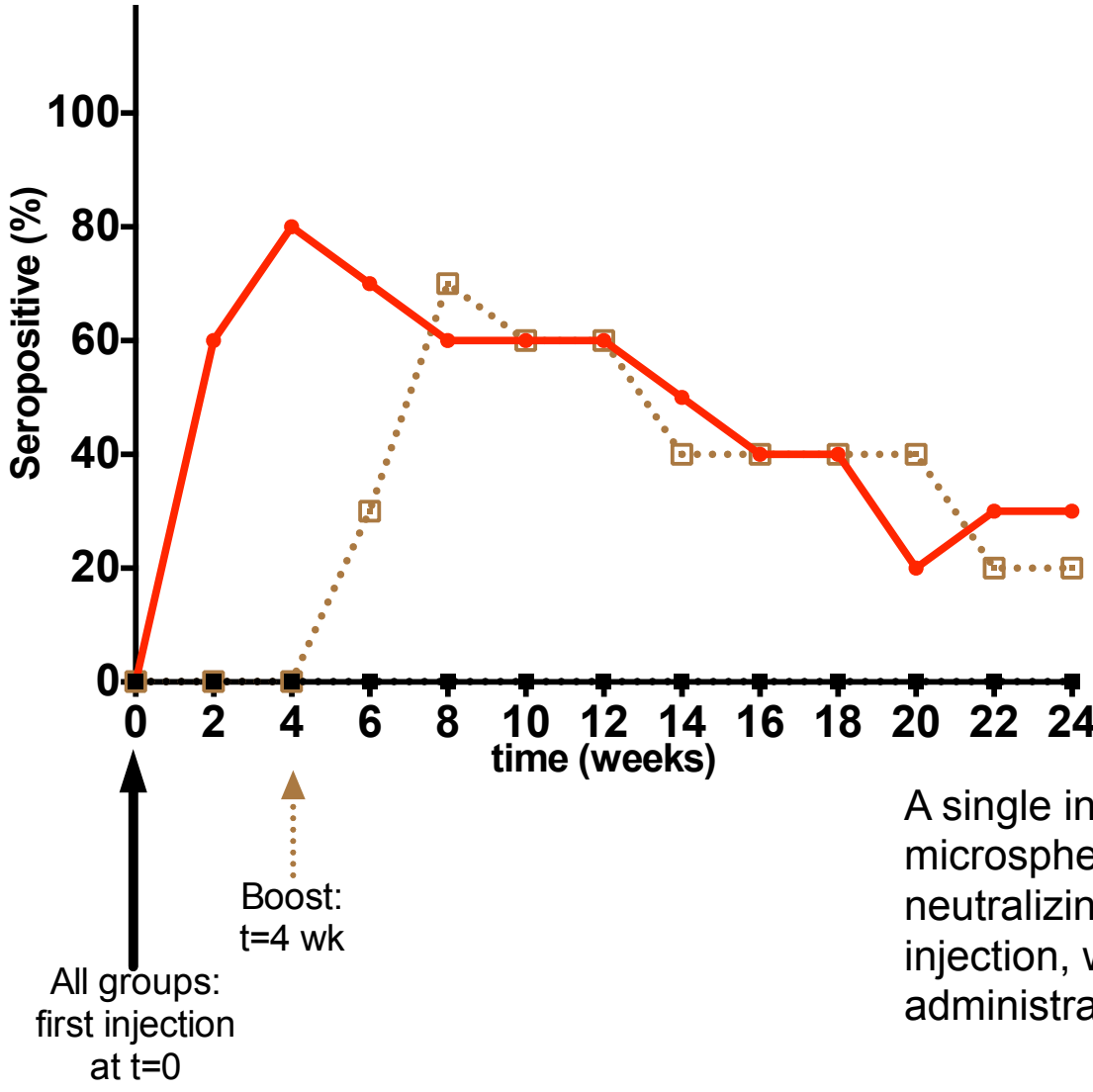
One bolus administration of IPV elicits no detectable neutralizing antibodies against serotype 1.

