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Effect of isotonic solutions and peptide adsorption on zeta potential of porous silicon nanoparticle drug delivery formulations

Martti Kaasalainen[a], Ermei Mäkilä[a], Joakim Riikonen[b], Miia Kovalainen[c], Kristiina Järvinen[c], Karl-Heinz Herzig[d,e], Vesa-Pekka Lehto[b], Jarno Salonen[a,f,*]

[a] Department of Physics and Astronomy, University of Turku, FI-20014 Turku, Finland
[b] Department of Physics, University of Eastern Finland, FI-70211 Kuopio, Finland
[c] School of Pharmacy, Pharmaceutical Technology, University of Eastern Finland, FI-70211 Kuopio, Finland
[d] Institute of Biomedicine, Biocenter of Oulu, University of Oulu, FI-90014 Oulu, Finland
[e] Department of Psychiatry, Kuopio University Hospital, FI-70211 Kuopio, Finland
[f] Turku University Centre for Materials and Surfaces (MatSurf), Turku, Finland

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ABSTRACT

Recently, highly promising results considering the use of porous silicon (PSi) nanoparticles as a controlled and targeted drug delivery system have been published. Drugs are typically loaded into PSi nanoparticles by electrostatic interactions, and the drug-loaded nanoparticles are then administered parenterally in isotonic solutions. Zeta potential has an important role in drug adsorption and overall physical stability of nanosuspensions. In the present study, we used zeta potential measurements to study the impact of the formulation components to the nanosuspension stability. The impact of medium was studied by measuring isoelectric points (IEP) and zeta potentials in isotonic media. The role of drug adsorption was demonstrated with gastrointestinal peptides GLP-1(7-37) and PYY (3-36) and the selection of isotonic additive was demonstrated with peptide-loaded PSi nanoparticles. The results show the notable effect of isotonic solutions and peptide adsorption on zeta potential of PSi nanosuspensions. As a rule of thumb, the sugars (sucrose, dextrose and mannitol) seem to be good media for negatively charged peptide-loaded particles and weak acids (citric- and lactic acid) for positively charged particles. Nevertheless, perhaps the most important rule can be given for isotonic salt solutions which all are very poor media when the stability of nanosuspension is considered.

1. Introduction

The advantages of porous nanoparticles are generating growing interest in the research of drug delivery devices (Park et al., 2009; Bimbo et al., 2010; Zhao et al., 2011). These advantages can be divided into two categories, those arising from the porosity of the material and those from the particle size. In porous silicon (PSi), both properties can be tuned by controlling electrochemical etching parameters, which make it a very versatile material for many different biomedical applications (Canham, 1995; Low et al., 2009; Park et al., 2009; Wang et al., 2010).

Enhanced dissolution behavior (Salonen et al., 2005), sustained release (Kilpeläinen et al., 2009; Kovalainen et al., 2012) and avoidance of enzymatic degradation of the active substance (De Rosa et al., 2011) can be achieved by loading the drug molecules into the pores. The latter two are of great importance in peptide delivery because of the sensitivity of the molecule to the medium properties (i.e. pH, ionic strength and temperature) and to the enzymatic reactions. Poor stability leads to a short half-live *in vivo*, thus necessitating frequent administration of high doses (Allen and Cullis, 2004). The stability of peptide formulations can be enhanced with excipients like buffers, antioxidants, sterilizers and preservatives. Many excipients are also used to optimize the formulation for different administration routes (Jorgensen et al., 2009).

The size benefit of nanoparticles, compared for example with microparticles, is self-evident because of the dimensions of the biological system (Arruebo et al., 2007). Nanoparticles, which are in general in the form of aqueous suspension (nanosuspension), could be administered as injections, and they are small enough to travel in the circulatory system. Also interesting is the possibility to target nanoparticles to a certain tissue, cell or even a part of a cell. This is possible because of specific pharmacokinetic behavior achieved with modifications of the particles instead of sensitive peptides (Gu et al., 2011). For example, cell membrane impermeable proteins can be hidden within the more permeable nanoparticles (Slowing et al., 2007).

* Corresponding author at: Department of Physics and Astronomy, University of Turku, FI-20014 Turku, Finland. Tel.: +358 2 333 6562; fax: +358 2 333 5070.
E-mail address: jarno.salonen@utu.fi (J. Salonen).

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Particle interaction with a cell membrane is the most important mechanism affecting particle behavior in the biological system from the targeting and toxicity perspective. In this interaction, the properties of the particle surface play a key role (Huang et al., 2010; Zhao et al., 2011). Porous silicon is a widely studied biomaterial, which is particularly interesting because its intrinsic properties, such as surface area, pore size and particle size, can be controlled in the fabrication process (Salonen et al., 2008). After fabrication, PSi surface chemistry can be modified in many different ways, and the resulting surface chemistry greatly affects the nanosuspension properties. In the present study, we focus on the suspension property referred to as zeta potential.

