



Short Communication

Studies on Stability of Alprostadil (Lipo-PGE1 and DN-PGE1) Targeting Preparation

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ABSTRACT

Objective of this study was to investigate the compatibility and stability of alprostadil (lipo-PGE1 and DN-PGE1) after compatibility with different volume of 0.9% sodium chloride or 5% glucose, and to provide a basis for drug safe use in the clinic. PGE1 lipid microspheres (lipo-PGE1) and alprostadil dried emulsion (DN-PGE1) were compatibility with different volumes of 0.9% sodium chloride and 5% glucose, respectively. After the compatibility of different concentrations, the changes of pH, particle size distributions, the particles greater than 5µm and encapsulation rate of the compatibility solutions were observed within 8 h by using electronic acidimeter, Malvern zeta sizer nanoparticle size analyzer, accuser APS 780 particle size analyzer and Agilent 6460 LC-MS/MS mass spectrometer. From the experimental results, 5 µg:50ml group kept good targeting within 6 h. The encapsulation rate of 5 µg:100ml group decreased significantly after 6 h, and the percentage of fat globules > 5 µm (PFAT₂) in 10 µg:10ml group was relatively high. The risk of embolism was easily caused by injection or small pot dripping, which may be related to the insufficient amount of solvent and the incomplete dispersion of lipid microspheres. As for the two dosage forms of lipo-PGE1 and DN-PGE1, the average particle size and PFAT₂ after the preparation of DN-PGE1 met the USP standard, and the stability indexes were more advantageous. The clinical treatment of alprostadil targeted injection can be prepared by pharmacy intravenous admixture service. The drug concentration should be more than 0.05 µg/ml, and the drug concentration 0.1 µg/ml is the most appropriate. Lipo-PGE1 and DN-PGE1 injection can be administered by intravenous drip. The finished infusion should be used up within 6 h after the dispensing.

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Authors' Contribution

FX and SZ conceived the idea of the research and designed the experiments. FX conducted the experiments. FX and CW analyzed the results. All authors discussed the results and wrote the manuscript.

Key words

Alprostadil injections, Lipo-PGE1, DN-PGE1, Stability

Prostaglandin E1 (PGE1), another name as alprostadil, is an autacoid drug. It is effective in treating patients with various peripheral vascular occlusive disorders (Martin and Tooke, 1982) and with spontaneous pain and sensory disturbance due to diabetic neuropathy and diabetic ulcers (Low *et al.*, 1986). However, the clinical application of PGE1 is limited due to its adverse reactions to frequently causes local pain on administration and low bioavailability. To overcome these problems, several new alternative dosage forms of PGE1, such as lipid microspheres (Mizushima *et al.*, 1983), cyclodextrin clathrate (Yabek and Mann, 1979), nanoparticles (Ishihara *et al.*, 2008) and nano emulsion (Preece, 2017) can prevent PGE1 from inactivation in blood and improve the efficacy of PGE1. Among these new dosage forms, cyclodextrin clathrate prostaglandin E1 (PGE1-CD) injected intravenously is rapidly inactivated in the lungs and a high dose is necessary for

the treatment of these disorders furthermore renal toxicity of beta-cyclodextrin limited its clinical use (Lewis *et al.*, 1981; Peled *et al.*, 1992). PGE1 lipid microspheres (lipo-PGE1) which incorporated PGE1 into lipid microspheres were established by Mizushima *et al.* (1983) and alprostadil dried emulsion (DN-PGE1) which incorporated PGE1 into nanoemulsion and freeze-dried at low temperature by Chongqing Yaoyou Pharmaceutical Co., Ltd. Since they can deliver the encapsulated PGE1 efficiently to disease sites, lipo-PGE1 and DN-PGE1 were used widely in the clinic.

This type of alprostadil targeted formulation clearly states in its instructions: Alprostadil injection is administered once a day for adults, 1-2ml (5-10µg of alprostadil) + 10ml of normal saline (or 5% Glucose) intravenously (Okuno *et al.*, 2020; Fan *et al.*, 2019). In clinical application, how to formulate this type of infusion to ensure the safety and effectiveness of alprostadil is a matter of great concern. In this study, the stability of alprostadil targeted formulations (alprostadil lipid microsphere injection and alprostadil lipid microsphere

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dry emulsion) in different volumes and different kinds of solvent were investigated by using comparative experimental methods. We aimed to provide a basis for the safe use of this formulation in clinical practice.

Materials and methods

Alprostadil (99%) was purchased from Taiwan Yongguang Chemical Co., China; E0018506 and Alprostadil injection from Beijing Teide Pharmaceutical Co., China; 1B058H, 1B068H, 1B078H and Alprostadil emulsion from Chongqing Youyao Pharmaceutical Co., China; 18220960, 18220190, 18220020 were used for the experiment.

For measurement of precision and accuracy Alprostadil was dissolved in methanol and diluted to 20, 5, and 1 ng/ml and used for injection. Each concentration was measured six times and was injected on different days. Three batches were prepared and measured to calculate intra-batch and inter-batch precision and accuracy. The results showed that the intra-precisions among three concentrations (RSD) were 8.52%, 5.92%, and 7.12%. The inter-precisions among three concentrations (RSD) were 11.14%, 6.83%, and 9.52%. The accuracy of three concentrations (RE) was 12.14%, 6.83%, and -5.52%. The results show that the precision and accuracy of alprostadil is good under the conditions of this study.

For measurement of stability alprostadil was dissolved in methanol and diluted to 20 and 1 ng/ml. Each concentration was prepared for 6 samples. Three samples were placed in the sampler, and measured after being placed for 0 h, 2h, 4h, and 8h; Other three samples were placed at room temperature and measured after being placed for 0 h, 2 h, 4 h, and 8 h. The results showed that the stability of alprostadil was good.