IPV Type 1 Neutralizing Antibodies in Serum



Two boluses administered 4 weeks apart are able to elicit neutralizing antibodies against type 1 poliovirus, although the proportion of animals protected against the virus decreased over time.

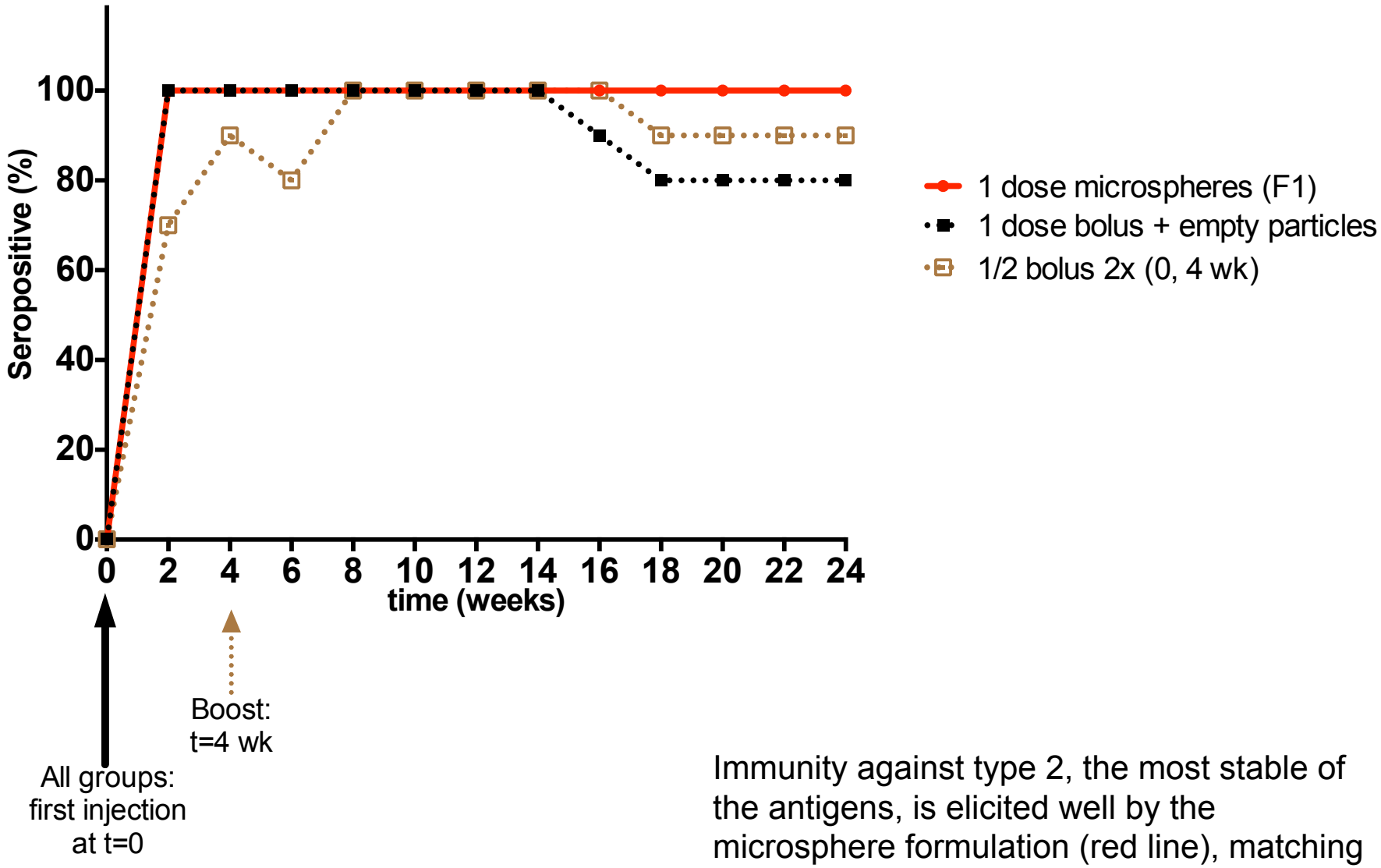
IPV Type 1 Neutralizing Antibodies in Serum



A single injection of IPV-containing microspheres (formulation F1) elicits a strong neutralizing response immediately after injection, without needed a second administration.