Zeta potential is of great interest because of its importance in several different phenomena related to nanoparticle-based drug delivery systems, especially in the stability of the suspension (Hunter, 2001). The high absolute value of zeta potential generates a repulsive electrostatic force between the particles, which is a key property of an agglomeration resistant suspension. The importance of suspension stability has led to wide research activity in the field of colloid science, and it has become evident that a complex suspension medium might have a great effect on zeta potential, even greater than the native surface charge. Therefore, the choice of the formulation excipients should be made by carefully studying its effect on the properties of the nanosuspension.

Zeta potential is sensitive to the surrounding medium basically in two different ways. The surface charge of nanoparticles in most cases originates from the pH dependent ionization of the surface groups. The influence of pH changes can be characterized by determining the isoelectric point (IEP), which is pH value where zeta potential reaches the value zero (Kosmulski, 2009). Another impact of the medium arises from the fact that zeta potential is calculated from particle mobility measurement, in which the adsorbed surface layer cannot be distinguished from the moving body. Sensitivity to the medium, together with various measurement methods, makes zeta potential a powerful indicator for the characterization of nanoparticles surface chemistry (Rosenholm and Lindén, 2007), drug molecule adsorption (Nieto et al., 2010) and suspension stability in a biologically relevant medium (Bergman et al., 2008).

Zeta potential also has intrinsic value in biological systems. The renal clearance of nanoparticles or uptake by macrophages in the mononuclear phagocyte system (MPS) is a problem when considering parenteral administration (Park et al., 2009; Bimbo et al., 2010). The immune system reacts to foreign particles through protein adsorption, which enables macrophages to recognize the particles. This so-called opsonization is affected by zeta potential, surface polarity and particle size (Patil et al., 2007; Serda et al., 2009). PEGylation of nanoparticle surface is one of the best known opsonization-reducing treatments and its efficiency is attributed to its capability to neutralize zeta potential, to make surfaces hydrophilic and to cause a steric hindrance for adsorption (Zhang et al., 2002; Owens and Peppas, 2006). Furthermore, zeta potential has been noted to play a key role in non-specific nanoparticle–cell membrane interaction. Positive zeta potential enhances the uptake (Slowing et al., 2006; Serda et al., 2009; Asati et al., 2010), which is attributed to the fact that, despite the small and fragmented positively charged domains, most of the cell membranes are overall negatively charged (Wilhelm et al., 2003).

The main objective of the present study was to demonstrate the influence of medium composition on the zeta potential of PSi nanoparticles. This was done by measuring zeta potential in different isotonic media that are commonly used when formulating nanosuspensions (Budavari et al., 2001). We have also demonstrated how therapeutic peptide molecules can affect zeta potential. The experiments were made with the gastrointestinal peptides GLP-1 (7-37) and PYY (3-36), both of which are released endogenously (Karhunen et al., 2008) and have promising applications in obesity treatment (Wren and Bloom, 2007). These two peptides were chosen because of the difference in pH-dependent charging behavior. As a basic characterization procedure and as a support for the reasoning in excipient studies, the pH-dependent behaviors of PSi nanoparticles of different surface chemistries were also studied. Finally, the rational selection of isotonic medium was demonstrated with peptide-loaded PSi nanoparticles.

2. Materials and methods

2.1. Porous silicon fabrication and stabilization

Porous silicon (PSi) was anodized from boron doped p^+-type Si(100)-wafers (Cemat Silicon S. A.) with a resistivity of 0.01–0.02 Ω cm. A solution of hydrofluoric acid (HF, 38%) and ethanol was used as an electrolyte with a 1:1 volumetric ratio. Electrochemical etching of multilayer PSi-film was carried out by applying two different current densities successively. The smaller etching current density of 50 mA/cm^2 was used as a work pulse to form a mesoporous structure for nanoparticles. The work pulse was followed by a high current density pulse of 200 mA/cm^2, which is expected to lead to the formation of a brittle fracture layer in between the mesoporous layers. After the higher current pulse, a brief pause was applied to allow the stabilization of concentration gradients in the electrolyte.