For measurement of appearance and pH three new prostol preparations Kaishi, Mencito, and Youdier were thoroughly mixed with saline injection or 5% glucose injection, to prepare three concentrations of 1.0, 0.1, and 0.05 µg/ml. This operation was reported for 3 batches of each alprostadil targeted preparation product. The appearance of compatibility solution was observed and then pH was recorded using Mettler Seven Easy pH meter, Mettler Toledo Instruments, Switzerland.

Two ml samples of the compatibility solution of prostol preparations Kaishi and Youdier were taken at 0, 1, 2, 3, 4, 6, and 8h, after preparation and the changes in average particle size and 90% cumulative particle size were measured with the Malvern Zetasizer nano particle size analyzer (Malvern Instruments, UK). Three parallel tests were conducted for each rinse solution. Particles greater than 5 µm were detected by Accusizer 780/ APS (PSS particle size analyzer company, USA). Three parallel

tests were conducted for each punch.

For measurement of encapsulation rate at different times, 1ml of each rinse solution was sampled on the gel column (Hitrap gel column, 5ml), and then eluted with acetic acid buffer at pH 4.5 (see Chinese pharmacopeia buffer). 5 ml was dissolved by isopropanol into 25 ml. After shaking, the sample solution 1 was obtained, and the enveloped PGE1 was determined by LC-MS/MS (Agilent 6460 LC -MS/MS mass spectrometer, USA). Another 1 ml sample was dissolved by isopropanol into 25 ml. After shaking, the sample solution 2 was obtained, and the enveloped PGE1 was also determined by LC-MS/MS. The encapsulation rate is calculated according to the following formula:

Encapsulation rate % = encapsulation PGE1 / total PGE1 × 100

For determination of alprostadil and its degradation product PGA1 at different times, 1 ml of each rinse solution was dissolved by isopropanol into 10 ml. After shaking, the sample solution was obtained. LC-MS/MS was used to determine alprostadil and PGA1 in the sample solution.

For LC-MS/MS chromatographic conditions column were Agilent XDB C18 (2.1 × 50mm, 1.8µm); mobile phase: A is 0.05% glacial acetic acid aqueous solution, B is 0.05% glacial acetic acid methanol solution; flow rate: 0.3ml / min; injection volume: 20µl; with gradient wash in LC-MS/MS as shown below.

Time (min): 0, 4, 4.01, 6; A% 35, 0, 35, 35. MS conditions ESI ion source; dry gas flow rate: 8 L/min, dry gas temperature: 350 °C, atomizing gas pressure: 25 psi, capillary voltage: -3500 V; negative ion detection. Scanning method is Multiple Reaction Monitoring (MRM). The ions are: parent ion 353.2, daughter ion 317.2, fragmentation voltage 110 V, and collision energy 16 V.

Results and discussion

Comparing the pH and average particle size difference of two different dosage forms of alprostadil at different diluent volumes in two different solvents, the results showed that the difference of solvent type and placement time was not statistically significant. There were differences in pH and average particle size between dosage forms, and the pH value of lipo-PGE1 was lower after punching and the mean particle size of the lipo-PGE1 was larger after punching. The influence of dilution volume on pH and the mean particle size were different. The pH of 5µg:100ml was lower and the mean particle size was greater than 10µg:10ml, and the difference between 5µg:50ml and 10µg:10ml was not statistically significant. There was no difference in dosage, solvent, and volume over time (Table I).

Comparing the PFAT₅ and encapsulation rates changes of two different dosage forms of alprostadil in two different

solvents with different dilution volume, the results showed that the difference of dosage type, solvent type, dilution volume and placement time was not statistically significant. There was no difference in the type of solvent over time. Compared with DN-PGE1, there were more particles larger than 5µm in lipo-PGE1 with the extension of the placement time. 5µg:50ml had the least number of particles greater than 5 µm over time, followed by 5µg:100ml and 10µg:10ml (Table I).

Table I. Analysis results of pH mean particle size, particles greater than 5µm and encapsulation rate variation mixed linear model.

Effect	Num DF	Den DF	F value	Pr > F
pH				
Dosage	1	62	17.23	0.0001
Solvent	1	62	2.19	0.1440
Dilution volume	2	62	5.01	0.0096
Time of placement	1	62	0.00	0.9768
Time* Dosage	1	62	0.12	0.7265
Time* Solvent	1	62	0.00	0.9920
Time* Dilution volume	2	62	0.03	0.9731
Mean particle size				
Dosage	1	62	199.49	<.0001
Solvent	1	62	2.12	0.1504
Dilution volume	2	62	4.17	0.0200
Time of placement	1	62	0.01	0.9324
Time * Dosage	1	62	6.45	0.0136
Time * Solvent	1	62	0.05	0.8280
Time * Dilution volume	2	62	0.45	0.6419
Particles greater than 5µm				
Dosage	1	62	0.95	0.3331
Solvent	1	62	0.06	0.8003
Dilution volume	2	62	0.11	0.8927
Time of placement	1	62	0.06	0.8053
Time * Dosage	1	62	7.13	0.0097
Time * Solvent	1	62	0.38	0.5373
Time * Dilution volume	2	62	8.87	0.0004
Encapsulation rate				
Dosage	1	62	0.64	0.4282
Solvent	1	62	0.46	0.4982
Dilution volume	2	62	7.50	0.0012
Time of placement	1	62	0.06	0.8152
Time * Dosage	1	62	3.29	0.0747
Time * Solvent	1	62	3.57	0.0635
Time * Dilution volume	2	62	13.08	<.0001

Note: SAS 9.4 is adopted to take time as a random effect; dose, solvent, volume as fixed effect.

The encapsulation rate of 5µg:100ml was significantly lower than that of 10µg:10ml in different diluted volumes,

and the difference between 5µg:50ml and 10µg:10ml was not statistically significant (Table I).