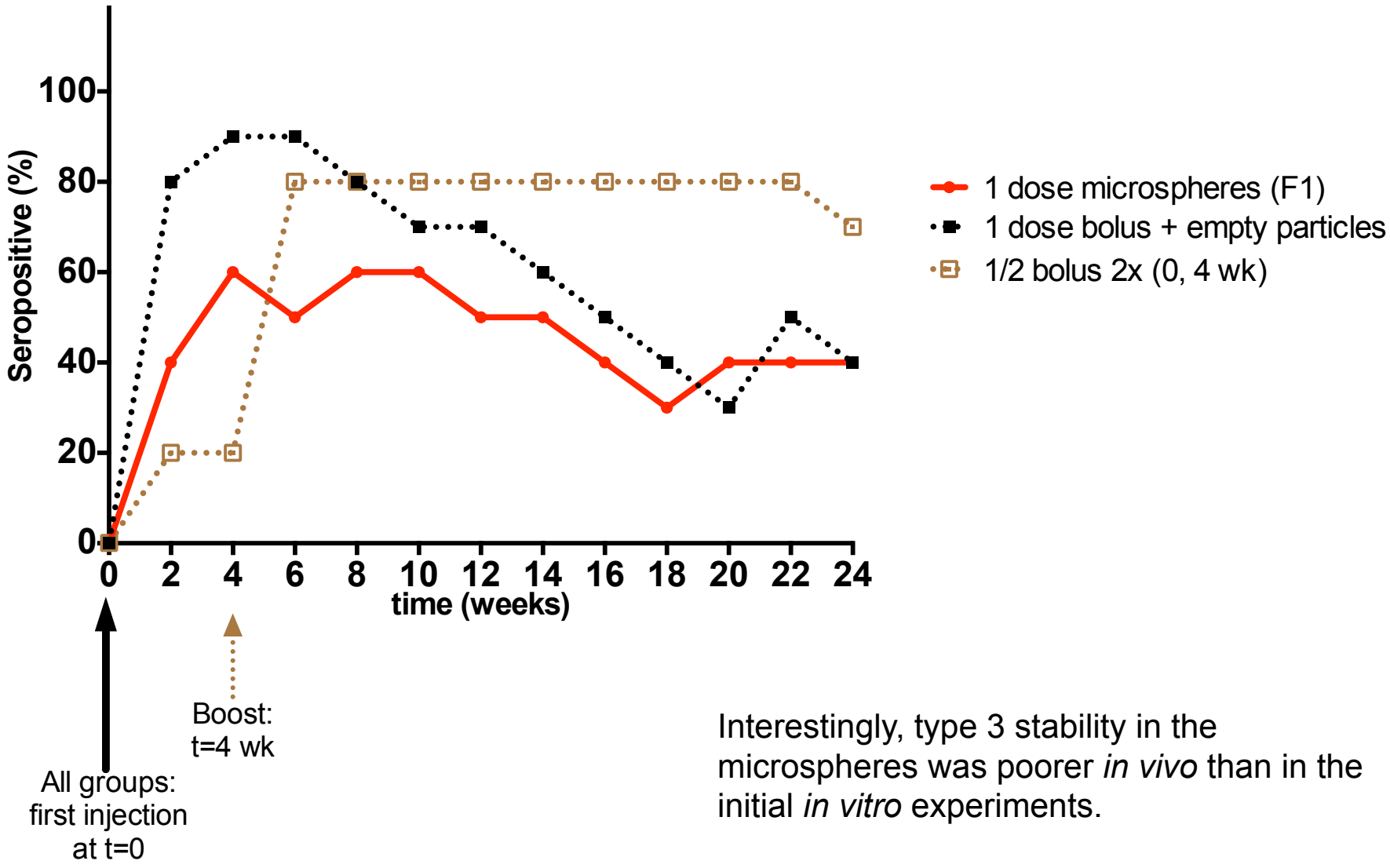
A single injection of microspheres is equal or superior to two bolus injections and appears to have even longer duration of immunity.

