The potential of chitosan-tripolyphosphate microparticles in the visualisation of latent fingermarks

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Abstract

Chitosan (CS) is a cationic polymer with excellent film, gel and particle-forming properties. This polymer has been investigated widely for its potential in the development of food and drug delivery systems and pharmaceutical applications, however it has not generally been considered in forensic applications for example fingerprints (fingermarks). Fingerprints are a very common form of physical evidence. The most commonly used procedure for revealing the ridge pattern is powder dusting, which relies on the mechanical adherence of fingerprint formulation to the fatty components of the skin deposit that are secreted by sweat pores that exist on friction ridges. Cross-linking between oppositely charged molecules can be used to prepare chitosan microparticles. Tripolyphosphate (TPP) is a nontoxic polyanion; it can form particles by ionic interaction between positively charged amino groups of CS and negatively charged counter ions of TPP. In the present study chitosan microparticles (CSMPs) were prepared under four different processing/ formulation conditions. The development of latent fingermarks using CSMPs was analysed by using a 2^3 factorial design, which considered simultaneously three main factors: pH, ionic strength and CS: TPP (v/v) ratio. In this study CS: TPP ratio has the strongest effect on fingerprint quality. The best conditions for fingerprint visualisation were pH 4.8, CS: TPP of 2:1 and 0.2 M of ionic strength in buffer (AB-12).

Keywords

Latent fingermark development; chitosan; microparticles; non-porous surfaces; formulation engineering

Highlights

- Chitosan-TPP nanoparticles show potential in latent finger mark visualisation
- Fingerprint quality depends on formulation conditions
- The best conditions were pH 4.8, CS: TPP of 2:1 and 0.2 M of ionic strength

1. Introduction

Since the late 1800s, fingermark or fingerprint recognition has formed the central pillar of forensic science, taking advantage of the fact that no two individuals possess identical fingerprints (Hazarika, Jickells & Russell, 2009). Fingerprints, or fingermarks, are made when the tip of the finger comes into physical contact with a surface and leaves an impression of the ridges. These ridges contain a complex mixture of natural secretions of the body, and external contaminations from the environment (Champod, Lennard, Margot & Stoilovic, 2004). The dermis, which is the bottom layer of the skin, contains three types of secretory glands including eccrine, apocrine and sebaceous glands, whose secretions reach the skin surface through epidermal pores (Choi, McDonagh, Maynard & Roux, 2008). These secretions are transferred, depending on a number of factors including temperature of the surface, surface structure, electrostatic forces of the receptor surface, and humidity. These factors play significant roles in the visualisation and/ or development of fingermarks. A sebaceous compound adheres better to a surface that is cooler than the human body. Moreover, a rough surface will have more adhesion forces (Weyermann, Roux & Champod, 2011). Visible fingerprints can be enhanced by dusting with a powder for example flaked aluminium - that sticks to the eccrine gland residues. Invisible or latent prints (Wang, Yang, Wang, Shi & Liu, 2009) require visualisation techniques such as physical (*e.g.*, powdering), or chemical (*e.g.*, ninhydrin), or optical (*e.g.*, ultraviolet imaging) to develop (enhance) the fingermark in order for it to be readily visible and to be recovered for comparison purposes (Becue, Scoundrianos, Champod & Margot, 2008; Hazarika, Jickells & Russell, 2009; James & Nordby, 2003). Selection of the technique for fingermark development/visualisation is dependent on the composition of latent print residue (Choi, McDonagh, Maynard & Roux, 2008). However, often latent prints are difficult to develop, this will depend on their age or the surface on to which they have been deposited, and forensic scientists are continually searching for new improved methods to enhance them (Hadlington, 2012). Chitosan due to its potential as a bioadhesive (Islam, Ahmed, Sugunan & Dutta, 2007) has been investigated widely for its potential in the development of drug delivery systems and pharmaceutical applications (Morris, Kök, Harding & Adams, 2010) and more recently for its forensic applications (Il Dueik & Morris, 2013).

Chitosans are a family of linear copolymer polysaccharides consisting of β (1-4)-linked 2-amino-2-deoxy-D-glucopyranose (D-glucosamine) and 2-acetamido-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine) units with different fractions of acetylated units (Sailaja, Amareshwar