The instructions limit the solvent to 10ml and require immediate use. This is mainly because the use of large amounts of solvent will destroy the structure of lipid microspheres in the targeted drugs of alprostadil injection, which will dissolve the drug into the solvent. On the one hand, it will increase the incidence of adverse drug reactions (phlebitis); on the other hand, it will also destroy the targeting of the drug. However, other clinical studies on the treatment of different diseases by intravenous infusion of 50-100ml 0.9% sodium chloride after infusion of proprolid targeting preparations and achieved good therapeutic effects (Liang *et al.*, 2018; Hao *et al.*, 2017; Wang *et al.*, 2016). Tian *et al.* (2017) conducted a systematic evaluation of phlebitis caused by different administration methods of alprostadil injection. Intravenous infusion of alprostadil was more recommended by the conclusion.

However, from the perspective of infection control and infusion safety, the safety of intravenous drug dispensing centers for treatment infusion is much higher than that of nurses in an exposed environment. Intravenous injection not only increases the difficulty of clinical nursing but also increases the risk of infection. Therefore, the clinical application of alprostadil targeted injection by intravenous drip is relatively common, and the treatment is effective (Liang *et al.*, 2018; Yi-na *et al.*, 2014).

Since the inner diameter of the venous capillaries is 4~9 µm, in a clinical continuous infusion, excessive particles with a diameter of > 5 µm may block the capillaries after entering the systemic circulation, leading to the inflammatory response. Particle retention in the lungs will cause pulmonary embolism and liver function impairment (Yi-na *et al.*, 2014). Therefore, the size and size distribution of lipo-particles are important indexes to evaluate the stability and safety of injection. The United States Pharmacopoeia (USP) (Kelley *et al.*, 2012) stipulates that MDD < 500 nm and PFAT₅ ≤ 0.05%. The national drug standard (Hutt, 2008) stipulates that MDD should be 120~280 nm. In this study, the dynamic light scattering method was used to measure MDD, which has a low sensitivity to the ions with a diameter of >1 µm. Therefore, light shading-single particle optical sensing technology (LO/SPOS) was used to detect PFAT₅. LO/SPOS technology is highly sensitive to large liposomes. It can accurately measure the size and number of large liposomes by detecting and counting the large size particles one by one, instead of obtaining an approximate value based on mathematical transformation. Encapsulation rate is an important index to evaluate the preparation technology and quality of lipid microspheres, and it is also the key to improve drug treatment index, reduce adverse

drug reactions, and reduce drug dose. In this study, gel chromatography was used to separate lipid microspheres from free drugs by the difference in molecular weight and particle size. The larger size of the lipid microspheres was eluted first, and the smaller size of the free drug was eluted to achieve the separation effect. After separation, the enriched samples were completely dissolved with 4 times the volume of isopropanol. The content of PGE1 was determined by LC-MS/MS and the encapsulation rate was calculated.

According to the experimental results, the 5µg: 50ml group maintained good targeting of the preparation within 6h. The encapsulation rate of 5 µg:100ml group decreased significantly after 6h. The percentage of PFAT₅ in 10 µg:10ml group was higher. The risk of embolization is easily caused by push injection or small pot infusion, which may be related to the insufficient amount of solvent and the inability to completely disperse the lipid microspheres. As for lipo-PGE1 injection and DN-PGE1 injection, the average particle size and PFAT₅ percentage of DN-PGE1 are in line with USP standards, and the stability indicators are more dominant.

Conclusion

The clinical treatment of alprostadil targeted injection can be dispensed by pharmacy intravenous admixture service. The concentration of the drug should over 0.05 µg /ml, and the concentration of the drug 0.1 µg /ml is the most appropriate. The drug can be administered by intravenous infusion, and the finished infusion should be used within 6 h after deployment.

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Supplementary material

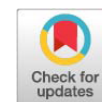
There is supplementary material associated with this article. Access the material online at: <https://dx.doi.org/10.17582/journal.pjz/20200722050704>

Statement of conflict of interest

The authors have declared no conflict of interest.

References

- Driscoll, D.F., Ling, P.-R., Silvestri, A. and Bistran, B., 2008. *Clin. Nutr.*, **27**: 889-894. <https://doi.org/10.1016/j.clnu.2008.08.011>
- Fan, Q., Zhang, L., Zhong, M. and Dong, L., 2019. *Interventional effect of off-label use of alprostadil lipid microsphere injection in neurosurgical patients: A before-after study*. Preprint, <https://doi.org/10.21203/rs.2.12797/v1>
- Hao, Y., Zhu, W., Yuhe, K., Zhihua, Y., Tian, L., Duan, G., Wen, F. and Zheng, Q., 2017. *Chinese J. Cardiovas. Rehab. Med.*, **26**: 402-405.
- Hutt, P.B., 2008. *Admin. L. Rev.*, **60**: 431.
- Ishihara, T., Takahashi, M., Higaki, M., Takenaga, M., Mizushima, T. and Mizushima, Y., 2008. *Pharm. Res.*, **25**: 1686-1695. <https://doi.org/10.1007/s11095-008-9549-8>
- Kelley, K.E., Hernández-Díaz, S., Chaplin, E.L., Hauser, R. and Mitchell, A.A., 2012. *Environ. Hlth. Perspect.*, **120**: 379-384. <https://doi.org/10.1289/ehp.1103998>
- Lewis, A.B., Freed, M.D., Heymann, M.A., Roehl, S.L. and Kensey, R.C., 1981. *Circulation*, **64**: 893-898. <https://doi.org/10.1161/01.CIR.64.5.893>
- Liang, M., Yang, S., Fu, N., Lu, C., Tian, F., Xing, X., Lin, W. and Liu, J., 2018. *Catheter. Cardiovasc. Interv.*, **91**: 742-750. <https://doi.org/10.1002/ccd.27353>
- Low, P., Schmelzer, J., Ward, K. and Yao, J., 1986. *Am. J. Physiol. Endocrinol. Metab.*, **250**: E94-E99. <https://doi.org/10.1152/ajpendo.1986.250.1.E94>
- Martin, M. and Tooke, J., 1982. *Br. med. J. (Clin. Res. Ed.)*, **285**: 1688-1690. <https://doi.org/10.1136/bmj.285.6356.1688>
- Mizushima, Y., Yanagawa, A. and Hoshi, K., 1983. *J. Pharm. Pharmacol.*, **35**: 666-667. <https://doi.org/10.1111/j.2042-7158.1983.tb02862.x>
- Okuno, T., Morimoto, S., Nishikawa, H., Haraguchi, T., Kojima, H., Tsujino, H., Arisawa, M., Yamashita, T., Nishikawa, J. and Yoshida, M., 2020. *Chem. Pharm. Bull.*, **68**: 234-243. <https://doi.org/10.1248/cpb.c19-00736>
- Peled, N., Dagan, O., Babyn, P., Silver, M. M., Barker, G., Hellmann, J., Scolnik, D. and Koren, G., 1992. *N. Engl. J. Med.*, **327**: 505-510. <https://doi.org/10.1056/NEJM199208203270801>
- Preece, K.E., 2017. *Protein extraction during soybase production: Enhancing yields using cavitation techniques*. University of Birmingham.
- Tian, F., Zou, M., Bin, W., Ting, X. and Jiang, X., 2017. *China Pharm.*, **28**: 2955-2958.
- Wang, W., Lu, Z.-Y., Ren, Z. and Zhang, G.-Z., 2016. *J. Hainan med. Univ.*, **22**: 40-43.
- Yabek, S.M. and Mann, J.S., 1979. *Chest*, **76**: 330-331. <https://doi.org/10.1378/chest.76.3.330>
- Yi-na, L., Shi-cheng, Y. and Nai-kuan, F., 2014. *Chinese J. Geriat. Heart Brain, Vessel Dis.*, 580-583.