The hydrogen-terminated surface of the anodized PSi was stabilized using three different surface treatments. Thermally oxidized porous silicon (TOPSi) was obtained by heating the anodized films in air at 300 °C for 2 h. Thermal hydro-carbonization of PSi (THCPSi) was made in constant acetylene/N_2 flow at 500 °C. By continuing the treatment and by annealing the material at 800 °C, surface hydrogen completely dissociates, and thermally carbonized porous silicon (TCPSi) is formed. For detailed description of treatments, see references Salonen et al. (2004, 2005) and Bimbo et al. (2010).

Further functionalization of the THCPSi films was carried out by attaching covalently 10-undecylenic acid to the surface hydrocarbons according to the method described by Kovalainen et al. (2012). Accordingly fresh THCPSi films were immersed in 120 °C undecylenic acid for 4 h. The functionalization of the TCPSi surface was made via silanization of the surface with (3-aminopropyl)-triethoxysilane (APTES) (Mäkilä et al., submitted). These functionalized particles are referred to as UnTHCPSi and APSTCPSi, respectively.

2.2. Porous silicon nanoparticles

After the surface treatments, the free-standing PSi films were wet ball-milled (Fritsch Pulverisette 7). Ethanol was used as a grinding medium for the UnTHCPSi, TCPSi and TOPSi films. In the case of APSTCPSi, the functionalization was carried out during the milling in 10% APTES-toluene solution. With THCPSi, particular attention was paid to avoiding oxidation by using 1-decene as a grinding medium. Fractioning of the nanoparticles from the polydisperse mixture of PSi and grinding medium was done with centrifuge (Thermo Scientific IEC MicroCL 17).

The diverse stability behavior arising from the different surface chemistries dictates the selection of centrifugation medium. An attempt was made to keep the particle size distributions of the PSi nanoparticles constant by altering the centrifugation parameters so that the particle size distribution of the supernatant was within the desired limits. Centripetal accelerations from 1500 × g to 2700 × g were applied for the fractioning, and a maximum acceleration of 17,000 × g was applied to change the dispersion medium.

The particles were redispersed with ultrasound when necessary, and ethanol was used as a storage medium.

Particle size was characterized by dynamic light scattering (DLS) measurements (Malvern Zetasizer Nano ZS) which are based on the intensity fluctuations of back-scattered (178°) laser light, caused by the spatial movement of nanoparticles. This so-called Brownian motion can be expressed by a diffusion coefficient that is inversely proportional to the diameter of the particle. The non-negative least squares (NNLS) algorithm (Morrison and Grabowski, 1985) with a higher smoothing parameter, the so-called general purpose mode, was used for the deconvolution of particle size distribution from the autocorrelation function of scattered light intensity. The cumulants algorithm (Koppel, 1972) was used for calculations of the average size (Z-average) and polydispersity index (PdI). All the measurements were made in ethanol, with the exception of the THCPSi nanoparticles which were measured in dimethyl-formamide (DMF) because of fast agglomeration in ethanol.

2.3. IEP measurements

The electrophoretic mobility of the PSi nanoparticles with different surface chemistries and nanosuspension media was measured with electrophoretic light scattering (ELS) using the Malvern Zetasizer Nano ZS. Zeta potential ζ was calculated from the electrophoretic mobility u_e with equation

$$u_e = \frac{2}{3}\frac{\varepsilon}{\eta}\zeta f_1(\kappa a), \quad (1)$$

where permittivity ε and viscosity η are properties of the medium, and $f_1(\kappa a)$ is the so-called Henry's function which depends on the particle radius a and the Debye length κ^{-1} (Henry, 1931). The Debye length can be understood as the thickness of the electric double layer which is inversely proportional to the ionic strength (Delgado et al., 2007). The value of $f_1(\kappa a)$ varies from 1.0 for low ionic strength and/or small particle size to 1.5 for high ionic strength and/or large particle size. Henry's function values for each medium are calculated with Ohshima's approximation (Ohshima, 1994).

$$f_1(\kappa a) = 1 + \frac{1}{2}\left\{1 + \left[\frac{5}{2\kappa a(1 + 2\exp(-\kappa a))}\right]\right\}^{-3}. \quad (2)$$

Zeta potential characterization of pure nanoparticle – water nanosuspension was made as a function of pH with the IEP titrations. The nanoparticle concentration was 50–100 μg/ml, and de-ionized water was used as a medium. HCl and NaOH were used as titrants, and the addition of new ions to the nanosuspension was taken into account in Henry's function. Each data point is the average of at least three individual measurements. The IEPs were determined by interpolating the titration curve in a linear fashion to the pH point at which zeta potential reaches the value zero.

2.4. Impact of the isotonic medium

The electrostatic stability of a suspension is widely considered to be sufficient when the absolute value of zeta potential is over 30 mV. According to our observations, this also holds true in the case of PSi nanoparticles, and the motivation to study isotonic media was to achieve good and stable nanosuspension in terms of zeta potential.