& Chakravarty, 2010), which determines the degree of deacetylation (DD). Moreover, the DD of commercial chitosan is approximately 66 - 95 %, and the molecular weight (M_W) approximately 10000 – 1000000 g/mol (Morris, Castile, Smith, Adams & Harding, 2009; Sonia & Sharma, 2011). The structural units of chitosan have one reactive primary amino group (-NH_2) on the C-2 position of each D-glucosamine unit, and two reactive free hydroxyl groups (-OH) for each C-6 and C-3 position building unit (glucosamine and *N*-acetyl-D-glucosamine). These groups (both amino and hydroxyl) can be modified to obtain different chitosan derivatives, and provide opportunities for chemical modification to impart useful physicochemical properties and distinctive biological functions (Chen, Mi, Liao & Sung, 2011; Giri, Thakur, Alexander, Badwaik & Tripathi, 2012). In addition, the advantage of chitosan over other polysaccharides is that its chemical structure allows specific modifications at the C-2 position without too many difficulties (Shweta & Sonia, 2013). Chitosan is present in solutions in a cationic polyelectrolyte form, which opens the possibility for interactions with negatively charged substances (anions and polyanions) (Il'ina & Varlamov, 2005) such as tripolyphosphate (TPP) (Giri, Thakur, Alexander, Badwaik & Tripathi, 2012; Hu, Li, Decker, Xiao & McClements, 2009; Ponnuraj, Janakiraman, Gopalakrishnan, Senthilnathan, Meganathan & Saravanan, 2015). Ionic cross-linking can occur inside the network via interactions between the negative charges of the cross-linker such as TPP and the positively charged amino groups of chitosan molecules (Berger, Reist, Mayer, Felt, Peppas & Gurny, 2004; Davis & Illum, 1999; Dyer et al., 2002; He, Davis & Illum, 1998; Janes, Calvo & Alonso, 2001; Morris, Castile, Smith, Adams & Harding, 2011; Shu & Zhu, 2000). Various techniques have been developed to prepare chitosan micro/nanoparticles, such as ionic gelation, emulsion droplet, spray drying, coacervation and self-assembly chemical modification (Jarudilokkul, Tongthammachat & Boonamnuayvittaya, 2011; Liu & Gao, 2009). Among those methods, the ionic gelation method (also known as ionotropic gelation) is the most widely used approach to physical cross-linking.

This method provides several advantages, such as its simple and mild method of preparation without the use of organic solvent, high temperatures or toxic materials (Baskar & Sampath Kumar, 2009; Chen, Mohanraj, Wang & Benson, 2007; Fan, Yan, Xu & Ni, 2012; Rampino, Borgogna, Blasi, Bellich & Cesaro, 2013; Sailaja, Amareshwar & Chakravarty, 2010). Knowledge of viscosity, zeta potential and particle size will have an influence on the mucoadhesion/ bioadhesion of chitosan-TPP microparticles and hence potential applications in

drug delivery (Wang et al., 2011) or in forensic applications such as the development of fingermarks (Il Dueik & Morris, 2013).

Traditionally the most widely used techniques for latent finger print development are powder dusting, ninhydrin dipping and iodine fuming and their effectiveness will depend upon the surface on to which the latent fingerprint has been deposited. However, these traditional methods for latent print detection are not always effective and researchers and practitioners are continually trying to improve upon these existing techniques. There are a number of different powders which have been used including for example, granular carbon particles, lead powder (Graham. 1969), Congo red dye (Sodhi, Kaur and Garg, 2003), eosin yellow dye (Sodhi and Kaur, 1999) (see Table 1 in Garg, Kumari and Kaur for more examples). Some of these chemical substances are toxic and pose potential health and environmental hazards, e.g. Congo red is a Group 1 carcinogen. In attempt to minimise these issues, we have proposed a novel fingerprint visualisation powder based on the naturally occurring positively charged polysaccharide chitosan which is cheap, readily available, non-toxic (Aramwit, Ekasit, Yamdech, 2015) and has shown potential in pharmaceutical applications (Morris, Kök, Harding & Adams, 2010) and drug delivery (Wang et al., 2011).

The purpose of the present study is to prepare different formulations of chitosan-TPP (CS-TPP) microparticles and optimisation using a 2^3 factorial factor design, with 8 experiments (in triplicate), to analyse the effects of the three selected factors (pH, ionic strength and CS: TPP ratio), in order to design particles of defined properties for latent fingerprint visualisation.

2. Materials and Methods

2.1. Materials

Chitosan of medium molecular weight (M_W ~295 000 g/mol) was obtained from Sigma–Aldrich (Gillingham, UK) and reported to have an average degree of deacetylation (DD) of ~75–85 %. Glacial acetic acid, sodium acetate trihydrate and tripolyphosphate (TPP) sodium salt were obtained from Sigma–Aldrich (Gillingham, UK) and red dye for enhanced visualisation from British Sugar (London, UK). All materials were used without any further purification.

157 **2.2. Factorial design experiment**

158 The experimental design applied in this study. The influence of three different parameters on
159 the ability of chitosan microparticles properties to enhance latent fingermark were evaluated
160 using a 2^3 factorial design composed of three factors (**Table 1a**). These factors including: pH
161 value, ionic strength, and CS: TPP ratio were selected as independent variables and set at two
162 levels each (upper and lower). The quality of fingerprint were response parameter or the
163 dependent variable (Y_4).

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165 **Table 1a**: Parameters used in the factorial design

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Factors	**Symbol**	**Lower level (-)**	**Upper level (+)**
pH value	**X_1**	3.8	4.8
Ionic strength	**X_2**	0.2	0.4
CS:TPP ratio	**X_3**	1:1	2:1
Dependent variables	**Y_4**	**Assessment quality fingerprint** (adapted from (Bandey, 2004)):	
		4: Full development – whole mark clear continuous ridge, which is very similar to granular carbon particles (control) **3**: >2/3 or mark continuous ridges, but not quite a perfect mark **2**: 1/3 – 2/3 or mark continuous ridges **1**: Signs of contact but < 1/3 of mark continuous ridges **0**: No development	

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168 The four different acetate buffers (AB) were prepared as described in **Table 1b**.