Supplementary Material

Studies on Stability of Alprostadi (Lipo-PGE1 and DN-PGE1) Targeting Preparation

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Supplementary Table I. Analysis results of pH variation mixed linear model.

pH of lipo-PGE1 injection in different solvents						
	5% glucose			0.9% saline		
	5ug:100ml	5ug:50ml	10ug:10ml	5ug:100ml	5ug:50ml	10ug:10ml
0h	4.32±0.09	4.51±0.08	4.62±0.18	5.10±0.19	4.91±0.09	4.91±0.09
1h	4.44±0.09	4.51±0.07	4.63±0.15	5.10±0.08	5.01±0.09	5.11±0.04
2h	4.41±0.02	4.54±0.09	4.90±0.03	5.11±0.10	5.02±0.04	5.02±0.08
4h	4.42±0.02	4.60±0.09	4.91±0.09	5.13±0.10	5.01±0.03	5.12±0.10
6h	4.42±0.01	4.52±0.09	4.81±0.03	5.02±0.09	4.81±0.19	4.91±0.08
8h	4.43±0.01	4.52±0.08	4.81±0.07	5.03±0.09	4.81±0.08	5.03±0.06
pH of DN-PGE1 injection in different solvents						
	5% glucose			0.9% saline		
	5ug:100ml	5ug:50ml	10ug:10ml	5ug:100ml	5ug:50ml	10ug:10ml
0h	4.61±0.09	5.51±0.04	6.51±0.17	5.21±0.04	5.51±0.11	6.04±0.01
1h	4.62±0.08	5.62±0.04	6.51±0.22	5.21±0.04	5.51±0.06	6.02±0.04
2h	4.54±0.02	5.61±0.04	6.53±0.16	5.41±0.04	5.52±0.04	6.10±0.04
4h	4.60±0.02	5.61±0.06	6.51±0.16	5.40±0.07	5.61±0.04	6.02±0.04
6h	4.60±0.02	5.54±0.06	6.53±0.18	5.40±0.06	5.71±0.04	6.11±0.04
8h	4.60±0.04	5.54±0.06	6.53±0.18	5.41±0.04	5.71±0.04	6.10±0.03
Analysis results of pH variation mixed linear model						
Effect	Num DF	Den DF	F Value	Pr > F		
Dosage	1	62	17.23	0.0001		
Solvent	1	62	2.19	0.1440		
Dilution volume	2	62	5.01	0.0096		
Time of placement	1	62	0.00	0.9768		
Time * Dosage	1	62	0.12	0.7265		
Time * Solvent	1	62	0.00	0.9920		

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pH of lipo-PGE1 injection in different solvents								
5% glucose			0.9% saline					
Time * Dilution volume	2		62		0.03		0.9731	
Refined analysis results of pH variation mixed linear model								
Effect	Dosage	Solvent	Dilution volume	Estimate	Standard Error	DF	t Value	Pr > t
Intercept				6.0417	0.3505	62	17.24	<.0001
Dosage	Lipo			-0.7667	0.1847	62	-4.15	0.0001
Dosage	DN			0	.	.	.	.
Solvent		glucose		-0.2733	0.1847	62	-1.48	0.1440
Solvent		saline		0	.	.	.	.
Dilution volume			100ml	-0.7150	0.2262	62	-3.16	0.0024
Dilution volume			50ml	-0.3900	0.2262	62	-1.72	0.0897
Dilution volume			10ml	0	.	.	.	.
Time of placement				0.02500	0.3373	62	0.07	0.9412
Time * Dosage	Lipo			-0.01667	0.04743	62	-0.35	0.7265
Time * Dosage	DN			0	.	.	.	.
Time * Solvent		glucose		-0.00048	0.04743	62	-0.01	0.9920
Time * Solvent		saline		0	.	.	.	.
Time * Dilution volume			100ml	-0.00643	0.05809	62	-0.11	0.9122
Time * Dilution volume			50ml	-0.01357	0.05809	62	-0.23	0.8160
Time * Dilution volume			10ml	0	.	.	.	.

Supplementary Table II. Analysis results of mean particle size variation mixed linear model.