IPV Type 2 Neutralizing Antibodies in Serum



Immunity against type 2, the most stable of the antigens, is elicited well by the microsphere formulation (red line), matching or exceeding the multiple-bolus controls.

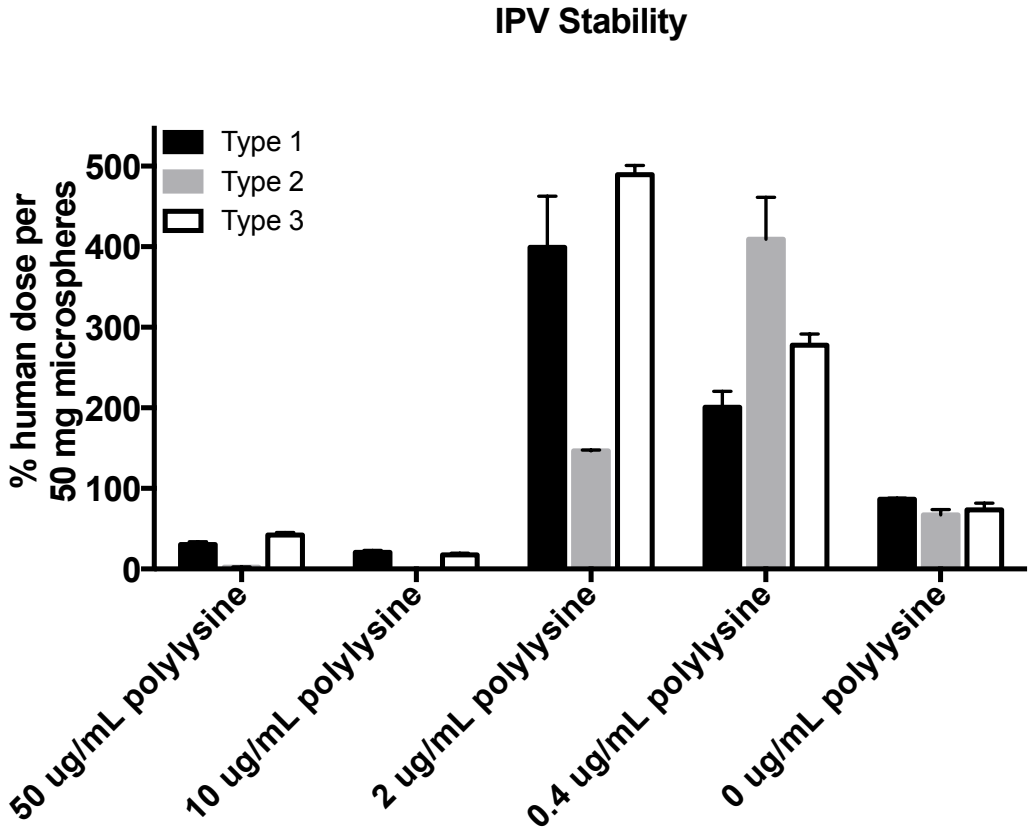
IPV Type 3 Neutralizing Antibodies in Serum