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Table 1b - Acetate buffers of varying ionic strength and pH

Acetate buffer (AB)	pH	Ionic strength (IS)
AB-10	3.8	0.2 M
AB-11	3.8	0.4 M
AB-12	4.8	0.2 M
AB-13	4.8	0.4 M

2.3.Preparation of chitosan and TPP microparticles at different ionic strengths and pH values (Acetate buffers AB-10 to AB-13)

Four different chitosan solutions were prepared by dissolving 2 g of chitosan powder in 1 L of acetate buffers (see **Table 1b**) to prepare chitosan solutions (2.0 g/L). The chitosan solutions were stirred overnight at room temperature using a magnetic stirrer. The TPP powder (1.680 g) was dissolved in 2 L of acetate buffers (AB) to prepare nine samples of TPP solution (0.84 g/L) (Dyer et al., 2002; Morris, Castile, Smith, Adams & Harding, 2011).

2.3.1. Microparticle preparation (CS:TPP)

In order to prepare an appropriate volume of the TPP solution was added drop wise to the appropriate volume of the chitosan solution make CS: TPP microparticles of ratios 6:1, 4:1, 2:1, 1:1, 1:2, 1:4 and 1:6, and the samples were then stirred at 600 rpm for 60 minutes at room temperature. The resultant microparticles spontaneously formed due to the ionic crosslinking of chitosan by sodium tripolyphosphate. 30 drops of red dye (British Sugar, London, UK) were then added to make the particles clearly visible. The resultant microparticles were left standing overnight at room temperature before being subjected to further analysis. The CS: TPP microparticles were recovered by centrifugation (Heraeus Biofuge Primo R, Thermo Fisher Scientific, Loughborough, UK) at 8500 rpm for 60 minutes and then supernatant was discarded. The microparticles were washed three times with deionised water, followed by freeze drying for 24 hours (Alpha 1-4 LD2 freeze drier (Martin Christ GmbH, Osterode am Harz, Germany). After freeze-drying, the solid material was ground with a pestle and mortar to produce powder suitable for fingerprinting applications.

2.3.2 Fingerprint enhancement

To determine the sensitivity and capability of this technique after long time, traces of fingermark were left on a glass slides (non-porous surface) and pieces of paper (porous surface) overnight. The long-time allows drying and reducing the amount of residue, and then dusted with the CS: TPP powders.

2.3.3. Scanning electron microscopy (SEM)

The surface microparticle morphology was characterised using scanning electron microscopy (SEM). The microparticles were vacuum dried, coated with gold palladium and observed microscopically (JEOL JSM 6060 LV - Oxford instruments, Abingdon, UK). Images were taken by applying an electron beam accelerating voltage of 20 kV.

2.3.4. Light microscopy

Samples were imaged using Leica compound, DM 500 and Leica stereo low powered microscope (LPM), EZ4HD and Leica LAZ software for image manipulation (Leica Microsystems, Milton Keynes, UK). Samples were prepared for imaging by powder dusting the samples on microscope slide prior to examination under the microscope.

3. Results and Discussion

The physico-chemical properties of CS: TPP microparticles in terms of infra-red spectroscopy, x-ray diffraction, viscosity, zeta-potential and particle size have been fully discussed previously (Hejjaji, Smith and Morris, 2016) and a résumé of some of the important parameters are shown in **Table 2**.

3.1. Scanning electron microscopy (SEM)

The surface morphologies of chitosan microparticles are shown in **Figure 1**. SEM images allow observations on the morphology of obtained particles is dependent on CS: TPP ratio. The effectiveness with which the microparticle powder adheres to the ridges depends on the size and shape on the particles relative small, fine smooth microparticles probably adhere more easily to fingermark residues than rough lager, coarse ones (Choi, McDonagh, Maynard & Roux, 2008). As can be seen in **Figure 1a**, that the microparticles prepared with AB-12 (pH 4.8 and I.S 0.2 M) at the higher CS: TPP ratio 2:1 had smoother surface than those of microparticles prepared with the lower CS: TPP ratio 1:6 which had a rough surface (**Figure 1b**). Therefore, those samples (2: 1) were used for further studies in this work.

<Figure 1 here>

3.2. Latent fingerprint development using chitosan microparticles

A preliminary study using all seven microparticle formations available demonstrated that CS: TPP ratios of 2:1 and 1:1 gave good yields of microparticles and showed better potential in latent fingerprint development (results not shown). Based on those results obtained in preliminary experiments pH, ionic strength and CS: TPP ratio were selected to find the optimised conditions to obtain the best quality fingerprint visualisation using a 2^3 factorial design (see **Table 1**). The formulations (F1 - F8) were easily prepared based on the ionic gelation of positively charged amino groups of CS with TPP anions (**Table 2**).