The mean particle size of lipo-PGE1 injection in different solvent (nm)						
	5% glucose			0.9% saline		
	5ug:100ml	5ug:50ml	10ug:10ml	5ug:100ml	5ug:50ml	10ug:10ml
0h	186±7	183±9	183±17	187±8	177±16	182±14
1h	188±12	187±1	181±22	192±9	177±5	179±17
2h	187±27	192±1	182±11	190±18	179±18	179±14
4h	189±16	185±8	186±12	188±11	181±6	181±7
6h	183±13	191±6	190±8	190±11	183±13	185±11
8h	185±16	192±3	191±11	192±8	186±12	189±11
The mean particle size of DN-PGE1 injection in different solvent (nm)						
	5% glucose			0.9% saline		
	5ug:100ml	5ug:50ml	10ug:10ml	5ug:100ml	5ug:50ml	10ug:10ml
0h	155±4	153±11	144±3	149±5	139±2	147±6
1h	141±10	154±10	143±4	150±6	136±3	148±1
2h	152±2	154±5	141±3	158±10	140±2	139±7
4h	154±3	152±0	140±3	151±3	144±2	145±2
6h	155±2	149±3	141±3	143±9	143±2	145±2
8h	157±4	140±1	143±1	143±3	145±2	139±4

Table continue on next page.....

The mean particle size of lipo-PGE1 injection in different solvent (nm)								
5% glucose					0.9% saline			
Analysis results of mean particle size variation mixed linear model								
Effect	Num DF			Den DF	F Value		Pr > F	
Dosage	1			62	199.49		<.0001	
Solvent	1			62	2.12		0.1504	
Dilution volume	2			62	4.17		0.0200	
Time of placement	1			62	0.01		0.9324	
Time * Dosage	1			62	6.45		0.0136	
Time * Solvent	1			62	0.05		0.8280	
Time * Dilution volume	2			62	0.45		0.6419	
Refined analysis results of mean particle size variation mixed linear model								
Effect	Dosage	Solvent	Dilution volume	Estimate	Standard Error	DF	t Value	Pr > t
Intercept				143.14	4.5171	62	31.69	<.0001
Dosage	Lipo			33.6222	2.3805	62	14.12	<.0001
Dosage	DN			0	.	.	.	.
Solvent		glucose		3.4667	2.3805	62	1.46	0.1504
Solvent		saline		0	.	.	.	.
Dilution volume			100ml	7.8167	2.9155	62	2.68	0.0094
Dilution volume			50ml	1.2000	2.9155	62	0.41	0.6821
Dilution volume			10ml	0	.	.	.	.
Time of placement				-0.2024	4.3470	62	-0.05	0.9630
Time * Dosage	Lipo			1.5524	0.6113	62	2.54	0.0136
Time * Dosage	DN			0	.	.	.	.
Time * Solvent		glucose		-0.1333	0.6113	62	-0.22	0.8280
Time * Solvent		saline		0	.	.	.	.
Time * Dilution volume			100ml	-0.5429	0.7486	62	-0.73	0.4711
Time * Dilution volume			50ml	0.1214	0.7486	62	0.16	0.8717
Time * Dilution volume			10ml	0	.	.	.	.

Typical map

Lipo-PGE1 injection
5µg:50ml
Saline 0h

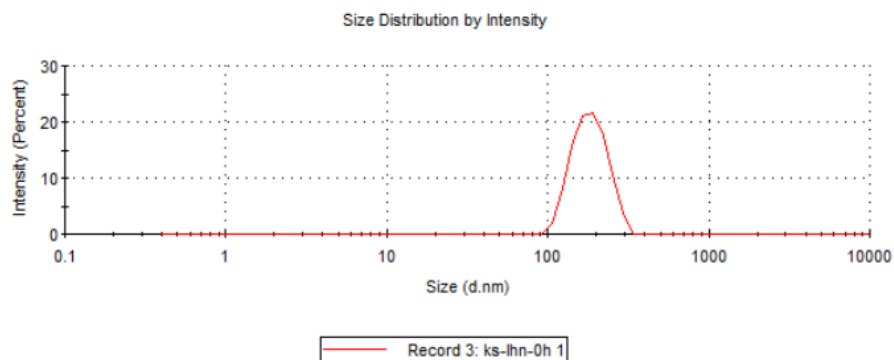
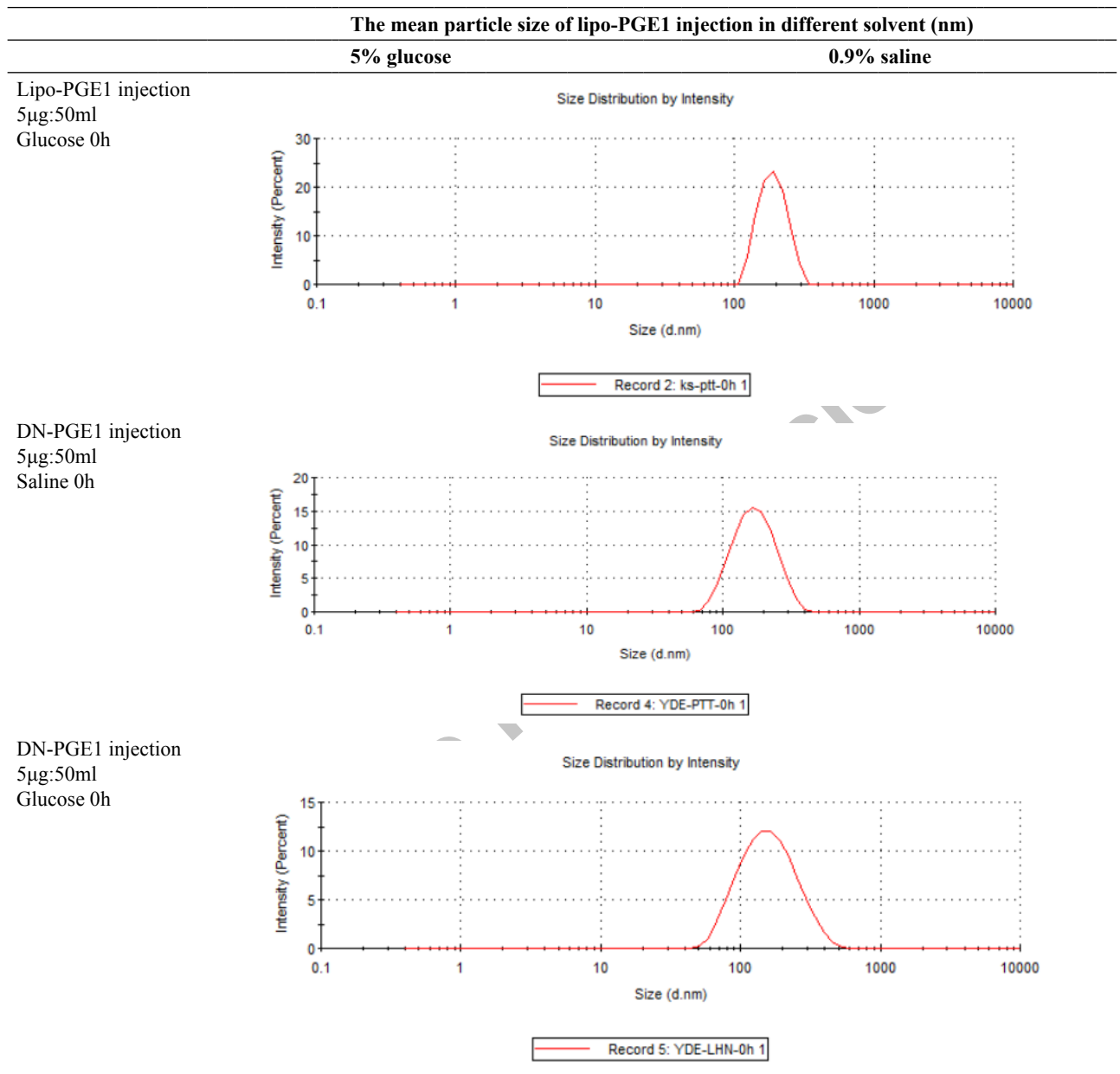