The isotonic concentration of an aqueous solution can be estimated by comparing its freezing point depression to an isotonic sodium chloride solution. Two solutions with the same freezing point depression have the same osmotic pressure, and they are referred to as isosmotic. It is well known that aqueous 0.9 w/v% sodium chloride solution is isotonic with blood, thus creating a minimal change to the osmotic pressure of blood when injected. Several other isotonic solutions are shown in Table 1 (Budavari et al., 2001).

Table 1
Isotonic concentrations (Budavari et al., 2001), viscosities (Lide, 2008) and measured pHs for the dispersion media. Henry's functions $f(\kappa a)$ were calculated with Eq. (2). Particle radius a was obtained from the DLS measurements (Fig. 1) and κ was calculated from the dissociation constants (Lide, 2008).

Isotonic medium		pH	Viscosity	$f(\kappa a)$
	%		mPa s	
TRIS	3.52	9.6	1.10	1.1
Sucrose	9.26	6.6	1.31	1.0
Dextrose	5.49	6.8	1.16	1.0
Mannitol	5.07	6.3	1.14	1.0
PEG 300	6.72	5.4	1.16	1.0
HEPES	6.80	5.1	1.00[a]	1.1
KCl	1.19	7.0	1.00	1.5
NaCl	0.90	–	1.02	1.5
$MgCl_2$	2.02	6.6	1.09	1.5
Citric acid	5.52	1.4	1.14	1.4
Lactic acid	2.30	1.8	1.06	1.4

[a] Viscosity of water is used.

Isotonic additives affect the viscosity and Henry's function $f_1(\kappa a)$, which are both used in zeta potential calculations. The average particle diameter used in Henry's function was measured with DLS. These properties are tabulated in Table 1.

2.5. Peptide adsorption and rational selection of the isotonic media

The peptides were purchased from BCN Peptides (Barcelona, Spain). Peptide YY (3-36) and glucagon-like peptide-1 (7-37) are referred to as PYY and GLP-1, respectively. In the case of peptides or proteins, the isoelectric point can be estimated to the pH point where the charges of amino acids cancel each other out and the total charge of the sequence is neutral.

In the present study, the isoelectric points were calculated with the Henderson–Hasselbalch equation as described in detail by Cameselle et al. (1986). Amino acid dissociation constants were obtained from Lehninger Principles of Biochemistry (Nelson and Cox, 2000). The isoelectric points for the peptides studied were pH 7.7 and 5.4 for PYY and GLP-1, respectively. This meant that the charge of peptides in neutral or slightly acidic pH is reverse, PYY was positively charged and GLP-1 was negatively charged.

In preliminary tests, zeta potential of the nanosuspension (PSi concentration 100 μg/ml) was measured as a function of the peptide concentration. Zeta potential change in the case of both peptides reached saturation level at a concentration of 100 μg/ml, which was used in the subsequent experiments.

Ethanol, as a nanosuspension storage medium, was changed to aqueous solution by pelleting nanoparticles with centrifuge and removing the supernatant. Next, the peptide solutions were made, added to the top of the PSi particles and dispersed with ultrasound. The dispersions were equilibrated at least 30 min before zeta potential measurements; pH of the PSi–peptide nanosuspension was also measured. Based on zeta potential measurements of the PSi–peptide nanosuspension, the isotonic medium was selected. A small volume of the selected stock solution was added to the formulations and zeta potentials were measured again.

3. Results and discussion

3.1. Porous silicon nanoparticles

The particle sizes were measured with DLS in order to standardize the size of nanoparticles with different surface chemistries. Particle diameters (Z-average), calculated with a cumulants algorithm, were 132 nm, 129 nm, 164 nm, 146 nm and 153 nm for TOPSi, THCPSi, UnTHCPSi, TCPSi and APSTCPSi, respectively.