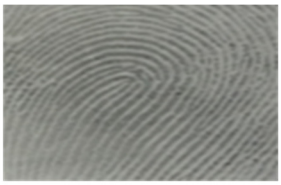
An important parameter in the characterization of microparticles is the surface charge of the chitosan microparticles indicated by zeta potential. The higher zeta potential may be related to stronger positive charges of the amino group of chitosan at high level in the factorial design experiment. The remaining amine groups (non-interacting) would be responsible for the positive zeta potential on microparticles.

Table 2: Characteristics of the chitosan microparticles obtained by the factorial design 2^3 for different formulation F1 to F8. Fingerprint quality was assessed using chitosan microparticles on glass slides.

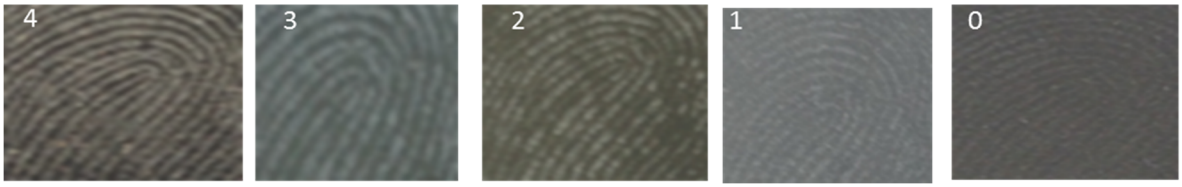
Formulation code	Dependent variables			Independent variables, mean ± SD (N = 3)			
	X_1: pH	**X_2: I.S**	**X_3: CS:TPP Ratio**	**Y_1: relative viscosity[a]**	**Y_2: zeta potential (mV)[a]**	**Y_3: particle size (µm) $D_{[4,3]}$[a]**	**Y_4: fingerprint quality[b]**
F1	3.8 (-)	0.2 (-)	1:1 (-)	1.11 ± 0.01	11.8 ± 0.9	111 ± 3	1
F2	4.8 (+)	0.2 (-)	1:1 (-)	1.03 ± 0.01	9.7 ± 0.5	135 ± 2	0
F3	3.8 (-)	0.4 (+)	1:1 (-)	1.00 ± 0.01	10.0 ± 0.7	121 ± 2	1
F4	4.8 (+)	0.4 (+)	1:1 (-)	1.02 ± 0.01	9.0 ± 0.5	158 ± 8	0
F5	3.8 (-)	0.2 (-)	2:1 (+)	1.07 ± 0.01	19.0 ± 1.5	135 ± 6	2
F6	4.8 (+)	0.2 (-)	2:1 (+)	1.09 ± 0.01	14.3 ± 1.1	171 ± 4	4
F7	3.8 (-)	0.4 (+)	2:1 (+)	1.04 ± 0.01	17.0 ± 0.6	146 ± 5	3
F8	4.8 (+)	0.4 (+)	2:1 (+)	1.06 ± 0.01	10.3 ± 0.3	194 ± 11	0

[a]Adapted from Hejjaji, Smith and Morris, 2016

[b]Y_4: Assessment quality fingerprint: (Bandey, 2004).



Latent fingerprint development using black powder consist of granular carbon particles (Control)



Where fingermarks are rated in terms of quality from 0 – 4 as per **Table 1a** and representative fingermarks from the 5 categories are shown above.

As shown in **Table 2**, the optimum quality fingerprint was obtained for three formulations: F5, F6, and F7. In addition, all the chitosan microparticle formulations are positively charged, but the values of charges for F5, F6, and F7 are higher than those of the other formulations. The ionic strength of solution in formulation F7 was at a higher level (**Table 2**) and caused an increase in quality of fingerprint compared to F5. Moreover, with an increased ionic strength at 0.4 M, the $–NH^{3+}$ on the chitosan molecules are more shielded by acetate ions (CH_3COO^-) leading to a decreased zeta potential (charge). Increase zeta potential diminished the electrostatic repulsion between the chitosan particles. In general, quality fingerprint increased with increased positive zeta potential (**Table 2**) and those samples with a zeta potential of less than +12 mV (F1, F2, F3, F4 and F8) produced prints of poor quality (1 or less on the Bandey scale (Bandey, 2004)). Of the 3 formulations which produced fingerprints of better quality F6 was the best performing (fingerprint quality of 4) and as this sample has a lower zeta potential than both F5 and F7 this suggests that the overall charge on the particles is not the only factor which affects fingerprint quality and that other interactions such as van der Waals with lipid residues of the latent fingerprint are also important. F6 also had a smoother surface, larger particle size and great viscosity than both F5 and F7, which should lead to decreased van der Waals interactions between particles and therefore potentially stronger van der Waals interactions with lipid residues than either F5 or F7.

In addition, the main (the largest) effect on quality fingerprint (Y_4) is the CS: TPP ratio (**Figure 2a**). The fingerprint quality increases as we move from low level (1:1) to higher level (2:1) of

the factor (CS: TPP ratio). However, the main effects plots also indicate that both pH and I.S have similar effects to each other on quality fingerprint. For both factors, the fingerprint quality decreases when we move from the low level to the high level pH/ I.S which indicates that the net charge on the particles (zeta potential) is important, which is evident from **Table 2**. Based on all these interpretations resulting from the factorial design, it is possible to say that under these specific conditions that the parameters at pH 4.8, ionic strength of 0.2 M and ratio CTS: TPP of 2:1 to present the best (clearly visible) quality fingerprint.