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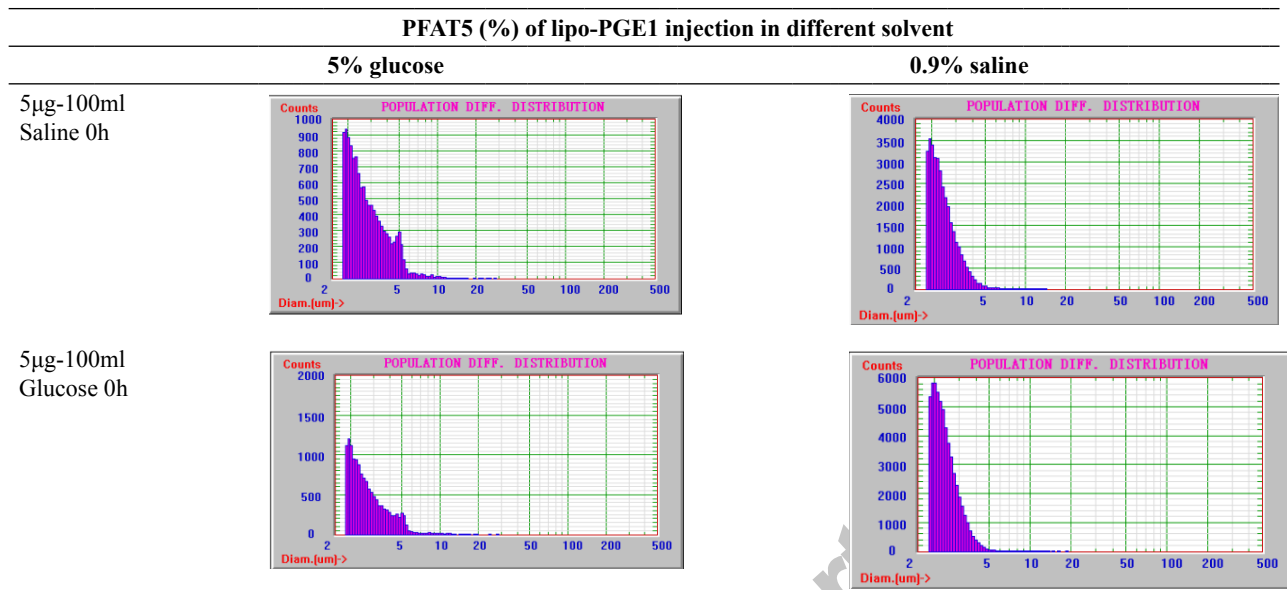
Supplementary Table III. Analysis results of particles greater than 5 μ m variation mixed linear model.

PFAT5 (%) of lipo-PGE1 injection in different solvent						
5% glucose			0.9% saline			
	5 μ g:100ml	5 μ g:50ml	10 μ g:10ml	5 μ g:100ml	5 μ g:50ml	10 μ g:10ml
0h	0.067 $\pm$ 0.002	0.043 $\pm$ 0.005	0.108 $\pm$ 0.008	0.040 $\pm$ 0.013	0.050 $\pm$ 0.003	0.073 $\pm$ 0.010
1h	0.037 $\pm$ 0.014	0.053 $\pm$ 0.009	0.107 $\pm$ 0.002	0.058 $\pm$ 0.008	0.046 $\pm$ 0.009	0.142 $\pm$ 0.008
2h	0.065 $\pm$ 0.010	0.056 $\pm$ 0.010	0.105 $\pm$ 0.002	0.048 $\pm$ 0.010	0.059 $\pm$ 0.006	0.195 $\pm$ 0.020
4h	0.046 $\pm$ 0.013	0.058 $\pm$ 0.004	0.294 $\pm$ 0.079	0.050 $\pm$ 0.012	0.048 $\pm$ 0.005	0.397 $\pm$ 0.080
6h	0.067 $\pm$ 0.006	0.055 $\pm$ 0.002	0.284 $\pm$ 0.037	0.057 $\pm$ 0.012	0.049 $\pm$ 0.002	0.365 $\pm$ 0.023

Table continue on next page.....