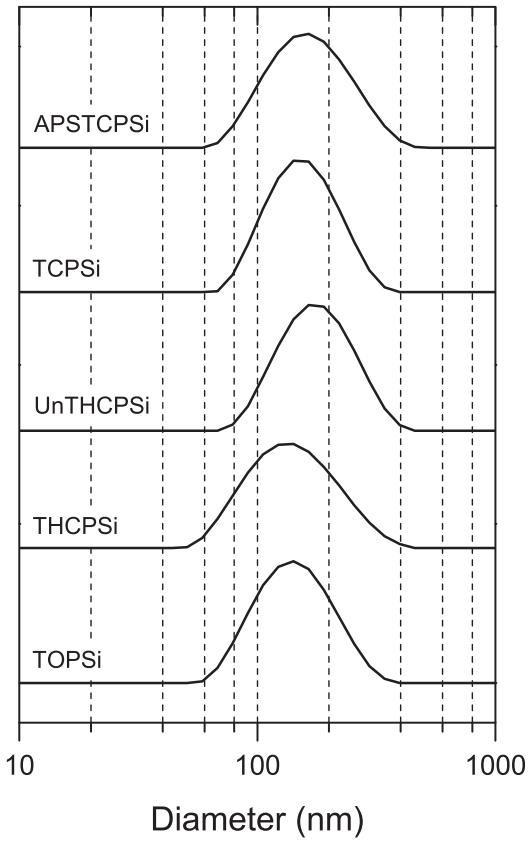

Fig. 1. Particle size distributions of porous silicon nanoparticles with all studied surface chemistries measured from dynamic light scattering. THCPSi nanoparticles were measured in DMF and the rest in ethanol.

Polydispersity indices (PdI) were in between 0.090 and 0.167. Particle size distributions, calculated with the NNLS algorithm, are shown in Fig. 1. Fairly similar average sizes and almost monodisperse size distributions were achieved. The average sizes and PSDs are typical of PSi nanoparticles prepared in this fashion (Bimbo et al., 2010, 2011), and no agglomeration was observed. Z-average particle diameters were used when calculating the values of Henry's functions for isotonic media (Table 1).

3.2. IEP measurements

The effect of surface chemistry and pH on zeta potential was studied with acid-base titrations. The titrations were made with several different nanoparticle batches out of which the IEPs obtained are reported in Fig. 2. Typical titration curves can be seen in Fig. 3. The results show differences in the IEPs between PSi nanoparticles with different surface chemistries.

The lowest IEPs, 2.6 and 3.3, can be found from TOPSi and TCPSi, respectively. The high treatment temperature of TCPSi enables the dissociated acetylene to be absorbed into the PSi crystal structure leading to the formation of a thin surface oxide layer on top of a silicon carbide layer (Sarparanta et al., 2011). The IEPs for TOPSi and TCPSi are consistent with the IEP of silica, which is generally assumed to be between 2 and 3, due to the presence of surface silanol groups (Parks, 1965; Rosenholm et al., 2007). The observed differences between the IEPs of TOPSi and TCPSi could be due to the differences in the surface oxide structure (Sarparanta et al., 2011).

Interesting IEP results were also obtained in the case of the THCPSi nanoparticles. The surface of this material is terminated by hydrocarbon groups (Salonen et al., 2004), which cannot be ionized, thus a pH independent behavior of surface charge should have

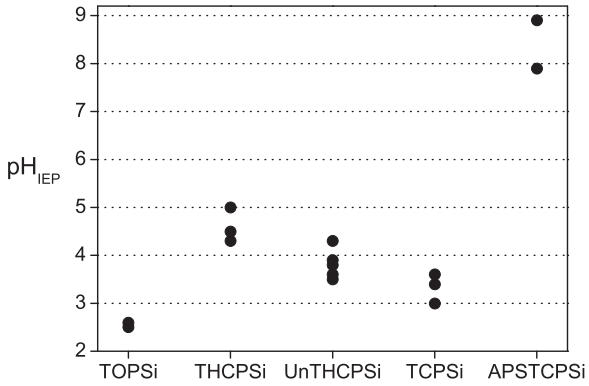

Fig. 2. Isoelectric points of porous silicon nanoparticles with different surface chemistries.

been observed. However, we managed to measure the IEP of 4.6 for the THCPSi in a repeatable fashion. Successful IEP measurements of hydrophobic materials have also been reported previously, in which the origins of the surface charge are attributed to impurities in the solution or to the specific adsorption of electrolyte ions or hydroxyl ions (Tandon et al., 2008). In our case, the measurements were made in deionized water, and a limited number of chemicals were used in the fabrication. These facts give reason to speculate that the adsorption of –OH ions could be the reason for these results. Another option is the formation of unwanted surface silanol in the ball-milling of PSi films.

The functionalization of TCPSi was made by attaching amine terminated organosilane (APTES) to the HF-activated surface silanols via a siloxane bond that increased the IEP significantly from 3.3 to 8.4. This behavior is also typical of similarly functionalized silica and is explained by the decrease in the number of free silanol groups and by the presence of the primary amine groups on the surface (Rosenholm and Lindén, 2007).

A shift of the IEP to pH 3.8 was observed with UnTHCPSi when compared to THCPSi. Much of the hydrophobic and neutral groups on the particle surface are replaced by a hydrocarbon chain terminated with a –COOH functional group as described by Kovalainen et al. (2012). This promotes pH-dependence and turns the surface charge and zeta potential to be more negative in neutral pH range.