In brief, an interaction plot basically reveals whether there is an interaction between two different extraction conditions for a certain response in the fingerprint quality. When the lines are parallel, interaction effects are zero. The more different the slopes, the more influence the interaction effect has on the results (Israel, Lellouche, Kenett, Green, Michaeli & Lellouche, 2014). In **Figure 2b** all of the lines are non-parallel indicating there are interactions between the different extraction conditions, however the interaction between pH and ionic strength (I.S.) is the most significant. The 2-factor interactions are -1.25, -0.75 and 0.25 for pH*I.S, I.S*Ratio and pH*Ratio, respectively.

<Figure 2 here>

3.3. Proposed mechanism for interaction

Many researchers have investigated the ability of CS:TPP microparticles to associate with organic compounds such as peptides and proteins for pharmaceutical applications (Hu, Pan, Sun, Hou, Ye & Zeng, 2008). However, they have not been considered in forensic applications. It is proposed that chitosan microparticles deposit on to fingermarks due to the lipophilic interactions with the lipid residues in fingerprint ridges. Polycationic chitosan molecules with long carbon chains forms an ionotropic gel with the TPP polyanion which results in partially lipophilic microparticles. Then steric and van der Waals interactions occur between the lipophilic (hydrophobic) ends of long carbon chain and the lipid residues of the latent fingerprint (**Figure 3**) (Islam, Ahmed, Sugunan & Dutta, 2007).

<Figure 3 here>

Latent fingerprint developed using this technique (chitosan microparticles as a powder) on glass microscope slides obtained satisfactory results (depending on pH, ionic strength and CS:

TPP ratio). This technique relies on the chitosan microparticles adherence in the fingerprint powder to the oily component of the skin ridge deposits. The effectiveness with which the powder adheres to the ridge depends on the factors such as particle size and the charge on the particles (Sodhi & Kaur, 2001). Latent fingermarks developed using AB-12 (pH = 4.8 and I.S = 0.2 M), CS: TPP powder ratio at 2:1 are shown in **Figure 4**. This ratio formulated as a powder had high capability to enhance the fingermark. It is thought that these microparticles adsorb onto the ridges as a result of lipophilic (hydrophobic) interactions. Moreover, the attachment of CS: TPP microparticles to residues of the fingerprint can easily be seen, and revealed clearly visible marks at this ratio resulting in a high quality fingerprint image(**Figure 4c**) where fingerprints are clear enough and have significant details for comparison and identification (**Figure 4d**).

<Figure 4 here>

As can be seen from **Figure 5** the latent fingerprint development using chitosan microparticles at ratio (2:1) in buffer AB-12 is very similar to control black fingerprint, which consisted of granular carbon particles.

<Figure 5 here>

Figure 6 shows the comparison of microscope images from the ridge area of samples developed with CS: TPP at 2:1 using acetate buffers AB-12 and AB-13, where it is clear that more chitosan microparticles are deposited on fingermark ridges using CS: TPP microparticles prepared using AB-12 (**Figure 6a**). Moreover, the microparticles aggregate on the fingermark ridges creating large clusters, probably due to hydrophobic interactions between the CS: TPP microparticles and the fatty residues of the latent print. On the other hand, very little chitosan microparticles were deposited between the ridges for AB-13 (**Figure 6b**).

<Figure 6 here>

Figure 7 shows a comparison between two fingerprints, one which is 24 hours old, that had clear continuous ridges across the whole mark, and the other has been taken after six months, which retains most of the details and ridges. As a result, this method allowed the developed marks to be seen by naked eye for long periods of time. Therefore, one further advantage of this technique is that they do not quickly fade.

<Figure 7 here>

4. Conclusions

In this study chitosan microparticles were successfully obtained from the ionotropic gelation method using different processing conditions. This novel method gives us the ability to design tuneable CS-TPP microparticles for specific forensic applications. It is proposed the CS-TPP deposit onto fingerprints due to the lipophilic interaction with the fatty components in fingerprint ridges. Latent fingerprint developed using chitosan microparticles as a powder technique on glass microscope slides obtained variable degrees of success depending on how the microparticles were prepared. A clear relationship between size and charge on the microparticles and the fingerprint quality was found. In the present study it was demonstrated that CS: TPP has the strongest effect on quality fingerprint. Microparticles were obtained with average diameter of 171.3 µm and a zeta potential of 14.3 mV which may have excellent potential for applications in fingerprint development. The advantages of using chitosan microparticles as a powder technique are that they are non-toxic (Aramwit, Ekasit, Yamdech, 2015) sustainable (Yan and Chen, 2015), quick, easy to apply and able to produce good quality fingerprints under the conditions studied. As well as the developed marks can be easily visualised and remain visible for a long period of time (at least 6 months) there is therefore no requirement that the fingerprints need to be photographed immediately. To our knowledge this is the first time that particle size, shape, viscosity and zeta potential have been used as a way of predicting latent fingerprint quality. Furthermore by making small changes to the formulation conditions (pH, ionic strength, CS:TPP ratio for example) this could potentially enable the fine tuning of nanoparticles in terms of size and charge to produce better or even bespoke particles for specific applications rather than one size fits all approach.