PFAT5 (%) of lipo-PGE1 injection in different solvent								
	5% glucose			0.9% saline				
8h	0.078±0.009	0.068±0.004	0.293±0.008	0.062±0.017	0.059±0.003	0.425±0.023		
PFAT5 (%) of DN-PGE1 injection in different solvent								
	5% glucose			0.9% saline				
	5ug:100ml	5ug:50ml	10ug:10ml	5ug:100ml	5ug:50ml	10ug:10ml		
0h	0.017±0.002	0.003±0.002	0.014±0.004	0.014±0.002	0.003±0.001	0.014±0.002		
1h	0.021±0.004	0.003±0.001	0.053±0.013	0.015±0.002	0.003±0.001	0.016±0.001		
2h	0.016±0.002	0.002±0.001	0.018±0.006	0.019±0.004	0.003±0.000	0.029±0.002		
4h	0.017±0.003	0.002±0.001	0.020±0.008	0.022±0.003	0.003±0.000	0.039±0.002		
6h	0.020±0.006	0.006±0.002	0.050±0.008	0.020±0.003	0.003±0.002	0.040±0.002		
8h	0.021±0.004	0.007±0.002	0.062±0.010	0.022±0.003	0.004±0.001	0.053±0.005		
Analysis results of particles greater than 5µm variation mixed linear model								
Effect	Num DF		Den DF	F Value		Pr > F		
Dosage	1		62	0.95		0.3331		
Solvent	1		62	0.06		0.8003		
Dilution volume	2		62	0.11		0.8927		
Time of placement	1		62	0.06		0.8053		
Time * Dosage	1		62	7.13		0.0097		
Time * Solvent	1		62	0.38		0.5373		
Time * Dilution volume	2		62	8.87		0.0004		
Refined analysis results of particles greater than 5µm variation mixed linear model								
Effect	Dosage	Solvent	Dilution volume	Estimate	Standard Error	DF	t Value	Pr > t
Intercept				-0.00412	0.05360	62	-0.08	0.9390
Dosage	Lipo			0.02755	0.02824	62	0.98	0.3331
Dosage	DN			0	.	.	.	.
Solvent		glucose		0.007174	0.02824	62	0.25	0.8003
Solvent		saline		0	.	.	.	.
Dilution volume			100ml	0.01634	0.03459	62	0.47	0.6384
Dilution volume			50ml	0.01017	0.03459	62	0.29	0.7698
Dilution volume			10ml	0	.	.	.	.
Time of placement				0.02680	0.05158	62	0.52	0.6051
Time * Dosage	Lipo			0.01937	0.007252	62	2.67	0.0097
Time * Dosage	DN			0	.	.	.	.
Time * Solvent		glucose		-0.00450	0.007252	62	-0.62	0.5373
Time * Solvent		saline		0	.	.	.	.
Time * Dilution volume			100ml	-0.03202	0.008882	62	-3.61	0.0006
Time * Dilution volume			50ml	-0.03277	0.008882	62	-3.69	0.0005
Time * Dilution volume			10ml	0	.	.	.	.
Typical map								
Lipo-PGE1 injection				DN-PGE1 injection				

Table continue on next page.....



Supplementary Table IV. Analysis results of encapsulation rate variation mixed linear model.

The encapsulation rate (%) of lipo-PGE1 injection in different solvent						
	5% glucose			0.9% saline		
	5 μ g:100ml	5 μ g:50ml	10 μ g:10ml	5 μ g:100ml	5 μ g:50ml	10 μ g:10ml
0h	98.1 $\pm$ 0.2	98.2 $\pm$ 0.2	97.6 $\pm$ 0.1	98.8 $\pm$ 0.1	96.2 $\pm$ 0.2	96.7 $\pm$ 0.2
1h	98.6 $\pm$ 0.4	98.5 $\pm$ 0.1	97.1 $\pm$ 0.8	99.3 $\pm$ 0.3	98.4 $\pm$ 0.5	97.2 $\pm$ 0.4
2h	97.2 $\pm$ 0.1	98.5 $\pm$ 0.0	96.8 $\pm$ 0.5	98.0 $\pm$ 0.1	99.0 $\pm$ 1.1	98.6 $\pm$ 0.2
4h	95.0 $\pm$ 0.1	99.5 $\pm$ 0.4	96.7 $\pm$ 0.4	97.1 $\pm$ 0.1	99.5 $\pm$ 0.9	98.3 $\pm$ 0.2
6h	94.1 $\pm$ 0.7	99.5 $\pm$ 0.9	96.2 $\pm$ 0.3	95.7 $\pm$ 0.0	97.7 $\pm$ 0.1	98.7 $\pm$ 0.1
8h	91.1 $\pm$ 1.4	98.5 $\pm$ 0.1	95.2 $\pm$ 0.6	94.7 $\pm$ 0.0	96.7 $\pm$ 0.1	96.7 $\pm$ 0.2
The encapsulation rate (%) of DN-PGE1 injection in different solvent						
	5% glucose			0.9% saline		
	5 μ g:100ml	5 μ g:50ml	10 μ g:10ml	5 μ g:100ml	5 μ g:50ml	10 μ g:10ml
0h	98.5 $\pm$ 0.0	99.6 $\pm$ 0.4	97.9 $\pm$ 0.4	98.5 $\pm$ 0.2	97.2 $\pm$ 0.1	97.5 $\pm$ 0.3
1h	97.6 $\pm$ 0.2	98.6 $\pm$ 0.2	94.5 $\pm$ 1.4	99.2 $\pm$ 0.2	97.4 $\pm$ 0.4	97.7 $\pm$ 0.3
2h	99.3 $\pm$ 0.2	98.0 $\pm$ 0.4	95.7 $\pm$ 1.2	98.5 $\pm$ 0.4	98.9 $\pm$ 0.2	98.0 $\pm$ 0.0
4h	97.9 $\pm$ 0.8	99.7 $\pm$ 0.1	95.5 $\pm$ 1.1	97.3 $\pm$ 0.4	97.9 $\pm$ 0.2	99.7 $\pm$ 0.4
6h	97.3 $\pm$ 0.8	98.9 $\pm$ 0.2	98.0 $\pm$ 0.4	98.5 $\pm$ 0.4	100.0 $\pm$ 0.7	99.3 $\pm$ 0.1
8h	94.3 $\pm$ 0.6	96.9 $\pm$ 0.9	97.0 $\pm$ 0.6	96.5 $\pm$ 0.4	97.0 $\pm$ 0.1	98.3 $\pm$ 0.1
Analysis results of encapsulation rate variation mixed linear model						
Effect	Num DF	Den DF		F Value	Pr > F	
Dosage	1	62		0.64	0.4282	
Solvent	1	62		0.46	0.4982	
Dilution volume	2	62		7.50	0.0012	
Time of placement	1	62		0.06	0.8152	