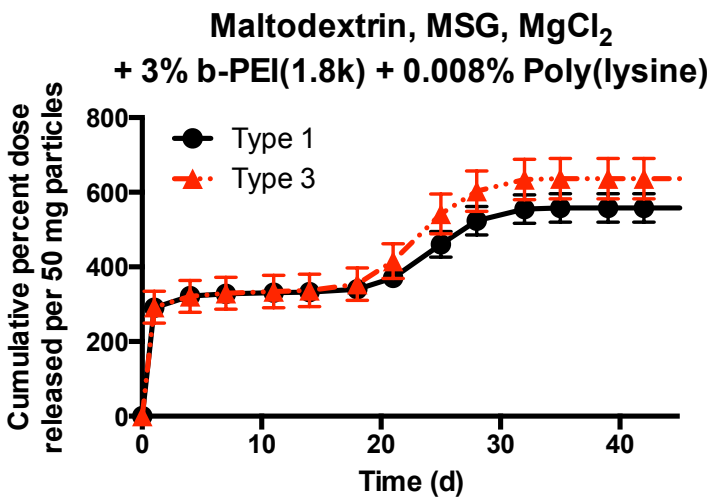


Interestingly, type 3 stability in the microspheres was poorer *in vivo* than in the initial *in vitro* experiments.

Further stability studies were conducted to optimize type 3 stability.