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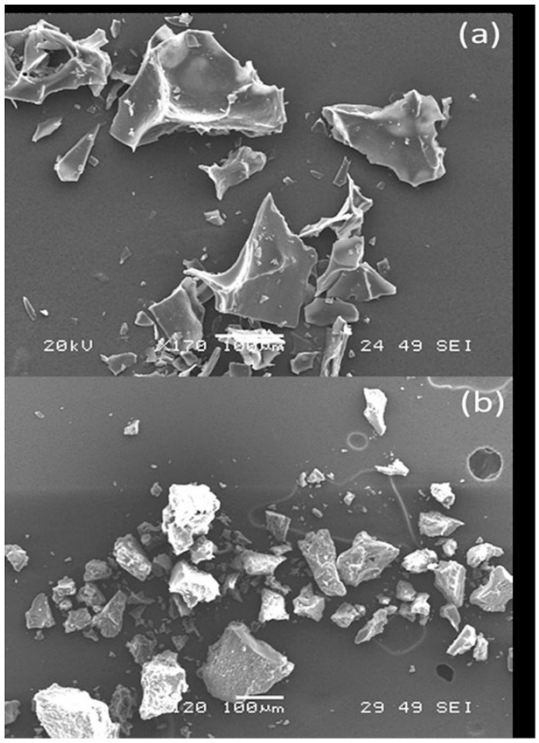

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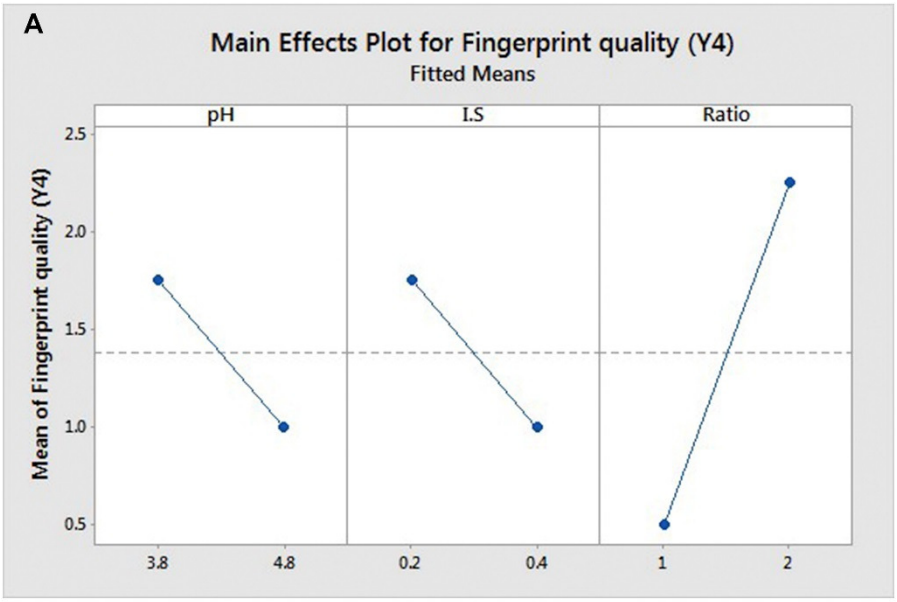
475 **Figure 1.** SEM images at 20 kV of chitosan microparticles CS: TPP using AB-12 (a) 2:1 (b)

476 1:6.



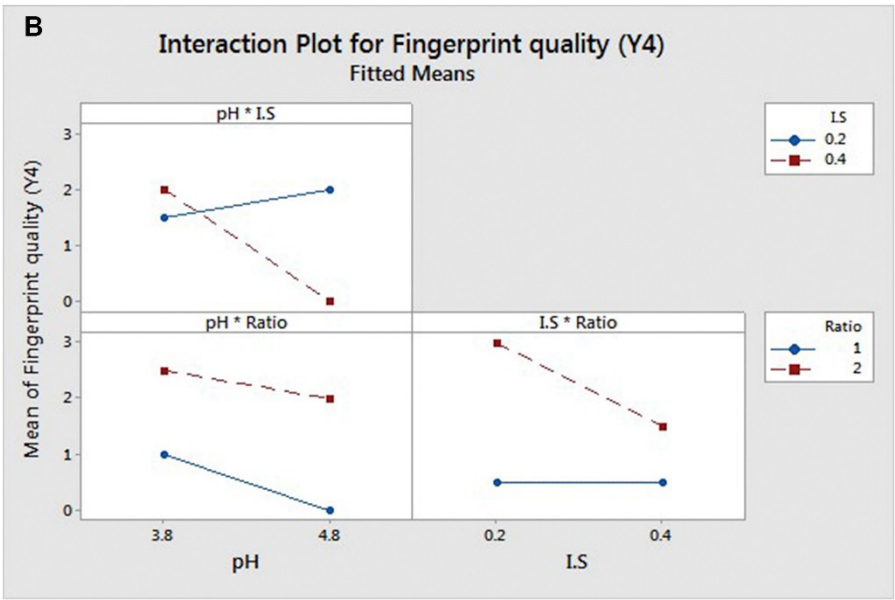


Figure 2a Error! No text of specified style in document.. The main effect plots for quality fingerprint (Y_4): pH; I.S and CS: TPP ratio. The overall mean (~1.4) is shown as dotted line and the steeper the slope the greater the effect of a particular parameter.