Table continue on next page.....

The encapsulation rate (%) of lipo-PGE1 injection in different solvent								
5% glucose			0.9% saline					
Time * Dosage	1	62	3.29	0.0747				
Time * Solvent	1	62	3.57	0.0635				
Time * Dilution volume	2	62	13.08	<.0001				
Refined analysis results of encapsulation rate variation mixed linear model								
Effect	Dosage	Solvent	Dilution volume	Estimate	Standard Error	DF	t Value	Pr > t
Intercept				96.6783	1.1976	62	80.73	<.0001
Dosage	Lipo			0.5033	0.6311	62	0.80	0.4282
Dosage	DN			0	.	.	.	.
Solvent		glucose		0.4300	0.6311	62	0.68	0.4982
Solvent		saline		0	.	.	.	.
Dilution volume			100ml	2.9717	0.7730	62	3.84	0.0003
Dilution volume			50ml	1.1683	0.7730	62	1.51	0.1357
Dilution volume			10ml	0	.	.	.	.
Time of placement				0.3407	1.1525	62	0.30	0.7685
Time * Dosage	Lipo			-0.2938	0.1621	62	-1.81	0.0747
Time * Dosage	DN			0	.	.	.	.
Time * Solvent		glucose		-0.3062	0.1621	62	-1.89	0.0635
Time * Solvent		saline		0	.	.	.	.
Time * Dilution volume			100ml	-0.8943	0.1985	62	-4.51	<.0001
Time * Dilution volume			50ml	-0.03143	0.1985	62	-0.16	0.8747
Time * Dilution volume			10ml	0	.	.	.	.

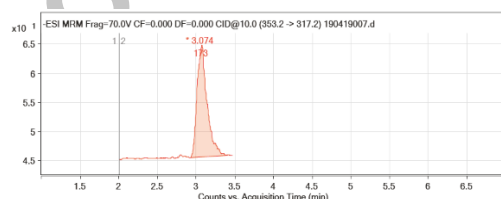
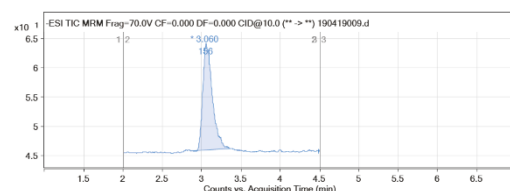
Typical map

Lipo-PGE1 injection

Total PGE1

5µg-100ml

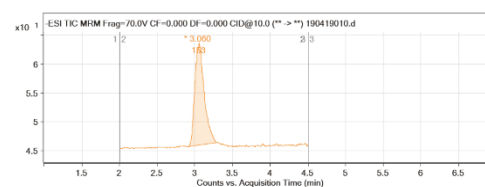
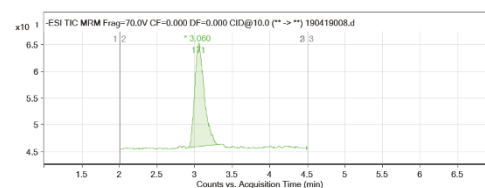
Saline 0h

5µg-100ml
Glucose 0h

DN-PGE1 injection

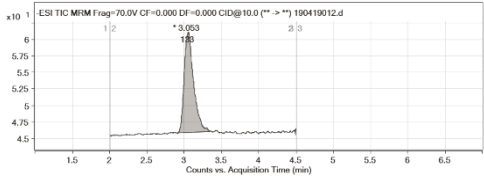
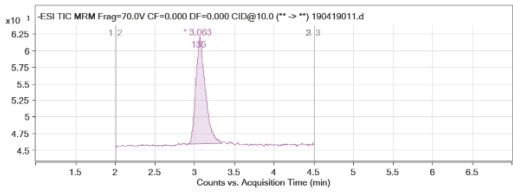
Total PGE1

PGE1 before encapsulation

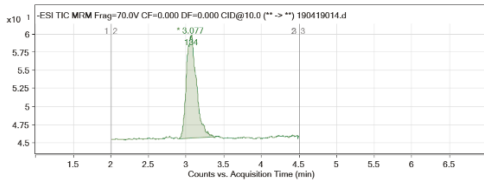
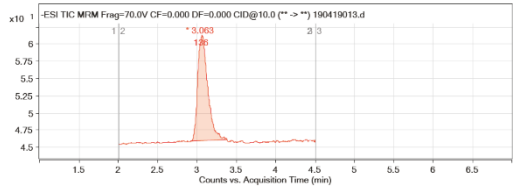


PGE1 before encapsulation

5µg-100ml
Saline 0h



5µg-100ml
Glucose 0h



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