Despite uncertainty in the case of the THCPSi nanoparticles, the IEP results indicate the maintenance of the special characteristics of surface chemistries during the complex fabrication process of PSi nanoparticles. The results also indicate successful surface

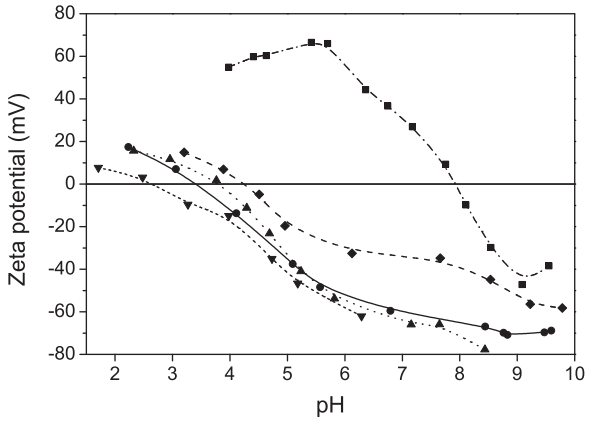

Fig. 3. Typical titration curve for PSi nanoparticles with different surface chemistries (■) APSTCPSi (♦) THCPSi (●)TCPSi (▲)UnTHCPSi (▼) TOPSi. Titrations were made in deionized water. HCl and NaOH were used as titrants. Lines are included to guide the eye.

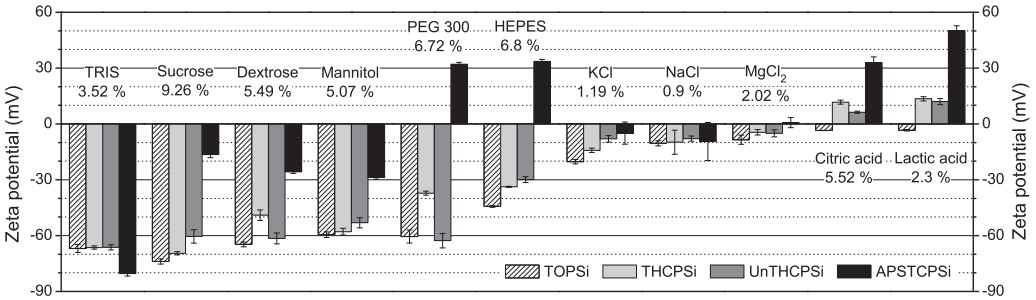

Fig. 4. Zeta potential of the PSi nanoparticles with different surface chemistries dispersed in isotonic media.

functionalizations, highlighting the importance of biofunctionalization when controlling the zeta potential of PSi nanoparticles in a biologically relevant pH area.

3.3. Impact of the isotonic medium

The isotonic media were found to have a significant effect on the zeta potential of the PSi nanoparticles (Fig. 4). TRIS (2-Amino-2-hydroxymethyl-propane-1,3-diol) as a highly basic compound increases nanosuspension pH to the area where zeta potential reaches its saturation value in every surface chemistry, and differences in between different surface chemistries disappear. Slightly acidic sucrose, dextrose and mannitol generate a good isotonic medium (in terms of electrostatic stability) for negatively charged nanoparticles, but for positively charged APSTCPSi these additives reverse zeta potential. This could be due to the adsorption of the molecules to the surface which could be seen also as a slight decrease of zeta potential to more negative values in case of hydrophobic THCPSi particles.

A somewhat unexpected result was the almost indistinguishable effect of PEG 300 on zeta potential of the nanoparticles. Adsorption of neutral molecules on the charged surface should cause the movement of the slip plane away from the surface causing a decrease in the absolute value of zeta potential (Koopal et al., 1988). We have noted that this holds also true in case of PSi nanoparticles, and the decrease of zeta potential depends also on the size of molecule (Rytkönen et al., submitted). The PEG molecule that was used in this study was very small thus insufficient to decrease zeta potential significantly.

All salt solutions are very poor media for PSi nanoparticles because the high ion concentration neutralizes the surface charge and shifts zeta potential practically to zero. HEPES also shifted the zeta potential of TOPSi and UnTHCPSi toward zero, although to a lesser extent than the salt solutions.

More acidic solutions of citric and lactic acid maintain the positive zeta potential of the APSTCPSi nanoparticles, but are at the same time very poor media for negatively charged particles. The reason for this is clearly the shift of pH to more acid and toward the IEP of the negatively charged particles.