Figure 2b Error! No text of specified style in document.. The interactions plots for quality fingerprint (Y_4). To visualize these effects, the Y axis scale is always the same for each combination of factors. This graph shows that the pH*I.S interaction effect is the largest.

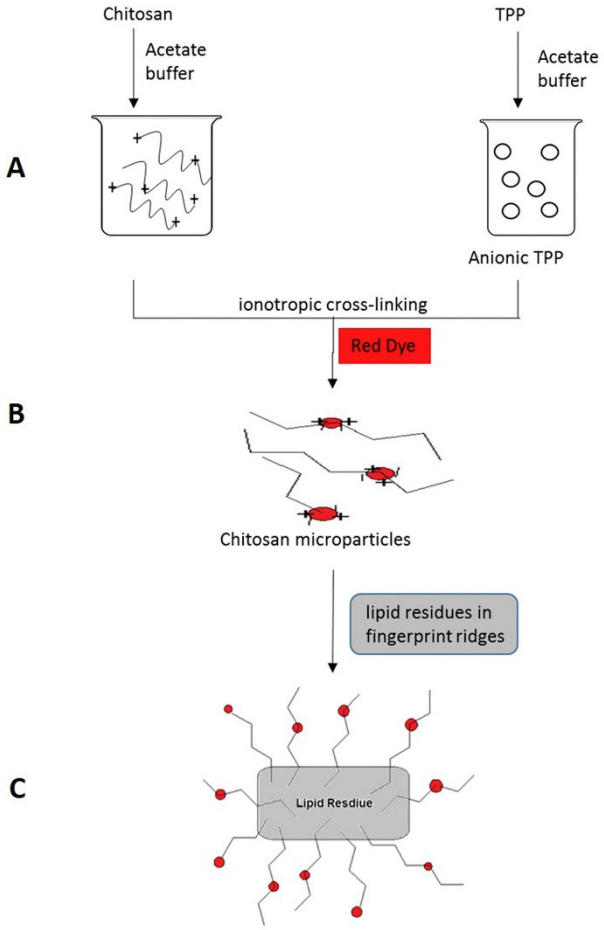


Figure 3. Schematic representation of third technique (a) chitosan carbon chains with ionic ends and TPP anions (b) chitosan polycations attraction with TPP polyanions making them lipophilic (c) the hydrophobic (lipophilic) ends of long carbon chains from chitosan microparticles burying themselves into the lipid residues of the latent fingerprint (Islam, Ahmed, Sugunan & Dutta, 2007).

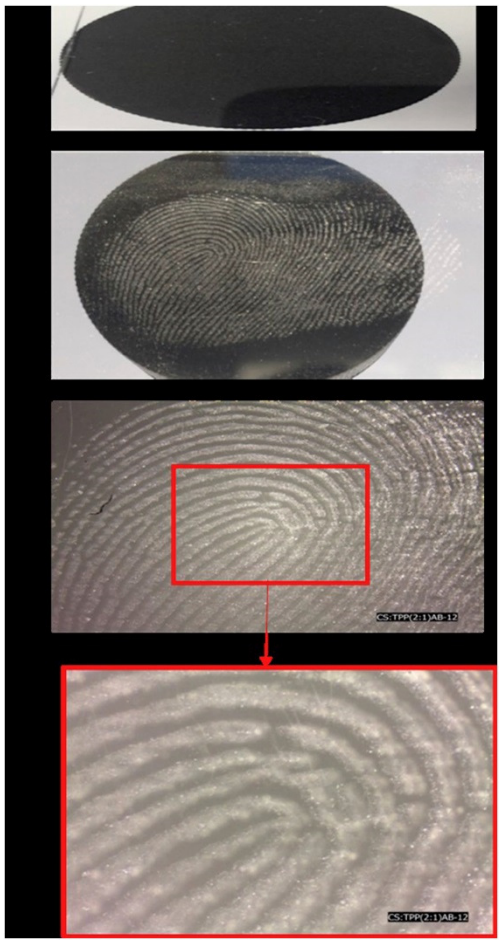


Figure 4. A developed latent fingerprint on glass slide using chitosan microparticle as a powder at CS: TPP (2:1) AB-12 (a) Before powder dusting, (b) After powder dusting (Naked eye) (c) fingerprint details under microscope, magnification 8x and (d) fingerprint details under microscope, magnification 20x.