PLGA microspheres: hydrophilic polycations for improved type 3 stability



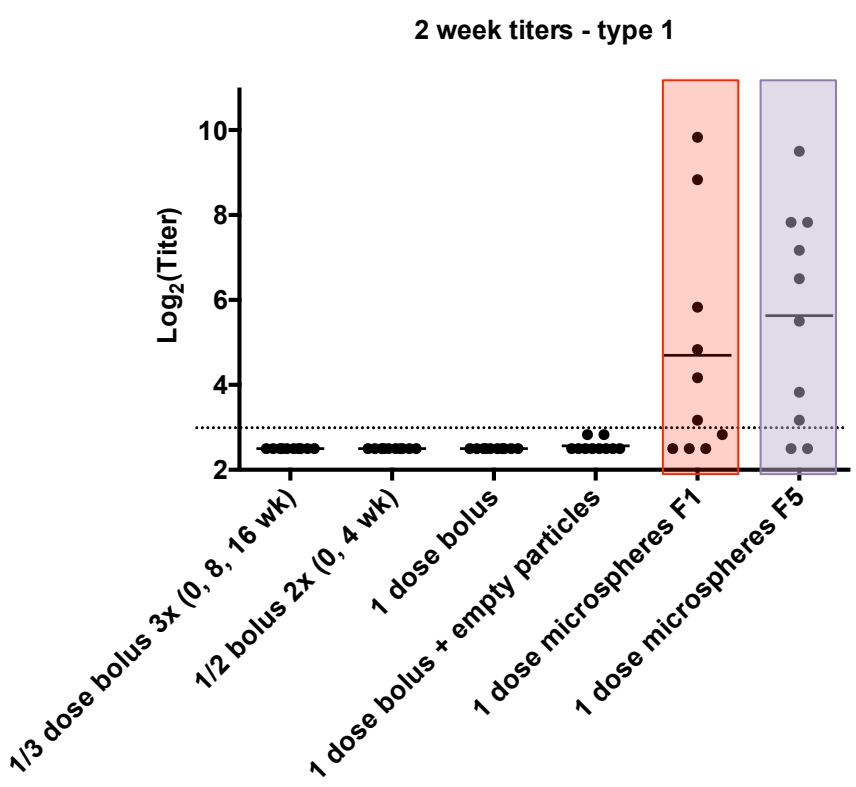


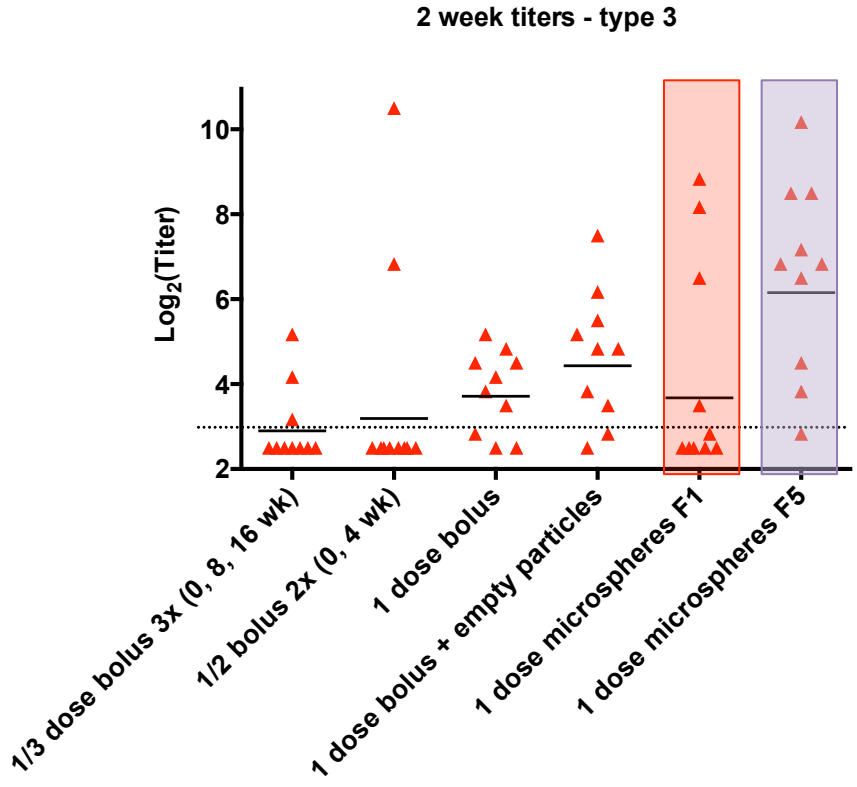
Human doses released/50 mg

Burst Time (d)	Type 1	Type 2	Type 3
0-4	3.2	9.1	3.2
18-28	2.2	7.7	2.8

- An optimized concentration of poly(L-lysine) (PLL) improves IPV stability, particularly for types 1 and 3.
- Higher overall release and higher late type 3 release are observed from microspheres that co-encapsulate polycations, such as PLL and low MW polyethylenimine (PEI).

IPV *in vivo* experiment (next iteration): Neutralizing antibodies (2 weeks)





Type 1: F1 microspheres (red) elicited neutralizing antibodies within 2 weeks, while none of the bolus controls did. The new formulation F5 (purple) is superior to F1 at the early time point.

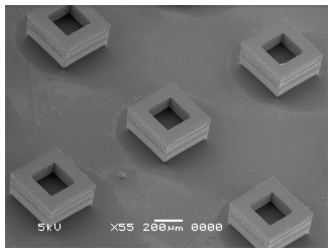
Type 3: The new formulation F5 (purple) is superior to the previous formulation F1 and, importantly, superior to the bolus controls. Importantly, the low dose of F5, in which the dosage was calculated by theoretical loading (i.e. assuming no loss of IPV doses due to processing or stability) is still superior to all controls and previous formulations.

Summary

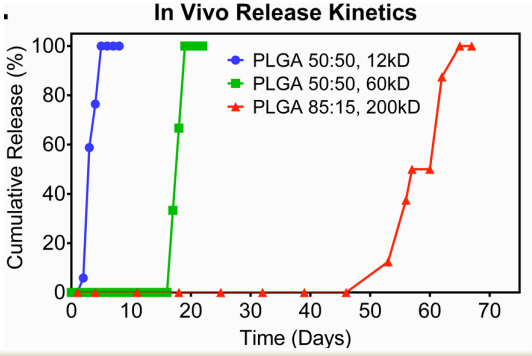
- Excipients can stabilize IPV against thermal and physical stresses over time
- IPV co-encapsulated with stabilizers and pH-modulators can be released in a pulsatile manner from PLGA particles
- Encapsulated IPV elicits a more potent neutralizing antibody response *in vivo* than free IPV injected as a bolus

Future Directions

- Expand pulsatile or continuous release platform to other vaccines
  - Sabin IPV (sIPV), experimental HIV vaccines, etc.
- Investigate the effect of types of release kinetics on immune response
- Increase the number of pulses that are released *in vivo*
- Pulsatile release of vaccines from core-shell particles



Poster #23



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