3.4. Peptide adsorption and rational selection of the isotonic media

The effect of peptide adsorption on the zeta potential of the PSi nanoparticles was studied with two different peptides, PYY and GLP-1. These peptides were selected because the overall charge of the sequences is the opposite in a neutral aqueous solution, but it should be noted that charge calculations does not take into account the tertiary structure of the peptide. Peptide zeta potential is dominated the charged groups located in the outer surface of folded peptide. Also structural changes during the adsorption and changes in orientation in the surface can change observed zeta potential (Jarvis et al., 2010).

Preliminary tests (Fig. 5) were made in order to determine the minimum peptide concentration, in which zeta potential reaches the plateau value. The effect of peptide adsorption on the zeta potential of PSi nanoparticles with different surface chemistries is presented in Table 2. The effect was the strongest for the THCPSi nanoparticles. The original zeta potential in the corresponding pH was slightly negative (−15 mV), but it increased to +40 mV when PYY was added and decreased to −44 mV when GLP-1 was added. This significant change in zeta potential as a result of peptide adsorption to the almost neutral surface can be attributed to hydrophobic interaction between the particle and the peptide (Ferstl et al., 2011; Florence and Attwood, 2011; Jarvis et al., 2010).

The more emphasized role of electrostatic interaction can be seen in the peptide adsorption to TCPSi, UnTHCPSi and APSTCPSi nanoparticles. The surface of these nanoparticles is more

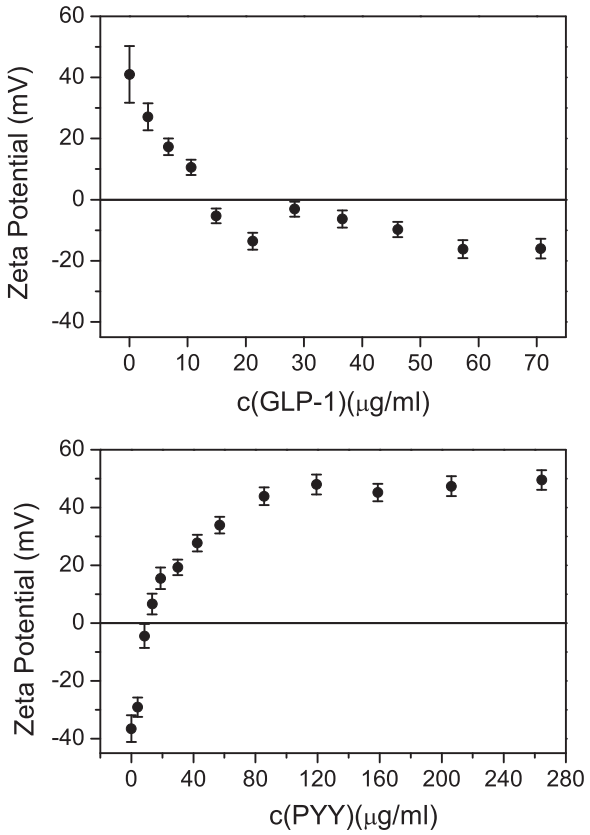

Fig. 5. Zeta potential of aqueous PSi nanosuspension (100 μg/ml) as a function of peptide concentration. Plot above shows the zeta potential behavior of APSTCPSi nanoparticles as a function of GLP-1 concentration and the one below shows the same behavior for THCPSi nanoparticles as a function of PYY concentration.

Table 2
Measured zeta potentials of PSi nanoparticles in peptide solutions (100 μg/ml). Left column represents a reference of pristine nanosuspension in corresponding pH conditions estimated from the pH titrations (Fig. 3).

Particle	Zeta potential (mV)		
	unloaded	PYY	GLP-1
THCPSi	−15 ± 6	40	−44
UnTHCPSi	−54 ± 4	13	−53
TCPSi	−46 ± 3	4.4	−53
APSTCPSi	>57	59	6.1

Table 3
Zeta potentials of PYY loaded PSi nanoparticles before and after the addition of isotonic medium.

Particle	Zeta potential (mV)		Isotonic medium
	Before[a]	After[a]	
THCPSi	40	72	Lactic acid
UnTHCPSi	13	61	Lactic acid
TCPSi	4.4	45	Lactic acid
APSTCPSi	59	66	Lactic acid

[a] Addition of isotonic medium reported in last column.

Table 4
Zeta potentials of GLP-1 loaded PSi nanoparticles before and after the addition of isotonic medium.