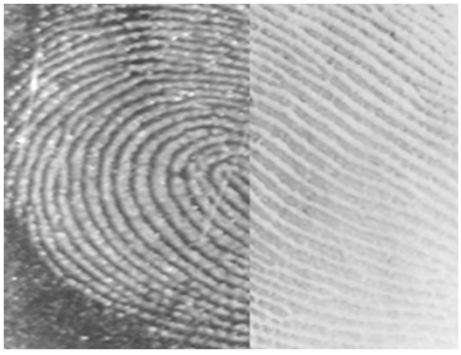


Figure 5. Comparison of latent fingerprint development on a glass slide between chitosan particles at CS-TPP (2:1) AB-12 (left half) and carbon particles as a control (right half).

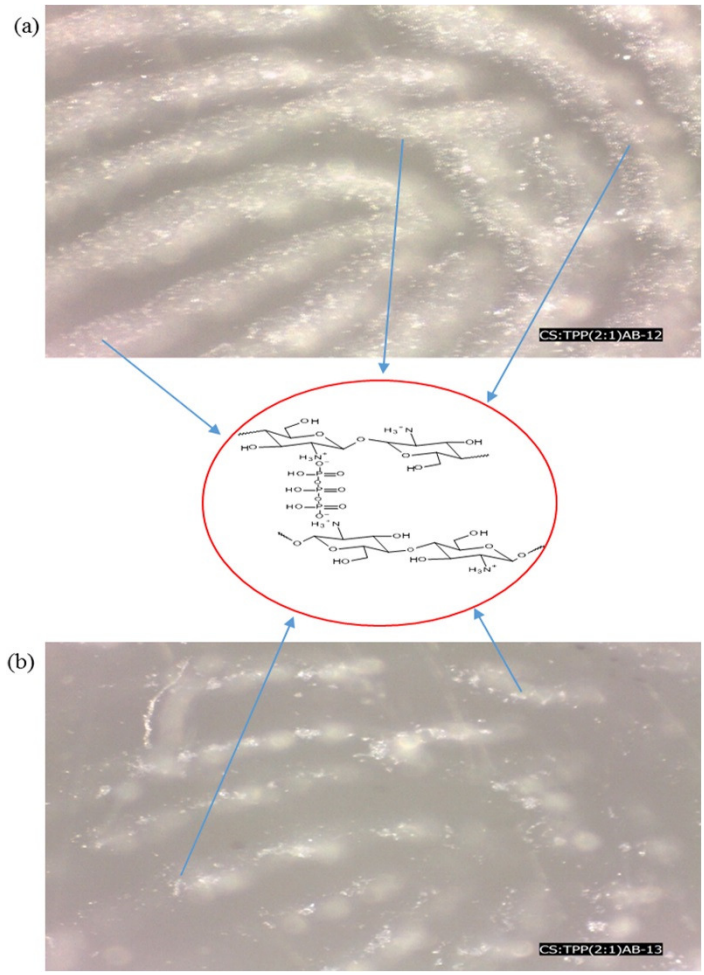


Figure 6. Chitosan microparticles at 2:1 ratio as a powder on slide adhere to the residues (fatty components) in the latent fingerprint deposit (Magnification 35x) (a) Significantly more chitosan microparticles using AB-12, (b) Very little chitosan microparticles using AB-13.

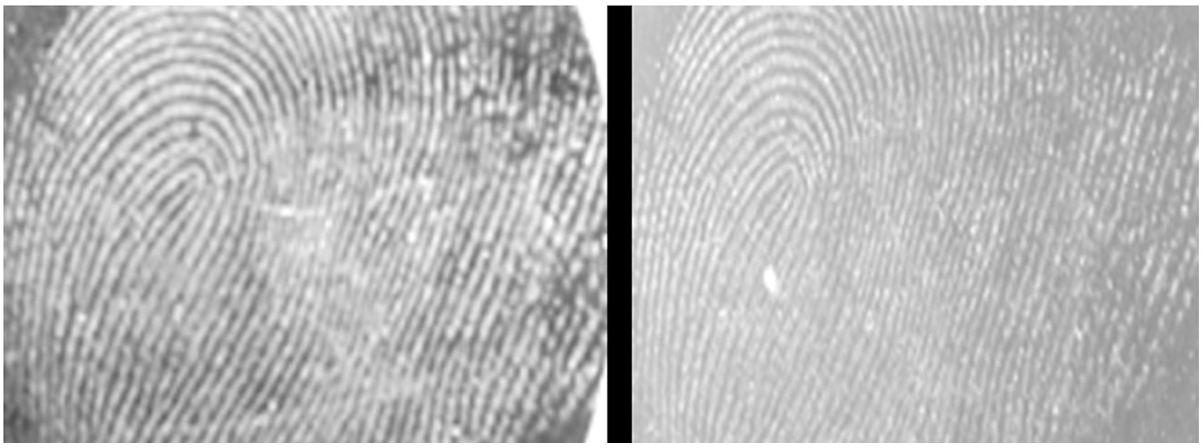


Figure 7. Latent fingerprint deposited on glass slide and developed by following the new procedure described in **Figure 3** using chitosan microparticle as a powder at CS: TPP (2:1) AB-12. Those pictures have been observed and taken: after the 24 hour (left) and after six months (right).