Particle	Zeta potential (mV)		Isotonic medium
	Before[a]	After[a]	
THCPSi	−44	−40	Dextrose
UnTHCPSi	−53	−50	Dextrose
TCPSi	−53	−47	Dextrose
APSTCPSi	6.1	69	Lactic acid

[a] Addition of isotonic medium reported in last column.

hydrophilic and more charged due to silanol and carboxyl groups. Positively charged PYY increased the zeta potential of the negative TCPSi and UnTHCPSi to near zero, while it kept the zeta potential of the positive APSTCPSi almost constant. The same applies to the negatively charged GLP-1, which did not significantly affect the negatively charged particles, but decreased the zeta potential of positive APSTCPSi.

In order to achieve good agglomeration-resistant suspension (absolute zeta potential more than 30 mV), the selection of the isotonic medium for each peptide-loaded nanoparticle could be made based on the previously obtained information about the effect of each isotonic medium on nanoparticles. All the salt solutions can be directly rejected because the high ionic strength causes the drop of zeta potentials practically to zero. Both sugars and mannitol are good isotonic additives for the formulation of negatively charged nanoparticles, although the adsorption effect of the molecules, especially toward the hydrophobic surface, should be considered carefully. Lactic and citric acids are good media for nanoparticles with high IEP, but the decrease in formulation pH is not always a desirable effect. Short length PEG and HEPES also seem to provide the sufficient zeta potential for a stable formulation.

The selection of an isotonic additive was demonstrated with two different media. We chose lactic acid for the peptide-loaded nanoparticles with a positive zeta potential and dextrose for peptide-loaded nanoparticles with a negative zeta potential. With these selections we were able to keep the absolute zeta potential of the formulations above 40 mV, which is sufficient to resist agglomeration. There results can be seen in Tables 3 and 4.

PYY-loading increased zeta potential of negatively charged nanoparticles to positive and the addition of lactic acid even increased it further. When the zeta potential of these same particles without peptide-loading but with lactic acid as a isotonic medium (Fig. 4) gave the near zero value, it can be deduced that the PYY stays in the particle surface and the increase in zeta potential is due the positive charge of both, peptide and nanoparticle as a result of the decreased pH. The same observation can be made also from positively charged APSTCPSi in the case of both peptides.

After GLP-loading, zeta potential of negatively charged nanoparticles decreased or did not change so that the zeta potential differences between THCPSi, UnTHCPSi and TCPSi surfaces almost diminished. We chose dextrose for isotonic medium to these particles because it has only minor effect on pH and thus, also on zeta potential.

4. Conclusions

The effect of surface chemistry on the zeta potential of PSi nanosuspension is considerable as can be observed from the differences in the acid–base titrations. We obtained IEPs of 2.6, 3.3, 3.8, 4.6 and 8.4 for TOPSi, TCPSi, UnTHCPSi, THCPSi and APSTCPSi nanoparticles, respectively. These results can also be seen as additional evidence of success in the surface treatments and in the maintenance of specific characteristics of different surfaces through the nanoparticle fabrication process.

It is important to characterize the zeta potential of the nanosuspension because of its role in agglomeration and toxicity. It must be kept in mind that zeta potential is not an intrinsic property of a nanoparticle, but as we have demonstrated, it is also affected by the properties of other formulation components like the isotonic medium and the loaded peptide. The peptides studied, GLP-1 (7-37) and PYY (3-36), had the opposite effects on the zeta potential of the PSi nanoparticles. The negatively charged GLP-1 decreased the zeta potential of the positively charged nanoparticles to neutral, and the positively charged PYY increased the negative zeta potential to neutral or positive. When the particles and peptides had the same charge, no change in zeta potential was observed. For the neutral and hydrophobic THCPSi nanoparticles, zeta potential was completely determined by the adsorbed peptide due to strong adsorption caused by the hydrophopic interactions.

In the case of particle-mediated drug delivery, the selection of a proper carrier particle has to be made considering also its loading and release properties. At the same time, the adjustment of zeta potential to a desired value is difficult. As we have shown, drug loading can turn zeta potential of a particle to neutral or even reverse it. By choosing the isotonic nanosuspension medium properly, the unwanted effect of the neutral zeta potential may be avoided and injection can be made safely. As a rule of thumb, the sugars (sucrose, dextrose and mannitol) seem generate good isotonic media for negatively charged peptide-loaded particles and weak acids (citric- and lactic acid) for positively charged ones. Nevertheless, perhaps the most important rule can be given for isotonic salt solutions which all are very poor media when the stability of nanosuspension is considered.

These results highlight the importance of the rational selection of the isotonic medium for nanoparticle–peptide formulation. In addition to the isotonicity of the medium, parameters such as medium pH and the surface chemistry of nanoparticles are of great interest and need to be studied carefully when designing stable and functional nanosuspensions for parenteral peptide delivery.

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